FILE HISTORY

13/266,862

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| INVENTORS: | Gregory Porreca<br>Uri Laserson<br>Jin Billy Li<br>E. Robert Wassman |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| APPLICATION NO: | 13/266,862 |
| FILED: | 13 MAR 2012 |
| COMPILED: | 21 AUG 2024 |

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
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| | 13266862 | PORRECA ET AL. |
| | Examiner | Art Unit |
| | STEVEN POHNERT | 1634 |

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| US CLASSIFICATION SEARCHED | | | |
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| Class | Subclass | Date | Examiner |
| | none | | |

| SEARCH NOTES | | |
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Petitioner Illumina, Inc., EX1002, p. 17

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Petitioner Illumina, Inc., EX1002, p. 18

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| consulted Kim Aron on 112 issues and art | 3/9/2017 | SCP |
| Searched EAST | 3/9/2017 | SCP |
| Inventor search EAST and PALM | 3/9/2017 | SCP |
| Assignee search: EAST | 3/9/2017 | SCP |
| consulted Joe Woitach on 112 issues and art | 11/2/2017 | SCP |
| consulted Kevin Orwig on 112 issues and art | 11/2/2017 | SCP |
| Searched EAST | 11/3/2017 | SCP |
| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |
| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| | | |
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| consulted Joe Woitach on claim interpretation, arguments and art | 02/12/2019 | SCP |
| Searched EAST | 02/12/2019 | SCP |
| Inventor search EAST and PALM | 02/12/2019 | SCP |
| Assignee search: EAST | 02/12/2019 | SCP |

| Interference Search | | | |
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| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
| | | | |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| CPC Combination Sets - Searched* | | |
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| Symbol | Date | Examiner |
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| US Classification - Searched* | | | |
|---|---|---|---|
| Class | Subclass | Date | Examiner |
| | none | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| Search Notes | Date | Examiner |
| Searched EAST and Plus | 1/9/2013 | SCP |
| Inventor search EAST and PALM | 1/9/2013 | SCP |
| Assignee search: EAST | 1/9/2013 | SCP |
| consulted Katherine Salmon on claim interpretation and 112 issues | 1/9/2013 | SCP |
| Searched EAST | 5/30/2013 | SCP |
| Inventor search EAST and PALM | 5/30/2013 | SCP |
| Assignee search: EAST | 5/30/2013 | SCP |
| consulted Steve Kapushoc on claim interpretation and 112 issues | 5/30/2013 | SCP |
| consulted Katherine Salmon on 112 issues and art | 10/23/2014 | SCP |
| consulted Joe woitach on art | 10/23/2014 | SCP |
| Consulted Kevin Orwig on art and 112 issues | 10/23/2014 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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| Searched EAST | 10/24/2014 | SCP |
| Inventor search EAST and PALM | 10/24/2014 | SCP |
| Assignee search: EAST | 10/24/2014 | SCP |
| consulted Katherine Salmon on 112 issues and art | 3/6/2015 | SCP |
| Consulted Ben Blumel on 112 issues | 3/6/2015 | SCP |
| Searched EAST | 3/6/2015 | SCP |
| Inventor search EAST and PALM | 3/6/2015 | SCP |
| Assignee search: EAST | 3/6/2015 | SCP |
| consulted Kevin Orwig on 112 issues and art | 1/12/2016 | SCP |
| consulted Joe Woitach on 112 issues and art | 1/12/2016 | SCP |
| Searched EAST | 1/12/2016 | SCP |
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| consulted Kevin Orwig on 112 issues and art | 6/7/2016 | SCP |
| Searched EAST | 6/7/2016 | SCP |
| Inventor search EAST and PALM | 6/7/2016 | SCP |
| Assignee search: EAST | 6/7/2016 | SCP |
| consulted Joe Woitach on 112 issues and art | 3/9/2017 | SCP |
| consulted Kim Aron on 112 issues and art | 3/9/2017 | SCP |
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| Inventor search EAST and PALM | 3/9/2017 | SCP |
| Assignee search: EAST | 3/9/2017 | SCP |
| consulted Joe Woitach on 112 issues and art | 11/2/2017 | SCP |
| consulted Kevin Orwig on 112 issues and art | 11/2/2017 | SCP |
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| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |
| consulted Joe Woitach on claim interpretation, arguments and art | 02/12/2019 | SCP |
| Searched EAST | 02/12/2019 | SCP |
| Inventor search EAST and PALM | 02/12/2019 | SCP |
| Assignee search: EAST | 02/12/2019 | SCP |
| consulted Dave Nguyen on claim interpretation and art | 11/25/2019 | SCP |
| consulted Joe Woitach on claim interpretation and art | 12/02/2019 | SCP |
| Searched EAST | 12/02/2019 | SCP |
| Inventor search EAST and PALM | 12/02/2019 | SCP |
| Assignee search: EAST | 12/02/2019 | SCP |
| google searched MIP, CIP, SNP, phenotype, region | 12/02/2019 | SCP |
| consulted Dave Nguyen on claims and art of record | 01/06/2020 | SCP |

| Interference Search | | | |
|---|---|---|---|
| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| Symbol | Date | Examiner |
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| CPC Combination Sets - Searched* | | |
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| Symbol | Date | Examiner |
| | | |

| US Classification - Searched* | | | |
|---|---|---|---|
| Class | Subclass | Date | Examiner |
| | none | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| Search Notes | Date | Examiner |
| Searched EAST and Plus | 1/9/2013 | SCP |
| Inventor search EAST and PALM | 1/9/2013 | SCP |
| Assignee search: EAST | 1/9/2013 | SCP |
| consulted Katherine Salmon on claim interpretation and 112 issues | 1/9/2013 | SCP |
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| Consulted Kevin Orwig on art and 112 issues | 10/23/2014 | SCP |

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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| Searched EAST | 10/24/2014 | SCP |
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| Assignee search: EAST | 10/24/2014 | SCP |
| consulted Katherine Salmon on 112 issues and art | 3/6/2015 | SCP |
| Consulted Ben Blumel on 112 issues | 3/6/2015 | SCP |
| Searched EAST | 3/6/2015 | SCP |
| Inventor search EAST and PALM | 3/6/2015 | SCP |
| Assignee search: EAST | 3/6/2015 | SCP |
| consulted Kevin Orwig on 112 issues and art | 1/12/2016 | SCP |
| consulted Joe Woitach on 112 issues and art | 1/12/2016 | SCP |
| Searched EAST | 1/12/2016 | SCP |
| Inventor search EAST and PALM | 1/12/2016 | SCP |
| Assignee search: EAST | 1/12/2016 | SCP |
| consulted Kevin Orwig on 112 issues and art | 6/7/2016 | SCP |
| Searched EAST | 6/7/2016 | SCP |
| Inventor search EAST and PALM | 6/7/2016 | SCP |
| Assignee search: EAST | 6/7/2016 | SCP |
| consulted Joe Woitach on 112 issues and art | 3/9/2017 | SCP |
| consulted Kim Aron on 112 issues and art | 3/9/2017 | SCP |
| Searched EAST | 3/9/2017 | SCP |
| Inventor search EAST and PALM | 3/9/2017 | SCP |
| Assignee search: EAST | 3/9/2017 | SCP |
| consulted Joe Woitach on 112 issues and art | 11/2/2017 | SCP |
| consulted Kevin Orwig on 112 issues and art | 11/2/2017 | SCP |
| Searched EAST | 11/3/2017 | SCP |
| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |
| consulted Joe Woitach on claim interpretation, arguments and art | 02/12/2019 | SCP |
| Searched EAST | 02/12/2019 | SCP |
| Inventor search EAST and PALM | 02/12/2019 | SCP |
| Assignee search: EAST | 02/12/2019 | SCP |
| consulted Dave Nguyen on claim interpretation and art | 11/25/2019 | SCP |
| consulted Joe Woitach on claim interpretation and art | 12/02/2019 | SCP |
| Searched EAST | 12/02/2019 | SCP |
| Inventor search EAST and PALM | 12/02/2019 | SCP |
| Assignee search: EAST | 12/02/2019 | SCP |
| google searched MIP, CIP, SNP, phenotype, region | 12/02/2019 | SCP |
| consulted Dave Nguyen on claims and art of record | 01/06/2020 | SCP |
| consulted Joe Woitach on claim interpretation and art | 06/04/2020 | SCP |
| consulted Katherine Salmon on art of record and claim interpretation | 06/04/2020 | SCP |
| consulted Kevin Orwig on art of record and claim interpretation | 06/04/2020 | SCP |
| Searched EAST | 06/04/2020 | SCP |
| Inventor search EAST and PALM | 06/04/2020 | SCP |
| Assignee search: EAST | 06/04/2020 | SCP |
| google searched mip, cip padlock, molecular inversion probe, stagger | 06/04/2020 | SCP |

| Interference Search | | | |
|---|---|---|---|
| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| Symbol | Date | Examiner |
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| CPC Combination Sets - Searched* | | |
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| Symbol | Date | Examiner |
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| US Classification - Searched* | | | |
|---|---|---|---|
| Class | Subclass | Date | Examiner |
| | none | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| Search Notes | Date | Examiner |
| Searched EAST and Plus | 1/9/2013 | SCP |
| Inventor search EAST and PALM | 1/9/2013 | SCP |
| Assignee search: EAST | 1/9/2013 | SCP |
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| Inventor search EAST and PALM | 5/30/2013 | SCP |
| Assignee search: EAST | 5/30/2013 | SCP |
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| consulted Katherine Salmon on 112 issues and art | 10/23/2014 | SCP |
| consulted Joe woitach on art | 10/23/2014 | SCP |
| Consulted Kevin Orwig on art and 112 issues | 10/23/2014 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/24/2014 | SCP |
| Inventor search EAST and PALM | 10/24/2014 | SCP |
| Assignee search: EAST | 10/24/2014 | SCP |
| consulted Katherine Salmon on 112 issues and art | 3/6/2015 | SCP |
| Consulted Ben Blumel on 112 issues | 3/6/2015 | SCP |
| Searched EAST | 3/6/2015 | SCP |
| Inventor search EAST and PALM | 3/6/2015 | SCP |
| Assignee search: EAST | 3/6/2015 | SCP |
| consulted Kevin Orwig on 112 issues and art | 1/12/2016 | SCP |
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| Inventor search EAST and PALM | 1/12/2016 | SCP |
| Assignee search: EAST | 1/12/2016 | SCP |
| consulted Kevin Orwig on 112 issues and art | 6/7/2016 | SCP |
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| Inventor search EAST and PALM | 6/7/2016 | SCP |
| Assignee search: EAST | 6/7/2016 | SCP |
| consulted Joe Woitach on 112 issues and art | 3/9/2017 | SCP |
| consulted Kim Aron on 112 issues and art | 3/9/2017 | SCP |
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| Inventor search EAST and PALM | 3/9/2017 | SCP |
| Assignee search: EAST | 3/9/2017 | SCP |
| consulted Joe Woitach on 112 issues and art | 11/2/2017 | SCP |
| consulted Kevin Orwig on 112 issues and art | 11/2/2017 | SCP |
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| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |
| consulted Joe Woitach on claim interpretation, arguments and art | 02/12/2019 | SCP |
| Searched EAST | 02/12/2019 | SCP |
| Inventor search EAST and PALM | 02/12/2019 | SCP |
| Assignee search: EAST | 02/12/2019 | SCP |
| consulted Dave Nguyen on claim interpretation and art | 11/25/2019 | SCP |
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| Searched EAST | 12/02/2019 | SCP |
| Inventor search EAST and PALM | 12/02/2019 | SCP |
| Assignee search: EAST | 12/02/2019 | SCP |
| google searched MIP, CIP, SNP, phenotype, region | 12/02/2019 | SCP |
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| consulted Joe Woitach on claim interpretation and art | 06/04/2020 | SCP |
| consulted Katherine Salmon on art of record and claim interpretation | 06/04/2020 | SCP |
| consulted Kevin Orwig on art of record and claim interpretation | 06/04/2020 | SCP |
| Searched EAST | 06/04/2020 | SCP |
| Inventor search EAST and PALM | 06/04/2020 | SCP |
| Assignee search: EAST | 06/04/2020 | SCP |
| google searched mip, cip padlock, molecular inversion probe, stagger | 06/04/2020 | SCP |
| consulted Joe Woitach on112, claim interpretation and art | 04/22/2021 | SCP |
| consulted Kevin Orwig on art of record and claim interpretation | 04/22/2021 | SCP |
| Searched EAST | 04/22/2021 | SCP |
| Inventor search EAST and PALM | 04/22/2021 | SCP |
| Assignee search: EAST | 04/22/2021 | SCP |

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| Interference Search | | | |
|---|---|---|---|
| US Class/CPC Symbol | US Subclass/CPC Group | Date | Examiner |
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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| Symbol | Date | Examiner |
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| CPC Combination Sets - Searched* | | |
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| Symbol | Date | Examiner |
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| US Classification - Searched* | | | |
|---|---|---|---|
| Class | Subclass | Date | Examiner |
| | none | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| Search Notes | Date | Examiner |
| Searched EAST and Plus | 1/9/2013 | SCP |
| Inventor search EAST and PALM | 1/9/2013 | SCP |
| Assignee search: EAST | 1/9/2013 | SCP |
| consulted Katherine Salmon on claim interpretation and 112 issues | 1/9/2013 | SCP |
| Searched EAST | 5/30/2013 | SCP |
| Inventor search EAST and PALM | 5/30/2013 | SCP |
| Assignee search: EAST | 5/30/2013 | SCP |
| consulted Steve Kapushoc on claim interpretation and 112 issues | 5/30/2013 | SCP |
| consulted Katherine Salmon on 112 issues and art | 10/23/2014 | SCP |
| consulted Joe woitach on art | 10/23/2014 | SCP |
| Consulted Kevin Orwig on art and 112 issues | 10/23/2014 | SCP |



Petitioner Illumina, Inc., EX1002, p. 32

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/24/2014 | SCP |
| Inventor search EAST and PALM | 10/24/2014 | SCP |
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| Consulted Ben Blumel on 112 issues | 3/6/2015 | SCP |
| Searched EAST | 3/6/2015 | SCP |
| Inventor search EAST and PALM | 3/6/2015 | SCP |
| Assignee search: EAST | 3/6/2015 | SCP |
| consulted Kevin Orwig on 112 issues and art | 1/12/2016 | SCP |
| consulted Joe Woitach on 112 issues and art | 1/12/2016 | SCP |
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| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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| Searched EAST | 10/05/2018 | SCP |
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| Assignee search: EAST | 10/05/2018 | SCP |
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| Assignee search: EAST | 04/22/2021 | SCP |
| consulted Joe Woitach on112, claim interpretation and art | 09/21/2021 | SCP |
| consulted Kevin Orwig on112, claim interpretation and art | 09/21/2021 | SCP |
| consulted  Katherine Salmon on112, claim interpretation and art | 09/21/2021 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 09/21/2021 | SCP |
| Inventor search EAST and PALM | 09/21/2021 | SCP |
| Assignee search: EAST | 09/21/2021 | SCP |
| google search | 09/21/2021 | SCP |

| Interference Search | | | |
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| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| Symbol | Date | Examiner |
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| CPC Combination Sets - Searched* | | |
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| Symbol | Date | Examiner |
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| US Classification - Searched* | | | |
|---|---|---|---|
| Class | Subclass | Date | Examiner |
| | none | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| Search Notes | Date | Examiner |
| Searched EAST and Plus | 1/9/2013 | SCP |
| Inventor search EAST and PALM | 1/9/2013 | SCP |
| Assignee search: EAST | 1/9/2013 | SCP |
| consulted Katherine Salmon on claim interpretation and 112 issues | 1/9/2013 | SCP |
| Searched EAST | 5/30/2013 | SCP |
| Inventor search EAST and PALM | 5/30/2013 | SCP |
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| consulted Joe woitach on art | 10/23/2014 | SCP |
| Consulted Kevin Orwig on art and 112 issues | 10/23/2014 | SCP |

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/24/2014 | SCP |
| Inventor search EAST and PALM | 10/24/2014 | SCP |
| Assignee search: EAST | 10/24/2014 | SCP |
| consulted Katherine Salmon on 112 issues and art | 3/6/2015 | SCP |
| Consulted Ben Blumel on 112 issues | 3/6/2015 | SCP |
| Searched EAST | 3/6/2015 | SCP |
| Inventor search EAST and PALM | 3/6/2015 | SCP |
| Assignee search: EAST | 3/6/2015 | SCP |
| consulted Kevin Orwig on 112 issues and art | 1/12/2016 | SCP |
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| Inventor search EAST and PALM | 1/12/2016 | SCP |
| Assignee search: EAST | 1/12/2016 | SCP |
| consulted Kevin Orwig on 112 issues and art | 6/7/2016 | SCP |
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| Inventor search EAST and PALM | 6/7/2016 | SCP |
| Assignee search: EAST | 6/7/2016 | SCP |
| consulted Joe Woitach on 112 issues and art | 3/9/2017 | SCP |
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| Inventor search EAST and PALM | 3/9/2017 | SCP |
| Assignee search: EAST | 3/9/2017 | SCP |
| consulted Joe Woitach on 112 issues and art | 11/2/2017 | SCP |
| consulted Kevin Orwig on 112 issues and art | 11/2/2017 | SCP |
| Searched EAST | 11/3/2017 | SCP |
| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |
| consulted Joe Woitach on claim interpretation, arguments and art | 02/12/2019 | SCP |
| Searched EAST | 02/12/2019 | SCP |
| Inventor search EAST and PALM | 02/12/2019 | SCP |
| Assignee search: EAST | 02/12/2019 | SCP |
| consulted Dave Nguyen on claim interpretation and art | 11/25/2019 | SCP |
| consulted Joe Woitach on claim interpretation and art | 12/02/2019 | SCP |
| Searched EAST | 12/02/2019 | SCP |
| Inventor search EAST and PALM | 12/02/2019 | SCP |
| Assignee search: EAST | 12/02/2019 | SCP |
| google searched MIP, CIP, SNP, phenotype, region | 12/02/2019 | SCP |
| consulted Dave Nguyen on claims and art of record | 01/06/2020 | SCP |
| consulted Joe Woitach on claim interpretation and art | 06/04/2020 | SCP |
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| Searched EAST | 06/04/2020 | SCP |
| Inventor search EAST and PALM | 06/04/2020 | SCP |
| Assignee search: EAST | 06/04/2020 | SCP |
| google searched mip, cip padlock, molecular inversion probe, stagger | 06/04/2020 | SCP |
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| consulted Kevin Orwig on112, claim interpretation and art | 09/21/2021 | SCP |
| consulted  Katherine Salmon on112, claim interpretation and art | 09/21/2021 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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| Searched EAST | 09/21/2021 | SCP |
| Inventor search EAST and PALM | 09/21/2021 | SCP |
| Assignee search: EAST | 09/21/2021 | SCP |
| google search | 09/21/2021 | SCP |
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| Inventor search EAST and PALM | 06/28/2022 | SCP |
| Assignee search: EAST | 06/28/2022 | SCP |
| google search | 06/28/2022 | SCP |
| reviewed global dossier | 06/28/2022 | SCP |

| Interference Search | | | |
|---|---|---|---|
| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| CPC Combination Sets - Searched* | | |
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| Symbol | Date | Examiner |
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| US Classification - Searched* | | | |
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| Class | Subclass | Date | Examiner |
| | none | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
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| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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| Assignee search: EAST | 11/3/2017 | SCP |
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| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |
| consulted Joe Woitach on claim interpretation, arguments and art | 02/12/2019 | SCP |
| Searched EAST | 02/12/2019 | SCP |
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| consulted Dave Nguyen on claim interpretation and art | 11/25/2019 | SCP |
| consulted Joe Woitach on claim interpretation and art | 12/02/2019 | SCP |
| Searched EAST | 12/02/2019 | SCP |
| Inventor search EAST and PALM | 12/02/2019 | SCP |
| Assignee search: EAST | 12/02/2019 | SCP |
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| consulted Kevin Orwig on art of record and claim interpretation | 06/04/2020 | SCP |
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| Inventor search EAST and PALM | 06/04/2020 | SCP |
| Assignee search: EAST | 06/04/2020 | SCP |
| google searched mip, cip padlock, molecular inversion probe, stagger | 06/04/2020 | SCP |
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| Inventor search EAST and PALM | 04/22/2021 | SCP |
| Assignee search: EAST | 04/22/2021 | SCP |
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| consulted  Katherine Salmon on112, claim interpretation and art | 09/21/2021 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 09/21/2021 | SCP |
| Inventor search EAST and PALM | 09/21/2021 | SCP |
| Assignee search: EAST | 09/21/2021 | SCP |
| google search | 09/21/2021 | SCP |
| consulted Joe Woitach on112, claim interpretation and art | 06/28/2022 | SCP |
| consulted Katherine Salmon on112, claim interpretation and art | 06/28/2022 | SCP |
| Searched EAST | 06/28/2022 | SCP |
| Inventor search EAST and PALM | 06/28/2022 | SCP |
| Assignee search: EAST | 06/28/2022 | SCP |
| google search | 06/28/2022 | SCP |
| reviewed global dossier | 06/28/2022 | SCP |
| consulted Joe Woitach onclaim interpretation and art | 01/18/2023 | SCP |
| consulted Katherine Salmon on claim interpretation and art | 01/18/2023 | SCP |
| searched P2E2 | 01/18/2023 | SCP |
| inventor search P2E2 and PALM | 01/18/2023 | SCP |
| Assignee search P2E2 | 01/18/2023 | SCP |
| google search | 01/18/2023 | SCP |
| reviewed global dossier | 01/18/2023 | SCP |

| Interference Search | | | |
|---|---|---|---|
| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
| | | | |



Petitioner Illumina, Inc., EX1002, p. 43

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| Symbol | Date | Examiner |
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| CPC Combination Sets - Searched* | | |
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| Symbol | Date | Examiner |
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| US Classification - Searched* | | | |
|---|---|---|---|
| Class | Subclass | Date | Examiner |
| | none | | |

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

| Search Notes | | |
|---|---|---|
| Search Notes | Date | Examiner |
| Searched EAST and Plus | 1/9/2013 | SCP |
| Inventor search EAST and PALM | 1/9/2013 | SCP |
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| consulted Steve Kapushoc on claim interpretation and 112 issues | 5/30/2013 | SCP |
| consulted Katherine Salmon on 112 issues and art | 10/23/2014 | SCP |
| consulted Joe woitach on art | 10/23/2014 | SCP |
| Consulted Kevin Orwig on art and 112 issues | 10/23/2014 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/24/2014 | SCP |
| Inventor search EAST and PALM | 10/24/2014 | SCP |
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| Assignee search: EAST | 3/9/2017 | SCP |
| consulted Joe Woitach on 112 issues and art | 11/2/2017 | SCP |
| consulted Kevin Orwig on 112 issues and art | 11/2/2017 | SCP |
| Searched EAST | 11/3/2017 | SCP |
| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |
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| Assignee search: EAST | 12/02/2019 | SCP |
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| consulted  Katherine Salmon on112, claim interpretation and art | 09/21/2021 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

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|---|---|---|
| Searched EAST | 09/21/2021 | SCP |
| Inventor search EAST and PALM | 09/21/2021 | SCP |
| Assignee search: EAST | 09/21/2021 | SCP |
| google search | 09/21/2021 | SCP |
| consulted Joe Woitach on112, claim interpretation and art | 06/28/2022 | SCP |
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| Inventor search EAST and PALM | 06/28/2022 | SCP |
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| reviewed global dossier | 06/28/2022 | SCP |
| consulted Joe Woitach onclaim interpretation and art | 01/18/2023 | SCP |
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| Assignee search P2E2 | 01/18/2023 | SCP |
| google search | 01/18/2023 | SCP |
| reviewed global dossier | 01/18/2023 | SCP |
| consulted Joe Woitach onclaim interpretation,112, and art | 08/29/2023 | SCP |
| searched P2E2 | 08/29/2023 | SCP |
| inventor search P2E2 and PDP | 08/29/2023 | SCP |
| google search | 08/29/2023 | SCP |
| reviewed global dossier | 08/29/2023 | SCP |

| Interference Search | | | |
|---|---|---|---|
| **US Class/CPC Symbol** | **US Subclass/CPC Group** | **Date** | **Examiner** |
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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| CPC - Searched* | | |
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| CPC Combination Sets - Searched* | | |
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| US Classification - Searched* | | | |
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| Class | Subclass | Date | Examiner |
| | none | | |

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| Inventor search EAST and PALM | 3/9/2017 | SCP |
| Assignee search: EAST | 3/9/2017 | SCP |
| consulted Joe Woitach on 112 issues and art | 11/2/2017 | SCP |
| consulted Kevin Orwig on 112 issues and art | 11/2/2017 | SCP |
| Searched EAST | 11/3/2017 | SCP |
| Inventor search EAST and PALM | 11/3/2017 | SCP |
| Assignee search: EAST | 11/3/2017 | SCP |
| consulted Joe Woitach on claim interpretation and art | 10/05/2018 | SCP |
| consulted Kevin Orwig on claim interpretation and art | 10/05/2018 | SCP |



Petitioner Illumina, Inc., EX1002, p. 49

| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| | | |
|---|---|---|
| Searched EAST | 10/05/2018 | SCP |
| Inventor search EAST and PALM | 10/05/2018 | SCP |
| Assignee search: EAST | 10/05/2018 | SCP |
| consulted Joe Woitach on claim interpretation, arguments and art | 02/12/2019 | SCP |
| Searched EAST | 02/12/2019 | SCP |
| Inventor search EAST and PALM | 02/12/2019 | SCP |
| Assignee search: EAST | 02/12/2019 | SCP |
| consulted Dave Nguyen on claim interpretation and art | 11/25/2019 | SCP |
| consulted Joe Woitach on claim interpretation and art | 12/02/2019 | SCP |
| Searched EAST | 12/02/2019 | SCP |
| Inventor search EAST and PALM | 12/02/2019 | SCP |
| Assignee search: EAST | 12/02/2019 | SCP |
| google searched MIP, CIP, SNP, phenotype, region | 12/02/2019 | SCP |
| consulted Dave Nguyen on claims and art of record | 01/06/2020 | SCP |
| consulted Joe Woitach on claim interpretation and art | 06/04/2020 | SCP |
| consulted Katherine Salmon on art of record and claim interpretation | 06/04/2020 | SCP |
| consulted Kevin Orwig on art of record and claim interpretation | 06/04/2020 | SCP |
| Searched EAST | 06/04/2020 | SCP |
| Inventor search EAST and PALM | 06/04/2020 | SCP |
| Assignee search: EAST | 06/04/2020 | SCP |
| google searched mip, cip padlock, molecular inversion probe, stagger | 06/04/2020 | SCP |
| consulted Joe Woitach on112, claim interpretation and art | 04/22/2021 | SCP |
| consulted Kevin Orwig on art of record and claim interpretation | 04/22/2021 | SCP |
| Searched EAST | 04/22/2021 | SCP |
| Inventor search EAST and PALM | 04/22/2021 | SCP |
| Assignee search: EAST | 04/22/2021 | SCP |
| consulted Joe Woitach on112, claim interpretation and art | 09/21/2021 | SCP |
| consulted Kevin Orwig on112, claim interpretation and art | 09/21/2021 | SCP |
| consulted  Katherine Salmon on112, claim interpretation and art | 09/21/2021 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| | | |
|---|---|---|
| Searched EAST | 09/21/2021 | SCP |
| Inventor search EAST and PALM | 09/21/2021 | SCP |
| Assignee search: EAST | 09/21/2021 | SCP |
| google search | 09/21/2021 | SCP |
| consulted Joe Woitach on112, claim interpretation and art | 06/28/2022 | SCP |
| consulted Katherine Salmon on112, claim interpretation and art | 06/28/2022 | SCP |
| Searched EAST | 06/28/2022 | SCP |
| Inventor search EAST and PALM | 06/28/2022 | SCP |
| Assignee search: EAST | 06/28/2022 | SCP |
| google search | 06/28/2022 | SCP |
| reviewed global dossier | 06/28/2022 | SCP |
| consulted Joe Woitach onclaim interpretation and art | 01/18/2023 | SCP |
| consulted Katherine Salmon on claim interpretation and art | 01/18/2023 | SCP |
| searched P2E2 | 01/18/2023 | SCP |
| inventor search P2E2 and PALM | 01/18/2023 | SCP |
| Assignee search P2E2 | 01/18/2023 | SCP |
| google search | 01/18/2023 | SCP |
| reviewed global dossier | 01/18/2023 | SCP |
| consulted Joe Woitach on claim interpretation,112, and art | 08/29/2023 | SCP |
| searched P2E2 | 08/29/2023 | SCP |
| inventor search P2E2 and PDP | 08/29/2023 | SCP |
| google search | 08/29/2023 | SCP |
| reviewed global dossier | 08/29/2023 | SCP |
| consulted Joe Woitach on claim interpretation, and art | 02/08/2024 | SCP |
| searched P2E2 | 02/08/2024 | SCP |
| inventor search P2E2 and PDP | 02/08/2024 | SCP |
| google search | 02/08/2024 | SCP |
| reviewed global dossier | 02/08/2024 | SCP |



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| Search Notes | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 13/266,862 | Porreca et al. |
| | Examiner | Art Unit |
| | STEVEN C POHNERT PhD | 1634 |

| Interference Search | | | |
|---|---|---|---|
| US Class/CPC Symbol | US Subclass/CPC Group | Date | Examiner |
| | | | |



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## PE2E SEARCH - Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | British Equivalents | Time Stamp |
|---|---|---|---|---|---|---|---|
| L1 | 1442715 | krishnakumar.in. or zheng.in. or wilhelmy.in. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 01:34 PM |
| L2 | 19 | large near2 padlock near2 probe | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:09 PM |
| L3 | 0 | smart with L2 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:11 PM |
| L4 | 18 | smart with (padlock near2 probe) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:12 PM |
| L5 | 65 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2021/09/21 03:55 PM |
| L6 | 91 | (molecular near2 loop near2 biosolutions).as. or (good near2 start near2 genetics).as. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 03:55 PM |
| L8 | 1025 | (padlock or mip or (molecular near2 inversion near2 probe)) same (nest$4 or concentric or stagger$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:06 PM |
| L9 | 3135 | (padlock or mip or (molecular near2 inersion near2 probe)) and (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:06 PM |
| L10 | 2850 | L9 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L11 | 1464 | (padlock or mip or (molecular near2 inersion near2 probe)) same (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L12 | 2850 | L10 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; | OR | ON | ON | 2021/09/21 04:07 PM |



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| | | | IBM_TDB) | | | | |
| L13 | 1422 | L11 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L14 | 0 | L13 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:08 PM |
| L15 | 0 | L11 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:09 PM |
| L16 | 1548 | (padlock or mip or (molecular near2 inversion near2 probe)) same (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:10 PM |
| L17 | 0 | L16 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:11 PM |
| L18 | 95833 | (next near2 generation near2 sequenc$4) ane avaerag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L19 | 95843 | (next near2 generation near2 sequenc$4) ane averag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L20 | 0 | (next near2 generation near2 sequenc$4) and averag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L21 | 15541 | (next near2 generation near2 sequenc$4) and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:16 PM |
| L22 | 23 | L21 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:17 PM |
| L23 | 14 | (L5 or L6) and L11 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:58 PM |
| L24 | 7442 | (padlock OR mip OR (molecular near2 inersion near2 probe)) AND (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, | OR | ON | ON | 2023/01/18 11:18 AM |

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|---|---|---|---|---|---|---|---|
| | | | IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | | | | |
| L25 | 1410 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2023/01/18 11:19 AM |
| L26 | 200 | (molecular near2 loop near2 biosolutions).as. OR (good near2 start near2 genetics).as. OR (good near2 staart near2 genetics).as. OR (capital near2 royalty near2 partners).as.or (bridge near2 bank).as. OR (western near2 alliance near2 bank).as. OR (invitae near2 corporation).as. OR combimatrix.as. OR (inn near2 saa).as. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 11:22 AM |
| L27 | 27 | (L25 OR L26) AND L11 | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 11:23 AM |
| L28 | 28 | smart WITH (padlock near2 probe) | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, | OR | ON | ON | 2023/01/18 11:25 AM |



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|---|---|---|---|---|---|---|---|
| | | | MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | | | | |
| L29 | 2 | "20070212704".pn. | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 02:29 PM |
| L30 | 1454 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2023/08/29 09:51 AM |
| L31 | 23 | (L30 OR L26) AND L16 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/08/29 09:51 AM |
| L32 | 9670 | (padlock OR mip OR (molecular near2 inversion near2 probe)) AND (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/08/29 09:52 AM |
| L33 | 53 | (L30 OR L26) AND L32 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/08/29 09:52 AM |
| L34 | 200737 | ((("PORRECA") near3 ("Gregory")) OR | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, | OR | ON | ON | 2024/02/08 11:24 AM |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO; DERWENT) | | | | |
| L35 | 209 | (molecular near2 loop near2 biosolutions).as. OR (good near2 start near2 genetics).as. OR (good near2 staart near2 genetics).as. OR (capital near2 royalty near2 partners).as.or (bridge near2 bank).as. OR (western near2 alliance near2 bank).as. OR (invitae near2 corporation).as. OR combimatrix.as. OR (inn near2 saa).as. | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2024/02/08 11:24 AM |
| L36 | 82 | (L35 OR L34) AND L32 | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2024/02/08 11:24 AM |

## PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.



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# Web Search History

| date, time | web site | search string |
|---|---|---|
| 2/8/2024 11:27:14 AM | Google | mips tiling |
| 2/8/2024 11:27:30 AM | Google | molecular inversion probe tiling |
| 2/8/2024 11:27:33 AM | Google Scholar | molecular inversion probe tiling |
| 2/8/2024 11:30:54 AM | Google Scholar | molecular inversion probe tiling [before:2009] |
| 2/8/2024 11:32:26 AM | Google Scholar | [before:2009] "molecular inversion probe" tiling |
| 2/8/2024 11:32:43 AM | Google Scholar | [before:2009] "padlock probes" tiling |

## PE2E SEARCH - Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | British Equivalents | Time Stamp |
|---|---|---|---|---|---|---|---|
| L1 | 1442715 | krishnakumar.in. or zheng.in. or wilhelmy.in. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 01:34 PM |
| L2 | 19 | large near2 padlock near2 probe | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:09 PM |
| L3 | 0 | smart with L2 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:11 PM |
| L4 | 18 | smart with (padlock near2 probe) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:12 PM |
| L5 | 65 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2021/09/21 03:55 PM |
| L6 | 91 | (molecular near2 loop near2 biosolutions).as. or (good near2 start near2 genetics).as. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 03:55 PM |
| L8 | 1025 | (padlock or mip or (molecular near2 inversion near2 probe)) same (nest$4 or concentric or stagger$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:06 PM |
| L9 | 3135 | (padlock or mip or (molecular near2 inersion near2 probe)) and (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:06 PM |
| L10 | 2850 | L9 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L11 | 1464 | (padlock or mip or (molecular near2 inersion near2 probe)) same (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L12 | 2850 | L10 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |

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|---|---|---|---|---|---|---|---|
| L13 | 1422 | L11 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L14 | 0 | L13 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:08 PM |
| L15 | 0 | L11 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:09 PM |
| L16 | 1548 | (padlock or mip or (molecular near2 inversion near2 probe)) same (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:10 PM |
| L17 | 0 | L16 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:11 PM |
| L18 | 95833 | (next near2 generation near2 sequenc$4) ane avaerag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L19 | 95843 | (next near2 generation near2 sequenc$4) ane averag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L20 | 0 | (next near2 generation near2 sequenc$4) and averag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L21 | 15541 | (next near2 generation near2 sequenc$4) and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:16 PM |
| L22 | 23 | L21 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:17 PM |
| L23 | 14 | (L5 or L6) and L11 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:58 PM |
| L24 | 7442 | (padlock OR mip OR (molecular near2 inersion near2 probe)) AND (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, | OR | ON | ON | 2023/01/18 11:18 AM |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | | | | |
| L25 | 1410 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2023/01/18 11:19 AM |
| L26 | 200 | (molecular near2 loop near2 biosolutions).as. OR (good near2 start near2 genetics).as. OR (good near2 staart near2 genetics).as. OR (capital near2 royalty near2 partners).as.or (bridge near2 bank).as. OR (western near2 alliance near2 bank).as. OR (invitae near2 corporation).as. OR combimatrix.as. OR (inn near2 saa).as. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 11:22 AM |
| L27 | 27 | (L25 OR L26) AND L11 | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 11:23 AM |
| L28 | 28 | smart WITH (padlock near2 probe) | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, | OR | ON | ON | 2023/01/18 11:25 AM |



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|---|---|---|---|---|---|---|---|
| | | | RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | | | | |
| L29 | 2 | "20070212704".pn. | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 02:29 PM |
| L30 | 1454 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2023/08/29 09:51 AM |
| L31 | 23 | (L30 OR L26) AND L16 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/08/29 09:51 AM |
| L32 | 9670 | (padlock OR mip OR (molecular near2 inversion near2 probe)) AND (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/08/29 09:52 AM |
| L33 | 53 | (L30 OR L26) AND L32 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/08/29 09:52 AM |

## PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.



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# Web Search History

| date, time | web site | search string |
|---|---|---|
| 8/29/2023 9:13:55 AM | Google | molecular inversion probes |
| 8/29/2023 9:14:06 AM | Google | molecular inversion probe tiling method |
| 8/29/2023 9:14:10 AM | Google Scholar | molecular inversion probe tiling method |
| 8/29/2023 9:21:22 AM | Google Scholar | molecular inversion probe tiling method [before:2009] |
| 8/29/2023 9:21:28 AM | Google Scholar | [before:2009] "molecular inversion probe" tiling method |
| 8/29/2023 9:33:25 AM | Google Scholar | "molecular inversion probe" tiling method [before:2008] |
| 8/29/2023 9:40:10 AM | Google Scholar | [before:2008] "molecular inversion probe" tiling |
| 8/29/2023 9:49:14 AM | Google Scholar | [before:2008] "molecular inversion probe" resequencing |
| 8/29/2023 9:49:21 AM | Google Scholar | [before:2008] "molecular inversion probe" overlapping |
| 8/29/2023 10:08:32 AM | Google Scholar | [before:2008] "molecular inversion probe" umi |
| 8/29/2023 10:09:33 AM | Google | ADVANCED SEQUENCING TECHNOLOGIES: METHODS AND GOALS |
| 8/29/2023 10:09:51 AM | Google Scholar | [before:2008] "molecular inversion probe" overlap |
| 8/29/2023 10:10:08 AM | Google Scholar | [before:2008] "molecular inversion probe" unique |
| 8/29/2023 10:10:23 AM | Google Scholar | [before:2008] "molecular inversion probe" unique molecular |
| 8/29/2023 10:11:40 AM | Google Scholar | [before:2008] "molecular inversion probe" "unique molecular" |
| 8/29/2023 10:11:50 AM | Google Scholar | [before:2008] "molecular inversion probe" "unique tag" |
| 8/29/2023 11:48:50 AM | Google | Mockler (Genomics (2005) volume 85, pages 1-15) |

# Web Search History

| date, time | web site | search string |
|---|---|---|
| 1/18/2023 3:12:12 PM | Google | molecular inversion probes (mips) |
| 1/18/2023 3:12:16 PM | Google Scholar | molecular inversion probes (mips) |
| 1/18/2023 3:12:32 PM | Google Scholar | molecular inversion probes (mips) tiling |
| 1/18/2023 3:21:47 PM | Google Scholar | molecular inversion probes (mips) tiling [before:2009] |
| 1/18/2023 3:24:40 PM | Google Scholar | [before:2009] molecular inversion probes (mips) overlapping |
| 1/18/2023 3:38:34 PM | Google | Krishnakumar mips |
| 1/18/2023 3:38:57 PM | Google | krishnakumar molecular inversion mips |
| 1/18/2023 3:38:59 PM | Google Scholar | krishnakumar molecular inversion mips |

## PE2E SEARCH - Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | British Equivalents | Time Stamp |
|---|---|---|---|---|---|---|---|
| L1 | 1442715 | krishnakumar.in. or zheng.in. or wilhelmy.in. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 01:34 PM |
| L2 | 19 | large near2 padlock near2 probe | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:09 PM |
| L3 | 0 | smart with L2 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:11 PM |
| L4 | 18 | smart with (padlock near2 probe) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 02:12 PM |
| L5 | 65 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2021/09/21 03:55 PM |
| L6 | 91 | (molecular near2 loop near2 biosolutions).as. or (good near2 start near2 genetics).as. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 03:55 PM |
| L8 | 1025 | (padlock or mip or (molecular near2 inversion near2 probe)) same (nest$4 or concentric or stagger$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:06 PM |
| L9 | 3135 | (padlock or mip or (molecular near2 inersion near2 probe)) and (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:06 PM |
| L10 | 2850 | L9 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L11 | 1464 | (padlock or mip or (molecular near2 inersion near2 probe)) same (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L12 | 2850 | L10 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| L13 | 1422 | L11 and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:07 PM |
| L14 | 0 | L13 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:08 PM |
| L15 | 0 | L11 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:09 PM |
| L16 | 1548 | (padlock or mip or (molecular near2 inversion near2 probe)) same (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:10 PM |
| L17 | 0 | L16 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:11 PM |
| L18 | 95833 | (next near2 generation near2 sequenc$4) ane avaerag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L19 | 95843 | (next near2 generation near2 sequenc$4) ane averag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L20 | 0 | (next near2 generation near2 sequenc$4) and averag?4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:15 PM |
| L21 | 15541 | (next near2 generation near2 sequenc$4) and averag$4 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:16 PM |
| L22 | 23 | L21 and @pd<"20070430" | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:17 PM |
| L23 | 14 | (L5 or L6) and L11 | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2021/09/21 04:58 PM |
| L24 | 7442 | (padlock OR mip OR (molecular near2 inersion near2 probe)) AND (next near2 generation near2 sequenc$4) | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, | OR | ON | ON | 2023/01/18 11:18 AM |

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|---|---|---|---|---|---|---|---|
| | | | MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | | | | |
| L25 | 1410 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | (US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT) | OR | ON | ON | 2023/01/18 11:19 AM |
| L26 | 200 | (molecular near2 loop near2 biosolutions).as. OR (good near2 start near2 genetics).as. OR (good near2 staart near2 genetics).as. OR (capital near2 royalty near2 partners).as.or (bridge near2 bank).as. OR (western near2 alliance near2 bank).as. OR (invitae near2 corporation).as. OR combimatrix.as. OR (inn near2 saa).as. | (US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 11:22 AM |
| L27 | 27 | (L25 OR L26) AND L11 | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 11:23 AM |
| L28 | 28 | smart WITH (padlock near2 probe) | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, | OR | ON | ON | 2023/01/18 11:25 AM |

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|---|---|---|---|---|---|---|---|
| | | | RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | | | | |
| L29 | 2 | "20070212704".pn. | (US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB) | OR | ON | ON | 2023/01/18 02:29 PM |

## PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.



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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 1,678,520 | krishnakumar.in. or zheng.in. or wilhelmy.in. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:05 |
| L2 | 22 | large near2 padlock near2 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:05 |
| L3 | 12,261,676 | next generation sequencing | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:05 |
| L4 | 20 | smart with (padlock near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:05 |
| L5 | 157 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri"))).INV. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:06 |
| L6 | 95 | (molecular near2 loop near2 biosolutions).as. or (good near2 start near2 genetics).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:06 |
| L7 | 3,617 | (padlock or mip or (molecular near2 inersion near2 probe)) and (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:06 |
| L8 | 4,023 | (padlock or mip or (molecular near2 inversion near2 probe)) and (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:06 |
| L9 | 49 | (l5 or l6) and l8 | US-PGPUB; USPAT; USOCR; | OR | ON | 2022/06/28 11:07 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | FPRS; EPO; JPO; DERWENT; IBM_TDB | | | |
| L10 | 3,625 | l8 and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:07 |
| L11 | 0 | l10 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2022/06/28 11:07 |
| S1 | 1,442,715 | krishnakumar.in. or zheng.in. or wilhelmy.in. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 13:34 |
| S2 | 19 | large near2 padlock near2 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 14:09 |
| S3 | 0 | smart with S2 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 14:11 |
| S4 | 18 | smart with (padlock near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 14:12 |
| S5 | 65 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri"))).INV. | US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT | OR | ON | 2021/09/21 15:55 |
| S6 | 91 | (molecular near2 loop near2 biosolutions).as. or (good near2 start near2 genetics).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 15:55 |
| S8 | 1,025 | (padlock or mip or (molecular near2 inversion near2 probe)) same (nest$4 or concentric or stagger$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:06 |

| | | | | | | |
|---|---|---|---|---|---|---|
| S9 | 3,135 | (padlock or mip or (molecular near2 inersion near2 probe)) and (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:06 |
| S10 | 2,850 | S9 and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| S11 | 1,464 | (padlock or mip or (molecular near2 inersion near2 probe)) same (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| S12 | 2,850 | S10 and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| S13 | 1,422 | S11 and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| S14 | 0 | S13 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:08 |
| S15 | 0 | S11 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:09 |
| S16 | 1,548 | (padlock or mip or (molecular near2 inversion near2 probe)) same (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:10 |
| S17 | 0 | S16 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:11 |
| S18 | 95,833 | (next near2 generation near2 sequenc$4) ane avaerag?4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; | OR | ON | 2021/09/21 16:15 |

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|---|---|---|---|---|---|---|
| | | | DERWENT; IBM_TDB | | | |
| S19 | 95,843 | (next near2 generation near2 sequenc$4) ane averag?4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:15 |
| S20 | 0 | (next near2 generation near2 sequenc$4) and averag?4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:15 |
| S21 | 15,541 | (next near2 generation near2 sequenc$4) and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:16 |
| S22 | 23 | S21 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:17 |
| S23 | 14 | (S5 or S6) and S11 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:58 |

6/28/2022 11:08:17 AM
C:\Users\spohnert\Documents\EAST\Workspaces\13266862.wsp

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 65 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri"))).INV. | US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT | OR | ON | 2021/09/21 15:55 |
| L2 | 91 | (molecular near2 loop near2 biosolutions).as. or (good near2 start near2 genetics).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 15:55 |
| L4 | 1,025 | (padlock or mip or (molecular near2 inversion near2 probe)) same (nest$4 or concentric or stagger$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:06 |
| L5 | 3,135 | (padlock or mip or (molecular near2 inersion near2 probe)) and (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:06 |
| L6 | 2,850 | l5 and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| L7 | 1,464 | (padlock or mip or (molecular near2 inersion near2 probe)) same (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| L8 | 2,850 | l6 and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| L9 | 1,422 | l7 and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:07 |
| L10 | 0 | l9 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; | OR | ON | 2021/09/21 16:08 |

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| | | | DERWENT; IBM_TDB | | | |
| L11 | 0 | l7 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:09 |
| L12 | 1,548 | (padlock or mip or (molecular near2 inversion near2 probe)) same (next near2 generation near2 sequenc$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:10 |
| L13 | 0 | l12 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:11 |
| L14 | 95,833 | (next near2 generation near2 sequenc$4) ane avaerag?4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:15 |
| L15 | 95,843 | (next near2 generation near2 sequenc$4) ane averag?4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:15 |
| L16 | 0 | (next near2 generation near2 sequenc$4) and averag?4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:15 |
| L17 | 15,541 | (next near2 generation near2 sequenc$4) and averag$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:16 |
| L18 | 23 | l17 and @pd<"20070430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:17 |
| L19 | 14 | (l1 or l2) and l7 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 16:58 |

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|---|---|---|---|---|---|---|
| S1 | 1,442,715 | krishnakumar.in. or zheng.in. or wilhelmy.in. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 13:34 |
| S2 | 19 | large near2 padlock near2 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 14:09 |
| S3 | 0 | smart with S2 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 14:11 |
| S4 | 18 | smart with (padlock near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/09/21 14:12 |

**EAST Search History (Interference)**

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9/21/2021 4:59:15 PM
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# Web Search History

| date, time | web site | search string |
|---|---|---|
| 9/21/2021 3:58:20 PM | Google Scholar | "Molecular inversion probe" "tandem repeat" |
| 9/21/2021 3:58:31 PM | Google Scholar | "Molecular inversion probe" concentric |
| 9/21/2021 3:59:48 PM | Google Scholar | Porecca(Nature methods (2007) volume 4, pages 931-936). |
| 9/21/2021 3:59:51 PM | Google Scholar | Porreca(Nature methods (2007) volume 4, pages 931-936). |
| 9/21/2021 4:03:31 PM | Google Scholar | MIP next generation sequencing |
| 9/21/2021 4:03:41 PM | Google Scholar | MIP next generation sequencing [before:2006] |
| 9/21/2021 4:03:55 PM | Google Scholar | [before:2006] "Molecular inversion probe" next generation sequencing |
| 9/21/2021 4:09:15 PM | Google Scholar | [before:2006] ""Molecular inversion probe" "next generation sequencing |
| 9/21/2021 4:10:12 PM | Google Scholar | [before:2006] ""Molecular inversion probe" ngs |
| 9/21/2021 4:11:47 PM | Google Scholar | "allele quantification" molecular inversion probes |
| 9/21/2021 4:12:04 PM | Google Scholar | sequencing molecular inversion probes |
| 9/21/2021 4:12:53 PM | Google Scholar | sequencing molecular inversion probes [before:2006] |
| 9/21/2021 4:13:06 PM | Google Scholar | [before:2006] "next generation sequencing" |
| 9/21/2021 4:14:38 PM | Google Scholar | [before:2006] "next generation sequencing" average calls |
| 9/21/2021 4:20:52 PM | Google Scholar | [before:2006] "next generation sequencing" MIP |
| 9/21/2021 4:21:04 PM | Google Scholar | [before:2006] "next generation sequencing" MIP inversion |

| date, time | web site | search string |
|---|---|---|
| 9/21/2021 4:21:23 PM | Google Scholar | [before:2006] "next generation sequencing" padlock inversion |



Petitioner Illumina, Inc., EX1002, p. 77

# Web Search History

| date, time | web site | search string |
|---|---|---|
| 4/21/2021 4:40:08 PM | Google Scholar | nested molecular inversion probes |
| 4/21/2021 4:41:06 PM | Google Scholar | nested "molecular inversion probes" |
| 4/21/2021 4:42:47 PM | Google Scholar | nested "molecular inversion probes" [before:2008] |
| 4/22/2021 12:57:21 PM | Google Scholar | "molecular inversion probe" tiling overlapping |
| 4/22/2021 12:57:30 PM | Google Scholar | "molecular inversion probe" staggered |
| 4/22/2021 12:57:38 PM | Google Scholar | "molecular inversion probe" nested |
| 4/22/2021 12:58:26 PM | Google Scholar | "molecular inversion probe" concentric |
| 4/22/2021 12:58:40 PM | Google Scholar | "molecular inversion probe" |
| 4/22/2021 12:58:50 PM | Google Scholar | "molecular inversion probe" insertions |
| 4/22/2021 12:59:05 PM | Google Scholar | "molecular inversion probe" insertions [before:2008] |

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
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| S2 | 144 | ("20090127589" \| "20090129647" \| "20090156412" \| "20090163366" \| "20090191565" \| "20090192047" \| "20090203014" \| "20090226975" \| "20090233814" \| "20090298064" \| "20090318310" \| "20100035243" \| "20100035252" \| "20100063742" \| "20100069263" \| "20100105107" \| "20100137143" \| "20100137163" \| "20100188073" \| "20100197507" \| "20100216151" \| "20100248984" \| "20100282617" \| "20100285578" \| "20100297626" \| "20100300559" \| "20100300895" \| "20100301042" \| "20100301398" \| "20100304982" \| "20100330619" \| "20110004413" \| "20110009278" \| "20110015863" \| "20110021366" \| "20110034342" \| "20110098193" \| "20110159499" \| "20110166029" \| "20110230365" \| "20110257889" \| "20110301042" \| "20120015050" \| "20120021930" \| "20120059594" \| "20120165202" \| "20120179384" \| "20120252020" \| "20120252684" \| "20130275103" \| "20130344096" \| "4683195" \| "4683202" \| "4988617" \| "5234809" \| "5242794" \| "5494810" \| "5583024" \| "5604097" \| "5636400" \| "5674713" \| "5695934" \| "5700673" \| "5701256" \| "5830064" \| "5846719" \| "5863722" \| "5866337" \| "5869252" \| "5871921" \| "5993611" \| "6100099" \| "6138077" \| "6150516" \| "6172214" \| "6172218" \| "6197508" \| "6210891" \| "6235472" \| "6235475" \| "6235501" \| "6258568" \| "6274320" \| "6306597" \| "6352828" \| "6361940" \| "6403320" \| "6489105" \| "6558928" \| "6582938" \| "6714874" \| "6719449" \| "6818395" \| "6828100" \| "6833246" \| "6858412" \| "6911345" \| "6913879" \| "6948843" \| "7034143" \| "7041481" \| "7049077" \| "7057026" \| "7071324" \| "7074564" \| "7115400" \| "7169560" \| "7211390" \| "7232656" \| "7244559" \| "7264929" \| "7282337" \| "7297518" \| "7320860" \| "7323305" \| "7335762" \| "7351528" \| "7393665" \| "7510829" \| "7537897" \| "7544473" \| "7582431" \| "7598035" \| "7666593" \| | US-PGPUB; USPAT | OR | ON | 2021/04/16 09:59 |

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| S3 | 2,755 | Krishnakumar.in. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/04/16 11:54 |
| S4 | 4 | S3 and padlock | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/04/16 11:54 |
| S5 | 216 | ("10066259" \| "10202637" \| "10604799" \| "10683533" \| "20020042052" \| "20020172954" \| "20040161773" \| "20050250147" \| "20060030536" \| "20060246500" \| "20060281098" \| "20060286577" \| "20070009925" \| "20070212704" \| "20080125324" \| "20090035777" \| "20090220955" \| "20100076185" \| "20100086914" \| "20130129755" \| "20130268206" \| "20130274146" \| "20130337447" \| "20140255931" \| "20140308667" \| "20150111208" \| "20150258170" \| "20150310163" \| "20150354003" \| "20160003812" \| "20160068889" \| "20160251719" \| "20170183731" \| "20170275676" \| "20180371533" \| "20190233881" \| "20200181696" \| "5434049" \| "5491224" \| "6020127" \| "6223128" \| "8738300" \| "20040053275" \| "20040121373" \| "20110288780" \| "20120270739" \| "20140228226" \| "20140342354" \| "20150051085" \| "20170044610" \| "8114027" \| "10227635" \| "9115387" \| "9677124" \| "20060008824" \| "20060263789" \| "20020040216" \| "20020091666" \| "20030175709" \| "20030208454" \| | US-PGPUB; USPAT | OR | ON | 2021/04/21 16:49 |

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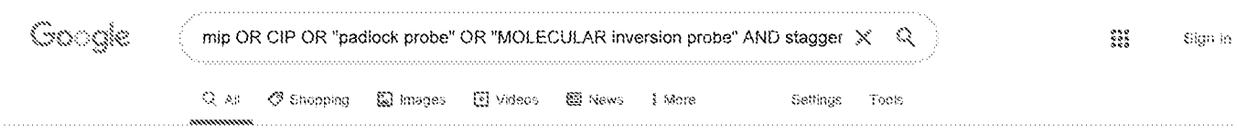
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| S6 | 46 | S5 and (MIP or (molecular near2 inversion near2 probe)) | US-PGPUB; USPAT | OR | ON | 2021/04/21 16:49 |
| S7 | 34 | S6 and (nested or staggered) | US-PGPUB; USPAT | OR | ON | 2021/04/21 16:50 |
| S8 | 5 | S7 and @pd<"20080528" | US-PGPUB; USPAT | OR | ON | 2021/04/21 16:50 |
| S9 | 429 | (MIP or (molecular near2 inversion near2 probe)) with (insertion or repeat) | US-PGPUB; USPAT | OR | ON | 2021/04/21 16:56 |
| S10 | 87 | S9 and @pd<"20080528" | US-PGPUB; USPAT | OR | ON | 2021/04/21 16:56 |
| S11 | 43 | S10 and hybridiz$5 | US-PGPUB; USPAT | OR | ON | 2021/04/21 16:57 |
| S12 | 775 | nested near2 sequencing | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2021/04/22 09:58 |
| S13 | 265 | S12 and @pd<"20080528" | US-PGPUB; USPAT | OR | ON | 2021/04/22 09:59 |
| S14 | 0 | 2005/0250147.pn. | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:36 |
| S15 | 1 | "20050250147".pn. | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:36 |
| S16 | 305 | mip with (sequenced or sequencing) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:42 |
| S17 | 58 | S16 and @pd<"20080528" | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:42 |

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| S18 | 78,847 | (MIP or (molecular near2 inversion near2 probe)) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:44 |
| S19 | 1,701 | S18 with (sequenced or sequencing) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:44 |
| S20 | 1,006 | (MIP and (molecular near2 inversion near2 probe)) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:44 |
| S21 | 2,857 | (molecular near2 inversion near2 probe) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:45 |
| S22 | 1,548 | S21 with (sequenced or sequencing) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:45 |
| S23 | 3 | S22 and @pd<"20080528" | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:45 |
| S24 | 1,194 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT | OR | ON | 2021/04/22 10:53 |
| S25 | 73 | (good near2 start near2 genetics).as. or (captial near2 royalty near2 partners).as. or (parallel near2 investment near2 opportunities).as. or (bridge near2 bank).as. or (western near2 alliance).as. or (molecular near2 loop near2 biosolutions).as. or (invitae near2 corporation ).as. or combimatrix.as. or (inn near2 sa).as. | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:57 |
| S26 | 43 | (S24 or S25) and (padlock or (molecular near2 inversion near2 probe)) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:57 |
| S27 | 25 | S26 and (stagger$2 or flanking) | US-PGPUB; USPAT | OR | ON | 2021/04/22 10:58 |

**EAST Search History (Interference)**

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4/22/2021 12:45:03 PM
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About 4,920 results (0.43 seconds)

**WO2010126614A2 - Methods and compositions for evaluating ...**
https://patents.google.com › patent
In some embodiments, **molecular inversion probe** capture of a target **nucleic** acid is ... 2 illustrates a non-limiting embodiment of a **staggered** probe layout; FIG.

**EP3126524A1 - Improved compositions and methods for ...**
https://patents.google.com › patent
Probes include elements that allow MIP ends to abut for ligation while avoiding the ... C12Q1/6837 Enzymatic or biochemical coupling of **nucleic** acids to a solid ... Place the plates on thermal cyclers and **stagger**-start the anneal program 98°C, ...

**Tracking the processing of damaged DNA double-strand ...**
https://www.ncbi.nlm.nih.gov › pmc › articles › PMC3650721
by K Akopiants - 2014 - Cited by 11 - Related articles
Dec 25, 2013 - ... the chemical structure of the termini as well as the **stagger** between breaks in opposite ... Omission of NOS-C, CIP, the anchor, DNA ligase or either of the primers ... In whole-cell or **nuclear** extracts of TDP1-mutant SCAN1 ...

**Library construction for next-generation sequencing ...**
https://www.ncbi.nlm.nih.gov › pmc › articles › PMC4351865
by SR Head - 2014 - Cited by 402 - Related articles
Feb 1, 2014 - Three approaches are available to fragment **nucleic** acid chains: ... to the 5′ end to offset/ **stagger** the order of sequencing when adapters are ...
Missing: **mip** | Must include: **mip**

**Activity and structure of EcoKMcrA | Nucleic Acids Research ...**
https://academic.oup.com › nar › article
by H Czapinska - 2018 - Cited by 9 - Related articles
Aug 16, 2018 - **Nucleic** Acids Research, Volume 46, Issue 18, 12 October 2018, Pages ... most likely with a single **nucleotide stagger** (leading to 3′ overhangs). ... to M.B.}; Research Council of Lithuania [MIP-027/2012 to G.S.]; Part of this ...

**Solution structure of a dsDNA:LNA triplex | Nucleic Acids ...**
https://academic.oup.com › nar › article
by JJ Sørensen - 2004 - Cited by 38 - Related articles
Jan 1, 2004 - **Nucleic** Acids Research, Volume 32, Issue 20, 15 October 2004, Pages ... from the **stagger** between the cytosine and thymine nucleobases.

**[PDF] METHODS FOR ANALYZING NUCLEIC ACIDS - EPO**
https://data.epo.org › rest › publication-dates › patents › document
May 8, 2019 - ficiency of the MIP to the target sequence on the **nucleic** acid fragment ... designed to 'stagger' the exact positions of the hybridi- zation regions ...

**WO2006028517A2 - Comparative genomic resequencing ...**
www.google.com › patents
C12Q1/689 **Nucleic** acid products used in the analysis of **nucleic** acids, e.g. ... below the first set of microarrays were tiled **staggered** by seven nucleotides. Then ...

**US20050266423A1 - Novel nucleic acids and polypeptides ...**
https://www.google.com › patents
The present invention provides novel **nucleic** acids, novel polypeptide ... e.g., by employing blunt-ended or **stagger**-ended termini for ligation, restriction ... to IL-6, macrophage inflammatory protein 1-alpha (MIP-1-alpha), G-CSF, GM-CSF, ...

WO2002022660A2 - Novel nucleic acids and polypeptides ...
https://www.google.com › patents
The present invention provides nucleic acids, novel polypeptide sequences ... e.g., by employing blunt-ended or stagger-ended termini for ligation, restriction ... IL-8, macrophage inflammatory protein 1 -alpha (MIP- 1 -alpha), G-CSF, GM-CSF, ...

Searches related to mip OR CIP OR "padlock probe" OR "MOLECULAR inversion probe" AND stagger nucleic

**ngs library preparation ppt**
**ngs library definition**
**library construction for next-generation sequencing: overviews and challenges**
**sequencing library id**
**illumina sequencing protocol**
**ngs library prep comparison**
**illumina library prep method**
**ngs sample preparation**

1 2 3 4 5 6 7 8 9 Next

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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 3 | "8,778,609".pn. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:12 |
| L2 | 3 | "9,677,124".pn. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:14 |
| L3 | 7 | "10202637".pn. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:15 |
| L4 | 1,131 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT | OR | ON | 2020/06/04 11:37 |
| L5 | 299,360 | (mip or cip or padlock or (molecular near2 inversion near2 probe)) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:42 |
| L6 | 6,528 | l5 and (stagger$5 or tiling) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:42 |
| L7 | 98 | l5 with (stagger$5 or tiling) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:43 |
| L8 | 59 | l7 and stagger$5 | US-PGPUB; USPAT; | OR | ON | 2020/06/04 11:43 |

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|---|---|---|---|---|---|---|
| | | | USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | | | |
| L9 | 12 | l8 and @pd<"20080518" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:43 |
| L10 | 23,571 | l5 and hybridi$6 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:45 |
| L11 | 2,051 | l10 and (stagger$5 or tiling) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:45 |
| L12 | 45 | l7 and hybridi$6 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:46 |
| L13 | 0 | l12 and @pd<"20080518" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:46 |
| L14 | 6,528 | (l4 or l5) and l6 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:47 |
| L15 | 187 | (good near2 start near2 genetics).as. or (capital near2 royalty).as. or (parallel near2 investment).as. or (bridge near2 bank).as. or (western near2 alliance).as. or (molecular near2 loop near2 biosolutions).as. or Invitae.as. or combimatrix.as. or (inn near2 sa).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:47 |

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|---|---|---|---|---|---|---|
| L16 | 27 | (l4 or l15) and l6 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:48 |
| L17 | 13 | l16 and stagger$5 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:48 |
| L18 | 0 | l16 and stagger$5.clm. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 11:48 |
| S1 | 2 | "20050250147".pn. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2020/06/04 09:33 |

**EAST Search History (Interference)**

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6/4/2020 11:49:40 AM
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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 1093 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT | OR | ON | 2019/12/02 14:09 |
| L2 | 32 | (good near2 start near genetics).as. (capital near2 royalty near2 partners).as. or (parallel near2 investment near2 opportunities).as. or (bridge near2 bank).as. or (western near2 aliiance near2 bank).as. | US-PGPUB; USPAT | OR | ON | 2019/12/02 14:12 |
| L3 | 45 | (l1 or l2) and (mip or cip or (molecular near2 inversion near2 probe)) | US-PGPUB; USPAT | OR | ON | 2019/12/02 14:12 |
| L4 | 21 | l3 and (stagger$4 or tiling) | US-PGPUB; USPAT | OR | ON | 2019/12/02 14:13 |
| L5 | 3 | l3 and (stagger$4 or tiling).clm. | US-PGPUB; USPAT | OR | ON | 2019/12/02 14:13 |
| S1 | 3 | ("10227635" \| "9115387" \| "9677124").PN. | US-PGPUB; USPAT | OR | ON | 2019/11/28 06:12 |
| S2 | 2 | ("20060008824" \| "20060263789").PN. | US-PGPUB; USPAT | OR | ON | 2019/11/28 06:12 |
| S3 | 16 | 13/266862 | US-PGPUB; USPAT | OR | ON | 2019/12/02 09:03 |
| S4 | 16 | S3 and flank$4 | US-PGPUB; USPAT | OR | ON | 2019/12/02 09:03 |
| S5 | 16 | S3 and target | US-PGPUB; USPAT | OR | ON | 2019/12/02 09:06 |
| S6 | 16 | S3 and stagger$4 | US-PGPUB; USPAT | OR | ON | 2019/12/02 09:12 |

**EAST Search History (Interference)**

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**12/2/2019 2:16:35 PM**
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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L6 | 14 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 10:32 |
| L7 | 14 | L6 and (hybridization near2 region) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 10:32 |
| L8 | 1031 | ((("PORRECA") near3 ("Gregory")) OR (("LASERSON") near3 ("Uri")) OR (("LI") near3 ("Jin")) OR (("WASSMAN") near3 ("E"))).INV. | US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT | OR | ON | 2019/02/12 11:27 |
| L9 | 110 | (GOOD NEAR2 START NEAR2 GENETICS).AS. OR (CAPITAL NEAR2 ROYALTY NEAR2 PARTNERS).AS. OR (PARALLEL NEAR2 INVESTMENT NEAR2 OPPORTUNITIES).AS. OR (BRIDGE NEAR2 BANK).AS. OR (WESTERN NEAR2 ALLIANCE).AS. OR (INVITAE NEAR2 CORPORATION).AS. OR (COMBIMATRIX NEAR2 CORPORATION).AS. OR (INN NEAR2 SA NEAR2 LLC).AS. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 11:31 |
| L10 | 58 | (L8 OR L9) AND (MIP OR (MOLECULAR NEAR2 INVERSION NEAR2 PROBE) OR CIP OR CIPER) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 11:32 |
| L11 | 11 | (L8 OR L9) AND (MIP OR (MOLECULAR NEAR2 INVERSION NEAR2 PROBE) OR CIP OR CIPER).CLM. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 11:32 |
| S1 | 14 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 08:06 |
| | | | | | | |

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|---|---|---|---|---|---|---|
| S2 | 14 | S1 and stagger$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 08:06 |
| S3 | 1378 | stagger$4 with probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 08:08 |
| S4 | 9 | stagger$4 with (molecular near2 inversion near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 08:09 |
| S5 | 517 | S3 and @pd< "20080501" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 08:10 |
| S6 | 41 | S5 and hybridi$6 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2019/02/12 08:14 |

**EAST Search History (Interference)**

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**2/ 12/ 2019 11:33:14 AM**

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| S1 | 13 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2018/10/05 08:06 |
| S2 | 12 | S1 and overlap$4 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2018/10/05 08:06 |
| S3 | 13 | S1 and (target near2 nucleic ) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2018/10/05 08:22 |
| S4 | 31 | ((("UMBARGER") near3 ("Mark"))).INV. | US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT | OR | ON | 2018/10/05 10:52 |
| S5 | 72 | (good near2 start near2 genetics).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2018/10/05 10:52 |
| S6 | 42 | (S4 or S5) and ( overlap$4) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2018/10/05 10:53 |
| S7 | 9 | (S4 or S5) and ( overlap$4).clm. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2018/10/05 10:53 |

**10/ 5/ 2018 3:05:47 PM**

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 31 | (("PORRECA") near3 ("Gregory")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/11/03 14:42 |
| L2 | 2 | (("LASERSON") near3 ("Uri")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/11/03 14:42 |
| L3 | 822 | (("LI") near3 ("Jin")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/11/03 14:42 |
| L4 | 1 | (("WASSMAN") near3 ("E")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/11/03 14:42 |
| L5 | 69 | (good near2 start near2 genetics).as. or (capial near royalty near2 partners).as. or (parallel near2 investment near2 oppotunities).as. or (bridge near2 bank).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/03 14:44 |
| L6 | 1236 | (molecular near2 inversion near2 probes) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/03 14:44 |
| L7 | 73 | (molecular near2 inversion near2 probes).clm. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/03 14:44 |
| L8 | 73 | (l1 or l2 or l3 or l4 or l5 or l6) and l7 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/03 14:45 |
| L9 | 10 | (l1 or l2 or l3 or l4 or l5 ) and l7 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/03 14:45 |
| S1 | 13 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 11:36 |
| S2 | 13 | S1 and target | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 12:14 |
| S3 | 13 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:02 |

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|---|---|---|---|---|---|---|
| S4 | 0 | S3 and "entire target" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:02 |
| S5 | 1103 | staggered with probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:45 |
| S6 | 108 | S5 and hybridi$6 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:45 |
| S7 | 38 | S6 and @pd<"20080518" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:46 |
| S8 | 821 | staggered with primer | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:56 |
| S9 | 660 | S8 and hybridi$6 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:56 |
| S10 | 350 | S9 and @pd<"20080518" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:56 |
| S11 | 16046 | nested near2 pcr | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:58 |
| S12 | 0 | multiply near2 nested near2 pcr | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:59 |
| S13 | 6821 | S11 and @pd<"20080518" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:59 |
| S14 | 245 | S11 with advant$5 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 14:59 |
| S15 | 0 | S12 and @pd<"20080518" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 15:00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| S16 | 73 | S14 and @pd<"20080518" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/11/02 15:00 |

**11/3/2017 2:51:27 PM**
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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| S1 | 11 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 09:39 |
| S2 | 0 | S1 and (additonal near2 flanking) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 09:41 |
| S3 | 8 | S1 and (additional near2 flanking) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 09:41 |
| S4 | 1 | S1 and (entirely near2 overlaps) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 09:47 |
| S5 | 9 | S1 and (residues) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 09:56 |
| S6 | 1 | S1 and (nucleotide near2 flanking) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 10:36 |
| S7 | 29 | (("PORRECA") near3 ("Gregory")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/03/09 11:46 |
| S8 | 2 | (("LASERSON") near3 ("Uri")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/03/09 11:46 |
| S9 | 773 | (("LI") near3 ("Jin")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/03/09 11:46 |
| S10 | 1 | (("WASSMAN") near3 ("E")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2017/03/09 11:46 |
| S11 | 72 | (good near2 start near2 genetics).as. or (capital near2 royal nea2 prtners).as. (parallel near2 investment near2 opportunities).as. or (bridge near2 bacnk).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 11:48 |
| S12 | 34 | (S8 or S9 or S10 or S11 or S7) and (molecular near2 inversion near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 11:48 |

| | | | | | | |
|---|---|---|---|---|---|---|
| S13 | 21 | S12 and overlap | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2017/03/09 11:49 |

**3/9/2017 6:23:33 PM**
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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 51 | (good near2 start near2 genetics).as. or (captial near2 roylaty near2 partners).as. or (parallel near2 investment near2 opportunitities).as. or (bridge near2 bank).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2016/06/07 13:55 |
| L2 | 26 | ((Gregory) near2 (Porreca)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 14:04 |
| L3 | 2 | ((Uri) near2 (Laserson)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 14:04 |
| L4 | 734 | ((Jin) near2 (Li)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 14:04 |
| L5 | 1 | ((E) near2 (Wassman)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 14:04 |
| L6 | 31 | (L2 or L3 or L4 or L5 or l1) and (molecular near2 inversion near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2016/06/07 14:04 |
| S1 | 26 | ((Gregory) near2 (Porreca)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 12:19 |
| S2 | 2 | ((Uri) near2 (Laserson)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 12:19 |
| S3 | 734 | ((Jin) near2 (Li)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 12:19 |
| S4 | 1 | ((E) near2 (Wassman)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/06/07 12:19 |

**6/7/2016 2:06:32 PM**
**C:\Users\spohnert\Documents\EAST\Workspaces\13266862.wsp**

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 24 | (("Porreca") near2 ("Gregory")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/01/13 19:49 |
| L2 | 2 | (("Laserson") near2 ("Uri")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/01/13 19:49 |
| L3 | 1 | (("Li") near2 ("Jin") near2 ("Billy")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/01/13 19:49 |
| L4 | 1 | (("Wassman") near2 ("E") near2 ("Robert")).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2016/01/13 19:49 |
| L5 | 784 | (good near2 start near2 genetics).as. or (captial near2 royalty near2 partners).as. or (parallel near2 investment near2 opportunities).as. or (bridge near2 bank) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2016/01/13 19:51 |
| L6 | 26 | (l1 or l2 or l3 or l4 or l5) and (molecular near2 inversion near2 probes) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2016/01/13 19:52 |

**1/13/2016 7:54:21 PM**

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
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| L1 | 3 | "20060019304".pn. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 09:50 |
| L2 | 5 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 10:40 |
| L3 | 5 | L2 and reaction | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 10:40 |
| L4 | 5 | L2 and mixture | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 10:40 |
| L5 | 18 | ((Gregory) near2 (Porreca)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2015/03/06 10:42 |
| L6 | 2 | ((Uri) near2 (Laserson)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2015/03/06 10:43 |
| L7 | 634 | ((Jin) near2 (Li)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2015/03/06 10:43 |
| L8 | 1 | ((E) near2 (Wassman)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2015/03/06 10:43 |
| L9 | 33 | (good near2 start near2 genetics).as. or (capital near2 royalty near2 partners).as. or(parallel near2 investment near2 opportunities).as. or (bridge near2 bank).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 10:45 |
| L10 | 19 | (l5 or l6 or l7 or l8 or l9) and (inversion near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 10:45 |
| L11 | 5 | (l5 or l6 or l7 or l8 or l9) and (inversion near2 probe).clm. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 10:46 |
| S1 | 2 | "20060246500".pn. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 05:37 |

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| S2 | 5 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 08:21 |
| S3 | 3119 | "I5" and loci | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 08:21 |
| S4 | 5 | S2 and loci | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2015/03/06 08:21 |

**3/6/2015 10:48:14 AM**
**C:\Users\spohnert\Documents\EAST\Workspaces\13266862.wsp**

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| S1 | 2222966 | molecular newar2 inversion near2 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 08:02 |
| S2 | 593 | molecular near2 inversion near2 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 08:02 |
| S3 | 533 | S2 and (amount or concentration) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 08:03 |
| S4 | 95 | S3 and @pd<"20090430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 08:03 |
| S5 | 248 | S2 and ((amount or concentration or quantit$4) with probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 08:34 |
| S6 | 51 | S5 and @pd<"20090430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 08:34 |
| S7 | 18 | ((Gregory) near2 (Porreca)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2014/10/23 08:40 |
| S8 | 2 | ((Uri) near2 (Laserson)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2014/10/23 08:40 |
| S9 | 605 | ((Jin) near2 (Li)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2014/10/23 08:41 |
| S10 | 1 | ((E) near2 (Wassman)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2014/10/23 08:41 |
| S11 | 32 | (good near2 start near2 genetics).as. or (capital near2 royalty near2 partners).as. or (parallel near2 investment near2 opportunities).as. or (bridge near2 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; | OR | ON | 2014/10/23 08:43 |

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|---|---|---|---|---|---|---|
| | | bank).as. | IBM_TDB | | | |
| S12 | 20 | (S7 or S8 or S9 or S10 or S11) and (S2 or (padlock near2 probe)) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 08:44 |
| S13 | 593 | molecular near2 inversion near2 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:12 |
| S14 | 91 | spik$4 and S13 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:13 |
| S15 | 10 | S14 and @pd<"20090430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:13 |
| S16 | 10805 | (spik$4 or excess) near3 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:16 |
| S17 | 29 | S16 and S13 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:16 |
| S18 | 6 | S17 and @pd<"20090430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:16 |
| S19 | 6913 | S16 and @pd<"20090430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:29 |
| S20 | 6194 | S16 and @pd<"20080430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:29 |
| S21 | 13345 | (molecular near2 inversion near2 probe) or (rolling near2 circle) or (padlock near2 probe) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; | OR | ON | 2014/10/23 13:31 |

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| | | | IBM_TDB | | | |
| S22 | 456 | S20 and S21 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:31 |
| S23 | 1037 | (molecular near2 inversion near2 probe) or (rolling near2 circle) and S22 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:38 |
| S24 | 447 | ((molecular near2 inversion near2 probe) or (rolling near2 circle) ) and S22 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:38 |
| S25 | 705 | (spik$4 ) near3 probe | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:49 |
| S26 | 0 | S25 and 1l2 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:49 |
| S27 | 0 | S25 and 1l1 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:49 |
| S28 | 10 | S14 and @pd<"20090430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:50 |
| S29 | 484 | S25 and @pd<"20090430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:50 |
| S30 | 437 | S29 and @pd<"20080430" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 13:51 |
| S31 | 5 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; | OR | ON | 2014/10/23 15:34 |

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|---|---|---|---|---|---|---|
| | | | IBM_TDB | | | |
| S32 | 5 | S31 and locus | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2014/10/23 15:34 |

**EAST Search History (Interference)**

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**10/24/2014 3:34:53 PM**
**C:\Users\spohnert\Documents\EAST\Workspaces\13448961.wsp**

**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 10 | (good near2 start near2 genetics).as. or (capital near2 royalty near2 partners).as. or (parallel near2 investment near2 opportunitities).as. or (bridge near2 bank).as. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2013/05/30 15:50 |
| L2 | 10 | ((GREGORY) near2 (PORRECA)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:51 |
| L3 | 1 | ((URI) near2 (LASERSON)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:51 |
| L4 | 520 | ((JIN) near2 (LI)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:51 |
| L5 | 1 | ((E) near2 (WASSMAN)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:51 |
| L6 | 22586 | (l1 or L2 or L3 or L4 or L5) annd ((molecular near2 inversion) or padlock) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2013/05/30 15:51 |
| L7 | 10 | (l1 or L2 or L3 or L4 or L5) and ((molecular near2 inversion) or padlock) | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2013/05/30 15:51 |
| L8 | 4 | (l1 or L2 or L3 or L4 or L5) and ((molecular near2 inversion) or padlock).clm. | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2013/05/30 15:51 |
| S1 | 1 | 13/266862 | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2013/05/30 14:20 |
| S2 | 1 | S1 and "molecular inversion probe" | US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2013/05/30 14:21 |
| S3 | 10 | ((GREGORY) near2 (PORRECA)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:13 |
| S4 | 1 | ((URI) near2 (LASERSON)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:13 |
| S5 | 520 | ((JIN) near2 (LI)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:13 |
| S6 | 1 | ((E) near2 (WASSMAN)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/05/30 15:14 |

**EAST Search History (Interference)**

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**5/ 30/ 2013 3:54:42 PM**
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**EAST Search History**

**EAST Search History (Prior Art)**

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 4 | (good near2 start near2 genetics).as. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 13:32 |
| S1 | 10 | ((GREGORY) near2 (PORRECA)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 08:55 |
| S2 | 1 | ((URI) near2 (LASERSON)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 08:55 |
| S3 | 498 | ((JIN) near2 (LI)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 08:55 |
| S4 | 1 | ((E) near2 (WASSMAN)).INV. | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 08:55 |
| S6 | 1 | 13/266862 | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 09:04 |
| S7 | 0 | S6 and http | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 09:04 |
| S8 | 0 | S6 and (red or green or blue or color) | US-PGPUB; USPAT; USOCR | OR | ON | 2013/01/09 09:04 |

**1/9/2013 1:33:26 PM**
**C:\Users\spohnert\Documents\EAST\Workspaces\13266862.wsp**

PLUS Search Results for S/N 13266862, Searched Thu Dec 13 09:04:01 EST 2012

The Patent Linguistics Utility System (PLUS) is a USPTO automated search system for U.S. Patents from 1971 to the present PLUS is a query-by-example search system which produces a list of patents that are most closely related linguistically to the application searched. This search was prepared by the staff of the Scientific and Technical Information Center, SIRA.

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# BIB DATA SHEET

**CONFIRMATION NO. 6082**

| SERIAL NUMBER | FILING or 371(c) DATE<br>RULE | CLASS | GROUP ART UNIT | ATTORNEY DOCKET NO. |
|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | 435 | 1634 | GSGE-002/01US<br>29258/20 |

**APPLICANTS**

Gregory Porreca, Cambridge, MA;
Uri Laserson, Boston, MA;
Jin Billy Li, Medford, MA;
E. Robert Wassman, Marblehead, MA;

**** CONTINUING DATA ************************

This application is a 371 of PCT/US10/01293 04/30/2010
which claims benefit of 61/174,470 04/30/2009
and claims benefit of 61/174,923 05/01/2009
and claims benefit of 61/179,358 05/18/2009
and claims benefit of 61/182,089 05/28/2009

**** FOREIGN APPLICATIONS ************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
03/19/2012

| Foreign Priority claimed ☐ Yes ☑ No<br>35 USC 119(a-d) conditions met ☐ Yes ☑ No<br>Verified and Acknowledged /STEVEN C POHNERT/ Examiner's Signature | ☐ Met after Allowance<br>scp Initials | STATE OR COUNTRY | SHEETS DRAWINGS | TOTAL CLAIMS | INDEPENDENT CLAIMS |
|---|---|---|---|---|---|
| | | MA | 16 | 39 | 3 |

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**TITLE**

METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

| FILING FEE RECEIVED | | |
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| 1540 | FEES: Authority has been given in Paper<br>No._____ to charge/credit DEPOSIT ACCOUNT<br>No._____ for following: | ☐ All Fees<br>☐ 1.16 Fees (Filing)<br>☐ 1.17 Fees (Processing Ext. of time)<br>☐ 1.18 Fees (Issue)<br>☐ Other _____<br>☐ Credit |

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 11287372 |
| **Application Number:** | 13266862 |
| **International Application Number:** | PCT/US10/01293 |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Franziska Salcedo |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 28-OCT-2011 |
| **Filing Date:** | |
| **Time Stamp:** | 13:06:25 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $1165 |
| RAM confirmation Number | 10803 |
| Deposit Account | 500369 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

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|---|---|
| Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees) | |
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| Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges) | |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Transmittal Letter | GSGE_002_01US_371_Transmittal_Letter.pdf | 264065<br>4f2a6082de0cb5ff5bd3fe05fc157f4132e1e57a | no | 4 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Preliminary Amendment | GSGE_002_01US_Preliminary_Amendment.pdf | 44927<br>a9806a51a0ce385e5ef9cccdf1d20f3ed949f268 | no | 10 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Documents submitted with 371 Applications | GSGE_002_01US_WO2010126614A2.pdf | 2503161<br>e24c4bc05c7d1768909adc957100fa5487a93a12 | no | 116 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Application Data Sheet | GSGE-002-01US_SB14_Non-Provisional_ADS.pdf | 2145297<br>1cd733bce81f401a27c44dfb6ed25b1330eaafc0 | no | 6 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Fee Worksheet (SB06) | fee-info.pdf | 40137<br>82e81c4ee971c9f27efe64a4731ae17d6de8a847 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 4997587 | | |

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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

PTO-1390 (09-11)
Approved for use through 4/30/2013. OMB 0651-0021
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371

| ATTORNEY'S DOCKET NUMBER | U.S. APPLICATION NO. (If known, see 37 CFR 1.5) |
|---|---|
| GSGE-002/01US 29258/20 | |

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| PCT/US2010/001293 | 30 April 2010 | 30 April 2009 |

TITLE OF INVENTION
Methods and compositions for evaluating genetic markers

APPLICANT(S) FOR DO/EO/US
Porreca et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a submission under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a submission under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☑ The US has been elected (Article 31).
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☑ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11 to 20 below concern document(s) or information included:**

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☑ A preliminary amendment.
14. ☑ An Application Data Sheet under 37 CFR 1.76.
15. ☐ A substitute specification.
16. ☐ A power of attorney and/or change of address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.3 and 37 CFR 1.821- 1.825.
18. ☑ A second copy of the published International Application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).

This collection of information is required by 37 CFR 1.414 and 1.491-1 .492. The information is required to obtain or retain a b enefit by the public, w hich is to file (and by the USPTO to pro cess) an application . Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 15 minutes to complete, including gathering information, preparing, and submitting the completed form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEE S OR COMPLE TED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

PTO-1390 (09-11)
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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

| U.S. APPLICATION NO. (if known, see 37 CFR 1.5) | INTERNATIONAL APPLICATION NO. | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| | PCT/US2010/001293 | GSGE-002/01US 29258/20 |

20. Other items or information:

| The following fees have been submitted | CALCULATIONS | PTO USE ONLY |
|---|---|---|
| 21. ☑ Basic national fee (37 CFR 1.492(a)) ..... **$380** | $ 380 | |
| 22. ☑ Examination fee (37 CFR 1.492(c))<br>If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4)..... **$0**<br>All other situations..... **$250** | $ 250 | |
| 23. ☑ Search fee (37 CFR 1.492(b))<br>If the written opinion of the ISA/US or the International preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4)..... **$0**<br>Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority..... **$120**<br>International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB..... **$490**<br>All other situations..... **$620** | $ 120 | |
| **TOTAL OF 21, 22 and 23 =** | 750 | |

☑ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)).
The fee is **$310** for each additional 50 sheets of paper or fraction thereof.

| Total Sheets | Extra Sheets | Number of each additional 50 or fraction thereof (round **up** to a whole number) | RATE | |
|---|---|---|---|---|
| 116 - 100 = | 16 /50 = | 1 | x $**310** | $ 310 |

| | | |
|---|---|---|
| Surcharge of **$130.00** for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)). | $ 130 | |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | $ | |
|---|---|---|---|---|---|
| Total claims | 39 - 20 = | 19 | x $ **60** | $1140 | |
| Independent claims | 3 - 3 = | 0 | x **$250** | $0 | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | + **$450** | $ 0 | |
| | | | **TOTAL OF ABOVE CALCULATIONS =** | $2330 | |
| ☑ Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by ½. | | | | | |
| | | | **SUBTOTAL =** | $ 1165 | |
| Processing fee of **$130.00** for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). + | | | | $ 0 | |
| | | | **TOTAL NATIONAL FEE =** | $ 1165 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property + | | | | $ 0 | |
| | | | **TOTAL FEES ENCLOSED =** | $ 1165 | |
| | | | **Amount to be refunded:** | | $ 0 |
| | | | **Amount to be charged** | | $1165 |

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☑ Please charge my Deposit Account No. 500369 in the amount of $ 1165 to cover the above fees.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 500369.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

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**NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.**

SEND ALL CORRESPONDENCE TO:

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111

/s/Thomas C. Meyers/
SIGNATURE

Thomas C. Meyers
NAME

36,989
REGISTRATION NUMBER

# Privacy Act Statement

The **Privacy Act of 1974** (**P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANT: | Porreca et al. | ART UNIT: | Not yet assigned |
| SERIAL NUMBER: | 35 U.S.C. §371 national phase application of PCT/US2010/001293 | CONFIRMATION NO.: | Not yet assigned |
| FILING DATE: | October 28, 2011 | EXAMINER: | Not yet assigned |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

**FILED ELECTRONICALLY**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

**PRELIMINARY AMENDMENT**

Prior to examination of the above utility patent application, please amend the specification and claims and consider the following remarks.

Applicants believe that no additional fee is due with respect to this Preliminary Amendment. However, Applicants authorize the Commissioner to charge any fees that may be due to Deposit Account Number 500369.

**Amendments to the Specification** begin on p. 2.

**Amendments to the Claims** begin on p. 3.

**Remarks** begin on p. 10.

Amendments to the specification

Please amend the "RELATED APPLICATIONS" section at p. 1 of the application as filed as follows:

This application is a national phase application and claims the benefit of and priority to PCT/US2010/001293, filed April 30, 2010, which claims the benefit under 35 U.S.C. §119(e) of U.S. provisional application serial number 61/174,470, filed April 30, 2009, U.S. provisional application serial number 61/178,923, filed May 15, 2009, U.S. provisional application serial number 61/179,358, filed May 18, 2009, and U.S. provisional application serial number 61/182,089, filed May 28, 2009, the entire contents of each of which are incorporated herein by reference.



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Amendments to the claims

Please amend claims 14-16, 22, 24, 28, 33, 36-37, 41-44, 50, 52, and 56 and cancel claims 5-11, 17-19, 31-32, 34-35, 39, 48-49, and 57-67 as follows. This listing of claims will replace all prior versions and listings of claims in the application.

1. (Original) A method of analyzing a plurality of genetic loci, the method comprising:

contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion,

isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

2. (Original) The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion.

3. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.

4. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5-11. (Canceled)

12. (Original) A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. (Original) The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

14. (Currently amended) The method of claim 12 ~~or 13~~, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

15. (Currently amended) The method of ~~any one of claims 12-14~~ claim 12, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. (Currently amended) The method of ~~any one of claims 12-14~~ claim 12, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

17-19. (Canceled)

20. (Original) A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. (Original) The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. (Currently amended) The method of claim 20 ~~-21~~, wherein the capture efficiency is lower than the reference capture efficiency.

23. (Original) The method of claim 22, wherein the subject is identified as having an insertion in the target region.

24. (Currently amended) The method of claim 20 ~~-21~~, wherein the capture efficiency is higher than the reference capture efficiency.

25. (Original) The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. (Original) The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.



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27. (Original) The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. (Currently amended) The method of ~~any prior~~ claim 20, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. (Original) The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

30. (Original) The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31-32. (Canceled)

33. (Currently amended) The method of ~~any one of claims 29-31~~ claim 29, wherein the first and second targeting arms have the same length.

34-35. (Canceled)

36. (Currently amended) The method of claim ~~34~~ 29, wherein the hybridization Tms of the first and second targeting arms are identical.



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37. (Currently amended) The method of ~~any one of claims 28-36~~ claim 28, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. (Original) The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. (Canceled)

40. (Original) The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. (Currently amended) The method of ~~any one of claims 29-36~~ claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.

42. (Currently amended) The method of ~~any one of claims 29-36~~ claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.

43. (Currently amended) The method of ~~any one of claims 29-36~~ claim 29, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. (Currently amended) The method of ~~any of the prior claims~~ claim 20, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. (Original) The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. (Original) The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. (Original) The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48-49. (Canceled)

50. (Currently amended) The method of ~~any prior~~ claim 20, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. (Original) The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.

52. (Currently amended) The method of claim 50 ~~51~~, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.

53. (Original) The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. (Original) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.



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55. (Original) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. (Currently amended) The method of ~~any prior~~ claim 20, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57-67 (Canceled)



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Remarks

Applicants submit herewith a 35 U.S.C. §371 national phase application. This Preliminary Amendment amends claims 14-16, 22, 24, 28, 33, 36-37, 41-44, 50, 52, and 56 to remove the multiple dependency in those claims and cancels claims 5-11, 17-19, 31-32, 34-35, 39, 48-49, and 57-67. Additionally, the specification of this national phase application is herein amended by this Preliminary Amendment to add a benefit claim to PCT international patent application number PCT/US2010/001293, filed April 30, 2010. No new matter is added and no new material presented that would necessitate an additional search on the part of the examiner.

Summary

Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested. If there are any questions regarding these remarks, the Examiners are invited and encouraged to contact Applicants' representatives at the telephone number provided.

Dated: October 28, 2011

Respectfully submitted,
**BROWN RUDNICK LLP**

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8434
Fax: (617) 856-8201

/s/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicants
Email: tmeyers@brownrudnick.com

1864781



Petitioner Illumina, Inc., EX1002, p. 129

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| 61/179,358 | 18 May 2009 (18.05.2009) | US |
| 61/182,089 | 28 May 2009 (28.05.2009) | US |

(71) **Applicant** *(for all designated States except US)*: **GOOD START GENETICS, INC.** [US/US]; 8 St. Mary's Street, Suite 621, Boston, MA 02215 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **PORRECA, Gregory** [US/US]; 911 Garden Parkway, Ocean City, NJ 08226 (US). **LASERSON, Uri** [US/US]; 645 Beacon Street #215, Boston, MA 02215 (US). **LI, Jin, Billy** [CN/US]; 100 Station Landing, Unit 204, Medford, MA 02155 (US). **WASSMAN, E., Robert** [US/US]; 90 West Shore Drive, Marblehead, MA 01945 (US).

(74) **Agent**: **WALLER, Patrick, R., H.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

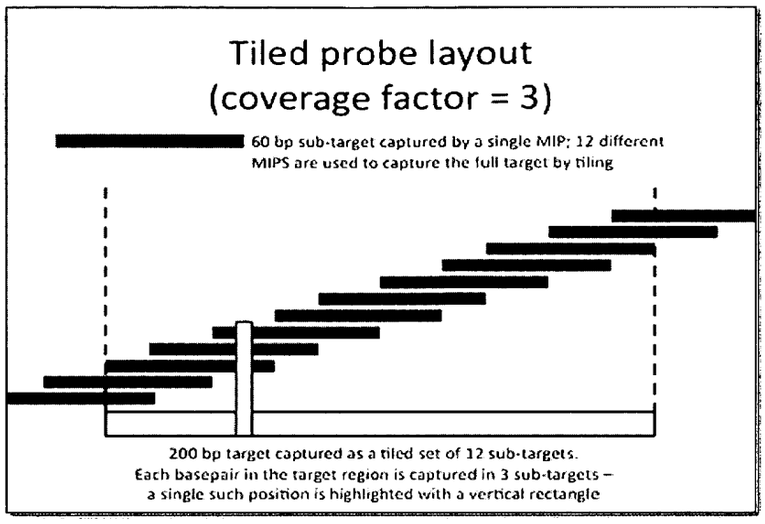
(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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*[Continued on next page]*

(54) **Title**: METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

**Figure 1**




(57) **Abstract**: Aspects of the invention relates to methods and compositions that are useful to reduce bias and increase the reproducibility of multiplex analysis of genetic loci. In some configurations, predetermined preparative steps and/or nucleic acid sequence analysis techniques are used in multiplex analyses for a plurality of genetic loci in a plurality of samples.

WO 2010/126614 A3

WO 2010/126614 A3

**Published**:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

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(71) Applicant *(for all designated States except US)*: **GOOD START GENETICS, INC.** [US/US]; 8 St. Mary's Street, Suite 621, Boston, MA 02215 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PORRECA, Gregory** [US/US]; 911 Garden Parkway, Ocean City, NJ 08226 (US). **LASERSON, Uri** [US/US]; 645 Beacon Street #215, Boston, MA 02215 (US). **LI, Jin, Billy** [CN/US]; 100 Station Landing, Unit 204, Medford, MA 02155 (US). **WASSMAN, E., Robert** [US/US]; 90 West Shore Drive, Marblehead, MA 01945 (US).

(74) Agent: **WALLER, Patrick, R., H.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

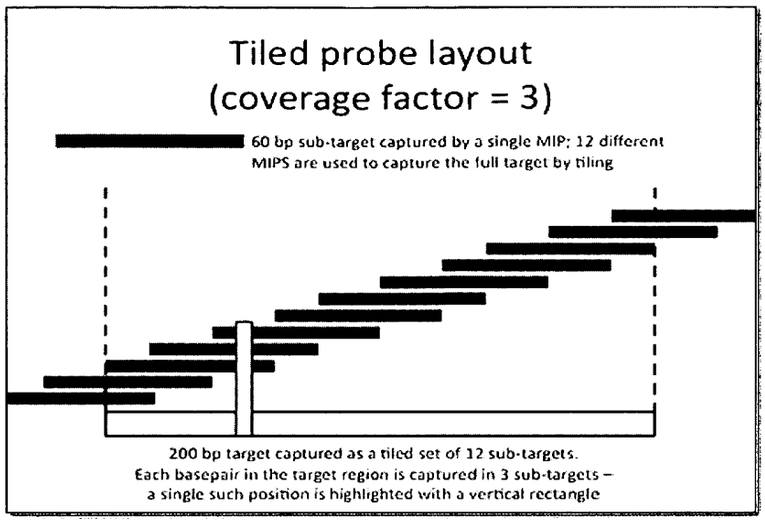
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(54) Title: METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

**Figure 1**




(57) Abstract: Aspects of the invention relates to methods and compositions that are useful to reduce bias and increase the reproducibility of multiplex analysis of genetic loci. In some configurations, predetermined preparative steps and/or nucleic acid sequence analysis techniques are used in multiplex analyses for a plurality of genetic loci in a plurality of samples.

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# METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

## RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. §119(e) of U.S. provisional application serial number 61/174,470, filed April 30, 2009, U.S. provisional application serial number 61/178,923, filed May 15, 2009, U.S. provisional application serial number 61/179,358, filed May 18, 2009, and U.S. provisional application serial number 61/182,089, filed May 28, 2009, the entire contents of each of which are incorporated herein by reference.

## FIELD OF INVENTION

The invention relates to methods and compositions for determining genotypes in patient samples.

## BACKGROUND OF THE INVENTION

Information about the genotype of a subject is becoming more important and relevant for a range of healthcare decisions as the genetic basis for many diseases, disorders, and physiological characteristics is further elucidated. Medical advice is increasingly personalized, with individual decisions and recommendations being based on specific genetic information. Information about the type and number of alleles at one or more genetic loci impacts disease risk, prognosis, therapeutic options, and genetic counseling amongst other healthcare considerations.

For cost-effective and reliable medical and reproductive counseling on a large scale, it is important to be able correctly and unambiguously identify the allelic status for many different genetic loci in many subjects.

Numerous technologies have been developed for detecting and analyzing nucleic acid sequences from biological samples. These technologies can be used to genotype subjects and determine the allelic status of any locus of interest. However, they are not sufficiently robust and cost-effective to be scaled up for reliable high throughput analysis of many genetic loci in large numbers of patients. The frequency of incorrect or ambiguous calls is too high for current technology to manage large numbers of patient

samples without involving expensive and time-consuming steps to resolve uncertainties and provide confidence in the information output.

## SUMMARY OF THE INVENTION

Aspects of the invention relate to preparative and analytical methods and compositions for evaluating genotypes, and in particular, for determining the allelic identity (or identities in a diploid organism) of one or more genetic loci in a subject.

Aspects of the invention are based, in part, on the identification of different sources of ambiguity and error in genetic analyses, and, in part, on the identification of one or more approaches to avoid, reduce, recognize, and/or resolve these errors and ambiguities at different stages in a genetic analysis.

According to aspects of the invention, certain types of genetic information can be under-represented or over-represented in a genetic analysis due to a combination of stochastic variation and systematic bias in any of the preparative stages (e.g., capture, amplification, etc.), determining stages (e.g., allele-specific detection, sequencing, etc.), data interpretation stages (e.g., determining whether the assay information is sufficient to identify a subject as homozygous or heterozygous), and/or other stages.

According to aspects of the invention, error or ambiguity may be apparent in a genetic analysis, but not readily resolved without running additional samples or more expensive assays (*e.g.*, array-based assays may report no-calls due to noisy/low signal). According to further aspects of the invention, error or ambiguity may not be accounted for in a genetic analysis and incorrect base calls may be made even when the evidence for them is limited and/or not statistically significant (*e.g.*, next-generation sequencing technologies may report base calls even if the evidence for them is not statistically significant). According to further aspects of the invention error or ambiguity may be problematic for a multi-step genetic analysis because it is apparent but not readily resolved in one or more steps of the analysis and not apparent or accounted for in other steps of the analysis.

In some embodiments, sources of error and ambiguity in one or more steps can be addressed by capturing and/or interrogating each target locus of interest with one or more sets of overlapping probes that are designed to overcome any systematic bias or stochastic effects that may impact the complexity and/or fidelity of the genetic information that is generated.



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In some embodiments, sources of error and ambiguity in one or more steps can be addressed by capturing and/or interrogating each target locus of interest with at least one set of probes, wherein different probes are labeled with different identifiers that can be used to track the assay reactions and determine whether certain types of genetic information are under-represented or over-represented in the information that is generated.

In some embodiments, errors and ambiguities associated with the analysis of regions containing large numbers of sequence repeats are addressed by systematically analyzing frequencies of certain nucleic acids at particular stages in an assay (e.g., at a capture, sequencing, or detection stage). It should be appreciated that such techniques may be particularly useful in the context of a standardized protocol that is designed to allow many different loci to be evaluated in parallel without requiring different assay procedures for each locus. In some embodiments, the use of a single detection modality (*e.g.*, sequencing) to assay multiple types of genetic lesions (*e.g.*, point mutations, insertions/deletions, length polymorphisms) is advantageous in the clinical setting. In some embodiments of the invention, methods are provided that facilitate the use of multiple sample preparation steps in parallel, coupled with multiple analytical processes following sequence detection. Thus, in some embodiments of the invention, an improved workflow is provided that reduces error and uncertainty when simultaneously assaying different types of genetic lesions across multiple loci in multiple patients.

In some embodiments, aspects of the invention provide methods for overcoming preparative and/or analytical bias by combining two or more techniques, each having a different bias (e.g., a known bias towards under-representation or over-representation of one or more types of sequences), and using the resulting data to determine a genetic call for a subject with greater confidence.

It should be appreciated that in some embodiments, aspects of the invention relate to multiplex diagnostic methods. In some embodiments, multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel (e.g., one or more genetic loci from Table 1). In some embodiments, the genetic loci possess one or more polymorphisms (e.g., one or more polymorphisms from Table 2) the genotypes of which correspond to disease causing alleles. Accordingly, in some embodiments, the disclosure provides methods for assessing multiple heritable disorders in parallel. In some



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embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample) as aspects of the invention are not limited in this respect.

Other samples may include tumor tissue or circulating tumor cells. In some embodiments, a patient sample (*e.g.*, a tumor tissue or cell sample) is mosaic for one or more mutations of interest, and thus, may require higher sensitivity than is needed for a germline mutation analysis. In some embodiments, a sample comprises cells from a non-host organism (*e.g.*, bacterial or viral infections in a human subject) or a sample for environmental monitoring (*e.g.*, bacterial, viral, fungal composition of a soil, water, or air sample).

Accordingly, in some embodiments, aspects of the methods disclosed herein relate to genotyping a polymorphism of a target nucleic acid. In some embodiments, the genotyping may comprise determining that one or more alleles of the target nucleic acid are heterozygous or homozygous. In further embodiments, the genotyping may comprise determining the sequence of a polymorphism and comparing that sequence to a control sequence that is indicative of a disease risk. In some embodiments, the polymorphism is selected from a locus in Table 1 or Table 2. However, it should be appreciated that any locus associated with a disease or condition of interest may be used.

In some embodiments, a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject). In some embodiments, an assessment is provided to a couple, based on their respective genotypes at one or more genetic loci, of the risk of their having one or more children having a genotype associated with a disease or condition (e.g., a homozygous or heterozygous genotype associated with a disease or condition). In some embodiments, a subject or a couple may seek genetic or reproductive counseling in connection with a genotype determined according to embodiments of the invention. In some embodiments, genetic information from a tumor or circulating tumor cells is used to determine prognosis and guide selection of appropriate drugs/treatments.



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It should be appreciated that any of the methods or compositions described herein may be used in combination with any of the medical evaluations associated with one or more genetic loci as described herein.

In some embodiments, aspects of the invention provide effective methods for overcoming challenges associated with systematic errors (bias) and/or stochastic effects in multiplex genomic capture and/or analysis (including sequencing analysis). In some embodiments, aspects of the invention are useful to avoid, reduce and/or account for variability in one or more sampling and/or analytical steps. For example, in some embodiments, variability in target nucleic acid representation and unequal sampling of heterozygous alleles in pools of captured target nucleic acids can be overcome.

Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the detection of target nucleic acids in multiplex capture methods. In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of different sets of multiple probes (e.g., molecular inversion probes MIPs) that capture overlapping regions of a target nucleic acid to achieve a more uniform representation of the target nucleic acids in a capture pool compared with methods of the prior art. In other embodiments, methods reduce bias, or the risk of bias, associated with large scale parallel capture of genetic loci, e.g., for diagnostic purposes. In other embodiments, methods are provided for increasing reproducibility (e.g., by reducing the effect of polymorphisms on target nucleic acid capture) in the detection of a plurality of genetic loci in parallel. In further embodiments, methods are provided for reducing the effect of probe synthesis and/or probe amplification variability on the analysis of a plurality of genetic loci in parallel.

According to some aspects, methods of analyzing a plurality of genetic loci are provided. In some embodiments, the methods comprise contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion, isolating a plurality of nucleic acids each having a nucleic acid sequence of a different



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subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids.

In other embodiments, methods comprise contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the sequence of the 3' region of a different probe, isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids.

Aspects of the disclosure are based, in part, on the discovery of methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, namely high variability in the capture and amplification of nucleic acids and disproportionate representation of heterozygous alleles in sequencing libraries. Accordingly, in some embodiments, the disclosure provides methods that reduce errors associated with the variability in the capture and amplification of nucleic acids. In other embodiments, the methods improve allelic representation in sequencing libraries and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of differentiator tag sequences to uniquely tag individual nucleic acid molecules. In some embodiments, the differentiator tag sequence permit the detection of bias based on the occurrence of combinations of differentiator tag and target sequences observed in a sequencing reaction. In other embodiments, the methods reduce errors caused by bias, or the risk of bias, associated with the capture, amplification and sequencing of genetic loci, e.g., for diagnostic purposes.

Aspects of the invention relate to providing sequence tags (referred to as differentiator tags) that are useful to determine whether target nucleic acid sequences identified in an assay are from independently isolated target nucleic acids or from multiple copies of the same target nucleic acid molecule (e.g., due to bias in a preparative step, for example, amplification). This information can be used to help analyze a



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threshold number of independently isolated target nucleic acids from a biological sample in order to obtain sequence information that is reliable and can be used to make a genotype conclusion (e.g., call) with a desired degree of confidence. This information also can be used to detect bias in one or more nucleic acid preparative steps.

In some embodiments, the methods disclosed herein are useful for any application where reduction of bias, e.g., associated with genomic isolation, amplification, sequencing, is important. For example, detection of cancer mutations in a heterogeneous tissue sample, detection of mutations in maternally-circulating fetal DNA, and detection of mutations in cells isolated during a preimplantation genetic diagnostic procedure.

Accordingly, in some aspects, methods of genotyping a subject are provided. In some embodiments, the methods comprise determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject.

In some embodiments, the isolated nucleic acids are products of a circularization selection-based preparative method, e.g., molecular inversion probe capture products. In other embodiments, the isolated nucleic acids are products of an amplification-based preparative methods. In other embodiments, the isolated nucleic acids are products of hybridization-based preparative methods.

Circularization selection-based preparative methods selectively convert regions of interest (target nucleic acids) into a covalently-closed circular molecule which is then isolated typically by removal (usually enzymatic, e.g. with exonuclease) of any non-circularized linear nucleic acid. Oligonucleotide probes (e.g., molecular inversion probes) are designed which have ends that flank the region of interest (target nucleic acid) and, optionally, primer sites, e.g., sequencing primer sites. The probes are allowed to hybridize to the genomic target, and enzymes are used to first (optionally) fill in any gap between probe ends and second ligate the probe closed. Following circularization, any remaining (non-target) linear nucleic acid is typically removed, resulting in isolation



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(capture) of target nucleic acid. Circularization selection-based preparative methods include molecular inversion probe capture reactions and 'selector' capture reactions. In some embodiments, molecular inversion probe capture of a target nucleic acid is indicative of the presence of a polymorphism in the target nucleic acid.

In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using one or more oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or more of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and differentiator tag sequences. Amplification-based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel).

Hybridization-based preparative methods involve selectively immobilizing target nucleic acids for further manipulation. It is to be understood that one or more oligonucleotides (immobilization oligonucleotides), which comprise differentiator tag sequences, and which may be from 15 to 170 nucleotides in length, are used which hybridize along the length of a target region of a genetic locus to immobilize it. In some embodiments, immobilization oligonucleotides, are either immobilized before hybridization is performed (e.g., Roche/Nimblegen 'sequence capture'), or are prepared such that they include a moiety (e.g. biotin) which can be used to selectively immobilize the target nucleic acid after hybridization by binding to e.g., streptavidin-coated microbeads (e.g. Agilent 'SureSelect').

It should be appreciated that any of the circularization, amplification, and/or hybridization based methods described herein may be used in connection with one or more of the tiling/staggering, tagging, size-detection, and/or sensitivity enhancing algorithms described herein.

In some embodiments, the methods disclosed herein comprise determining the sequence of molecular inversion probe capture products, each comprising a molecular



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inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, and wherein the target nucleic acid is a captured genomic locus of a subject, and genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products.

In some embodiments, the methods disclosed herein comprise obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products. In certain embodiments, obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence. In specific embodiments, capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

In one embodiment, the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype. In some embodiments, the methods further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

According to some aspects, methods of analyzing a plurality of genetic loci are provided. In some embodiments, the methods comprise obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (e.g., a sequence that is complementary to the sequence of a nucleic acid that is used as a primer for sequencing or other extension reaction), amplifying the plurality of molecular



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inversion probe capture products, determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. In some embodiments, the methods further comprise genotyping target sequences in the plurality, wherein the genotyping comprises correcting for bias, if detected.

In some embodiments, the target nucleic acid is a gene (or portion thereof) selected from Table 1. In some embodiments, the genotyping comprises determining the sequence of a target nucleic acid (e.g., a polymorphic sequence) at one or more (both) alleles of a genome (a diploid genome) of a subject. In certain embodiments, the genotyping comprises determining the sequence of a target nucleic acid at both alleles of a diploid genome of a subject, wherein in the target nucleic acid comprises, or consists of, a sequence of Table 1, Table 2, or other locus of interest.

In some embodiments, aspects of the invention provide methods and compositions for identifying nucleic acid insertions or deletions in genomic regions of interest without determining the nucleotide sequences of these regions. Aspects of the invention are particularly useful for detecting nucleic acid insertions or deletions in genomic regions containing nucleic acid sequence repeats (e.g., di- or tri-nucleotide repeats). However, the invention is not limited to analyzing nucleic acid repeats and may be used to detect insertions or deletions in any target nucleic acid of interest. Aspects of the invention are particularly useful for analyzing multiple loci in a multiplex assay.

In some embodiments, aspects of the invention relate to determining whether an amount of target nucleic acid that is captured in a genomic capture assay is higher or lower than expected. In some embodiments, a statistically significant deviation from an expected amount (e.g., higher or lower) is indicative of the presence of a nucleic acid insertion or deletion in the genomic region of interest. In some embodiments, the amount is a number of nucleic acid molecules that are captured. In some embodiments, the amount is a number of independently captured nucleic acid molecules in a sample. It should be appreciated that the captured nucleic acids may be literally captured from a sample, or their sequences may be captured without actually capturing the original nucleic acids in the sample. For example, nucleic acid sequences may be captured in an



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assay that involves a template-based extension of nucleic acids having the region of interest, in the sample.

Aspects of the invention are based on the recognition that the efficiency of certain capture techniques is affected by the length of the nucleic acid being captured. Accordingly, an increase or decrease in the length of a target nucleic acid (e.g., due to an insertion or deletion of a repeated sequence) can alter the capture efficiency of that nucleic acid. In some embodiments, a difference in the capture efficiency (e.g., a statistically significant difference in the capture efficiency) of a target nucleic acid is indicative of an insertion or deletion in the target nucleic acid. It should be appreciated that the capture efficiency for a target nucleic acid may be evaluated based on an amount of captured nucleic acid (e.g., number of captured nucleic acid molecules) relative to a control amount (e.g., based on an amount of control nucleic acid that is captured). However, the invention is not limited in this respect and other techniques for evaluating capture efficiency also may be used.

According to aspects of the invention, evaluating the capture efficiency as opposed to determining the sequence of the entire repeat region reduces errors associated with sequencing through repeat regions. Repeat sequences often give rise to stutters or skips in sequencing reactions that make it very difficult to accurately determine the number of repeats in a target region without running multiple sequencing reactions under different conditions and carefully analyzing the results. Such procedures are cumbersome and not readily scalable in a manner that is consistent with high throughput analyses of target nucleic acids. In some embodiments, repeat regions may be longer than the length of the individual sequence read, making length determination on the basis of a single read impossible. For example, when using next-generation sequencing the repeat regions may be longer than the length of the individual sequence read, making length determination on the basis of a single read impossible. Accordingly, aspects of the invention are useful to increase the sensitivity of detecting insertions or deletions in target regions, particularly target regions containing repeated sequences.

In some embodiments, aspects of the invention relate to capturing genomic nucleic acid sequences using a molecular inversion probe (e.g., MIP or Padlock probe) technique, and determining whether the amount (e.g., number) of captured sequences is higher or lower than expected. In some embodiments, the amount (e.g., number) of captured sequences is compared to an amount (e.g., number) of sequences captured in a



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control assay. The control assay may involve analyzing a control sample that contains a nucleic acid from the same genetic locus having a known sequence length (e.g., a known number of nucleic acid repeats). However, a control may involve analyzing a second (e.g., different) genetic locus that is not expected to contain any insertions or deletions. The second genetic locus may be analyzed in the same sample as the locus being interrogated or in a different sample where its length has been previously determined. The second genetic locus may be a locus that is not characterized by the presence of nucleic acid repeats (and thus not expected to contain insertions or deletions of the repeat sequence).

In some embodiments, a target nucleic acid region that is being evaluated may be determined by the identity of the targeting arms of a probe that is designed to capture the target region (or sequence thereof). For example, the targeting arms of a MIP probe may be designed to be complementary (e.g., sufficiently complementary for selective hybridization and//or polymerase extension and/or ligation) to genomic regions flanking a target region suspected of containing an insertion or deletion. It should be appreciated that two targeting arms may be designed to be complementary (e.g., sufficiently complementary for selective hybridization and/or polymerase extension and/or ligation) to the two flanking regions that are immediately adjacent (e.g., immediately 5' and 3', respectively) to a region of a sequence repeat on one strand of a genomic nucleic acid. However, one or both targeting arms may be designed to hybridize several bases (e.g., 1-5, 5-10, 10-25, 25-50, or more) upstream or downstream from the repeat region in such a way that the captured sequence includes a region of unique genomic sequence that on one or both sides of the repeat region. This unique region can then be used to identify the captured target (e.g., based on sequence or hybridization information).

In some embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) different loci may be interrogated in parallel in a single assay (e.g., in a multiplex assay). In some embodiments, the ratio of captured nucleic acids for each locus may be used to determine whether a nucleic acid insertion or deletion is present in one locus relative to the other. For example, the ratio may be compared to a control ratio that is representative of the two loci when neither one has an insertion or deletion relative to control sequences (e.g., sequences that are normal or known to be associated with healthy phenotypes for those loci). However, the amount of captured nucleic acids may be compared to any suitable control as discussed herein.



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The locus of a captured sequence may be identified by determining a portion of unique sequence 5' and/or 3' to the repeat region in the target nucleic acid suspected of containing a deletion or insertion. This does not require sequencing the captured repeat region itself. However, some or all of the repeat region also could be sequenced as aspects of the invention are not limited in this respect.

Aspects of the invention may be combined with one or more sequence-based assays (e.g., SNP detection assays), for example in a multiplex format, to determine the genotype of one or more regions of a subject.

In some embodiments, methods of detecting a polymorphism in a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise evaluating the efficiency of capture at one or more loci and determining whether one or both alleles at that locus contain an insertion or deletion relative to a control locus (e.g., a locus indicative of a length of repeat sequence that is associated with a healthy phenotype).

Accordingly, aspects of the invention relate to methods for determining whether a target nucleic acid has an abnormal length by evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length. It should be appreciated that the term "abnormal" is a relative term based on a comparison to a "normal" length. In some embodiments, a normal length is a length that is associated with a normal (e.g., healthy or non-carrier phenotype). Accordingly, an abnormal length is a length that is either shorter or longer than the normal length. In some embodiments, the presence of an abnormal length is indicative of an increased risk that the locus is associated with a disease or a disease carrier phenotype. In some embodiments, the abnormal length is indicative that the subject is either has a disease or condition or is a carrier of a disease or condition (e.g., associated with the locus). However, it should be appreciated that the description of embodiments relating to detecting the presence of an abnormal length also support detecting the presence of a length that is different from an expected or control length.

In some embodiments, aspects of the invention relate to estimating the length of a target nucleic acid (e.g., of a sub-target region within a target nucleic acid). In some embodiments, aspects of the invention relate to methods for estimating the length of a



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target nucleic acid by contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid, wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid, wherein the first and second regions are on a common strand of the target nucleic acid, and wherein the nucleotide sequence of the target between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid; and capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes; and measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the sub-target nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. It should be appreciated that methods for estimating a nucleic acid length may involve comparing a capture efficiency for a target nucleic acid region to two or more reference efficiencies for known nucleic acid lengths in order to determine whether the target nucleic acid region is smaller, intermediate, or larger in size than the known control lengths. In some embodiments, a series of nucleic acids of known different lengths may be used to provide a calibration curve for evaluating the length of a target nucleic acid region of interest.

In some embodiments, the capture efficiency of a target region suspected of having a deletion or insertion is determined by comparing the capture efficiency to a reference indicative of a normal capture efficiency. In some embodiments, the capture efficiency is lower than the reference capture efficiency. In some embodiments, the subject is identified as having an insertion in the target region. In some embodiments, the capture efficiency is higher than the reference capture efficiency. In some embodiments, the subject is identified as having a deletion in the target region. In some embodiments, the subject is identified as being heterozygous for the insertion. In some embodiments, the subject is identified as being heterozygous for the deletion.

In some embodiments of any of the methods described herein (e.g., tiling/staggering, tagging, size-detection, and/or sensitivity enhancement) aspects of the invention relate to capturing a sub-target nucleic acid (or a sequence of a sub-target nucleic acid). In some embodiments, a molecular inversion probe technique is used. In some embodiments, a molecular inversion probe is a single linear strand of nucleic acid



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that comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid. In some embodiments, the first and second targeting arms are between about 10 and about 100 nucleotides long. In some embodiments, the first and second targeting arms are about 10-20, 20-30, 30-40, or 40-50 nucleotides long. In some embodiments, the first and second targeting arms are about 20 nucleotides long. In some embodiments, the first and second targeting arms have the same length. In some embodiments, the first and second targeting arms have different lengths. In some embodiments, each pair of first and second targeting arms in a set of probes has the same length. Accordingly, if one of the targeting arms is longer, the other one is correspondingly shorter. This allows for a quality control step in some embodiments to confirm that all captured probe/target sequence products have the same length after a multiplexed plurality of capture reactions. In some embodiments, a set of probes may be designed to have the same length if the intervening region is varied to accommodate any differences in the length of either one or both of the first and second targeting arms.

In some embodiments, the hybridization Tms of the first and second targeting arms are similar. In some embodiments, the hybridization Tms of the first and second targeting arms are within 2-5 °C of each other. In some embodiments, the hybridization Tms of the first and second targeting arms are identical. In some embodiments, the hybridization Tms of the first and second targeting arms are close to empirically-determined optima but not necessarily identical.

In some embodiments, the first and second targeting arms of a molecular inversion probe have different Tms. For example, the Tm of the first targeting arm (at the 5' end of the molecular inversion probe) may be higher than the Tm of the second targeting arm (at the 3' end of the molecular inversion probe). According to aspects of the invention, and without wishing to be bound by theory, a relatively high Tm for the first targeting arm may help avoid or prevent the first targeting arm from being displaced after hybridization by the extension product of the 3' end of the second targeting arm. It should be appreciated that a reference to the Tm of a targeting arm as used herein relates to the Tm of hybridization of the targeting arm to a nucleic acid having the



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complementary sequence (e.g., the region of the target nucleic acid that has a sequence that is complementary to the sequence of the targeting arm). It also should be appreciated that the Tms of the targeting arms described herein may be calculated using any appropriate method. For example, in some embodiments an experimental method (e.g., a gel shift assay, a hybridization assay, a melting curve analysis, for example in a PCR machine with a SYBR dye by stepping through a temperature ramp while monitoring signal level from an intercalating dye, for example, bound to a double-stranded DNA, etc.) may be used to determine one or more Tms empirically. In some embodiments, an optimal Tm may be determined by evaluating the number of products formed (e.g., for each of a plurality of MIP probes), and determining the optimal Tm as the center point in a histogram of Tm for all targeting arms. In some embodiments, a predictive algorithm may be used to determine a Tm theoretically. In some embodiments, a relatively simple predictive algorithm may be used based on the number of G/C and A/T base pairs when the sequence is hybridized to its target and/or the length of the hybridized product (e.g., for example, 64.9 + 41 * ([G+C] - 16.4) / (A + T + G + C), see for example, Wallace,R.B., Shaffer,J., Murphy,R.F., Bonner,J., Hirose,T., and Itakura,K. (1979) Nucleic Acids Res 6:3543-3557). In some embodiments, a more complex algorithm may be used to account for the effects of base stacking entropy and enthalpy, ion concentration, and primer concentration (see, for example, SantaLucia J (1998), Proc Natl Acad Sci USA, 95:1460-5). In some embodiments an algorithm may use modified parameters (e.g., nearest-neighbor parameters for basepair entropy/enthalpy values). It should be appreciated that any suitable algorithm may be used as aspects of the invention are not limited in this respect. However, it also should be appreciated that different methodologies may results in different calculated or predicted Tms for the same sequences. Accordingly, in some embodiments, the same empirical and/or theoretical method is used to determine the Tms of different sequences for a set of probes to avoid a negative impact of any systematic difference in the Tm determination or prediction when designing a set of probes with predetermined similarities or differences for different Tms.

In some embodiments, the Tm of the first targeting arm may be about 1 °C, about 2 °C, about 3 °C, about 4 °C, about 5 °C, or more than about 5 °C higher than the Tm of the second targeting arm. In some embodiments, each probe in a plurality of probes (e.g., each probe in a set of 5-10, each probe in a set of at least 10, each probe in a set of



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10-50, each probe in a set of 50-100, each probe in a set of 100-500, each probe in a set of 500-1,000, each probe in a set of 1,000-1,500, each probe in a set of 1,500-2,000, each probe in a set of 2,000-3,000, 3,000-5,000, 5,000-10,000 or each probe in a set of at least 5,000 different probes) has a unique first targeting arm (e.g., they all have different sequences) and a unique second targeting arm (e.g., they all have different sequences). In some embodiments, for at least 10% of the probes (e.g., at least 25%, 25%-50%, 50%-75%, 75%-90%, 90%-95% or over 95%, or all of the probes) the first targeting arm has a Tm for its complementary sequence that is higher (e.g., about 1 °C, about 2 °C, about 3 °C, about 4 °C, about 5 °C, or more than about 5 °C higher) than the Tm of the second targeting arm for its complementary sequence. In some embodiments, each of the first targeting arms have similar or identical Tms for their respective complementary sequences and each of the second targeting arms have similar or identical Tms for their respective complementary sequences (and the first targeting arms have higher Tms than the second targeting arms). For example, in some embodiments, the Tm of the first arm(s) may be about 58 °C and the Tm of the second arm(s) may be about 56 °C. In some embodiments, the Tm of the first arm(s) may be about 68 °C, and the Tm of the second arm(s) may be about 65 °C. It should be appreciated that in some embodiments the similarity (e.g., within a range of 1 °C, 2 °C, 3 °C, 4 °C, 5 °C) or identity of the Tms for the different targeting arms should be based either on empirical data for each arm or based on the same predictive algorithm for each arm (e.g., Wallace,R.B., Shaffer,J., Murphy,R.F., Bonner,J., Hirose,T., and Itakura,K. (1979) Nucleic Acids Res 6:3543-3557, SantaLucia J (1998), Proc Natl Acad Sci USA, 95:1460-5, or other algorithm).

In some embodiments, the Tm of the first targeting arm of a molecular inversion probe (at the 5' end of the molecular inversion probe) is selected to be sufficiently stable to prevent displacement of the first targeting arm from its complementary sequence on a target nucleic acid. In some embodiments, the Tm of the first targeting arm is 50-55 °C, at least 55 °C, 55-60 °C, at least 60 °C, 60-65 °C, at least 65 °C, at least 70 °C, at least 75 °C, or at least 80 °C. As discussed above, it should be appreciated that the for a particular targeting arm may be determined empirically or theoretically. Different theoretical models may be used to determine a Tm and it should be appreciated that the predicted Tm for a particular sequence may be different depending on the algorithm used for the prediction. In some embodiments, each probe in a plurality of probes (e.g., each probe in a set of 5-10, each probe in a set of at least 10, each probe in a set of 10-50, each



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probe in a set of 50-100, each probe in a set of 100-500, or each probe in a set of at least 500 different probes) has a different first targeting arm (e.g., different sequences) but each different first targeting arm has a similar or identical Tm for its complementary sequence on a target nucleic acid. It should be appreciated that in some embodiments the similarity (e.g., within a range of 1 C, 2 C, 3 C, 4 C, 5 C) or identity of the Tms for the different targeting arms should be based either on empirical data for each arm or based on the same predictive algorithm for each arm (e.g., Wallace,R.B., Shaffer,J., Murphy,R.F., Bonner,J., Hirose,T., and Itakura,K. (1979) Nucleic Acids Res 6:3543-3557, SantaLucia J (1998), Proc Natl Acad Sci USA, 95:1460-5, or other algorithm).

In some embodiments, the sub-target nucleic acid contains a nucleic acid repeat. In some embodiments, the nucleic acid repeat is a dinucleotide or trinucleotide repeat. In some embodiments, the sub-target nucleic acid contains 10-100 copies of the nucleic acid repeat in the absence of an abnormal increase or decrease in nucleic acid repeats. In some embodiments, the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat. In some embodiments, one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats. In some embodiments, one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats. In some embodiments, the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

In some embodiments, aspects of the invention relate to evaluating the length of a plurality of different target nucleic acids in a biological sample. In some embodiments, the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes. In some embodiments, each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends. In some embodiments, each different molecular inversion probe comprises the same primer-binding sequence.

In some embodiments, aspects of the invention relate to analyzing nucleic acid from a biological sample obtained from a subject. In some embodiments, the biological sample is a blood sample. In some embodiments, the biological sample is a tissue sample, specific cell population, tumor sample, circulating tumor cells, or environmental



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sample. In some embodiments, the biological sample is a single cell. In some embodiments, nucleic acids are analyzed in biological samples obtained from a plurality of different subjects. In some embodiments, nucleic acids from a biological sample are analyzed in multiplex reactions. It should be appreciated that a biological sample contains a plurality of copies of a genome derived from a plurality of cells in the sample. Accordingly, a sample may contain a plurality of independent copies of a target nucleic acid region of interest, the capture efficiency of which can be used to evaluate its size as described herein.

In some embodiments, aspects of the invention relate to evaluating a nucleic acid capture efficiency by determining an amount of target nucleic acid that is captured (e.g., an amount of sub-target nucleic acid sequences that are captured). In some embodiments, the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules (e.g., the amount of independently captured molecules that have the sequence of the sub-target region). In some embodiments, the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid. In some embodiments, the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid. In some embodiments, the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion. In some embodiments, the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction. As described herein, a number of independently captured nucleic acid sequences can be determined by contacting a nucleic acid sample with a preparation of a probe (e.g., a MIP probe as described herein). It should be appreciated that the preparation may comprise a plurality of copies of the same probe and accordingly a plurality of independent copies of the target region may be captured by different probe molecules. The number of probe molecules that actually capture a sequence can be evaluated by determining an amount or number of captured molecules using any suitable technique. This number is a reflection of both the number of target molecules in the sample and the efficiency of capture of those target molecules, which in turn is related to the size of the target molecules as described herein. Accordingly, the capture efficiency can be evaluated by controlling for the abundance of the target nucleic acid, for example by comparing the number or amount of captured target molecules to an appropriate control (e.g., a known size and



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amount of control nucleic acid, or a different locus that should be present in the same amount in the biological sample and is not expected to contain any insertions or deletions). It should be appreciated that other factors may affect the capture efficiency of a particular target nucleic acid region (e.g., the sequence of the region, the GC content, the presence of secondary structures, etc.). However, these factors also can be accounted for by using appropriate controls (e.g., known sequences having similar properties, the same sequences, other genomic sequences expected to be present in the biological sample at the same frequency, etc., or any combination thereof).

In some embodiments, aspects of the invention relate to identifying a subject as having an insertion or deletion in one or more alleles of a genetic locus if the capture efficiency for that genetic locus is statistically significantly different than a reference capture efficiency.

It should be appreciated that hybridization conditions used for any of the capture techniques described herein (e.g., MIP capture techniques) can be based on known hybridization buffers and conditions.

In some embodiments, the methods disclosed herein are useful for any application where the detection of deletions or insertions is important.

In some embodiments, aspects of the invention relate to basing a nucleic acid sequence analysis on results from two or more different nucleic acid preparatory techniques that have different systematic biases in the types of nucleic acids that they sample. According to the invention, different techniques have different sequence biases that are systematic and not simply due to stochastic effects during nucleic acid capture or amplification. Accordingly, the degree of oversampling required to overcome variations in nucleic acid preparation needs to be sufficient to overcome the biases (e.g., an oversampling of 2-5 fold, 5-10 fold, 5-15 fold, 15-20 fold, 20-30 fold, 30-50 fold, or intermediate to higher fold).

According to some embodiments, different techniques have different characteristic or systematic biases. For example, one technique may bias a sample analysis towards one particular allele at a genetic locus of interest, whereas a different technique would bias the sample analysis towards a different allele at the same locus. Accordingly, the same sample may be identified as being different depending on the type of technique that is used to prepare nucleic acid for sequence analysis. This effectively



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represents a sensitivity limitation, because each technique has different relative sensitivities for polymorphic sequences of interest.

According to aspects of the invention, the sensitivity of a nucleic acid analysis can be increased by combining the sequences from different nucleic acid preparative steps and using the combined sequence information for a diagnostic assay (e.g., for a making a call as to whether a subject is homozygous or heterozygous at a genetic locus of interest).

In some embodiments, the invention provides a method of increasing the sensitivity of a nucleic acid detection assay by obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample, obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample, assaying the sequences obtained in both first and second nucleic acid preparations, and using the sequence information from both first and second nucleic acid preparations to determine the genotype of the target nucleic acid in the biological sample, wherein the first and second preparative methods have different systematic sequence biases. In some embodiments, the first and second nucleic acid preparations are combined prior to performing a sequence assay. In some embodiments, separate sequence assays are performed on the first and second nucleic acid preparations and the sequence information from both assays are combined to determine the genotype of the target nucleic acid in the biological sample. In some embodiments, the first preparative method is an amplification-based, a hybridization-based, or a circular probe-based preparative method. In some embodiments, the second method is an amplification-based, a hybridization-based, or a circular probe-based preparative method. In some embodiments, the first and second methods are of different types (e.g., only one of them is an amplification-based, a hybridization-based, or a circular probe-based preparative method, and the other one is one or the other two types of method). Accordingly, in some embodiments the second preparative method is an amplification-based, a hybridization-based, or a circular probe-based preparative method, provided that the second method is different from the first method. However, in some embodiments, both methods may be of the same type, provided they are different methods (e.g., both are amplification based or hybridization-based, but are different types of amplification or hybridization methods, e.g., with different relative biases).



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In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using a pair of oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or both of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and identifier sequences (e.g., source sequences). PCR based methods may include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel).

Hybridization-based preparative may methods involve selectively immobilizing target nucleic acids for further manipulation. It is to be understood that one or more oligonucleotides (immobilization oligonucleotides), which in some embodiments may be from 10 to 200 nucleotides in length, are used which hybridize along the length of a target region of a genetic locus to immobilize it. In some embodiments, immobilization oligonucleotides are either immobilized before hybridization is performed (e.g., Roche/Nimblegen 'sequence capture'), or are prepared such that they include a moiety (e.g., biotin) which can be used to selectively immobilize the target nucleic acid after hybridization by binding to e.g., streptavidin-coated microbeads (e.g., Agilent 'SureSelect').

Circularization selection-based preparative methods selectively convert each region of interest into a covalently-closed circular molecule which is then isolated by removal (usually enzymatic, e.g., with exonuclease) of any non-circularized linear nucleic acid. Oligonucleotide probes are designed which have ends that flank the region of interest. The probes are allowed to hybridize to the genomic target, and enzymes are used to first (optionally) fill in any gap between probe ends and second ligate the probe closed. In some embodiments, following circularization, any remaining (non-target) linear nucleic acid can be removed, resulting in isolation (capture) of target nucleic acid. Circularization selection-based preparative methods include molecular inversion probe capture reactions and 'selector' capture reactions. However, other techniques may be



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used as aspects of the invention are not limited in this respect. In some embodiments, molecular inversion probe capture of a target nucleic acid is indicative of the presence of a polymorphism in the target nucleic acid.

A variety of methods may be used to evaluate and compare bias profiles of each preparative technique. Next-generation sequencing may be used to quantitatively measure the abundance of each isolated target nucleic acid obtained from a certain preparative method. This abundance may be compared to a control abundance value (e.g., a known starting abundance of the target nucleic acid) and/or with an abundance determined through the use of an alternative preparative method. For example, a set of target nucleic acids may be isolated by one or more of the three preparative methods; the target nucleic acid may be observed x times using the amplification technique, y times using the hybridization enrichment technique, and z times using the circularization selection technique. A pairwise correlation coefficient may be computed between each abundance value (e.g., x and y, x and z, and y and z) to assess bias in nucleic acid isolation between pairs of preparative methods. Since the mechanisms of isolation are different in each approach, the abundances will usually be different and largely uncorrelated with each other.

In some embodiments, the invention provides a method of obtaining a nucleic acid preparation that is representative of a target nucleic acid in a biological sample by obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample, obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample, and combining the first and second nucleic acid preparations to obtain a combined preparation that is representative of the target nucleic acid in the biological sample.

In some embodiments of any of the methods described herein, a third preparation of the target nucleic acid is obtained using a third preparative method that is different from the first and second preparative methods, wherein the first, second, and third preparative methods all have different systematic sequence biases. In some embodiments of any of the methods described herein, the different preparative methods are used for a plurality of different loci in the biological sample to increase the sensitivity of a multiplex nucleic acid analysis. In some embodiments, the target nucleic acid has a sequence of a gene selected from Table 1.



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However, it should be appreciated that a genotyping method of the invention may include several steps, each of which independently may involve one or more different preparative techniques described herein. In some embodiments, a nucleic acid preparation may be obtained using one or more (e.g., 2, 3, 4, 5, or more) different techniques described herein (e.g., amplification, hybridization capture, circular probe capture, etc., or any combination thereof) and the nucleic acid preparation may be analyzed using one or more different techniques (e.g., amplification, hybridization capture, circular probe capture, etc., or any combination thereof) that are selected independently of the techniques used for the initial preparation.

In some embodiments, aspects of the invention also provide compositions, kits, devices, and analytical methods for increasing the sensitivity of nucleic acid assays. Aspects of the invention are particularly useful for increasing the confidence level of genotyping analyses. However, aspects of the invention may be used in the context of any suitable nucleic acid analysis, for example, but not limited to, a nucleic acid analysis that is designed to determine whether more than one sequence variant is present in a sample.

In some embodiments, aspects of the invention relate to a plurality of nucleic acid probes (e.g., 10-50, 50-100, 100-250, 250-500, 500-1,000, 1,000-2,000, 2,000-5,000, 5,000-7,500, 7,500-10,000, or lower, higher, or intermediate number of different probes). In some embodiments, each probe or each of a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) has a different first targeting arm. In some embodiments, each probe or each probe of a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) has a different second targeting arm. In some embodiments, the first and second targeting arms are separated by the same intervening sequence. In some embodiments, the first and second targeting arms are complementary to target nucleic acid sequences that are separated by the same or a similar length (e.g., number of nucleic acids, for example, 0-25, 25-50, 50-100, 100-250, 250-500, 500-1,000, 1,000-2,500 or longer or intermediate number of nucleotides) on their respective target nucleic acids (e.g., genomic loci). In some embodiments, each probe or a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) includes a first primer binding sequence. In some embodiments, the primer binding sequence is the same (e.g., it can be used to prime sequencing or other extension reaction). In some embodiments, each probe or a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) includes a unique



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identifier sequence tag (e.g., that is predetermined and can be used to distinguish each probe).

In some embodiments, the methods disclosed herein are useful for any application where sensitivity is important. For example, detection of cancer mutations in a heterogenous tissue sample, detection of mutations in maternally-circulating fetal DNA, and detection of mutations in cells isolated during a preimplantation genetic diagnostic procedure.

According to some aspects of the invention, methods of detecting a polymorphism in a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise obtaining a nucleic acid preparation using a preparative method (e.g., any of the preparative methods disclosed herein) on a biological sample, and performing a molecular inversion probe capture reaction on the nucleic acid preparation, wherein a molecular inversion probe capture (e.g., using a mutation-detection MIP) of a target nucleic acid of the nucleic acid preparation is indicative of the presence of a mutation (polymorphism) in the target nucleic acid, optionally wherein the polymorphism is selected from Table 2.

According to some aspects of the invention, methods of genotyping a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise obtaining a nucleic acid preparation using a preparative method on a biological sample, sequencing a target nucleic acid of the nucleic acid preparation, and performing a molecular inversion probe capture reaction on the biological sample, wherein a molecular inversion probe capture of the target nucleic acid in the biological sample is indicative of the presence of a polymorphism in the target nucleic acid, genotyping the target nucleic acid based on the results of the sequencing and the capture reaction.

In some embodiments of the methods disclosed herein, the target nucleic acid has a sequence of a gene selected from Table 1.

It should be appreciated that any one or more embodiments described herein may be used for evaluating multiple genetic markers in parallel. Accordingly, in some embodiments, aspects of the invention relate to determining the presence of one or more markers (e.g., one or more alleles) at multiple different genetic loci in parallel. Accordingly, the risk or presence of multiple heritable disorders may be evaluated in parallel. In some embodiments, the risk of having offspring with one or more heritable



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disorders may be evaluated. In some embodiments, an evaluation may be performed on a biological sample of a parent or a child (e.g., at a pre-implantation, prenatal, perinatal, or postnatal stage). In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table I or 2) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample. A patient or subject may be a human. However, aspects of the invention are not limited to humans and may be applied to other species (e.g., mammals, birds, reptiles, other vertebrates or invertebrates) as aspects of the invention are not limited in this respect. A subject or patient may be male or female. In some embodiments, in connection with reproductive genetic counseling, samples from a male and female member of a couple may be analyzed. In some embodiments, for example, in connection with an animal breeding program, samples from a plurality of male and female subjects may be analyzed to determine compatible or optimal breeding partners or strategies for particular traits or to avoid one or more diseases or conditions. Accordingly, reproductive risks may be determined and/or reproductive recommendations may be provided based on information derived from one or more embodiments of the invention.

However, it should be appreciated that aspects of the invention may be used in connection with any medical evaluation where the presence of one or more alleles at a genetic locus of interest is relevant to a medical determination (e.g., risk or detection of disease, disease prognosis, therapy selection, therapy monitoring, etc.). Further aspects of the invention may be used in connection with detection, in tumor tissue or circulating tumor cells, of mutations in cellular pathways that cause cancer or predict efficacy of treatment regimens, or with detection and identification of pathogenic organisms in the environment or a sample obtained from a subject, *e.g.*, a human subject.

These and other aspects of the invention are described in more detail in the following description and non-limiting examples and drawings.

## BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout;

FIG. 2 illustrates a non-limiting embodiment of a staggered probe layout;

FIG. 3 illustrates a non-limiting embodiment of an alternating staggered probe layout;



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FIGs. 4A, B, and C depict various non-limiting methods for combining differentiator tag sequence and target sequences (NNNN depicts a differentiator tag sequence);

FIG. 5 depicts a non-limiting method for genotyping based on target and differentiator tag sequences;

FIG. 6 depicts non-limiting results of a simulation of a MIP capture reaction;

FIG. 7 depicts a non-limiting graph of sequencing coverage;

FIG. 8 illustrates that shorter sequences are captured with higher efficiency that longer sequences using MIPs;

FIG. 9 illustrates a non-limiting scheme of padlock (MIP) capture of a region that includes both repetitive regions (thick wavy line) and the adjacent unique sequence (thick strait line);

FIG. 10 illustrates a non-limiting hypothetical relationship between target gap size and the relative number of reads of the repetitive region;

FIG. 11A depicts MIP capture of FMR1 repeat regions from a diploid genome;

FIG. 11B depicts preparative methods for biallelic resolution of FMR1 repeat region lengths in a diploid genome using MIP capture probes and unique differentiator tags;

FIG. 11C depicts an analysis of FMR1 repeat region lengths in a diploid genome;

FIG. 12 is a schematic of an embodiment of an algorithm of the invention;

FIG. 13 illustrates a non-limiting example of a graph of per-target abundance with MIP capture; and,

FIG. 14 shows a non-limiting a graph of correlation between two MIP capture reactions.

## DETAILED DESCRIPTION

Aspects of the invention relate to preparative and analytical methods and compositions for evaluating genotypes, and in particular, for determining the allelic identity (or identities in a diploid organism) of one or more genetic loci in a subject. Aspects of the invention are based, in part, on the identification of different sources of ambiguity and error in genetic analyses, and, in part, on the identification of one or more approaches to avoid, reduce, recognize, and/or resolve these errors and ambiguities at different stages in a genetic analysis. Aspects of the invention relate to methods and



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compositions for addressing bias and/or stochastic variation associated with one or more preparative and/or analytical steps of a nucleic acid evaluation technology. In some embodiments, preparative methods can be adapted to avoid or reduce the risk of bias skewing the results of a genetic analysis. In some embodiments, analytical methods can be adapted to recognize and correct for data variations that may give rise to misinterpretation (e.g., incorrect calls such as homozygous when the subject is actually heterozygous or heterozygous when the subject is actually homozygous). Methods of the invention may be used for any type of mutation, for example a single base change (e.g., insertion, deletion, transversion or transition, etc.), a multiple base insertion, deletion, duplication, inversion, and/or any other change or combination thereof.

In some embodiments, additional or alternative techniques may be used to address loci characterized by multiple repeats of a core sequence where the length of the repeat is longer than a typical sequencing read thereby making it difficult to determine whether a deletion or duplication of one or more core sequence units has occurred based solely on a sequence read.

In some embodiments, increased confidence in an assay result may be obtained by i) selecting two or more different preparative and/or analytical techniques that have different biases (e.g., known to have different biases), ii) evaluating a patient sample using the two or more different techniques, iii) comparing the results from the two or more different techniques, and/or iv) determining whether the results are consistent for the two or more different techniques. In some embodiments, if determining in step (iv) indicates that the results are consistent (e.g., the same) then increased confidence in the assay result is obtained. In other embodiments, if determining in step (iv) indicates that the results are inconsistent (e.g., that the results are ambiguous) then one or more additional preparative and/or analytical techniques, which have a different bias (e.g., known to have a different bias) compared with the two or more different preparative and/or analytical techniques selected in step (i), are used to evaluate the patient sample, and the results of the one or more additional preparative and/or analytical techniques are compared with the results from step (ii) to resolve the inconsistency.

In some embodiments, two or more independent samples may be obtained from a subject and independently analyzed. In some embodiments, two or more independent samples are obtained at approximately the same time point. In some embodiments, two or more independent samples are obtained at multiple different time points. In some



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embodiments, the use of two or more independent sample facilitates the elimination, normalization, and/or quantification of stochastic measurement noise. It is to be appreciated that two or more independent samples may be obtained in connection with any of the methods disclosed herein, including, for example, methods for pathogen profiling in a human or other animal subjects, monitoring tumor progression/regression, analyzing circulating tumor cells, analyzing fetal cells in maternal circulation, and analyzing/monitoring/profiling of environmental pathogens.

In some embodiments, one or more of the techniques described herein may be combined in a single assay protocol for evaluating multiple patient samples in parallel.

It should be appreciated that aspects of the invention may be useful for high throughput, cost-effective, yet reliable, genotyping of multiple patient samples (e.g., in parallel, for example in multiplex reactions). In some embodiments, aspects of the invention are useful to reduce the error frequency in a multiplex analysis. Certain embodiments may be particularly useful where multiple reactions (e.g., multiple loci and/or multiple patient samples) are being processed. For example, 10-25, 25-50, 50-75, 75-100 or more loci may be evaluated for each subject out of any number of subject samples that may be processed in parallel (e.g., 1-25, 25-50, 50-100, 100-500, 500-1,000, 1,000-2,500, 2,500-5,000 or more or intermediate numbers of patient samples). It should be appreciated that different embodiments of the invention may involve conducting two or more target capture reactions and/or two or more patient sample analyses in parallel in a single multiplex reaction. For example, in some embodiments a plurality of capture reactions (e.g., using different capture probes for different target loci) may be performed in a single multiplex reaction on a single patient sample. In some embodiments, a plurality of captured nucleic acids from each one of a plurality of patient samples may be combined in a single multiplex analysis reaction. In some embodiments, samples from different subjects are tagged with subject-specific (e.g., patient-specific) tags (e.g., unique sequence tags) so that the information from each product can be assigned to an identified subject. In some embodiments, each of the different capture probes used for each patient sample have a common patient-specific tag. In some embodiments, the capture probes do not have patient-specific tags, but the captured products from each subject may be amplified using one or a pair of amplification primers that are labeled with a patient-specific tag. Other techniques for associating a patient-specific tag with the captured product from a single patient sample may be used as aspects of the



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invention are not limited in this respect. It should be appreciated that patient-specific tags as used herein may refer to unique tags that are assigned to identified patients in a particular assay. The same tags may be used in a separate multiplex analysis with a different set of patient samples (e.g., from different patients) each of which is assigned one of the tags. In some embodiments, different sets of unique tags may be used in sequential (e.g., alternating) multiplex reactions in order to reduce the risk of contamination from one assay to the next and allow contamination to be detected on the basis of the presence of tags that are not expected to be present in a particular assay.

Embodiments of the invention may be used for any of a number of different settings: reproductive settings, disease screening, identifying subjects having cancer, identifying subjects having increased risk for a disease, stratifying a population of subjects according to one or more of a number of factors, for example responsiveness to a particular drug, lack or not of an adverse reaction (or risk therefore) to a particular drug, and/or providing information for medical records (e.g., homozygosity, heterozygosity at one or more loci). It should be appreciated that the invention is not limited to genomic analysis of patient samples. For example, aspects of the invention may be useful for high throughput genetic analysis of environment samples to detect pathogens.

In some embodiments, the methods disclosed herein are useful for diagnosis of one or more heritable disorders. In some embodiments, a heritable disorder that may be diagnosed with the methods disclosed herein is a genetic disorder that is prevalent in the Ashkenazi Jewish population. In some embodiments, the heritable disorders are selected from: 21-Hydroxylase-Defiocient Congenital Adrenal Hyperplasia; ABCC8-Related Hyperinsulinism; Alpha-Thalassemia, includes Constant Spring, & MR associated; Arylsulfatase A Deficiency-Metyachromatic Leukodystrophy; Biotinidase Deficiency-Holocarboxylase Synthetase Deficiency; Bloom's Syndrome; Canavan Disease; CFTR-Related Disorders-cystic fibrosis; Citrullinemia Type I; Combined MMA & Homocystinuria-dblC; Dystrophinopathies (DMD & BMD); Familial Dysautonomia; Fanconi Anemia-FANCC; Galactosemia-Classical: Galactokinase Defiency & Galactose Epimerase Deficiency; Gaucher Disease; GJB2-Related DFNB 1 Nonsyndromic Hearing Loss and Deafness; Glutaric acidemia Type 1; Hemoglobinopathies beta-chain disorders; Glycogen Storage Disease Type 1A; Maple Syrup Urine Disease; Types 1A, 1B, 2, 3; Medium Chain Acyl-Coenzyme A; Dehydrogenase Deficiency-MCADD;



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Methylmalonic Acidemia; Mucolipidosis IV; Nemaline Myopathy; Nieman-Pick Type A-Acid Sphingomyelinase Deficiency; Non-Ketotic Hyperglycinemia-Glycine Encephalopathy; Ornithine Transcarbamylase Deficiency; PKU Phenylalanine Hydroxylase Deficiency; Propionic Acidemia; Short Chain Acyl-CoA Dehydrogenase Deficiency-SCADD; Smith-Lemli-Opitz Syndrome; Spinal Muscular Atrophy (SMN1)-SMA; Tay Sachs-HexA Deficiency; Usher Synbdrome-Type I (Type IB, Type IC, Type ID, Type IF, Type IG) ; X-Linked Mental Retardation ARX-Related Disorders; X-Linked Mental Retardation with Cerebellar Cypoplasia and sistinctive Facial Appearance; X-Linked Mental Retardation; includes 9, 21, 30, 46, 58, 63, 88, 89; X-linked mental retardation: FM1-Related Disorders-FRXA, Fragile X MR; X-linked SMR: Renpenning Syndrome 1; Zellweger Spectrum disorders - Peroxisomal Bifunctional Enzyme Deficiencies including Zellweger, NALD, and/or infantile Refsums. However, all of these, subsets of these, other genes, or combinations thereof may be used.

According to some aspects, the disclosure relates to multiplex diagnostic methods. In some embodiments, multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel (e.g., a genetic locus of Table 1). In some embodiments, genetic loci possess one or more polymorphisms (e.g., a polymorphism of Table 2) the genotypes of which correspond to disease causing alleles. Accordingly, in some embodiments, the disclosure provides methods for assessing multiple heritable disorders in parallel.

In some embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample. A patient or subject may be a human. However, aspects of the invention are not limited to humans and may be applied to other species (e.g., mammals, birds, reptiles, other vertebrates or invertebrates) as aspects of the invention are not limited in this respect. A subject or patient may be male or female. In some embodiments, in connection with reproductive genetic counseling, samples from a male and female member of a couple may be analyzed. In some embodiments, for example, in connection with an animal breeding program, samples from a plurality of male and female subjects may be analyzed to



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determine compatible or optimal breeding partners or strategies for particular traits or to avoid one or more diseases or conditions.

However, it should be appreciated that any other diseases may be studied and/or risk factors for diseases or disorders including, but not limited to allergies, responsiveness to treatment, cancer tumor profiling for treatment and prognosis, monitoring and identification of patient infections, and monitoring of environmental pathogens.

*1. Reducing representational bias in multiplex amplification reactions:*

In some embodiments, aspects of the invention relate to methods that reduce bias and increase reproducibility in multiplex detection of genetic loci, e.g., for diagnostic purposes.

Molecular inversion probe technology is used to detect or amplify particular nucleic acid sequences in potentially complex mixtures. Use of molecular inversion probes has been demonstrated for detection of single nucleotide polymorphisms (Hardenbol et al. 2005 Genome Res 15:269-75) and for preparative amplification of large sets of exons (Porreca et al. 2007 Nat Methods 4:931-6, Krishnakumar et al. 2008 Proc Natl Acad Sci USA 105:9296-301). One of the main benefits of the method is in its capacity for a high degree of multiplexing, because generally thousands of targets may be captured in a single reaction containing thousands of probes. However, challenges associated with, for example, amplification efficiency (See, e.g., Turner EH, et al., Nat Methods. 2009 Apr 6:1-2.) have limited the practical utility of the method in research and diagnostic settings.

Aspects of the disclosure are based, in part, on the discovery of effective methods for overcoming challenges associated with systematic errors (bias) in multiplex genomic capture and sequencing methods, namely high variability in target nucleic acid representation and unequal sampling of heterozygous alleles in pools of captured target nucleic acids (e.g., isolated from a biological sample). Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the detection of target nucleic acids in multiplex capture methods. In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of different sets of



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multiple probes (e.g., molecular inversion probes MIPs) that capture overlapping regions of a target nucleic acid to achieve a more uniform representation of the target nucleic acids in a capture pool compared with methods of the prior art. In other embodiments, methods reduce bias, or the risk of bias, associated with large scale parallel capture of genetic loci, e.g., for diagnostic purposes. In other embodiments, methods are provided for increasing reproducibility (e.g., by reducing the effect of polymorphisms on target nucleic acid capture) in the detection of a plurality of genetic loci in parallel. In further embodiments, methods are provided for reducing the effect of probe synthesis and/or probe amplification variability on the analysis of a plurality of genetic loci in parallel.

In some aspects, the disclosure provides probe sets that comprise a plurality of different probes. As used herein, a 'probe' is a nucleic acid having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of a target nucleic acid or subregion thereof. An exemplary probe is a molecular inversion probe (MIP). A 'target nucleic acid' may be a genetic locus. Exemplary genetic loci are disclosed herein in Table 1 (RefSeqGene Column).

While probes have been typically designed to meet certain constraints (e.g. melting temperature, G/C content, etc.) known to partially affect capture/amplification efficiency (Ball et al (2009) Nat Biotech 27:361-8 AND Deng et al (2009) Nat Biotech 27:353-60), a set of constraints which is sufficient to ensure either largely uniform or highly reproducible capture/amplification efficiency has not previously been achieved. As disclosed herein, uniformity and reproducibility can be increased by designing multiple probes per target, such that each base in the target is captured by more than one probe. In some embodiments, the disclosure provides multiple MIPs per target to be captured, where each MIP in a set designed for a given target nucleic acid has a central region and a 5' region and 3' region ('targeting arms') which hybridize to (at least partially) different nucleic acids in the target nucleic acid (immediately flanking a subregion of the target nucleic acid). Thus, differences in efficiency between different targeting arms and fill-in sequences may be averaged across multiple MIPs for a single target, which results in more uniform and reproducible capture efficiency.

In some embodiments, the methods involve designing a single probe for each target (a target can be as small as a single base or as large as a kilobase or more of contiguous sequence).



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It may be preferable, in some cases, to design probes to capture molecules (e.g., target nucleic acids or subregions thereof) having lengths in the range of 1 - 200 bp (as used herein, a bp refers to a base pair on a double-stranded nucleic acid – however, where lengths are indicated in bps, it should be appreciated that single-stranded nucleic acids having the same number of bases, as opposed to base pairs, in length also are contemplated by the invention). However, probe design is not so limited. For example, probes can be designed to capture targets having lengths in the range of up to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000, or more bps, in some cases.

It is to be appreciated that the length of a capture molecule (e.g., a target nucleic acid or subregion thereof) is selected based upon multiple considerations. For example, where analysis of a target involves sequencing, e.g., with a next-generation sequencer, the target length should typically match the sequencing read-length so that shotgun library construction is not necessary. However, it should be appreciated that captured nucleic acids may be sequenced using any suitable sequencing technique as aspects of the invention are not limited in this respect.

It is also to be appreciated that some target nucleic acids are too large to be captured with one probe. Consequently, it may be necessary to capture multiple subregions of a target nucleic acid in order to analyze the full target.

In some embodiments, a subregion of a target nucleic acid is at least 1 bp. In other embodiments, a subregion of a target nucleic acid is at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp or more. In other embodiments, a subregion of a target nucleic acid has a length that is up to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more percent of a target nucleic acid length.

The skilled artisan will also appreciate that consideration is made, in the design of MIPs, for the relationship between probe length and target length. In some embodiments, MIPs are designed such that they are several hundred basepairs (e.g., up to 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp or more) longer than corresponding target (e.g., subregion of a target nucleic acid, target nucleic acid).

In some embodiments, lengths of subregions of a target nucleic acid may differ. For example, if a target nucleic acid contains regions for which probe hybridization is not possible or inefficient, it may be necessary to use probes that capture subregions of



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one or more different lengths in order to avoid hybridization with problematic nucleic acids and capture nucleic acids that encompass a complete target nucleic acid.

Aspects of the invention involve using multiple probes, e.g., MIPs, to amplify each target nucleic acid. In some embodiments, the set of probes for a given target can be designed to 'tile' across the target, capturing the target as a series of shorter sub-targets. In some embodiments, where a set of probes for a given target is designed to 'tile' across the target, some probes in the set capture flanking non-target sequence). Alternately, the set can be designed to 'stagger' the exact positions of the hybridization regions flanking the target, capturing the full target (and in some cases capturing flanking non-target sequence) with multiple probes having different targeting arms, obviating the need for tiling. The particular approach chosen will depend on the nature of the target set. For example, if small regions are to be captured, a staggered-end approach might be appropriate, whereas if longer regions are desired, tiling might be chosen. In all cases, the amount of bias-tolerance for probes targeting pathological loci can be adjusted ('dialed in') by changing the number of different MIPs used to capture a given molecule.

In some embodiments, the 'coverage factor', or number of probes used to capture a basepair in a molecule, is an important parameter to specify. Different numbers of probes per target are indicated depending on whether one is using the tiling approach (see, e.g., FIG. 1) or one of the staggered approaches (see, e.g., FIGs. 2 or 3).

FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout showing ten captured sub-targets tiled across a single target. Each position in the target is covered by three sub-targets such that MIP performance per base pair is averaged across three probes.

FIG. 2 illustrates a non-limiting embodiment of a staggered probe layout showing the targets captured by a set of three MIPs. Each MIP captures the full target, shown in black, plus (in some cases) additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes. Targeting arms land immediately adjacent to the black or gray regions shown. It should be appreciated that in some embodiments, the targeting arms (not shown) can be designed so that they do not overlap with each other.

FIG. 3 illustrates a non-limiting embodiment of an alternating staggered probe layout showing the targets captured by a set of three MIPs. Each MIP captures the full



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target, shown in black, plus (in some cases) additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes. Targeting arms land immediately adjacent to the black or gray regions shown.

It should be appreciated that for any of the layouts, the targeting arms on adjacent tiled or staggered probes may be designed to either overlap, not overlap, or overlap for only a subset of the probes.

In certain embodiments for any of the layouts, a coverage factor of about 3 to about 10 is used. However, the methods are not so limited and coverage factors of up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or more may be used. It is to be appreciated that the coverage factor selected may depend the probe layout being employed. For example, in the tiling approach, for a desired coverage factor, the number of probes per target is typically a function of target length, sub-target length, and spacing between adjacent sub-target start locations (step size). For example, for a desired coverage factor of 3, a 200 bp target with a start-site separation of 20 bp and sub-target length of 60 bp may be encompassed with 12 MIPs (FIG. 1). Thus, a specific coverage factor may be achieved by varying the number of probes per target nucleic acid and the length of the molecules captured. In the staggered approach, a fixed-length target nucleic acid is captured as several subregions or as 'super-targets', which are molecules comprising the target nucleic acid and additional flanking nucleic acids, which may be of varying lengths. For example, a target of 50 bp can be captured at a coverage factor of 3 with 3 probes in either a 'staggered' (FIG. 2) or 'alternating staggered' configuration (FIG. 3).

The coverage factor will be driven by the extent to which detection bias is tolerable. In some cases, where the bias tolerance is small, it may be desirable to target more subregions of target nucleic acid with, perhaps, higher coverage factors. In some embodiments, the coverage factor is up to 2, 3, 4, 5, 6, 7, 8, 9, 10 or more.

In some embodiments, when a tiled probe layout is used, when the target length is greater than 1 bp and when a step size (distance between the 5'-end of a target and the 5' end of its adjacent target) is less than the length of a target or subregion thereof, it is possible to compute probe number for a particular target based on target length (T), sub-target length (S), and coverage factor (C), such that probe number = T/(S/C) + (C-1).



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In some aspects, the disclosure provides methods to increase the uniformity of amplification efficiency when multiple molecules are amplified in parallel; methods to increase the reproducibility of amplification efficiency; methods to reduce the contribution of targeting probe variability to amplification efficiency; methods to reduce the effect on a given target nucleic acid of polymorphisms in probe hybridization regions; and/or methods to simplify downstream workflows when multiplex amplification by MIPs is used as a preparative step for analysis by nucleic acid sequencing.

Polymorphisms in the target nucleic acid under the regions flanking a target can interfere with hybridization, polymerase fill-in, and/or ligation. Furthermore, this may occur for only one allele, resulting in allelic drop-out, which ultimately decreases downstream sequencing accuracy. In some embodiments, using a set of MIPs having multiple hybridization sites for the capture of any given target, the probability of loss from polymorphism is substantially decreased because not all targeting arms in the set of MIPs will cover the location of the mutation.

Probes for MIP capture reactions may be synthesized on programmable microarrays because of the large number of sequences required. Because of the low synthesis yields of these methods, a subsequent amplification step is required to produce sufficient probe for the MIP amplification reaction. The combination of multiplex oligonucleotide synthesis and pooled amplification results in uneven synthesis error rates and representational biases. By synthesizing multiple probes for each target, variation from these sources may be averaged out because not all probes for a given target will have the same error rates and biases.

Multiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs, or preparatively, often for next-generation sequencing or other sequencing techniques. In the preparative setting, the output of an amplification reaction is generally the input to a shotgun library protocol, which then becomes the input to the sequencing platform. The shotgun library is necessary in part because next-generation sequencing yields reads significantly shorter than amplicons such as exons. In addition to the bias-reduction afforded by the multi-tiled approach described here, tiling also obviates the need for shotgun library preparation. Since the length of the capture molecule can be specified when the probes, e.g., MIPs, are designed, it can be chosen to



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match the readlength of the sequencer. In this way, reads can 'walk' across an exon by virtue of the start position of each capture molecule in the probe set for that exon.

*2. Reducing analytical errors associated with bias in nucleic acid preparations:*

In some embodiments, aspects of the invention relate to preparative steps in DNA sequencing-related technologies that reduce bias and increase the reliability and accuracy of downstream quantitative applications.

There are currently many genomics assays that utilize next-generation (polony-based) sequencing to generate data, including genome resequencing, RNA-seq for gene expression, bisulphite sequencing for methylation, and Immune-seq, among others. In order to make quantitative measurements (including genotype calling), these methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids. The majority of these techniques require a preparative step to construct a high-complexity library of DNA molecules that is representative of a sample of interest. This may include chemical or biochemical treatment of the DNA (e.g., bisulphite treatment), capture of a specific subset of the genome (e.g., padlock probe capture, solution hybridization), and a variety of amplification techniques (e.g., polymerase chain reaction, whole genome amplification, rolling circle amplification).

Systematic and random errors are common problems associated with genome amplification and sequencing library construction techniques. For example, genomic sequencing library may contain an over- or under-representation of particular sequences from a source genome as a result of errors (bias) in the library construction process. Such bias can be particularly problematic when it results in target sequences from a genome being absent or undetectable in the sequencing libraries. For example, an under-representation of particular allelic sequences (e.g., heterozygotic alleles) from a genome in a sequencing library can result in an apparent homozygous representation in a sequencing library. As most downstream sequencing library quantification techniques depend on stochastic counting processes, these problems have typically been addressed by sampling enough (over-sampling) to obtain a minimum number of observations necessary to make statistically significant decisions. However, the strategy of oversampling is generally limited to elimination of low-count Poisson noise, and the



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approach wastes resources and increases the expense required to perform such experiments. Moreover, oversampling can result in a reduced statistical confidence in certain conclusions (e.g., diagnostic calls) based on the data. Accordingly, new approaches are needed for overcoming bias in sequencing library preparatory methods.

Aspects of the disclosure are based, in part, on the discovery of methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, namely high variability in the capture and amplification of nucleic acids and disproportionate representation of heterozygous alleles in sequencing libraries. Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the capture and amplification of nucleic acids. In other embodiments, the methods improve allelic representation in sequencing libraries and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of differentiator tag sequences to uniquely tag individual nucleic acid molecules. In some embodiments, the differentiator tag sequence permits the detection of bias based on the frequency with which pairs of differentiator tag and target sequences are observed in a sequencing reaction. In other embodiments, the methods reduce errors caused by bias, or the risk of bias, associated with the capture, amplification and sequencing of genetic loci, e.g., for diagnostic purposes.

Aspects of the invention relate to associating unique sequence tags (referred to as differentiator tag sequences) with individual target molecules that are independently captured and/or analyzed (e.g., prior to amplification or other process that may introduce bias). These tags are useful to distinguish independent target molecules from each other thereby allowing an analysis to be based on a known number of individual target molecules. For example, if each of a plurality of target molecule sequences obtained in an assay is associated with a different differentiator tag, then the target sequences can be considered to be independent of each other and a genotype likelihood can be determined based on this information. In contrast, if each of the plurality of target molecule sequences obtained in the assay is associated with the same differentiator tag, then they probably all originated from the same target molecule due to over-representation (e.g., due to biased amplification) of this target molecule in the assay. This provides less information than the situation where each nucleic acid was associated with a different



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differentiator tag. In some embodiments, a threshold number of independently isolated molecules (e.g., unique combinations of differentiator tag and target sequences) is analyzed to determine the genotype of a subject.

In some embodiments, the invention relates to compositions comprising pools (libraries) of preparative nucleic acids that each comprise "differentiator tag sequences" for detecting and reducing the effects of bias, and for genotyping target nucleic acid sequences. As used herein, a "differentiator tag sequence" is a sequence of a nucleic acid (a preparative nucleic acid), which in the context of a plurality of different isolated nucleic acids, identifies a unique, independently isolated nucleic acid. Typically, differentiator tag sequences are used to identify the origin of a target nucleic acid at one or more stages of a nucleic acid preparative method. For example, in the context of a multiplex nucleic acid capture reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, target nucleic acid capture events. Also, in the context of a multiplex nucleic acid amplification reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, primary amplicons of a target nucleic acid, for example. Thus, combinations of target nucleic acid and differentiator tag sequence (target:differentiator tag sequences) of an isolated nucleic acid of a preparative method provide a basis for identifying unique, independently isolated target nucleic acids. FIG. 4A-C depict various non-limiting examples of methods for combining differentiator tag sequence and target sequences.

It will be apparent to the skilled artisan that differentiator tags may be synthesized using any one of a number of different methods known in the art. For example, differentiator tags may be synthesized by random nucleotide addition. Differentiator tag sequences are typically of a predefined length, which is selected to control the likelihood of producing unique target:differentiator tag sequences in a preparative reaction (e.g., amplification-based reaction, a circularization selection-based reaction, e.g., a MIP reaction). Differentiator tag sequences may be, up to 5, up to 6, up to 7 up to 8, up to 9, up to 10, up to 11, up to 12, up to 13, up to 14, up to 15, up to 16, up to 17, up to 18, up to 19, up to 20, up to 21, up to 22, up to 23, up to 24, up to 25, or more nucleotides in length. For purposes of genotyping, isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and observance of threshold numbers of



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unique combinations of target nucleic acid and differentiator tag sequences provide a certain statistical confidence in the genotype.

During a library preparation process, each nucleic acid molecule may be tagged with a unique differentiator tag sequence in a configuration that permits the differentiator tag sequence to be sequenced along with the target nucleic acid sequence of interest (the nucleic acid sequence for which the library is being prepared, e.g., a polymorphic sequence). The incorporation of the nucleic acid comprising a differentiator tag sequence at a particular step allows the detection and correction of biases in subsequent steps of the protocol.

A large library of unique differentiator tag sequences may be created by using degenerate, random-sequence polynucleotides of defined length. The differentiator tag sequences of the polynucleotides may be read at the final stage of the sequencing. The observations of the differentiator tag sequences may be used to detect and correct biases in the final sequencing read-out of the library. For example, the total possible number of differentiator tag sequences, which may be produced, e.g., randomly, is $4^N$, where N is the length of the differentiator tag sequence. Thus, it is to be understood that the length of the differentiator tag sequence may be adjusted such that the size of the population of MIPs having unique differentiator tag sequences is sufficient to produce a library of MIP capture products in which identical independent combinations of target nucleic acid and differentiator tag sequence are rare. As used herein combinations of target nucleic acid and differentiator tag sequences, may also be referred to as "target:differentiator tag sequences".

In the final readout of a sequencing process, each read may have an additional unique differentiator tag sequence. In some embodiments, when differentiator tag sequences are distributed randomly in a library, all the unique differentiator tag sequences will be observed about an equal number of times. Accordingly, the number of occurrences of a differentiator tag sequence may follow a Poisson distribution.

In some embodiments, overrepresentation of target:differentiator tag sequences in a pool of preparative nucleic acids (e.g., amplified MIP capture products) is indicative of bias in the preparative process (e.g., bias in the amplification process). For example, target:differentiator tag sequence combinations that are statistically overrepresented are indicative of bias in the protocol at one or more steps between the incorporation of the



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differentiator tag sequences into MIPs and the actual sequencing of the MIP capture products.

The number of reads of a given target:differentiator tag sequence may be indicative (may serve as a proxy) of the amount of that target sequence present in the originating sample. In some embodiments, the numbers of occurrence of sequences in the originating sample is the quantity of interest. For example, using the methods disclosed herein, the occurrence of differentiator tag sequences in a pool of MIPs may be predetermined (e.g., may be the same for all differentiator tag sequences). Accordingly, changes in the occurrence of differentiator tag sequences after amplification and sequencing may be indicative of bias in the protocol. Bias may be corrected to provide an accurate representation of the composition of the original MIP pool, e.g., for diagnostic purposes.

According to some aspects, a library of preparative nucleic acid molecules (e.g., MIPs, each nucleic acid in the library having a unique differentiator tag sequence, may be constructed such that the number of nucleic acid molecules in the library is significantly larger than the number prospective target nucleic acid molecules to be captured using the library. This ensures that products of the preparative methods include only unique target:differentiator tag sequence; e.g., in a MIP reaction the capture step would undersample the total population of unique differentiator tag sequences in the MIP library. For example, an experiment utilizing 1 μg of genomic DNA will contain about ~150,000 copies of a diploid genome. For a MIP library, each MIP in the library comprising a randomly produced 12-mer differentiator tag sequence (~1.6 million possible unique differentiator tag sequences), there would be more than 100 unique differentiator tag sequences per genomic copy. For a MIP library, each MIP in the library comprising a randomly produced 15-mer differentiator tag sequence (~1 billion possible unique differentiator tag sequences), there would be more than 7000 unique differentiator tag sequences per genomic copy. Therefore, the probability of the same differentiator tag sequence being incorporated multiple times is incredibly small. Thus, it is to be appreciated that the length of the differentiator tag sequence is to be selected based on the amount of target sequence in a MIP capture reaction and the desired probability for having multiple, independent occurrences of target:differentiator tag sequence combinations.



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FIG. 5 depicts a non-limiting method for genotyping based on target and differentiator tag sequences. Sequencing reads of target and differentiator tags sequences are collapsed to make diploid genotype calls. FIG. 6 depicts non-limiting results of a simulation of a MIP capture reaction in which MIP probes, each having a differentiator tag sequence of 15 nucleotides, are combined with 10000 target sequence copies (e.g., genome equivalents). In this simulated reaction, the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is 0.05. The Y axis reflects the number of observations. The X axis reflects the number of independent occurrences of target:differentiator tag combinations. FIG. 7 depicts a non-limiting graph of sequencing coverage, which can help ensure that alleles are sampled to sufficient depth (e.g., either 10x or 20x minimum sampling per allele, assuming 1000 targets). In this non-limiting example, the X axis is total per-target coverage required, and the Y axis is the probability that a given total coverage will result in at least 10x or 20x coverage for each allele.

The skilled artisan will appreciate that as part of a MIP library preparation process, adapters may be ligated onto the ends of the molecules of interest. Adapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites. In addition, barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007/0020640 A1 (McCloskey et al.)

The actual incorporation of the random differentiator tag sequences can be performed through various methods known in the art. For example, nucleic acids comprising differentiator tag sequences may be incorporated by ligation. This is a flexible method, because molecules having differentiator tag sequence can be ligated to any blunt-ended nucleic acids. The sequencing primers must be incorporated subsequently such that they sequence both the differentiator tag sequence and the target sequence. Alternatively, the sequencing adaptors can be synthesized with the random differentiator tag sequences at their 3' end (as degenerate bases), so that only one ligation must be performed. Another method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags. Another



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method to incorporate the differentiator tag sequences is to synthesize them into a padlock probe prior to performing a gene capture reaction. The differentiator tag sequence is incorporated 3' to the targeting arm but 5' to the amplification primer that will be used downstream in the protocol. Another method to incorporate the differentiator tag sequences is as a tag on a gene-specific or poly-dT reverse-transcription primer. This allows the differentiator tag sequence to be incorporated directly at the cDNA level.

In some embodiments, at the incorporation step, the distribution of differentiator tag sequences can be assumed to be uniform. In this case, bias in any part of the protocol would change the uniformity of this distribution, which can be observed after sequencing. This allows the differentiator tag sequence to be used in any preparative process where the ultimate output is sequencing of many molecules in parallel.

Differentiator tag sequences may be incorporated into probes (e.g., MIPs) of a plurality when they are synthesized on-chip in parallel, such that degeneracy of the incorporated nucleotides is sufficient to ensure near-uniform distribution in the plurality of probes. It is to be appreciated that amplification of a pool of unique differentiator tag sequences may itself introduce bias in the initial pool. However, in most practical cases, the scale of synthesis (e.g., by column synthesis, chip based synthesis, etc.) is large enough that amplification of an initial pool of differentiator tag sequences is not necessary. By avoiding amplification or selection steps on the pool of unique differentiator tag sequences, potential bias may be minimized.

One example of the use of the differentiator tag sequences is in genome re-sequencing. Considering that the raw accuracy of most next-generation sequencing instruments is relatively low, it is crucial to oversample the genomic loci of interest. Furthermore, since there are two alleles at every locus, it is important to sample enough to ensure that both alleles have been observed a sufficient number of times to determine with a sufficient degree of statistical confidence whether the sample is homozygous or heterozygous. Indeed, the sequencing is performed to sample the composition of molecules in the originating sample. However, after multiple reads have been collected for a given locus, it is possible that due to bias (e.g., caused by PCR amplification steps), a large fraction of the reads are derived from a single originating molecule. This would skew the population of target sequences observed, and would affect the outcome of the genotype call. For example, it is possible that a locus that is heterozygous is called as



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homozygous, because there are only a few observations of the second allele out of many observations of that locus. However, if information is available on differentiator tag sequences, this situation could be averted, because the over-represented allele would be seen to also have an over-represented differentiator tag sequence (i.e., the sequences with the overrepresented differentiator tag sequence all originated from the same single molecule). Therefore, the sequences and corresponding distribution of differentiator tag sequences can be used as an additional input to the genotype-calling algorithm to significantly improve the accuracy and confidence of the genotype calls.

In some aspects, the disclosure provides methods for analyzing a plurality of target sequences which are genetic loci or portions of genetic loci (e.g., a genetic locus of Table 1). The genetic loci may be analyzed by sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs). Exemplary polymorphisms are disclosed in Table 2. The skilled artisan will appreciate that other polymorphisms are known in the art and may be identified, for example, by querying the Entrez Single Nucleotide Polymorphism database, for example, by searching with a GeneID from Table 1.

**Table 1: Target Nucleic Acids**

| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| CYP21A2 | 1589 | cytochrome P450, family 21, subfamily A, polypeptide 2 | CAH1; CPS1; CA21H; CYP21; CYP21B; P450c21B; MGC150536; MGC150537; CYP21A2 | 201910 | NG_008337.1 | 6p21.3 |
| ABCC8 | 6833 | ATP-binding cassette, sub-family C (CFTR/MRP), member 8 | HI; SUR; HHF1; MRP8; PHHI; SUR1; ABC36; HRINS; TNDM2; ABCC8 | 600509 | NG_008867.1 | 11p15.1 |
| ATRX | 546 | alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) | SHS; XH2; XNP; ATR2; SFMI; RAD54; MRXHF1; RAD54L; ZNF-HX; MGC2094; ATRX | 300032 | NG_008838.1 | Xq13.1-q21.1 |
| ARSA | 410 | arylsulfatase A | MLD; ARSA | 607574 | NG_009260.1 | 22q13.31-qter; 22q13.33 |
| PSAP | 5660 | Prosaposin | GLBA; SAP1; FLJ00245; MGC110993; PSAP | 176801 | NG_008835.1 | 10q21-q22 |
| BTD | 686 | Biotinidase | BTD | 609019 | NG_008019.1 | 3p25 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| HLCS | 3141 | holocarboxylase synthetase (biotin-(proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)) ligase) | HCS; HLCS | 609018 | NC_000021.7 | 21q22.1; 21q22.13 |
| BLM | 641 | Bloom syndrome, RecQ helicase-like | BS; RECQ2; RECQL2; RECQL3; MGC126616; MGC131618; MGC131620; BLM | 604610 | NG_007272.1 | 15q26.1 |
| ASPA | 443 | aspartoacylase (Canavan disease) | ASP; ACY2; ASPA | 608034 | NG_008399.1 | 17pter-p13 |
| CFTR | 1080 | cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) | CF; MRP7; ABC35; ABCC7; CFTR/MRP; TNR-CFTR; dJ760C5.1; CFTR | 602421 | NC_000007.12 | 7q31.2 |
| ASS1 | 445 | argininosuccinate synthetase 1 | ASS; CTLN1; ASS1 | 603470 | NG_011542.1 | 9q34.1 |
| MMACHC | 25974 | methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria | cblC; FLJ25671; DKFZp564I122; RP11-291L19.3; MMACHC | 609831 | NC_000001.9 | 1p34.1 |
| IKBKAP | 8518 | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein | FD; DYS; ELP1; IKAP; IKI3; TOT1; FLJ12497; DKFZp781H1425; IKBKAP | 603722 | NG_008788.1 | 9q31 |
| FANCC | 2176 | Fanconi anemia, complementation group C | FA3; FAC; FACC; FLJ14675; FANCC | 227645 | NG_011707.1 | 9q22.3 |
| GALK1 | 2584 | galactokinase 1 | GK1; GALK; GALK1 | 604313 | NG_008079.1 | 17q24 |
| GALT | 2592 | galactose-1-phosphate uridylyltransferase | GALT | 606999 | NC_000009.10 | 9p13 |
| GALE | 2582 | UDP-galactose-4-epimerase | SDR1E1; FLJ95174; FLJ97302; GALE | 606953 | NG_007068.1 | 1p36-p35 |
| GBA | 2629 | glucosidase, beta; acid (includes glucosylceramidase) | GCB; GBA1; GLUC; GBA | 606463 | NG_009783.1 | 1q21 |
| GJB2 | 2706 | gap junction protein, beta 2, 26kDa | HID; KID; PPK; CX26; DFNA3; DFNB1; NSRD1; DFNA3A; DFNB1A; GJB2 | 121011 | NG_008358.1 | 13q11-q12 |
| GCDH | 2639 | glutaryl-Coenzyme A dehydrogenase | GCD; ACAD5; GCDH | 608801 | NG_009292.1 | 19p13.2 |
| G6PC | 2538 | glucose-6-phosphatase, catalytic subunit | G6PT; GSD1; GSD1a; MGC163350; G6PC | 232200 | NG_011808.1 | 17q21 |
| HBB | 3043 | hemoglobin, beta | CD113t-C; beta-globin; HBB | 141900 | NG_000007.3 | 11p15.5 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| BCKDHA | 593 | branched chain keto acid dehydrogenase E1, alpha polypeptide | MSU; MSUD1; OVD1A; BCKDE1A; FLJ45695; BCKDHA | 608348 | NC_000019.8 | 19q13.1-q13.2 |
| BCKDHB | 594 | branched chain keto acid dehydrogenase E1, beta polypeptide | E1B; FLJ17880; dJ279A18.1; BCKDHB | 248611 | NG_009775.1 | 6q13-q15 |
| DBT | 1629 | dihydrolipoamide branched chain transacylase E2 | E2; E2B; BCATE2; MGC9061; DBT | 248610 | NG_011852.1 | 1p31 |
| DLD | 1738 | dihydrolipoamide dehydrogenase | E3; LAD; DLDH; GCSL; PHE3; DLD | 238331 | NG_008045.1 | 7q31-q32 |
| ACADM | 34 | acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain | MCAD; ACAD1; MCADH; FLJ18227; FLJ93013; FLJ99884; ACADM | 607008 | NG_007045.1 | 1p31 |
| MMAA | 166785 | methylmalonic aciduria (cobalamin deficiency) cblA type | cblA; MGC120010; MGC120011; MGC120012; MGC120013; MMAA | 607481 | NG_007536.1 | 4q31.22 |
| MMAB | 326625 | methylmalonic aciduria (cobalamin deficiency) cblB type | ATR; cblB; MGC20496; MMAB | 607568 | NG_007096.1 | 12q24 |
| MUT | 4594 | methylmalonyl Coenzyme A mutase | MCM; MUT | 609058 | NG_007100.1 | 6p12.3 |
| MCOLN1 | 57192 | mucolipin 1 | ML4; MLIV; MST080; TRPML1; MSTP080; TRP-ML1; TRPM-L1; MCOLN1 | 605248 | NC_000019.8 | 19p13.3-p13.2 |
| ACTA1 | 58 | actin, alpha 1, skeletal muscle | ACTA; ASMA; CFTD; MPFD; NEM1; NEM2; NEM3; CFTD1; CFTDM; ACTA1 | 102610 | NG_006672.1 | 1q42.13 |
| TPM3 | 7170 | tropomyosin 3 | TM3; TRK; NEM1; TM-5; TM30; TM30nm; TPMsk3; hscp30; MGC3261; FLJ41118; MGC14582; MGC72094; OK/SW-cl.5; TPM3 | 191030 | NG_008621.1 | 1q21.2 |
| TNNT1 | 7138 | troponin T type 1 (skeletal, slow) | ANM; TNT; STNT; TNTS; FLJ98147; MGC104241; TNNT1 | 191041 | NG_011829.1 | 19q13.4 |
| NEB | 4703 | nebulin | NEM2; NEB177D; FLJ11505; FLJ36536; FLJ39568; FLJ39584; DKFZp686C1456; NEB | 161650 | NG_009382.1 | 2q22 |
| SMPD1 | 6609 | sphingomyelin phosphodiesterase 1, acid lysosomal | ASM; NPD; SMPD1 | 607608 | NG_011780.1 | 11p15.4-p15.1 |
| GLDC | 2731 | glycine dehydrogenase (decarboxylating) | GCE; NKH; GCSP; HYGN1; MGC138198; MGC138200; GLDC | 238300 | NC_000009.10 | 9p22 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| GCSH | 2653 | glycine cleavage system protein H (aminomethyl carrier) | GCE; NKH; GCSH | 238330 | NC_000016.8 | 16q23.2 |
| AMT | 275 | aminomethyltransferase | GCE; NKH; GCST; AMT | 238310 | NC_000003.10 | 3p21.2-p21.1 |
| OTC | 5009 | ornithine carbamoyltransferase | OCTD; MGC129967; MGC129968; MGC138856; OTC | 300461 | NG_008471.1 | Xp21.1 |
| PAH | 5053 | phenylalanine hydroxylase | PH; PKU; PKU1; PAH | 612349 | NG_008690.1 | 12q22-q24.2 |
| DHPR | 5860 | quinoid dihydropteridine reductase | DHPR; PKU2; SDR33C1; FLJ42391; QDPR | 612676 | NG_008763.1 | 4p15.31 |
| PTS | 5805 | 6-pyruvoyltetrahydropterin synthase | PTPS; FLJ97081; PTS | 261640 | NG_008743.1 | 11q22.3-q23.3 |
| PCCA | 5095 | propionyl Coenzyme A carboxylase, alpha polypeptide | PCCA | 232000 | NG_008768.1 | 13q32 |
| PCCB | 5096 | propionyl Coenzyme A carboxylase, beta polypeptide | DKFZp451E113; PCCB | 232050 | NG_008939.1 | 3q21-q22 |
| ACADS | 35 | acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain | SCAD; ACAD3; ACADS | 606885 | NG_007991.1 | 12q22-qter |
| DHCR7 | 1717 | 7-dehydrocholesterol reductase | SLOS; DHCR7 | 602858 | NC_000011.8 | 11q13.2-q13.5 |
| SMN1 | 6606 | survival of motor neuron 1, telomeric | SMA; SMN; SMA1; SMA2; SMA3; SMA4; SMA@; SMNT; BCD541; T-BCD541; SMN1 | 600354 | NG_008691.1 | 5q13 |
| HEXA | 3073 | hexosaminidase A (alpha polypeptide) | TSD; MGC99608; HEXA | 606869 | NG_009017.1 | 15q23-q24 |
| MYO7A | 4647 | myosin VIIA | DFNB2; MYU7A; NSRD2; USH1B; DFNA11; MYOVIIA; MYO7A | 276903 | NG_009086.1 | 11q13.5 |
| USH1C | 10083 | Usher syndrome 1C (autosomal recessive, severe) | PDZ73; AIE-75; DFNB18; PDZ-45; PDZ-73; NY-CO-37; NY-CO-38; ush1cpst; PDZ-73/NY-CO-38; USH1C | 605242 | NC_000011.8 | 11p15.1-p14 |
| CDH23 | 64072 | cadherin-like 23 | USH1D; DFNB12; FLJ00233; FLJ36499; KIAA1774; KIAA1812; MGC102761; DKFZp434P2350; CDH23 | 605516 | NG_008835.1 | 10q21-q22 |
| PCDH15 | 65217 | protocadherin 15 | USH1F; DFNB23; DKFZp667A1711; PCDH15 | 605514 | NG_009191.1 | 10q21.1 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| SANS | 124590 | Usher syndrome 1G (autosomal recessive) | SANS; ANKS4A; FLJ33924; USH1G | 607696 | NG_007882.1 | 17q25.1 |
| ARX | 170302 | aristaless related homeobox | ISSX; PRTS; MRX29; MRX32; MRX33; MRX36; MRX38; MRX43; MRX54; MRX76; MRX87; MRXS1; ARX | 300382 | NG_008281.1 | Xp21 |
| OPHN1 | 4983 | oligophrenin 1 | OPN1; MRX60; OPHN1 | 300127 | NG_008960.1 | Xq12 |
| JARID1C | 8242 | lysine (K)-specific demethylase 5C | MRXJ; SMCX; MRXSJ; XE169; JARID1C; DXS1272E; KDM5C | 314690 | NG_008085.1 | Xp11.22-p11.21 |
| FTSJ1 | 24140 | FtsJ homolog 1 (E. coli) | JM23; MRX9; SPB1; TRM7; CDLIV; MRX44; FTSJ1 | 300499 | NG_008879.1 | Xp11.23 |
| SLC6A8 | 6535 | solute carrier family 6 (neurotransmitter transporter, creatine), member 8 | CRT; CT1; CRTR; MGC87396; SLC6A8 | 300036 | NC_000023.9 | Xq28 |
| DLG3 | 1741 | discs, large homolog 3 (Drosophila) | MRX; MRX90; NEDLG; NE-Dlg; SAP102; SAP-102; KIAA1232; DLG3 | 300189 | NC_000023.9 | Xq13.1 |
| TM4SF2 | 7102 | tetraspanin 7 | A15; MXS1; CD231; MRX58; CCG-B7; TM4SF2; TALLA-1; TM4SF2b; DXS1692E; TSPAN7 | 300096 | NG_009160.1 | Xp11.4 |
| ZNF41 | 7592 | zinc finger protein 41 | MRX89; MGC8941; ZNF41 | 314995 | NG_008238.1 | Xp11.23 |
| FACL4 | 2182 | acyl-CoA synthetase long-chain family member 4 | ACS4; FACL4; LACS4; MRX63; MRX68; ACSL4 | 300157 | NG_008053.1 | Xq22.3-q23 |
| PQBP1 | 10084 | polyglutamine binding protein 1 | SHS; MRX55; MRXS3; MRXS8; NPW38; RENS1; PQBP1 | 300463 | NC_000023.9 | Xp11.23 |
| PEX1 | 5189 | peroxisomal biogenesis factor 1 | ZWS1; PEX1 | 602136 | NG_008341.1 | 7q21.2 |
| PXMP3 | 5828 | peroxisomal membrane protein 3, 35kDa | PAF1; PEX2; PMP3; PAF-1; PMP35; RNF72; PXMP3 | 170993 | NG_008371.1 | 8q21.1 |
| PEX6 | 5190 | peroxisomal biogenesis factor 6 | PAF2; PAF-2; PXAAA1; PEX6 | 601498 | NG_008370.1 | 6p21.1 |
| PEX10 | 5192 | peroxisomal biogenesis factor 10 | NALD; RNF69; MGC1998; PEX10 | 602859 | NG_008342.1 | 1p36.32 |
| PEX12 | 5193 | peroxisomal biogenesis factor 12 | PAF-3; PEX12 | 601758 | NG_008447.1 | 17q12 |
| PEX5 | 5830 | peroxisomal biogenesis factor 5 | PXR1; PTS1R; PTS1-BP; FLJ50634; FLJ50721; FLJ51948; PEX5 | 600414 | NG_008448.1 | 12p13.31 |
| PEX26 | 55670 | peroxisomal biogenesis factor 26 | FLJ20695; PEX26M1T; Pex26pM1T; PEX26 | 608666 | NG_008339.1 | 22q11.21 |



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The mutations listed in Table 2 are documented polymorphisms in several disease-associated genes (CFTR is mutated in cystic fibrosis, GBA is mutated in Gaucher disease, ASPA is mutated in Canavan disease, HEXA is mutated in Tay Sachs disease). The polymorphisms are of several types: insertion/deletion polymorphisms which will cause frameshifts (and thus generally interrupt protein function) unless the insertion/deletion length is a multiple of 3bp, and substitutions which can alter the amino acid sequence of the protein and in some cases cause complete inactivation by introduction of a stop codon.

**Table 2: Non-limiting examples of polymorphisms**

| Gene name | GeneID | SNP ID | Mutation | SEQ ID NO: |
|---|---|---|---|---|
| CFTR | 1080 | rs63500661 | TCACATCACCAAGTTAAAAAAAAAAA[A/G]G GGGCGGGGGGGCAGAATGAAAATT | 1 |
| CFTR | 1080 | rs63107760 | AAACAAGGATGAATTAAGTTTTTTTT[-/T]AAAAAAGAAACATTTGGTAAGGGGA | 2 |
| CFTR | 1080 | rs62469443 | ATCACCAAGTTAAAAAAAAAAAAGGG[A/G]C GGGGGGGCAGAATGAAAATTGCAT | 3 |
| CFTR | 1080 | rs62469442 | CTATTGAACCAGAACCAAACAGGAAT[A/G]C CATAGCATTTTGTAAACTAAACTG | 4 |
| CFTR | 1080 | rs62469441 | CAGGAGTTCAAGACCAGCCTACTAAA[A/C]C ACACACACACACACACACACACAC | 5 |
| CFTR | 1080 | rs62469439 | GATTAAATAATAGTGTTTATGTACCC[C/G]GC TTATAGGAGAAGAGGGTGTGTGT | 6 |
| CFTR | 1080 | rs62469438 | ATTGTTATCTTTTCATATAAGGTAAC[A/T]GA GGCCCAGAGAGATTAAATAACAT | 7 |
| CFTR | 1080 | rs62469437 | TAATTTTAATTAAGTAAATTTAATTG[A/G]TA GATAAATAAGTAGATAAAAAATA | 8 |
| CFTR | 1080 | rs62469436 | GTATAAAAAAAAAAAAAAAAAAAGTT[A/T]G AATGTTTTCTTGCATTCAGAGCCT | 9 |
| CFTR | 1080 | rs62469435 | ATACTAAAAATTTAAAGTTCTCTTGC[A/G]AT ATATTTTCTTAATATCTTACATC | 10 |
| CFTR | 1080 | rs62469434 | TGCTGGGATTACAGGCGTGAGCCACC[A/G]C GCCTGGCCTGATGGGACATATTTT | 11 |
| CFTR | 1080 | rs62469433 | CTACAATATAAGTATAGTATTGCAAA[A/C]CC ATCAGGAAGGGTGTTAACTATTT | 12 |
| CFTR | 1080 | rs61763210 | GTTGTCTCCAAACTTTTTTTCAGGTG[-/AGA]AGGTGGCCAACCGAGCTTCGGAAAG | 13 |
| CFTR | 1080 | rs61720488 | TTTTTTCATAAAAGATTATATAAAGG[A/C]TA TTGCTTTTGAATCACAAACACTA | 14 |
| CFTR | 1080 | rs61481156 | ATCTAGTGAGCAGTCAGGAAAGAGAA[C/T]T TCCAGATCCTGGAAATCAGGGTTA | 15 |
| CFTR | 1080 | rs61443875 | TAGAGTATAAAAAAAAAAAAAAAAA[-/A]GTTTGAATGTTTTCTTGCATTCAGA | 16 |



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| | | | | |
|---|---|---|---|---|
| CFTR | 1080 | rs61312222 | TGCAAATGCCAACTATCAAAGATATT[C/G]GAGTATACTGTCAATAAACTTCATA | 17 |
| CFTR | 1080 | rs61159372 | TCCTCAACAGTTAGAAACAATATTTT[C/G]AGTGATTTCCCATGCCAACTTTACT | 18 |
| CFTR | 1080 | rs61094145 | TTTTTGGTATTGTTGTTAAATAAGTG[A/G]GAATTCAATACAGTATAATGTCTGT | 19 |
| CFTR | 1080 | rs61086387 | CTTGAAATCGGATATATATATATATA[-/TGTATATATATATATATATATATATATATATACATATATATATATA]GTATTATCCCTGTTTTCACAGTTTT | 20 |
| CFTR | 1080 | rs60996744 | AGAGGGGCTGTGAAGGACACCAAGGA[A/G]GAGACTAAGAGCCAGGAGGGAAAAC | 21 |
| CFTR | 1080 | rs60960860 | TAGAGTTTATTAGCTTTTACTACTCT[A/G]CTTAGTTACTTTGTGTTACAGAATA | 22 |
| CFTR | 1080 | rs60923902 | ACTAGTGATGATGAGCTTCTTTTCAT[-/AT]GTTTGTTGGCTGCATAAATGTCTTC | 23 |
| CFTR | 1080 | rs60912824 | GCAGAGAAAAGAGGGGCTGTGAAGGA[C/G]ACCAAGGAGGAGACTAAGAGCCAGG | 24 |
| CFTR | 1080 | rs60887846 | TTCAGAGGTCTACCACTGGTGCATAC[G/T]CTAATCACAGTGTCGAAAATTTTAC | 25 |
| CFTR | 1080 | rs60793174 | AAGAAAGAGCAAAAGAGGGCAAACTT[C/T]TCATACATTTTTGATGTCGAAACCA | 26 |
| CFTR | 1080 | rs60788575 | CCTAAAGTTTAAAAAGAAAAAAAAAA[-/A]GGAAGAAGGAATTAAAAATCCAAAG | 27 |
| CFTR | 1080 | rs60760741 | GTGTGTGTGTGTATATATATATATAT[A/T]TATATATTTTTTTTTTCCTGAGCCA | 28 |
| CFTR | 1080 | rs60456599 | AAACTGTTGATGTTTTCATTTATTTA[C/G]ATCATTGGAAAACTTTAGATTCTAG | 29 |
| CFTR | 1080 | rs60363249 | TTTATCCATTCTTAACCAGAACAGAC[A/G]TTTTTTCAGAGCTGGTCCAGGAAAA | 30 |
| CFTR | 1080 | rs60355115 | TTGAAATCGGATATATATATATATAT[A/G]TATATATATATATATATATATATAT | 31 |
| CFTR | 1080 | rs60308689 | TAGTTTTTTATTTCCTCATATTATTT[-/T]CAGTGGCTTTTTCTTCCACATCTTT | 32 |
| CFTR | 1080 | rs60271242 | ACATAGTTCTCAGTGGTACAACTACA[A/G]GTGATTTCTCTTTTCTTATTTCTGG | 33 |
| CFTR | 1080 | rs60010318 | AGAGCAATGGCATCCCTTGTCTTGTG[C/T]TATACAGGATGCAGCAATTTATAGG | 34 |
| CFTR | 1080 | rs59961323 | TTCTGTCTACATAAGATGTCATACTA[A/G]ATTATCTTTTCCAGCATGCATTCAG | 35 |
| CFTR | 1080 | rs59961270 | CAGGGTGGCATGTTAGGCAGTGCTTA[A/G]AATAAATGAGTTGGTTATACAAGTA | 36 |
| CFTR | 1080 | rs59837506 | AGGACACACACACACACACACACACA[-/CA]TGCACACACATTTAAATAGATGCAT | 37 |
| CFTR | 1080 | rs59572090 | TAAAAAATTGGTATAATGAAATTGCA[C/T]TTGTAGTCTTTGGACATTTAAATCC | 38 |
| CFTR | 1080 | rs59548252 | TTTCAATACTTAAGAGGTACGCAGAG[A/G]AAAGAGGGGCTGTGAAGGACACCAA | 39 |
| CFTR | 1080 | rs59519859 | CAGCAATGAATATTTTGAGGCTGAGG[C/T]GCTGAGGGGTAAAATTGCAGCCTGG | 40 |
| CFTR | 1080 | rs59509837 | TTATGGTTTATATTTTTGTGTCTTCT[-/CTTT]AACACATCTTTTCTAGCAGAATTCA | 41 |



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|---|---|---|---|---|
| CFTR | 1080 | rs59417037 | GTATTTTAGTTTTTTTTTTTTGTTTG[-/T]TTTGTTTTGTTTTGTTTTGTTTTTG | 42 |
| CFTR | 1080 | rs59159458 | TGGGTGACTCCATTTTTACTTTTAGT[C/T]TGGTCTGTTGAGGCCTCGTGAGAGA | 43 |
| CFTR | 1080 | rs59048119 | TATTTTCATGTATTTTAGTTTTTTTTT[-/TTTT]GTTTGTTTTGTTTTGTTTTGTTTTG | 44 |
| CFTR | 1080 | rs58970500 | GTGTGTGTGTATATATATATATATAT[A/T]TATATTTTTTTTTTCCTGAGCCAAA | 45 |
| CFTR | 1080 | rs58942292 | AACCTATTAGCATGTCTGGCAGAAAA[-/A]TAGATACTTAATAAATTTCTTAAAT | 46 |
| CFTR | 1080 | rs58917054 | GAGGCTTAGACAGTTTAAGTAACTCA[A/G]GCATGGTTACACAACTAGCTAGGGC | 47 |
| CFTR | 1080 | rs58837484 | GTGTGAGTATTATGAGACCATATGTT[A/G]GGAGATTTTATTTGGTATTGAGGAT | 48 |
| CFTR | 1080 | rs58829491 | GAAACCCCACCCCTTCTATAGTTTTC[C/T]CTTTAATATTTACAATGGAACCATT | 49 |
| CFTR | 1080 | rs58805195 | CATATATATATAGTGTGTGTGTGTGT[A/G]TATATATATATATATATATTTTT | 50 |
| GBA | 2629 | rs60866785 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC | 51 |
| GBA | 2629 | rs60239603 | GGCAGGTAATATCTAGTACCTTACTT[A/T]TATTTCCTGAGCACATTCTACATTT | 52 |
| GBA | 2629 | rs56310840 | GGCCAGGAATGGGAGTGCTTAGGTGC[A/G]GAGGTGGCACTGTTCCCGCAGCTGC | 53 |
| GBA | 2629 | rs41264927 | TGAAAACTCCATCCCCTCAGGGTCAT[C/T]AGATGAAGAGAAGACCACAGGGGTT | 54 |
| GBA | 2629 | rs41264925 | TGTAGGTAAGGGTCACATGTGGGAGA[C/G]GCAGCTGTGGGTAGGTCAGCCCTGT | 55 |
| GBA | 2629 | rs36024691 | CCAAGAAGGCGCCATTACACTCCAGC[-/C]TGGGCGACAGGGCGAGACTCCCTCA | 56 |
| GBA | 2629 | rs36024092 | TGCCACACCCAGCTAATTTGTGTGTG[-/G]TATGTGTGTGTATGTATGTGTGTGT | 57 |
| GBA | 2629 | rs35682967 | GTTCCTCCAGTAATTTTTTTTTTTTT[-/T]GGTTTTGAGACAGAGTCTTGCCCTG | 58 |
| GBA | 2629 | rs35033592 | ATCATGCCCAGATAATTTTTTTTTTT[-/T]GTATTTTAGTAGACACAGGGTTTCA | 59 |
| GBA | 2629 | rs34732744 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC | 60 |
| GBA | 2629 | rs34620635 | CCTGTGAGGGGCACATTCCTTAGTAG[-/C]TAAGGAGTTGGGGGTGTGAAGATCC | 61 |
| GBA | 2629 | rs34302637 | ACAGGCTACTGGCTGGGCCCAGGCAA[-/A]GGGGGCCTTGGCAGGAAAAGTTCCT | 62 |
| GBA | 2629 | rs33949225 | GCGAGAGAGAGAGAGAGAGAGAGAGG[-/AG]AGCCGGCGCGAGAACTACGCATGCG | 63 |
| GBA | 2629 | rs28678003 | AAGAAGAAAAATAAAAAGAAAGTGGG[C/T]CAGACCGAGAGAACAGGAAGCCTGA | 64 |
| GBA | 2629 | rs28559737 | AAGGACAAAGGCAAAGAGACAAAGGC[G/T]CAACACTGGGGGTCCCCAGAGAGTG | 65 |
| GBA | 2629 | rs28373017 | TACCTAGTCACTTCCTGCCTCCATGG[C/T]GCAAAAGGGGATGGGTGTGCCTCTT | 66 |
| GBA | 2629 | rs12752133 | CTCTTCCGAGGTTCCACCCTGAACAC[C/T]TTCCTGCTCCCTCGTGGTGTAGAGT | 67 |



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|---|---|---|---|---|
| GBA | 2629 | rs12747811 | TTCTGACTGGCAACCAGCCCCACTCT[C/T]TG GGAGCCCTCAGGAATGAACTTGC | 68 |
| GBA | 2629 | rs12743554 | gctcagcctcccaggctggagtgcag[A/T]ggcgcgatctcggctcac cgcaacc | 69 |
| GBA | 2629 | rs12041778 | CATGAACCACATCAAATGAGATTTAG[C/T]GG GAGTGGCACACACAGTCATGACC | 70 |
| GBA | 2629 | rs12034326 | AAGCAGCCCTGGGGAGTCGGGGCGGG[A/G]C CTGGATTGGAAAAGAGACGGTCAC | 71 |
| GBA | 2629 | rs11558184 | CTCCAAGTTCTGGGAGCAGAGTGTGC[A/G]G CTAGGCTCCTGGGATCGAGGGATG | 72 |
| GBA | 2629 | rs11430678 | GTTCCTCCAGTAAttttttttttt[- /G/T]gttttgagacagagtcttgccctgt | 73 |
| GBA | 2629 | rs11264345 | CTAGTACCTTACTTCCCTCAAGTTCA[A/T]TC ATCTCACAGATATTTCCTGAGCA | 74 |
| GBA | 2629 | rs10908459 | aattagccgtgcgtggtggcgggtgc[C/T]tgtaatcccacgtacttgg gaggct | 75 |
| GBA | 2629 | rs10796940 | CCATGGCCAGCCGGGGAGGGGACGGG[A/C]A CACACAGACCCACACAGAGACTCA | 76 |
| GBA | 2629 | rs10668496 | agcgagagagagagagagagagagag[- /AG]gagCCGGCGCGAGAACTACGCATGC | 77 |
| GBA | 2629 | rs7416991 | CGTAGCAGTTAGCAGATGATAGGCGG[C/G/T] GAAATCTTATTTCACAGGGCATTAA | 78 |
| GBA | 2629 | rs4024049 | CTGGCCCTGGTGACAGTGGGGCTGTG[C/T]GT GGGGCCAGAGCCTTCTCAGAGGT | 79 |
| GBA | 2629 | rs4024048 | CAGATACTGGCCCTGGTGACAGTGGG[A/G]C TGTGCGTGGGGCCAGAGCCTTCTC | 80 |
| GBA | 2629 | rs4024047 | GACAGATACTGGCCCTGGTGACAGTG[G/T]G GCTGTGCGTGGGGCCAGAGCCTTC | 81 |
| GBA | 2629 | rs3841430 | GGCTCctctctctctctctctctc[- /TC]gctcgctctctcgctctctcgctct | 82 |
| GBA | 2629 | rs3754485 | GTTTCAGACCAGCCTGGCCAACATAG[C/T]GA AACCCCATCTCTACTAAAAATAA | 83 |
| GBA | 2629 | rs3205619 | AGTGGGCGATTGGATGGAGCTGAGTA[C/T]G GGGCCCATCCAGGCTAATCACACC | 84 |
| GBA | 2629 | rs2990227 | CCGGGCTCCGTGAATGTTTGTCACAT[C/G]TC TGAAGAACGTATGAATTACATAA | 85 |
| GBA | 2629 | rs2990226 | GAATCCCAACCCCGACGCTCGTCGCC[C/G]G GCTCCGTGAATGTTTGTCACATGT | 86 |
| GBA | 2629 | rs2990225 | GCGAATCCCAACCCCGACGCTCGTCG[C/T]CG GGCTCCGTGAATGTTTGTCACAT | 87 |
| GBA | 2629 | rs2990224 | TGGGCAGAAGTCAGGGTCCAAAGAAA[G/T]G GCAAAGAAAAGTGTcagtggctca | 88 |
| ASPA | 443 | rs63751297 | TAAGAAAGACGTTTTTGATTTTTTTC[A/G]GA CTTCTCTGGCTCCACTACCCTGC | 89 |
| ASPA | 443 | rs62071301 | CTGATTCCTGGCCAGGAGCGGTGGCT[C/T]AC GCCTGTAATCCCAGCGCTTTGGG | 90 |
| ASPA | 443 | rs62071300 | TAAAAATGCTGATTCCTGGCCAGGAG[C/T]GG TGGCTCACGCCTGTAATCCCAGC | 91 |
| ASPA | 443 | rs62071299 | TTTAAAAATGCTGATTCCTGGCCAGG[A/C]GC GGTGGCTCACGCCTGTAATCCCA | 92 |
| ASPA | 443 | rs62071297 | CAAGACCTGTCAAAGATCTGAGAAAT[A/T]TT ACCCGACTTACAAGCTAACCATT | 93 |



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| | | | | |
|---|---|---|---|---|
| ASPA | 443 | rs61697033 | ACTGTAATAAGTGCTGTAAAAGAAAT[A/G]C ACAAAATAATATAGCAGAGGGTAT | 94 |
| ASPA | 443 | rs60743592 | CTTGAGGTCAGGAGTTCAAGACCAGT[C/T]TG GGCAACATGGGGAAAACCTTGTC | 95 |
| ASPA | 443 | rs60666840 | AGGTTGCAGTGAGCCGAGATCATGCC[A/G]TT GCACTCCAGCCGGGGCAACAAAA | 96 |
| ASPA | 443 | rs60147514 | ACAAGTGTCTTGAAATTATCTGTGAT[C/T]TG CTATAGAGCAATACTTTTGTAAA | 97 |
| ASPA | 443 | rs59930743 | GTGGGTATATGCAGCTCTATGCACTA[C/T]CT GCTCATTTATTTGGTAAATCTAA | 98 |
| ASPA | 443 | rs59690349 | TGTGTGTGTGTGCGTGTGTGTGTGTG[- /TGTGTGTG]ATCATAAGAGTGGCTGCAGCAA ACT | 99 |
| ASPA | 443 | rs59676360 | AGTCTGGAGTGCAATGGTGCAATCTC[A/G]GC TCACTGCAGCCTCCACCTCCGGG | 100 |
| ASPA | 443 | rs59335404 | CTCCTAATGGATATTTCCTAAATTTT[G/T]CTG AACAGAATTTAACTTGAGCTGG | 101 |
| ASPA | 443 | rs58879097 | ATTTAAAAATGGATTTCTAGAAAAAC[A/G]AT CACATACTTGAATATTTTAGCAA | 102 |
| ASPA | 443 | rs58686774 | CTATAAATGGGTAGCATGAGGGATTC[A/G]A GGAGGTGGCTGAAAGAAGCACGTA | 103 |
| ASPA | 443 | rs57511162 | AAGAAACCAAGCATAGTAGAGTGTTA[A/G]A AAACCAAAGCAACTAAACAACTGT | 104 |
| ASPA | 443 | rs55859596 | CGGGGCTCAGAACTTGTAACAGAAAA[A/T]T AAAATATACTCCACTCAAGGGAAT | 105 |
| ASPA | 443 | rs55742972 | TACTACACTTCACGGATACTGTACTT[- /GTACTT]TTTTTCCAAATTGAAGGTTTTTGGC | 106 |
| ASPA | 443 | rs55640436 | TTGTTTTTGTTTTTGTTTTTGTTTTT[- /GTTTTTGTTTTT]TGAGATGGAGTCTCGCTCT GTCGCC | 107 |
| ASPA | 443 | rs36225687 | TTTGCCTTACTACACTTCACGGATAC[- /TGTACT]TGTACTTTTTTTCCAAATTGAAGGT | 108 |
| ASPA | 443 | rs36051310 | GAGGTGGCTGAAAGAAGCACGTATCC[- /C]TGATGGCATGGTTGCGGGTTATATG | 109 |
| ASPA | 443 | rs36034906 | GAGAAAAGCAGTTCCTGGAACACCCC[- /C]ACCCCTTAACCCCTTATCTCTGCTT | 110 |
| ASPA | 443 | rs36033666 | TTACATATGTATACATGTGCCATGTT[- /T]GGTGTGCCGCACCCATTAACTCGTC | 111 |
| ASPA | 443 | rs35730123 | CTTTTTCCAGATTTTTTTTTTTTTTT[- /T]GAGACAGAGTTTCACTCTTGTTGCC | 112 |
| ASPA | 443 | rs35629100 | TTTGGAAATCTTAAGCTTTTATTTGG[- /G]TGTCACAGAGAAACAGGATCTGTAT | 113 |
| ASPA | 443 | rs35614631 | TACTTTAAGTTTTAGGGTACATGTGC[- /A]CCATGTGCAGGTTTGTTACATATGT | 114 |
| ASPA | 443 | rs35225782 | ATTCATGACCAGCCACATAAATGCAC[- /A]GTATTACTTCGCAAGCATGCCAATG | 115 |
| ASPA | 443 | rs35178659 | GTGCACTAGAATTAGCTAAAGTGGGG[- /G]AAAAAAAGATGCATTTGATGGTCTA | 116 |
| ASPA | 443 | rs35095578 | AACCTCCACCTCCCAGGTTCAAGAGA[- /A]TTCTCCTGCCTCAGCCTCCCAAGTA | 117 |
| ASPA | 443 | rs35002210 | CCTCCCTGTGATCCGAAGTAGCAGAC[A/G]TA CTTAACTTCCATGGTGGATTGTT | 118 |



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| | | | | |
|---|---|---|---|---|
| ASPA | 443 | rs34744839 | AAAACATTATTATATCTAGAAAAAAA[-/A]TGTATCTTAACCATTGTGGGAAGTG | 119 |
| ASPA | 443 | rs34680506 | TTGAAGGTAAAATCATAGGGAGTTGG[-/G]AGCTGTCCTCTTGCGCTGAATCAGT | 120 |
| ASPA | 443 | rs34365618 | ACTTGTGGCCTTTTTGGAGAGGTTAG[-/CA]ACTCTGAAAACTCTGTCCCTGGACC | 121 |
| ASPA | 443 | rs34275920 | GAAGGAGAAAAAGAGAGGAAATAAGT[-/T]AAAATAATAAACACAATTAATAAAG | 122 |
| ASPA | 443 | rs34109510 | TGTATACATGTGCCATGTTGGTGTGC[C/T]GCACCCATTAACTCGTCATTTAGCA | 123 |
| ASPA | 443 | rs34054576 | TCACCTGTCACCTCCTATAGAACTTT[-/C]CCCTGACCCTCCTCTATAGCATTAA | 124 |
| ASPA | 443 | rs34015272 | ATAAATGATCATCATTCACAGTAGGG[-/G]TTTTGTTTTGTTTTTTTTCTGGAAA | 125 |
| ASPA | 443 | rs34002091 | ACAGACATATCTACAAACACACTTTT[-/T]CACATATTTGTGTAAGTCATTTATG | 126 |
| ASPA | 443 | rs28940574 | AAAGACAACTAAACTAACGCTCAATG[A/C]AAAAAGTATTCGCTGCTGTTTACAT | 127 |
| ASPA | 443 | rs28940279 | TACCGTGTACCCCGTGTTTGTGAATG[A/C]GGCCGCATATTACGAAAAGAAAGAA | 128 |
| ASPA | 443 | rs17850703 | CAGGGCTGGAGGTAAAACCATTTATT[A/G]CTAACCCCAGAGCAGTGAAGAAGTG | 129 |
| ASPA | 443 | rs17222495 | TTCTTCATTGCCTATTGAAGAGAGAG[C/T]GGAATGCTTTGGTTGCCAGATATGG | 130 |
| ASPA | 443 | rs17175228 | CACAAGATCTCATTACTCAGGAGCTG[C/T]CCAAGTGTCTAATGTACTTAGTTAA | 131 |
| ASPA | 443 | rs16953074 | TTCTGTGTAACATTTCATTTAAGCAA[A/G]GGATTCGGCAAATCAAAAATTGTCA | 132 |
| ASPA | 443 | rs16953070 | TAAAACGTATTGAAGGTATTATTGAC[G/T]CTGTTGAAGCAAAGAGAACAAAACA | 133 |
| HEXA | 3073 | rs62022858 | ATCTGCTCTTCCAGTTGGATGACAAG[C/T]CTTGCTGTCTAACACCTGCTGCAGA | 134 |
| HEXA | 3073 | rs62022857 | CCATTTTTTGTTGTATTTTTTTTTTC[C/T]TGAATACTTTTTATCGCAGTTGGTT | 135 |
| HEXA | 3073 | rs62017872 | CCCTGTCTCTAAAAGAAAAAAAAAAA[A/G]AAAAAAAAAGAAAACAAAACCCAA | 136 |
| HEXA | 3073 | rs62017871 | AGTGGCTCCAAAAAGGTCATGGAACC[C/T]CTTGAGGATGATGCAAATTGACTCT | 137 |
| HEXA | 3073 | rs61662730 | TAAAGTTACTTTTCTTTTATTGACTT[C/T]CCCTTATTTTTTAACCTTATGCTTT | 138 |
| HEXA | 3073 | rs61329913 | CAGAGTTAAAAAAAAAAAAAAAAAA[-/A]GGAAGTAGCAGCAACAGCTTGGAAA | 139 |
| HEXA | 3073 | rs60920713 | GTTGCCCAGGGTTGAGTGCAGAGGCA[C/T]ATCTTGGCTCACAGCAACCTCTGCC | 140 |
| HEXA | 3073 | rs60783213 | AAGGCTTTTTTTTTTTTTTTTTTTT[-/TTTT]GAGACAGAGTCTTGCTGTGTCACCC | 141 |
| HEXA | 3073 | rs60644867 | GCCTACATTCTGCAAAGAGGAGGGAA[C/G]ATTCACAGCTCCATACTTGAACCCT | 142 |
| HEXA | 3073 | rs60288568 | CCAAAGGAGAATAGCTCTAGGGGAGG[C/G]AGGTGGATGAGTATGCATGGGGGAG | 143 |
| HEXA | 3073 | rs59888548 | GACTCCATCTCAAAAAAAAAAAAAA[-/A]TGCAGTCTAATGGCAGAATTAGACT | 144 |



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| | | | | |
|---|---|---|---|---|
| HEXA | 3073 | rs59733856 | TTATTTATTTATTTATTTATTTTTGA[A/G]ACA GGGTCTCTGTTGTCCAGGCTGG | 145 |
| HEXA | 3073 | rs59427837 | TTTTGAGGCAGGGTCTCACTCTGTTG[C/T]CC AGGGTTGAGTGCAGAGGCACATC | 146 |
| HEXA | 3073 | rs59171976 | CGCCTTGCGAAGGCCCCACAGCTTGC[C/T]TG TGACAAACGTTCATAGGCAAATG | 147 |
| HEXA | 3073 | rs58706602 | GGAGGTCTGTACAAAGCACCACCTAC[C/T]TC ATGGGTCAGTTTCCACAGCAGAA | 148 |
| HEXA | 3073 | rs58696963 | GAATCTTATAATTCACTGTGTACCTC[- /CCTC]TGTTTCATATTTTCGCAATTGAACT | 149 |
| HEXA | 3073 | rs58610850 | AACATAGTATCTAATATAGCTTTACA[C/T]CC AAAGCCAAAATATGAATACACTG | 150 |
| HEXA | 3073 | rs58016062 | TTGTTTTGTTTTGTTTGGGGGGGGGG[- /G]TTGTTTTTCTGAGAGGGAGTCTTGC | 151 |
| HEXA | 3073 | rs57733983 | CATACCAAAGGGCAGCTGGAGGGATA[C/T]A GACGGAAGTCATGTGGAGAGTGAA | 152 |
| HEXA | 3073 | rs57476645 | CAGGTGTGAGCCACCACGACCACCAA[A/T]T TAGCTCTTTTTACTCCTTCCCTTC | 153 |
| HEXA | 3073 | rs56870003 | AGTGGTAGCTGATTTTGCTTCTGGAT[A/C]CT TTGCCACCTTCCCACTCTTTAAT | 154 |
| HEXA | 3073 | rs56338339 | AAAGACCTGTTTCTTAAAAAAAAAAA[- /AGAAAAAAAAAAA]GAAAGAAAAGAAAAG AAAAAAACAG | 155 |
| HEXA | 3073 | rs55995352 | TAAAAAATCTTTCAATGAGGAGATGT[C/T]CC CAGAGCAAGACAGCTGTAGGATG | 156 |
| HEXA | 3073 | rs55860138 | AAAAGAAAAAAAAAAAAAAAAAAAA[- /A]GAAAACAAAACCCAAACCCATAAAG | 157 |
| HEXA | 3073 | rs55743646 | CCTGTCTCTAAAAGAAAAAAAAAAAA[A/G]A AAAAAAAAGAAAACAAAACCCAAA | 158 |
| HEXA | 3073 | rs55665666 | GTTATCATAGAAAAATATCACACTCT[- /GT]CTGTATCCCCACTTCCAGAAACTGT | 159 |
| HEXA | 3073 | rs36106892 | CAGGAGCTCATAGAATTACATACAAT[- /C]TTTTTTTTTTTTTTTTTGAGACAGCG | 160 |
| HEXA | 3073 | rs36091525 | TTGAGAATCTTATAATTCACTGTGTA[- /CCTC]CCTCTGTTTCATATTTTCGCAATTG | 161 |
| HEXA | 3073 | rs35949555 | CCACTACCACAGTGCCTAGAGAACAA[C/T]A TGTGTTTAATAATATTTAAATAAT | 162 |
| HEXA | 3073 | rs35827424 | CCCTGTCTCTAAAAGAAAAAAAAAAA[- /A]AAAAAAAAAAGAAAACAAAACCCAA | 163 |
| HEXA | 3073 | rs35729578 | CCATTATATCATTCATTTCCCACTCA[- /T]TTTCTTCATTCCAACCAAGATATAT | 164 |
| HEXA | 3073 | rs35649102 | TCCGTCTCAAAAAAAAAAAAAAAAAG[- /A]GAAAGGAATTATTCTCATGTATACA | 165 |
| HEXA | 3073 | rs35118677 | CTGGGGCAGTTAAAAAGAAAAACAAA[- /C]CCCTGGTCCCTGCCCTTGAGGAGAT | 166 |
| HEXA | 3073 | rs35005352 | CTCCAGGGTCCCATTCCAGGACCACA[- /C]GCCTGCTACCTCTGCAGCTCACTCA | 167 |
| HEXA | 3073 | rs34736306 | GGATTGACATATACCAGTTAGACGGA[- /T]TTTTTTTTTCCATAAACCAGGCTCA | 168 |
| HEXA | 3073 | rs34607939 | ACAAATAATTACTACATATCTACAAC[A/G]TT CCAGATACAGAAGAAATGGCCAA | 169 |



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|---|---|---|---|---|
| HEXA | 3073 | rs34496117 | TAAACACACTTGAAACATCATATAAA[-/ATG]ATATTACTACAAGACTTAACCGTAA | 170 |
| HEXA | 3073 | rs34300017 | ACACAGGTAATCCATGTTTATTATAG[-/A]AAAATGCCACATTACTCTTTATTGA | 171 |
| HEXA | 3073 | rs34206496 | AGTTATCATAGAAAAATATCACACTC[-/TG]TCTGTATCCCCACTTCCAGAAACTG | 172 |
| HEXA | 3073 | rs34110830 | AATGAACTTACAGGAAGGTAATATAT[-/G]GGAAATAAACATCTTATTGAATTTA | 173 |
| HEXA | 3073 | rs34093438 | GGACCCCTGAAAGGCACAAGACACCC[-/T]TTCAGGTTCACACTTCCTGAAAGCT | 174 |
| HEXA | 3073 | rs34085965 | CCACCAATCACCAGAGCCTTCTGCTC[A/G]GGGGTACCTGAGGGAAAACAAGCAA | 175 |
| HEXA | 3073 | rs34004907 | AAAGACTGAAAAAACATTCATAACTA[-/T]TTTTCTTGTTATCCTCGGAAATGTC | 176 |
| HEXA | 3073 | rs28942072 | TATCTTCATCTTGGAGGAGATGAGGT[C/T]GATTTCACCTGCTGGAAGTCCAACC | 177 |
| HEXA | 3073 | rs28942071 | TTGCCTATGAACGTTTGTCACACTTC[C/T]GCTGTGAGTTGCTGAGGCGAGGTGT | 178 |
| HEXA | 3073 | rs28941771 | GCTTGCTGTTGGATACATCTCGCCAT[C/T]ACCTGCCACTCTCTAGCATCCTGGA | 179 |
| HEXA | 3073 | rs28941770 | CCGGGGCTTGCTGTTGGATACATCTC[G/T]CCATTACCTGCCACTCTCTAGCATC | 180 |

*3. Nucleic acid target length evaluation:*

In some embodiments, aspects of the invention relate to methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats.

Genomic regions that contain nucleic acid sequence repeats are often the site of genetic instability due to the amplification or contraction of the number of sequence repeats (e.g., the insertion or deletion of one or more units of the repeated sequence). Instability in the length of genomic regions that contain high numbers of repeat sequences has been associated with a number of hereditary and non hereditary diseases and conditions.

For example, "Fragile X syndrome, or Martin-Bell syndrome, is a genetic syndrome which results in a spectrum of characteristic physical, intellectual, emotional and behavioral features which range from severe to mild in manifestation. The syndrome is associated with the expansion of a single trinucleotide gene sequence (CGG) on the X chromosome, and results in a failure to express the FMR-1 protein which is required for normal neural development. There are four generally accepted forms of Fragile X syndrome which relate to the length of the repeated CGG sequence; Normal (29-31 CGG



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repeats), Premutation (55-200 CGG repeats), Full Mutation (more than 200 CGG repeats), and Intermediate or Gray Zone Alleles (40 - 60 repeats)."

Other examples include cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26). The are currently many sequencing-based assays for determining the number of nucleic acid sequence repeats at a particular locus and identifying the presence of nucleic acid insertions or deletions. However, such techniques are not useful in a high throughput multiplex analysis where the entire length of a region may not be sequenced.

In contrast, in some embodiments, aspects of the invention relate to detecting the presence of an insertion or deletion at a genomic locus without requiring the locus to be sequenced (or without requiring the entire locus to be sequenced). Aspects of the invention are particularly useful for detecting an insertion or deletion in a nucleic acid region that contains high levels of sequence repeats. The presence of sequence repeats at a genetic locus is often associated with relatively high levels of polymorphism in a population due to insertions or deletions of one or more of the sequence repeats at the locus. The polymorphisms can be associated with diseases or predisposition to diseases (e.g., certain polymorphic alleles are recessive alleles associated with a disease or condition). However, the presence of sequence repeats often complicates the analysis of a genetic locus and increases the risk of errors when using sequencing techniques to determine the precise sequence and number of repeats at that locus.

In some embodiments, aspects of the invention relate to determining the size of a genetic locus by evaluating the capture frequency of a portion of that locus suspected of containing an insertion or deletion (e.g., due to the presence of sequence repeats) using a nucleic acid capture technique (e.g., a nucleic acid sequence capture technique based on molecular inversion probe technology). According to aspects of the invention, a statistically significant difference in capture efficiency for a genetic locus of interest in different biological samples (e.g., from different subjects) is indicative of different relative lengths in those samples. It should be appreciated that the length differences may be at one or both alleles of the genetic locus. Accordingly, aspects of the invention may be used to identify polymorphisms regardless of whether biological samples being interrogated at heterozygous or homozygous for the polymorphisms. According to



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aspects of the invention, subjects that contain one or more loci with an insertion or deletion can be identified by analyzing capture efficiencies for nucleic acids obtained from one or more biological samples using appropriate controls (e.g., capture efficiencies for known nucleic acid sizes, capture efficiencies for other regions that are not suspected of containing an insertion or deletion in the biological sample(s), or predetermined reference capture efficiencies, or any combination thereof. However, it should be appreciated that aspects of the invention are not limited by the nature or presence of the control. In some embodiments, if a statistically significant variation in capture efficiency is detected, a subject may be identified as being at risk for a disease or condition associated with insertions or deletions at that genetic locus. In some embodiments, the subject may be analyzed in greater detail in order to determine the precise nature of the insertion or deletion and whether the subject is heterozygous or homozygous for one or more insertions or deletions. For example, gel electrophoresis of an amplification (e.g., PCR) product of the locus, or Southern blotting, or any combination thereof can be used as an orthogonal approach to verify the length of the locus. In some embodiments, a more exhaustive and detailed sequence analysis of the locus can be performed to identify the number and types of insertions and deletions. However, other techniques may be used to further analyze a locus identified as having an abnormal length according to aspects of the invention.

Accordingly, aspects of the invention relate to detecting abnormal nucleic acid lengths in genomic regions of interest. In some embodiments, the invention aims to estimate the size of genomic regions that are hard to be accessed, such as repetitive elements. However, it should be appreciated that methods of the invention do not require that the precise length be estimated. In some embodiments, it is sufficient to determine that one or more alleles with abnormal lengths are present at a locus of interest (e.g., based on the detection of abnormal capture efficiencies).

In a non-limiting example, fragile X can be used to illustrate aspects of the invention where the size of trinucleotide repeats (genotype) is linked to a symptom (phenotype). However, it should be appreciated that fragile X is a non-limiting example and similar analyses may be performed for other genetic loci (e.g., independently or simultaneously in multiplex analyses).

Use of molecular inversion probes (MIPs) has been demonstrated for detection of single nucleotide polymorphisms (Hardenbol et al. 2005 Genome Res 15:269-75) and for



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preparative amplification of large sets of exons (Porreca et al. 2007 Nat Methods 4:931-6, Krishnakumar et al. 2008 Proc Natl Acad Sci USA 105:9296-301). In both cases, oligonucleotide probes are designed which have ends ('targeting arms') that hybridize up-stream and down-stream of the locus that is to be amplified.

In some embodiments, aspects of the invention are based on the recognition that the effect of length on probe capturing efficiency can be used in the context of an assay (e.g., a high throughput and/or multiplex assay) to allow the length of sequences to be determined without requiring sequencing of the entire region being evaluated. This is particularly useful for repeat regions that are prone to changes in size. As illustrated in FIG. 8, which is reproduced from Deng et al., Nature Biotech. 27:353-60, (see Supplemental Fig. 1G of Deng et al.,) illustrates that shorter sequences are captured with higher efficiency that longer sequences using MIPs. The statistical package R and its *effects* module were used for this analysis. A linear model was used, and each individual factor was assumed to be independent. The dashed lines represent a 95% confidence interval. Shorter target sequences were captured with higher efficiency than long target sequences ($p < 2x10^{-16}$). However, the use of this differential capture efficiency for systematic sequence length analysis was not previously recognized.

In some embodiments, following probe hybridization, polymerase fill-in and ligation reactions are performed to convert the hybridized probe to a covalently-closed, circular molecule containing the desired target. PCR or rolling circle amplification plus exonuclease digestion of non-circularized material is performed to isolate and amplify the circular targets from the starting nucleic acid pool. Since one of the main benefits of the method is the potential for a high degree of multiplexing, generally thousands of targets are captured in a single reaction containing thousands of probes.

According to aspects of the invention, repetitive regions are surrounded by non-repetitive unique sequences, which can be used to amplify the repeat-containing regions using, for example, PCR or padlock (MIP)-based method.

In addition to the repetitive regions, a probe (e.g., a MIP or padlock probe) can be designed to include at least a sequence that is sufficient to be uniquely identified in the genome (or target pool). After the probe is circularized and amplified, the amplicon can be end-sequenced so that the unique sequence can be identified and served as the "representative" of the repetitive region as illustrated in FIG. 9. FIG. 9 illustrates a non-limiting scheme of padlock (MIP) capture of a region that includes both repetitive



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regions (thick wavy line) and the adjacent unique sequence (thick strait line). The regions of the probe are indicated with the targeting arms shown as regions "1" and "3." An intervening region that may be, or include, a sequencing primer binding site is shown as "2." After the padlock is circularized and amplified, it can be end-sequenced to obtain the sequence of the unique sequence, which represents the repetitive region of interest. Although capturing efficiency is overall negatively correlated with target length, different probe sequences may have unique features. Therefore, multiple probes could be designed and tested so that an optimal one is chosen to be sensitive enough to differentiate repetitive sizes of roughly 0-150bp, 150-600bp, and beyond, which represent normal, premutation and full mutation of fragile X syndrome, respectively. However, it should be appreciated that other probe sizes and sequences can be designed, and optionally optimized, to distinguish a range of repeat region size differences (e.g., length differences of about 3-30 bases, about 30-60 bases, about 60-90 bases, about 90-120 bases, about 120-150 bases, about 150-300 bases, about 300-600 bases, about 600-900 bases, or any intermediate or longer length difference). It should be appreciated that a length difference may be an increase in size or a decrease in size.

In some embodiments, an initial determination of an unexpected capture frequency is indicative of the presence of size difference. In some embodiments, an increase in capture frequency is indicative of a deletion. In some embodiments, a decrease in capture frequency is indicative of an insertion. However, it should be appreciated that depending on specific sequence parameters and the relative sizes of the capture probes, the target region, and the deletions or insertions, a change in capture frequency can be associated with either an increase or decrease in target region length. In some embodiments, the precise nature of the change can be determined using one or more additional techniques as described herein.

Accordingly, in some aspects a MIP probe includes a linear nucleic acid strand that contains two hybridization sequences or targeting arms, one at each end of the linear probe, wherein each of the hybridization sequences is complementary to a separate sequence on a the same strand of a target nucleic acid, and wherein these sequences on the target nucleic acid flank the two ends of the target nucleic acid sequence of interest. It should be appreciated that upon hybridization, the two ends of the probe are inverted with respect to each other in the sense that both 5' and 3' ends of the probe hybridize to



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the same strand to separate regions flanking the target region (as illustrated in FIG. 9 for example).

In some embodiments, the hybridization sequences are between about 10-100 nucleotides long, for example between about 10-30, about 30-60, about 60-90, or about 20, about 30, about 40, or about 50 nucleotides long. However, other lengths may be used depending on the application. In some embodiments, the hybridization Tms of both targeting arms of a probe are designed or selected to be similar. In some embodiments, the hybridization Tms of the targeting arms of a plurality of probes designed to capture different target regions are selected or designed to be similar so that they can be used together in a multiplex reaction. Accordingly, a typical size of a MIP probe prior to fill-in is about 60-80 nucleotides long. However, other sizes can be used depending on the sizes of the targeting arms and any other sequences (e.g., primer binding or tag sequences) that are present in the MIP probe. In some embodiments, MIP probes are designed to avoid sequence-dependent secondary structures. In some embodiments, MIP probes are designed such that the targeting arms do not overlap with known polymorphic regions. In some embodiments, targeting arms that can be used for capturing the repeat region of the Fragile X locus can have the following sequences or complementary to these sequences depending on the strand that is captured.

left: CTCCGTTTCGGTTTCACTTC (SEQ ID NO: 181)

right: ATCTTCTCTTCAGCCCTGCT (SEQ ID NO: 182)

The typical captured size using these targeting arms is about 100 nucleotides in length (e.g., about 30 repeats of a tri-nucleotide repeat).

In some embodiments, the number of reads obtained for the "representative" of the repetitive region is not informative to estimate the target length because it is dependent on the total number of reads obtained. To overcome this, it is useful to include one or more probes that target other "control" regions where no or minimal polymorphism exists among populations. Because of the systematic consistency of capturing efficiency (see, e.g., FIG. 9), the ratio of reads obtained for the repetitive "representative" to reads obtained for the control region(s) will be tuned using DNA with defined numbers of repeats. Ultimately, the ratio can serve as a measure of the repeat length as illustrated in FIG. 10. FIG. 10 illustrates a non-limiting hypothetical relationship between target gap size and the relative number of reads of the repetitive



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region, which is measured by the ratio of the repeat "representative" reads vs. the "control" region reads. The unit of y-axis is arbitrary.

In some embodiments, to better tell targets with similar size range apart, the whole repetitive region can be sequenced by making a shotgun library (e.g., by making a shotgun library from a captured sequence, for example a sequence captured using a MIP probe). The longer the repeat is, the more short reads of repeats will be obtained. Therefore, the target length will contribute twice to the relative number of "repetitive" reads, which will gain better resolution of differentiating targets. In some embodiments, the expectation is that the number of reads from any given repeat will be a direct function of the number of repeats present. However, in some embodiments, a Poisson sampling-induced spread may need to be considered and in some embodiments may be sufficiently large to limit the resolution.

When a precise measurement of the length of both alleles from a diploid sample is desired, further manipulations may be required. This is because the capture efficiency measured will actually be the average efficiency of the two alleles. To effectively achieve separate measurements for each allele, barcodes (e.g., sequence tags) can be used that allow the efficiency of individual capture events (from individual genomic loci) to be followed. FIG. 11A-C shows the approach. For a given locus, MIPs are synthesized to contain one of a large number differentiator tags in their backbone such that the probability of any two MIPs in a reaction having the same differentiator tag sequence is low. MIP capture is performed on the sample; the reaction will be biased for shorter target lengths, and therefore the reaction product will be comprised of more 'short' circles than 'long' circles. Each circle should bear a unique differentiator tag sequence. Then, linear RCA (lRCA) is performed on the circles. In the lRCA reaction, circles are converted into long, linear concatemers of themselves. The lRCA reaction for a given circle stops when the concatemer has reached a 'fixed' length (based on the processivity/error rate of the polymerase). Concatemers derived from smaller circles will therefore contain more copies of the differentiator tag, and concatemers derived from larger circles will contain fewer copies of the differentiator tag. The number of each differentiator tag sequence is counted, for example, by next-generation sequencing. When number of occurrences is plotted against differentiator tag ID, the data will naturally cluster into two groups reflecting the lengths of the two alleles in the diploid sample. The allele lengths can therefore be read directly off this graph, after absolute



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length calibration using known standards. In some embodiments, a sequencing technique (e.g., a next-generation sequencing technique) is used to sequence part of one or more captured targets (e.g., or amplicons thereof) and the sequences are used to count the number of different barcodes that are present. Accordingly, in some embodiments, aspects of the invention relate to a highly-multiplexed qPCR reaction.

Other non-limiting examples of loci at which insertions or deletions or repeat sequences may be associated with a disease or condition are provided in Tables 3 and 4. It should be appreciated that the presence of an abnormal length at any one or more of these loci may be evaluated according to aspects of the invention. In some embodiments, two or more of these loci or other loci may be evaluated in a single multiplex reaction using different probes designed to hybridize under the same reaction conditions to different target nucleic acid in a biological sample.

Table 3.

**Polyglutamine (PolyQ) Diseases**

| Type | Gene | Normal/wildtype | Pathogenic |
|---|---|---|---|
| DRPLA (Dentatorubropallidoluysian atrophy) | ATN1 or DRPLA | 6 - 35 | 49 - 88 |
| HD (Huntington's disease) | HTT (Huntingtin) | 10 - 35 | 35+ |
| SBMA (Spinobulbar muscular atrophy or Kennedy disease) | Androgen receptor on the X chromosome. | 9 - 36 | 38 - 62 |
| SCA1 (Spinocerebellar ataxia Type 1) | ATXN1 | 6 - 35 | 49 - 88 |
| SCA2 (Spinocerebellar ataxia Type 2) | ATXN2 | 14 - 32 | 33 - 77 |
| SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease) | ATXN3 | 12 - 40 | 55 - 86 |
| SCA6 (Spinocerebellar ataxia Type 6) | CACNA1A | 4 - 18 | 21 - 30 |
| SCA7 (Spinocerebellar ataxia Type 7) | ATXN7 | 7 - 17 | 38 - 120 |
| SCA17 (Spinocerebellar ataxia Type 17) | TBP | 25 - 42 | 47 - 63 |

Table 4.



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**Non-Polyglutamine Diseases**

| Type | Gene | Codon | Normal/wildtype | Pathogenic |
|---|---|---|---|---|
| FRAXA (Fragile X syndrome) | *FMR1*, on the X-chromosome | **CGG** | 6 - 53 | 230+ |
| FXTAS (Fragile X-associated tremor/ ataxia syndrome) | *FMR1*, on the X-chromosome | **CGG** | 6 - 53 | 55-200 |
| FRAXE (Fragile XE mental retardation) | AFF2 or FMR2, on the X-chromosome | **GCC** | 6 - 35 | 200+ |
| FRDA (Friedreich's ataxia) | FXN or X25, (frataxin) | **GAA** | 7 - 34 | 100+ |
| DM (Myotonic dystrophy) | DMPK | **CTG** | 5 - 37 | 50+ |
| SCA8 (Spinocerebellar ataxia Type 8) | OSCA or SCA8 | **CTG** | 16 - 37 | 110 – 250 |
| SCA12 (Spinocerebellar ataxia Type 12) | PPP2R2B or SCA12 | **CAG** On 5' end | 7 - 28 | 66 - 78 |

The following examples illustrate aspects and embodiments of the invention and are not intended to be limiting or restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

*4. Increasing detection sensitivity:*

In some embodiments, aspects of the invention relate to methods for increasing the sensitivity of nucleic acid detection assays.

There are currently many genomic assays that utilize next-generation (e.g., polony-based) sequencing to generate data, including genome resequencing, RNA-seq for gene expression, bisulphite sequencing for methylation, and Immune-seq, among others. In order to make quantitative measurements (including genotype calling), these



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methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids. The majority of these techniques require a preparative step to construct a high-complexity library of DNA molecules that is representative of a sample of interest. Current assays use one of several alternative nucleic acid preparative techniques (e.g., amplification, for example PCR-based amplification; sequence-specific capture, for example, using immobilized capture probes; or target capture into a circularized probe followed by a sequence analysis step. In order to reduce errors associated with the unpredictability (stochastic nature) of nucleic acid isolation and sequence analysis techniques, current methods involve oversampling a target nucleic acid preparation in order to increase the likelihood that all sequences that are present in the original nucleic acid sample will be represented in the final sequence data. For example, a genomic sequencing library may contain an over- or under-representation of particular sequences from a source nucleic acid sample (e.g., genome preparation) as a result of stochastic variations in the library construction process. Such variations can be particularly problematic when they result in target sequences from a genome being absent or undetectable in a sequencing library. For example, an under-representation of particular allelic sequences (e.g., heterozygotic alleles) from a genome in a sequencing library can result in an apparent homozygous representation in a sequencing library.

In contrast, aspects of the invention relate to basing a nucleic acid sequence analysis on results from two or more different nucleic acid preparatory techniques that have different systematic biases in the types of nucleic acids that they sample rather than simply oversampling the target nucleic acid. According to some embodiments, different techniques have different sequence biases that are systematic and not simply due to stochastic effects during nucleic acid capture or amplification. Accordingly, in some embodiments, the degree of oversampling required to overcome variations in nucleic acid preparation needs to be sufficient to overcome the biases. In some embodiments, the invention provides methods that reduce the need for oversampling by combining nucleic acid and/or sequence results obtained from two or more different nucleic acid preparative techniques that have different biases.

According to the invention, different techniques have different characteristic or systematic biases. For example, one technique may bias a sample analysis towards one



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particular allele at a genetic locus of interest, whereas a different technique would bias the sample analysis towards a different allele at the same locus. Accordingly, the same sample may be identified as being different depending on the type of technique that is used to prepare nucleic acid for sequence analysis. This effectively represents a sensitivity issue, because each technique has a different relative sensitivities for polymorphic sequences of interest.

According to aspects of the invention, the sensitivity of a nucleic acid analysis can be increased by combining the sequences from different nucleic acid preparative steps and using the combined sequence information for a diagnostic assay (e.g., for a making a call as to whether a subject is homozygous or heterozygous at a genetic locus of interest).

Currently, the ability of DNA sequencing to detect mutations is limited by the ability of the upstream sample isolation (e.g., by amplification, immobilization enrichment, circularization capture, etc.) methods to reliably isolate the locus of interest. If one wishes to make heterozygote base-calls for a diploid genome (e.g. a human sample presented for molecular diagnostic sequencing), it is important in some embodiments that the isolation method produces near- or perfectly- uniform amounts of the two alleles to be sequenced (at least sufficiently uniform to be "called" unambiguously as a heterozygote or a homozygote for a locus of interest).

Sample preparative methods may fall into three classes: 1) single- or several-target amplification (e.g., uniplex PCR, 'multiplex' PCR), 2) multi-target hybridization enrichment (e.g., Agilent SureSelect 'hybrid capture' [Gnirke et al 2009, *Nature methods* 27:182-9], Roche/Nimblegen 'sequence capture' [Hodges et al 2007, *Nature genetics* 39:1522-7], and 3) multi-target circularization selection (e.g. molecular inversion probes or padlock probes, [Porreca et al 2007, *Nature methods* 4:931-6, Turner et al 2009, *Nature methods* 6:315-6], 'selectors' [Dahl et al 2005, *Nucleic acids research* 33:e71]). Each of these methods can result in a pool of isolated product that does not adequately represent the input abundance distribution. For example, the two alleles at a heterozygous position can become skewed far from their input 50:50 ratio to something that results in a missed basecall during downstream sequencing. For example, if the ratio was skewed from 50:50 to 10:90, and the sample was sequenced to 10X average coverage, there is a high probability that one of the two alleles would not be observed once in the ten sequencing reads. This would reduce the sensitivity of the sequencing



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method by converting a heterozygous position to homozygous (where potentially the 'mutant' allele was the one not observed). In some embodiments, a skewed ratio is a particular issue that decreases the sensitivity of detecting mutations present in a heterogeneous tumor tissue. For example, if only 10% of the cells analyzed in a heterogeneous sample harbored a heterozygous mutation, the mutation would be expected to be present in 5% of sequence reads, not 50%. In this scenario, the need for robust, sensitive detection may be even more acute.

The methods disclosed herein are based, in part, on the discovery that certain classes of isolation methods have different modes of bias. The disclosure provide methods for increasing the sensitivity of the downstream sequencing by using a combination of multiple isolation methods (e.g., one or more from at least two of the classes disclosed herein) for a sample. This is particularly important in molecular diagnostics where high sensitivity is required to minimize the chances of 'missing' a disease-associated mutation. For example, given a nominal false-negative error rate of $1x10^{-3}$ for sequencing following circularization selection, and a false-negative error rate of $1x10^{-3}$ for sequencing following hybridization enrichment, one can achieve a final false-negative rate of $1x10^{-6}$ by performing both techniques on the sample (assuming failures in each method are fully independent). For a recessive disease with carrier frequency of 0.1, caused by a single fully-penetrant mutant allele, the number of missed carrier diagnoses would decrease from 1000 per million patients tested to 1 per million patients tested. Furthermore, if the testing was used in the context of prenatal carrier screening, the number of affected children born as a result of missing the carrier call in one parent would decrease from 25 per million to 25 per billion born.

Additionally, the disclosure provides combinations of preparative methods to effectively increase sequencing coverage in regions containing disease-associated alleles. Since heterozygote error rate is largely tied to both deviations from 50:50 allele representation, and in the case of next-generation DNA sequencing deviations from average abundance (such that less abundant isolated targets are more likely to be undersampled at one or both alleles), selectively increasing coverage in these regions will also selectively increase sensitivity. Furthermore, MIPs that detect presence or absence of specific known disease-associated mutations can be used to increase sensitivity selectively. In some embodiments, these MIPs would have a targeting arm whose 3' –most region is complementary to the expected mutation, and has a fill-in



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length of 0 or more bp. Thus, the MIP will form only if the mutation is present, and its presence will be detected by sequencing.

Additionally, algorithms disclosed herein may be used to determine base identity with varying levels of stringency depending on whether the given position has any known disease-associated alleles. Stringency can be reduced in such positions by decreasing the minimum number of observed mutant reads necessary to make a consensus base-call. This will effectively increase sensitivity for mutant allele detection at the cost of decreased specificity.

An embodiment of the invention combines MIPs plus hybridization enrichment, plus optionally extra MIPs targeted to specific known, common disease-associated loci, e.g., to detect the presence of a polymorphism in a target nucleic acid. A non-limiting example is illustrated in FIG. 12 that illustrates a schematic using MIPs plus hybridization enrichment, plus optionally extra MIPs targeted to specific known, common disease-associated loci, e.g., to detect the presence of a polymorphism in a target nucleic acid.

FIGs. 13 and 14 illustrate different capture efficiencies for MIP-based captures. FIG. 13 shows a graph of per-target abundance with MIP capture. In this graph, bias largely drives the heterozygote error rate, since targets which are less abundant here are less likely to be covered in sufficient depth during sequencing to adequately sample both alleles. This is from Turner et al 2009, Nature methods 6:315-6. Hybridization enrichment results in a qualitatively similar abundance distribution, but the abundance of a given target is likely not correlated between the two methods. FIG. 14 shows a graph of correlation between two MIP capture reactions from Ball et al 2009, Nature biotechnology 27:361-8. Each point represents the target abundance in replicate 1 and replicate 2. Pearson correlation r=0.956. This indicates that MIP capture reproducibly biases targets to specific abundances. Hybridization enrichment is similarly correlated from one capture to the next.

According to aspects of the invention, such biases can be detected or overcome by systematically combining different capture and/or analytical techniques in an assay that interrogates a plurality of loci in a plurality of subject samples.

Accordingly, it should be appreciated that in any of the embodiments described herein (e.g., tiling/staggering, tagging, size-detection, sensitivity enhancing algorithms,



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or any combination thereof), aspects of the invention involve preparing genomic nucleic acid and/or contacting them with one or more different probes (e.g., capture probes, hybridization probes, MIPs, others etc.). In some embodiments, the amount of genomic nucleic acid used per subject ranges from 1 ng to 10 micrograms (e.g., 500 ng to 5 micrograms). However, higher or lower amounts (e.g., less than 1 ng, more than 10 micrograms, 10-50 micrograms, 50-100 micrograms or more) may be used. In some embodiments, for each locus of interest, the amount of probe used per assay may be optimized for a particular application. In some embodiments, the ratio (molar ratio, for example measured as a concentration ratio) of probe to genome equivalent (e.g., haploid or diploid genome equivalent, for example for each allele or for both alleles of a nucleic acid target or locus of interest) ranges from 1/100, 1/10, 1/1, 10/1, 100/1, 1000/1. However, lower, higher, or intermediate ratios may be used.

In some embodiments, the amount of target nucleic acid and probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios. In some embodiments, in order to normalize genomic DNA and probe, the genomic DNA concentration is read using a standard spectrophotometer or by fluorescence (e.g., using a fluorescent intercalating dye). The probe concentration may be determined experimentally or using information specified by the probe manufacturer.

Similarly, once a locus has been captured (e.g., on a MIP or other probe or in another form), it may be amplified and/or sequenced in a reaction involving one or more primers. The amount of primer added for each reaction can range from 0.1 pmol to 1 nmol, 0.15 pmol to 1.5 nmol (for example around 1.5 pmol). However, other amounts (e.g., lower, higher, or intermediate amounts) may be used.

In some embodiments, it should be appreciated that one or more intervening sequences (e.g., sequence between the first and second targeting arms on a MIP capture probe), identifier or tag sequences, or other probe sequences that are not designed to hybridize to a target sequence (e.g., a genomic target sequence) should be designed to avoid excessive complementarity (to avoid cross-hybridization) to target sequences or other sequences (e.g., other genomic sequences) that may be in a biological sample. For example, these sequences may be designed have a sufficient number of mismatches with any genomic sequence (e.g., at least 5, 10, 15, or more mismatches out of 30 bases) or as having a Tm (e.g., a mismatch Tm) that is lower (e.g., at least 5, 10, 15, 20, or more degrees C lower) than the hybridization reaction temperature.



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It should be appreciated that a targeting arm as used herein may be designed to hybridize (e.g., be complementary) to either strand of a genetic locus of interest if the nucleic acid being analyzed is DNA (e.g., genomic DNA). However, in the context of MIP probes, whichever strand is selected for one targeting arm will be used for the other one. However, in the context of RNA analysis, it should be appreciated that a targeting arm should be designed to hybridize to the transcribed RNA. It also should be appreciated that MIP probes referred to herein as "capturing" a target sequence are actually capturing it by template-based synthesis rather than by capturing the actual target molecule (other than for example in the initial stage when the arms hybridize to it or in the sense that the target molecule can remain bound to the extended MIP product until it is denatured or otherwise removed).

It should be appreciated that in some embodiments a targeting arm may include a sequence that is complementary to one allele or mutation (e.g., a SNP or other polymorphism, a mutation, etc.) so that the probe will preferentially hybridize (and capture) target nucleic acids having that allele or mutation. However, in many embodiments, each targeting arm is designed to hybridize (e.g., be complementary) to a sequence that is not polymorphic in the subjects of a population that is being evaluated. This allows target sequences to be captured and/or sequenced for all alleles and then the differences between subjects (e.g., calls of heterozygous or homozygous for one or more loci) can be based on the sequence information and/or the frequency as described herein.

It should be appreciated that sequence tags (also referred to as barcodes) may be designed to be unique in that they do not appear at other positions within a probe or a family of probes and they also do not appear within the sequences being targeted. Thus they can be used to uniquely identify (e.g., by sequencing or hybridization properties) particular probes having other characteristics (e.g., for particular subjects and/or for particular loci).

It also should be appreciated that in some embodiments probes or regions of probes or other nucleic acids are described herein as comprising or including certain sequences or sequence characteristics (e.g., length, other properties, etc.). However, it should be appreciated that in some embodiments, any of the probes or regions of probes or other nucleic acids consist of those regions (e.g., arms, central regions, tags, primer sites, etc., or any combination thereof) of consist of those sequences or have sequences with characteristics that consist of one or more characteristics (e.g., length, or other



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properties, etc.) as described herein in the context of any of the embodiments (e.g., for tiled or staggered probes, tagged probes, length detection, sensitivity enhancing algorithms or any combination thereof).

It should be appreciated that probes, primers, and other nucleic acids designed or used herein may be synthetic, natural, or a combination thereof. Accordingly, as used herein, the term "nucleic acid" refers to multiple linked nucleotides (i.e., molecules comprising a sugar (e.g., ribose or deoxyribose) linked to an exchangeable organic base, which is either a pyrimidine (e.g., cytosine (C), thymidine (T) or uracil (U)) or a purine (e.g., adenine (A) or guanine (G)). "Nucleic acid" and "nucleic acid molecule" may be used interchangeably and refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms shall also include polynucleosides (i.e., a polynucleotide minus a phosphate) and any other organic base containing nucleic acid. The organic bases include adenine, uracil, guanine, thymine, cytosine and inosine. Unless otherwise stated, nucleic acids may be single or double stranded. The nucleic acid may be naturally or non-naturally occurring. Nucleic acids can be obtained from natural sources, or can be synthesized using a nucleic acid synthesizer (i.e., synthetic). Harvest and isolation of nucleic acids are routinely performed in the art and suitable methods can be found in standard molecular biology textbooks. (See, for example, Maniatis' Handbook of Molecular Biology.) The nucleic acid may be DNA or RNA, such as genomic DNA, mitochondrial DNA, mRNA, cDNA, rRNA, miRNA, or a combination thereof. Non-naturally occurring nucleic acids such as bacterial artificial chromosomes (BACs) and yeast artificial chromosomes (YACs) can also be used.

The invention also contemplates the use of nucleic acid derivatives. As will be described herein, the use of certain nucleic acid derivatives may increase the stability of the nucleic acids of the invention by preventing their digestion, particularly when they are exposed to biological samples that may contain nucleases. As used herein, a nucleic acid derivative is a non-naturally occurring nucleic acid or a unit thereof. Nucleic acid derivatives may contain non-naturally occurring elements such as non-naturally occurring nucleotides and non-naturally occurring backbone linkages.

Nucleic acid derivatives may contain backbone modifications such as but not limited to phosphorothioate linkages, phosphodiester modified nucleic acids, phosphorothiolate modifications, combinations of phosphodiester and phosphorothioate nucleic acid, methylphosphonate, alkylphosphonates, phosphate esters,



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alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, carboxymethyl esters, methylphosphorothioate, phosphorodithioate, p-ethoxy, and combinations thereof. The backbone composition of the nucleic acids may be homogeneous or heterogeneous.

Nucleic acid derivatives may contain substitutions or modifications in the sugars and/or bases. For example, they include nucleic acids having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position (e.g., an 2'-O-alkylated ribose group). Nucleic acid derivatives may include non-ribose sugars such as arabinose. Nucleic acid derivatives may contain substituted purines and pyrimidines such as C-5 propyne modified bases, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, 2-thiouracil and pseudoisocytosine. In some embodiments, substitution(s) may include one or more substitutions/modifications in the sugars/bases, groups attached to the base, including biotin, fluorescent groups (fluorescein, cyanine, rhodamine, etc), chemically-reactive groups including carboxyl, NHS, thiol, etc., or any combination thereof.

A nucleic acid may be a peptide nucleic acid (PNA), locked nucleic acid (LNA), DNA, RNA, or co-nucleic acids of the same such as DNA-LNA co-nucleic acids. PNA are DNA analogs having their phosphate backbone replaced with 2-aminoethyl glycine residues linked to nucleotide bases through glycine amino nitrogen and methylenecarbonyl linkers. PNA can bind to both DNA and RNA targets by Watson-Crick base pairing, and in so doing form stronger hybrids than would be possible with DNA or RNA based oligonucleotides in some cases.

PNA are synthesized from monomers connected by a peptide bond (Nielsen, P.E. et al. Peptide Nucleic Acids, Protocols and Applications, Norfolk: Horizon Scientific Press, p. 1-19 (1999)). They can be built with standard solid phase peptide synthesis technology. PNA chemistry and synthesis allows for inclusion of amino acids and polypeptide sequences in the PNA design. For example, lysine residues can be used to introduce positive charges in the PNA backbone. All chemical approaches available for the modifications of amino acid side chains are directly applicable to PNA. Several types of PNA designs exist, and these include single strand PNA (ssPNA), bisPNA and pseudocomplementary PNA (pcPNA).



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The structure of PNA/DNA complex depends on the particular PNA and its sequence. ssPNA binds to single stranded DNA (ssDNA) preferably in antiparallel orientation (i.e., with the N-terminus of the ssPNA aligned with the 3' terminus of the ssDNA) and with a Watson-Crick pairing. PNA also can bind to DNA with a Hoogsteen base pairing, and thereby forms triplexes with double stranded DNA (dsDNA) (Wittung, P. et al., *Biochemistry* 36:7973 (1997)).

A locked nucleic acid (LNA) is a modified RNA nucleotide. An LNA form hybrids with DNA, which are at least as stable as PNA/DNA hybrids (Braasch, D.A. et al., *Chem & Biol.* 8(1):1-7(2001)). Therefore, LNA can be used just as PNA molecules would be. LNA binding efficiency can be increased in some embodiments by adding positive charges to it. LNAs have been reported to have increased binding affinity inherently.

Commercial nucleic acid synthesizers and standard phosphoramidite chemistry are used to make LNAs. Therefore, production of mixed LNA/DNA sequences is as simple as that of mixed PNA/peptide sequences. The stabilization effect of LNA monomers is not an additive effect. The monomer influences conformation of sugar rings of neighboring deoxynucleotides shifting them to more stable configurations (Nielsen, P.E. et al. Peptide Nucleic Acids, Protocols and Applications, Norfolk: Horizon Scientific Press, p. 1-19 (1999)). Also, lesser number of LNA residues in the sequence dramatically improves accuracy of the synthesis. Most of biochemical approaches for nucleic acid conjugations are applicable to LNA/DNA constructs.

These and other aspects of the invention are illustrated by the following non-limiting examples.

## EXAMPLES

The following examples illustrate non-limiting embodiments of the invention.

**Example 1: Design a set of capture probes for a human target exon**

All targets are captured as a set of partially-overlapping subtargets. For example, in the tiling approach, a 200 bp target exon might be captured as a set of 12 subtargets,



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each 60bp in length (FIG. 1). Each subtarget is chosen such that it partially overlaps two or three other targets.

In some embodiments, all probes are composed of three regions: 1) a 20 bp 'targeting arm' comprised of sequence which hybridizes immediately upstream from the sub-target, 2) a 30 bp 'constant region' comprised of sequence used as a pair of amplification priming sites, and 3) a second 20 bp 'targeting arm' comprised of sequence which hybridizes immediately downstream from the sub-target. Targeting arm sequences will be different for each capture probe in a set, while constant region sequence will be the same for all probes in the set, allowing all captured targets to be amplified with a single set of primers. Targeting arm sequences should be designed such that any given pair of 20 bp sequences is unique in the target genome (to prevent spurious capture of undesired sites). Additionally, melting temperatures should be matched for all probes in the set such that hybridization efficiency is uniform for all probes at a constant temperature (e.g., 60 C). Targeting arm sequences should be computationally screened to ensure they do not form strong secondary structure that would impair their ability to basepair with the genomic target.

***Hybridize capture probes to human genomic sample***

Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

***Convert hybridized probes into covalently-closed circular products containing subtargets***

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)



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- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

***Purify circularized probe / subtarget products from un-reacted probes and genomic DNA***

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubte at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

***Amplify circular material by PCR using primers specific to the 'constant region' of the probes***

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3') (SEQ ID NO: 183)
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3') (SEQ ID NO: 184)
- 
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

1. 95C for 5 min



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2. 95C for 30 sec
3. 58C for 60 sec
4. 72C for 60 sec
5. goto 2, N more times

***Prepare a shotgun next-generation sequencing library for analysis***

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

**Example 2: Use of differentiator tag sequences to detect and correct bias in a MIP-capture reaction of a set of exon targets**

The first step in performing the detection/correction is to determine how many differentiator tag sequences are necessary for the given sample. In this example, 1000 genomic targets corresponding to 1000 exons were captured. Since the differentiator tag sequence is part of the probe, it will measure/report biases that occur from the earliest protocol steps. Also, being located in the backbone, the differentiator tag sequence can easily be sequenced from a separate priming site, and therefore not impact the total achievable read-length for the target sequence. MIP probes are synthesized using standard column-based oligonucleotide synthesis by any number of vendors (e.g. IDT), and differentiator tag sequences are introduced as 'degenerate' positions in the backbone. Each degenerate position increases the total number of differentiator tag sequences synthesized by a factor of 4, so a 10 nt degenerate region implies a differentiator tag sequence complexity of ~1e6 species.

*Hybridize capture probes to human genomic sample*



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Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

*Convert hybridized probes into covalently-closed circular products containing subtargets*

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

*Purify circularized probe / subtarget products from un-reacted probes and genomic DNA*

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubate at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

*Amplify circular material by PCR using primers specific to the 'constant region' of the probes*

Prepare PCR mixture:



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- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3') (SEQ ID NO: 183)
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3') (SEQ ID NO: 184)
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

6. 95C for 5 min
7. 95C for 30 sec
8. 58C for 60 sec
9. 72C for 60 sec
10. goto 2, N more times

*Prepare a shotgun next-generation sequencing library for analysis*

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in lenth.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.



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*Perform sequencing of library according to manufacturer's directions (e.g. Illumina,ABI, etc), reading both the target sequence and the differentiator tag sequence.*

*Analyze data by correcting for any biases detected by quantitation of differentiator tag sequence abundance*

Construct a table of target:differentiator tag abundances from the read data, e.g.:

| Target ID | Differentiator tag sequence ID | Count |
|---|---|---|
| 1 | 3547 | 1 |
| 2 | 4762 | 1 |
| 1 | 9637 | 1 |
| 1 | 1078 | 5 |
| 3 | 4762 | 1 |
| 1 | 2984 | 1 |

All 'count' entries should be '1', since any particular target:differentiator tag mapping will not occur more than once by chance, and therefore will only be observed if bias was present somewhere in the sample preparation process. For any target:differentiator tag combination observed more than once, all such reads are 'collapsed' into a single read before consensus basecalls are determined. This will cancel the effect of bias on consensus basecall accuracy. FIG. 5 depicts a method for making diploid genotype calls in which repeat target:differentiator tag combination are collapsed.

**Example 3: Differentiator tag sequence design for MIP capture reactions**

For a set of targets, the number of differentiator tag sequences necessary to be confident (within some statistical bounds) that a certain differentiator tag sequence will not be observed more than once by chance in combination with a certain target sequence was determined. The total number of unique differentiator tag sequences for a certain differentiator tag sequence length is determined as $4^{(\text{Length in nucleotides of the differentiator tag sequence})}$. For a molecular inversion probe capture reaction that uses MIP probes having differentiator tag sequences, the probability of performing the capture reaction and capturing one or more copies of a target sequence having the same differentiator tag sequence is calculated as: p = 1 - [N! / (N-M)!] / [N^M], wherein N is the total number



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of possible unique differentiator tag sequences and M is the number of target sequence copies in the capture reaction. Thus, by varying the differentiator tag sequence length it is possible to perform a MIP capture reaction in which the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is set at a predetermined probability value.

For example, for a differentiator tag sequence of 15 nucleotides in length, there are 1,073,741,824 possible differentiator tag sequences. A MIP capture reaction in which MIP probes, each having a differentiator tag sequence of 15 nucleotides, are combined with 10000 target sequence copies (e.g., genome equivalents), the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is 0.05. In this example, the MIP reaction will produce very few (usually 0, but occasionally 1 or more) targets where multiple copies are tagged with the same differentiator tag sequence. FIG. 6 depicts results of a simulation for 100000 capture reactions having 15 nucleotide differentiator tag sequences and 10000 target sequences.

**Example 4: Assessment of the probability for obtaining enough sequencing reads to make accurate base-calls at multiple independent loci, as a function of sequencing coverage.**

Monte Carlo simulations were performed to determine sequencing coverage requirements. The simulations assume 10000 genomic copies of a given locus (target) half mom alleles and half dad alleles. The simulations further assume 1% efficiency of capture for the MIP reaction. The simulation samples from a capture mix 100 times without replacement to create a set of 100 capture products. The simulation then samples from the set of 100 capture products with replacement (assuming unbiased amplification) to generate 'reads' from either mom or dad. The number of reads sampled depends on the coverage. The number of independent reads from both mom and dad necessary to make a high-quality base-call (assumed to be 10 or 20 reads) were then determined. The process was repeated 1000 times for each coverage level, and the fraction of times that enough reads from both parents were successfully obtained was determined. This fraction was raised to the power 1000, assuming we have 1000 independent loci that must obtain successful base-calls, plotted (See FIG. 7). Result show that roughly 50x coverage is required to capture each allele >= 10x with > 0.95 probability.



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**Example 5: MIP capture of 'target' locus and 'control' loci**

In some embodiments, to accurately quantify the efficiency of target locus capture, at least three sets of control loci are captured in parallel that have *a priori* been shown to serve as proxies for various lengths of target locus. For example, if the target locus is expected to have a length between 50 and 1000 bp, then sets of control loci having lengths of 50, 250, and 1000 bp could be captured (e.g. 20 loci per set should provide adequate protection from outliers), and their abundance digitally measured by sequencing. These loci should be chosen such that minimal variation in efficiency between samples and on multiple runs of the same sample is observed (and are therefore 'efficiency invariant'). These will serve as 'reference' points that define the shape of the curve of abundance-vs-length. Determining the length of the target is then simply a matter of 'reading' the length from the appropriate point on the calibration curve.

In some embodiments, the statistical confidence one has in the estimate of target length from this method is driven largely by three factors: 1) reproducibility / variation of the abundance data used to generate the calibration curve; 2) goodness of fit of the regression to the 'control' datapoints; 3) reproducibility of abundance data for the target locus being measured. Statistical bounds on 1) and 2) will be known in advance, having been measured during development of the assay. Additionally, statistical bounds on 3) will be known in general in advance, since assay development should include adequate population sampling and measure of technical reproducibility. Standard statistical methods should be used to combine these three measures into a single *P* value for any given experimental measure of target abundance.

In some embodiments, given the set of calibration observations, and a linear regression fit to that data, the regression can be used to predict the length value for n observations of the target locus whose length is unknown. First, choose an acceptable range for the confidence interval of the length estimate. For example, in the case of distinguishing "normal" (87-93bp) from "premutation" (165-600bp) potential cases of Fragile X, the goal is to measure length to sufficient precision to distinguish 93 bp from 165 bp. The predicted response value, computed when n observations is substituted into the equation for the regressed line, will have arbitrary precision. However, if for example a 95% confidence level is desired, that 95% confidence interval must be sufficiently short that it does not overlap both the "normal" and "premutation" length



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ranges. Continuing the example, if one calculates a length of 190 from n=400 MIP observations, and based on the regression from calibration data, the 95% confidence interval is 190 +/- 20 bp, one can conclude the sample represents a "premutation" length with 95% certainty. Conversely, if the calibration data were less robust, error estimates of the regression would be higher, leading to larger confidence intervals on the predicted response value. In some embodiments, if the 95% CI were calculated as 190 +/- 100 bp from n=400, one could not determine whether the predicted response value corresponds to a "normal" or "premutation" length.

In some embodiments, the confidence interval for a predicted response is calculated as:

*The estimate for the response* $\hat{y}$ *is identical to the estimate for the mean of the response:* $\hat{y} = b_0 + b_1 x^*$. *The confidence interval for the predicted value is given by* $\hat{y} \pm t^* s_{\hat{y}}$, *where* $\hat{y}$ *is the fitted value corresponding to* $x^*$. *The value* $t^*$ *is the upper (1 - C)/2 critical value for the t(n - 2) distribution.*

In some embodiments, a technique for analyzing a locus of interest can involve the following steps.

***Convert hybridized probes into covalently-closed circular products containing subtargets***

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

***Purify circularized probe / subtarget products from un-reacted probes and genomic DNA***

Prepare exonuclease reaction mixture:



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- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubte at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

***Amplify circular material by PCR using primers specific to the 'constant region' of the probes***

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA-Ilmn (platform-specific amplification sequence plus 'circle constant region'-specific sequence)
- 1.5 ul 10 uM CP-2-RA-Ilmn (platform-specific amplification sequence plus 'circle constant region'-specific sequence)
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

11. 95C for 5 min
12. 95C for 30 sec
13. 58C for 60 sec
14. 72C for 60 sec
15. goto 2, N more times

***Perform sequencing (e.g., next-generation sequencing) on sample for digital quantitation according to manufacturer's instructions (e.g., Illumina, ABI)***

**Example 6: MIP-capture reaction of a set of exon target nucleic acids**



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MIP probes are synthesized using standard column-based oligonucleotide synthesis by any number of vendors (e.g. IDT).

*Hybridize capture probes to human genomic sample*

Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

*Convert hybridized probes into covalently-closed circular products containing target nucleic acids*

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

*Purify circularized probe/target nucleic acid products from un-reacted probes and genomic DNA*

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubate at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

*Amplify circular material by PCR using primers specific to the 'constant region' of the probes*

Prepare PCR mixture:



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- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3') (SEQ ID NO: 183)
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3') (SEQ ID NO: 184)
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

16. 95C for 5 min
17. 95C for 30 sec
18. 58C for 60 sec
19. 72C for 60 sec
20. goto 2, N more times

*Prepare a shotgun next-generation sequencing library for analysis*

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

*Perform sequencing of library according to manufacturer's directions (e.g. Illumina,ABI, etc), reading the target sequence to determine abundance of the target nucleic acid.*



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**Example 7: Use of MIPs, hybridization, and mutation-detection MIPs to genotype a set of 1000 targets**

MIPs, hybridization, and mutation-detection MIPs are used to genotype a set of 1000 targets. The protocol permits detection of any of 50 specific known point mutations

First, separate MIP, hybridization, and mutation-detection MIP reactions are performed on a biological sample. A MIP capture reaction is performed essentially as described in Turner et al 2009, Nature methods 6:315-6. A set of MIPs is designed such that each probe in the set flanks one of the 1000 targets. Separately, a hybridization enrichment reaction is performed using the Agilent SureSelect procedure. Prior to selection, the genomic DNA to be enriched is converted into a shotgun sequencing library using Illumina's 'Fragment Library' kit and protocol. Agilent's web interface is used to design a set of probes which will hybridize to the target nucleic acids. Separately, a set of probes are designed (mutation-detection MIPs) which will form MIPs only if mutations (e.g., specific polymorphisms) are present. Each mutation-detection MIP has a 3'-most base identity that is specific for a single known mutation. A reaction with this set of mutation-detection MIPs is performed to selectively detect the presence of any mutant alleles.

Once all three reactions have been performed, the two MIP reactions are combined (e.g., at potentially non-equimolar ratios to further increase sensitivity of mutation detection) into a single tube, and run as one sample on the next-generation DNA sequencing instrument. The hybridization-enriched reaction is run as a separate sample on the next-generation DNA sequencing instrument. Reads from each 'sample' are combined by a software algorithm which forms a consensus diploid genotype at each position in the target set by evaluating the total coverage at each position, the origin of each read in that total coverage, the quality score of each individual read, and the presence (or absence) of any reads derived from mutation-specific MIPs overlapping the region.

It should be appreciated that the preceding examples are non-limiting and aspects of the invention may be implemented as described herein using alternative techniques and/or protocols that are available to one or ordinary skill in the art.



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It will be clear that the methods may be practiced other than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present disclosure are possible in light of the above teachings and, therefore, are within the scope of the claims. Preferred features of each aspect of the disclosure are as for each of the other aspects mutatis mutandis. The documents including patents, patent applications, journal articles, or other disclosures mentioned herein are hereby incorporated by reference in their entirety. In the event of conflict, the disclosure of present application controls, other than in the event of clear error.



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## CLAIMS

What is claimed is:

1. A method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set,
wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion,
isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and
analyzing the isolated nucleic acids.

2. The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion.

3. The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.

4. The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5. A method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set,
wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe,



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isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

6. The method of claim 1 or 5, wherein the target nucleic acids are selected from Table 1.

7. The method of claim 1 or 5, wherein the analyzing comprises genotyping a polymorphism of the target nucleic acid.

8. The method of claim 7, wherein the polymorphism is selected from Table 2.

9. A method of genotyping a subject, the method comprising:

determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence,

wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject.

10. The method of claim 9., wherein the isolated nucleic acids are molecular inversion probe capture products.

11. A method of genotyping a subject, the method comprising:

determining the sequence of molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, and wherein the target nucleic acid is a captured genomic locus of a subject, and



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genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products.

12. A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject,

amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

14. The method of claim 12 or 13, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

15. The method of any one of claims 12-14, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. The method of any one of claims 12-14, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.



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17. A method of analyzing a plurality of genetic loci, the method comprising:

obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence,

amplifying the plurality of molecular inversion probe capture products,

determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and

if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination.

18. The method of claim 17, further comprising genotyping target sequences in the plurality, wherein the genotyping comprises correcting for bias, if detected.

19. The method of any of claims 9-18, wherein the target nucleic acid sequence is

a sequence of a gene selected from Table 1.

20. A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. The method of claim 20-21, wherein the capture efficiency is lower than the reference capture efficiency.



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23. The method of claim 22, wherein the subject is identified as having an insertion in the target region.

24. The method of claim 20-21, wherein the capture efficiency is higher than the reference capture efficiency.

25. The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.

27. The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. The method of any prior claim, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

30. The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31. The method of claim 30, wherein the first and second targeting arms are about 10-20, 20-30, 30-40, or 40-50 nucleotides long.



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32. The method of claim 31, wherein the first and second targeting arms are about 20 nucleotides long.

33. The method of any one of claims 29-31, wherein the first and second targeting arms have the same length.

34. The method of any one of claims 29-33, wherein the hybridization Tms of the first and second targeting arms are similar.

35. The method of claim 34, wherein the hybridization Tms of the first and second targeting arms are within 2-5 C of each other.

36. The method of claim 34, wherein the hybridization Tms of the first and second targeting arms are identical.

37. The method of any one of claims 28-36, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. The method of claim 37, wherein the sub-target nucleic acid contains 10-100 copies of the nucleic acid repeat in the absence of an abnormal increase or decrease in nucleic acid repeats.

40. The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. The method of any one of claims 29-36, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.



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42. The method of any one of claims 29-36, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.

43. The method of any one of claims 29-36, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. The method of any of the prior claims, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48. The method of any prior claim, wherein the biological sample is a blood sample.

49. The method of any prior claim, wherein the biological sample is a tissue sample.

50. The method of any prior claim, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.



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52. The method of claim 50-51, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.

53. The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.

55. The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. The method of any prior claim, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57. A method for estimating the length of a target nucleic acid, the method comprising:

(i) contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid,

wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid,

wherein the first and second regions are on a common strand of the target nucleic acid, and

wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid; and

(ii) capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes; and



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(iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the sub-target nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid.

58. A method of increasing the sensitivity of a nucleic acid detection assay, the method comprising:

obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample,

obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample,

assaying the sequences obtained in both first and second nucleic acid preparations,

using the sequence information from both first and second nucleic acid preparations to determine the genotype of the target nucleic acid in the biological sample, wherein the first and second preparative methods have different systematic sequence biases.

59. The method of claim 58, wherein the first and second nucleic acid preparations are combined prior to performing a sequence assay.

60. The method of claim 58, wherein separate sequence assays are performed on the first and second nucleic acid preparations and the sequence information from both assays are combined to determine the genotype of the target nucleic acid in the biological sample.

61. The method of claim 58, wherein the first preparative method is a PCR-based, a hybridization-based, or a circular probe-based preparative method.

62. The method of claim 61, wherein the second preparative method is a PCR-based, a hybridization-based, or a circular probe-based preparative method, provided that the second method is different from the first method.



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63. A method of obtaining a nucleic acid preparation that is representative of the nucleic acid in a biological sample, the method comprising

obtaining a first nucleic acid preparation using a first preparative method on a biological sample,

obtaining a second nucleic acid preparation using a second preparative method on the biological sample, and

combining the first and second nucleic acid preparations to obtain a combined preparation that is representative of nucleic acid in the biological sample.

64. The method of claim 1 or 6, further comprising performing a third nucleic acid preparation using a third preparative method that is different from the first and second preparative methods, wherein the first, second, and third preparative methods all have different systematic sequence biases.

65. The method of claim any of the prior claims, wherein the different preparative methods are used for a plurality of different loci in the biological sample to increase the sensitivity of a multiplex nucleic acid analysis.

66. A method of genotyping a nucleic acid in a biological sample, the method comprising:

obtaining a nucleic acid preparation using a preparative method on a biological sample,

sequencing a target nucleic acid of the nucleic acid preparation,

and performing a molecular inversion probe capture reaction on the biological sample, wherein a molecular inversion probe capture of the target nucleic acid in the biological sample is indicative of the presence of a polymorphism in the target nucleic acid,

genotyping the target nucleic acid based on the results of the sequencing and the capture reaction.

67. The method of any of the prior claims, wherein the target nucleic acid has a sequence of a gene selected from Table 1.



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**Figure 1**

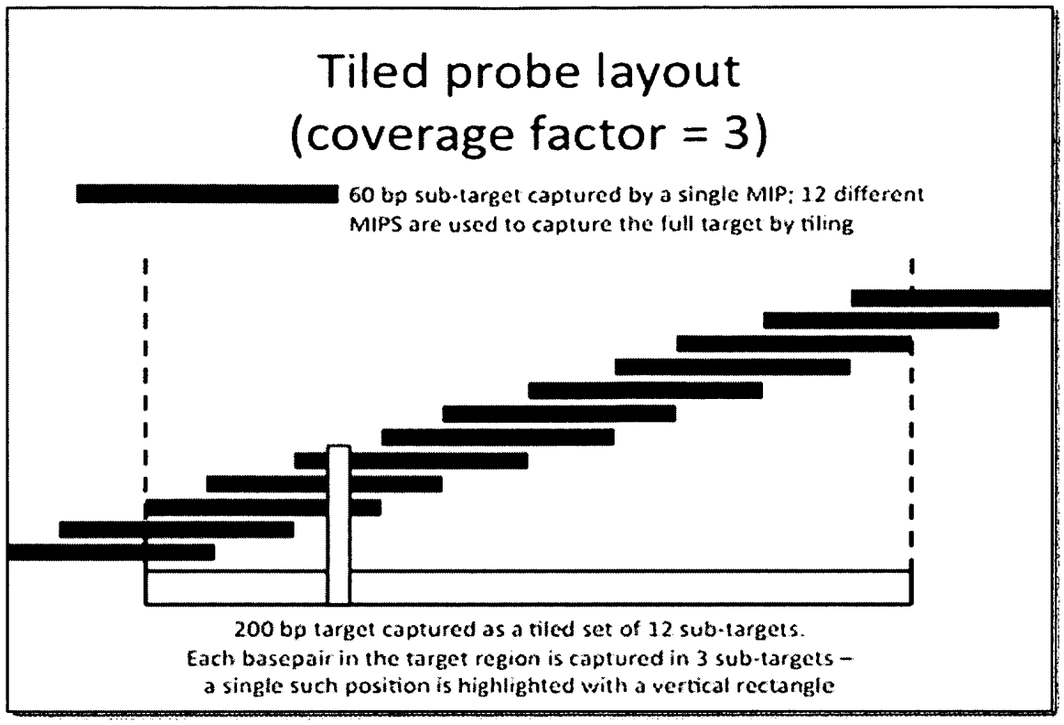

Tiled probe layout
(coverage factor = 3)
60 bp sub-target captured by a single MIP; 12 different MIPS are used to capture the full target by tiling
200 bp target captured as a tiled set of 12 sub-targets.
Each basepair in the target region is captured in 3 sub-targets –
a single such position is highlighted with a vertical rectangle

**Figure 2**

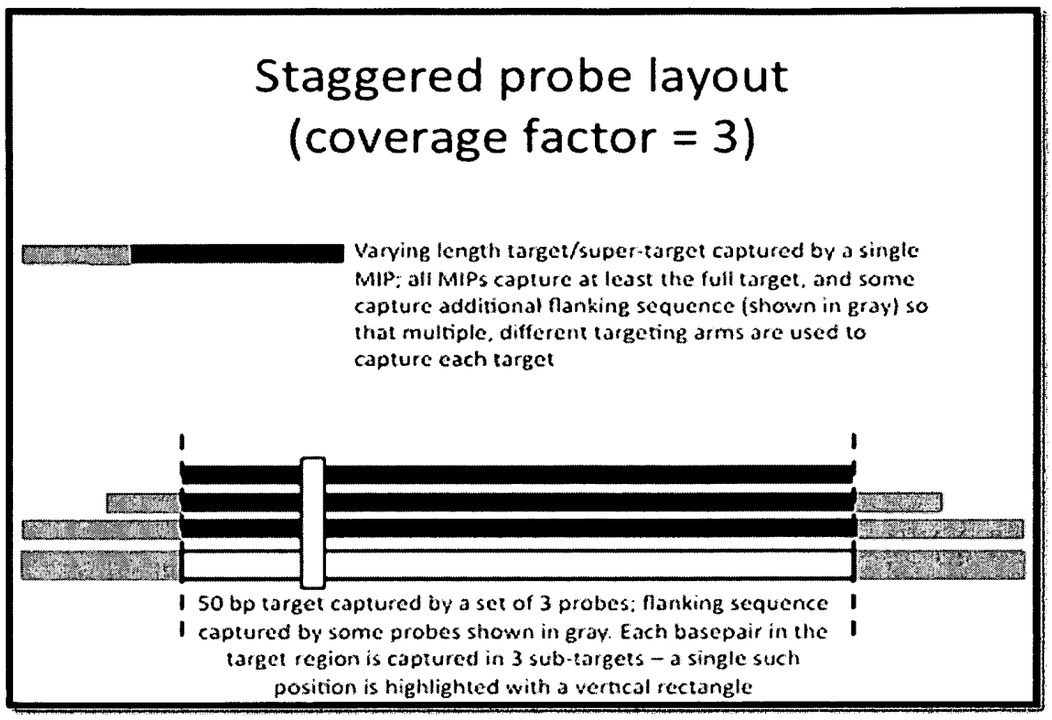

Staggered probe layout
(coverage factor = 3)
Varying length target/super-target captured by a single MIP; all MIPs capture at least the full target, and some capture additional flanking sequence (shown in gray) so that multiple, different targeting arms are used to capture each target
50 bp target captured by a set of 3 probes; flanking sequence captured by some probes shown in gray. Each basepair in the target region is captured in 3 sub-targets – a single such position is highlighted with a vertical rectangle

**Figure 3**

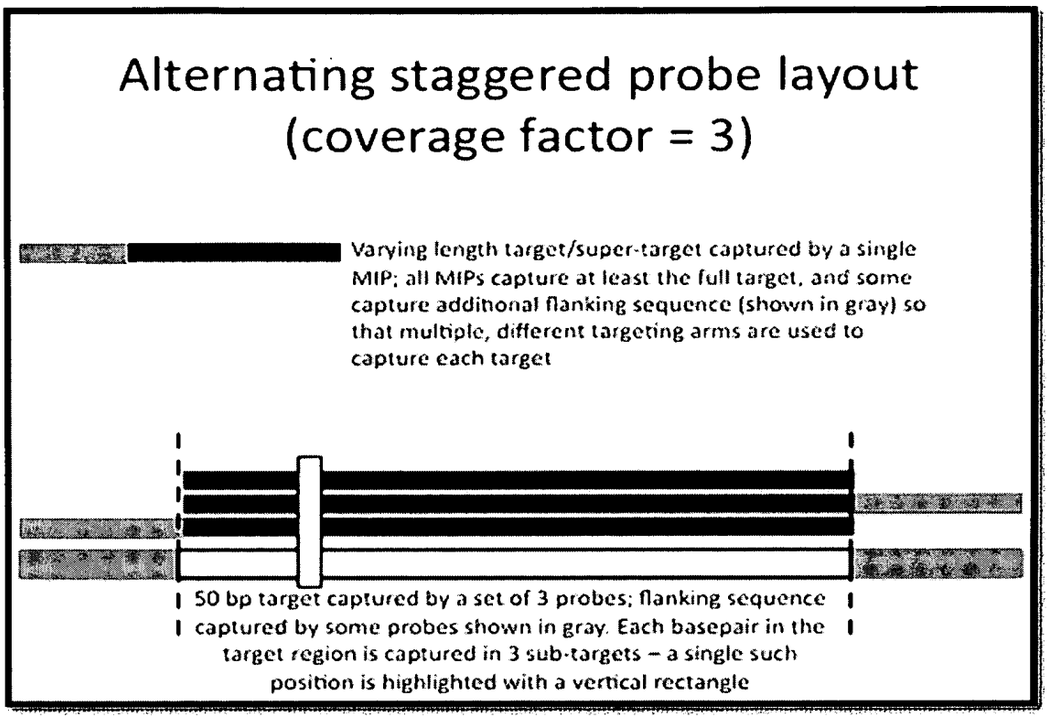

Alternating staggered probe layout
(coverage factor = 3)
Varying length target/super-target captured by a single MIP; all MIPs capture at least the full target, and some capture additional flanking sequence (shown in gray) so that multiple, different targeting arms are used to capture each target
50 bp target captured by a set of 3 probes; flanking sequence captured by some probes shown in gray. Each basepair in the target region is captured in 3 sub-targets – a single such position is highlighted with a vertical rectangle

FIGURE 4

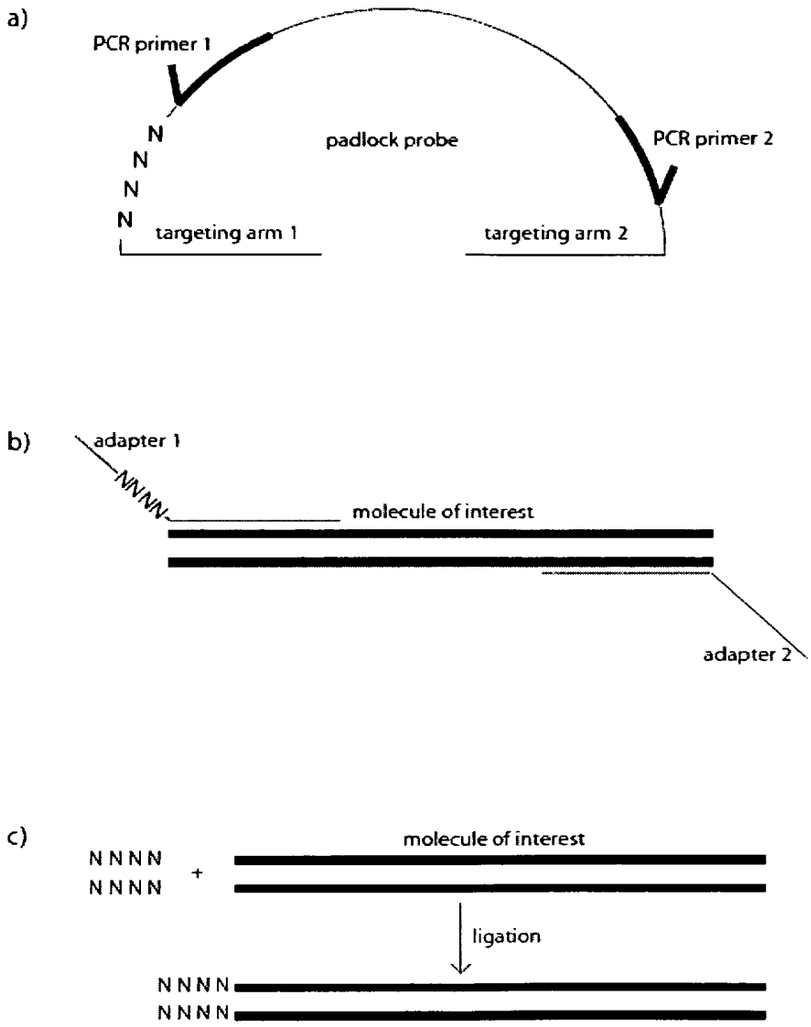

a)
PCR primer 1
N
N
N
N
padlock probe
PCR primer 2
targeting arm 1
targeting arm 2
b)
adapter 1
NNNN
molecule of interest
adapter 2
c)
molecule of interest
NNNN
NNNN
+
ligation
NNNN
NNNN

FIGURE 5

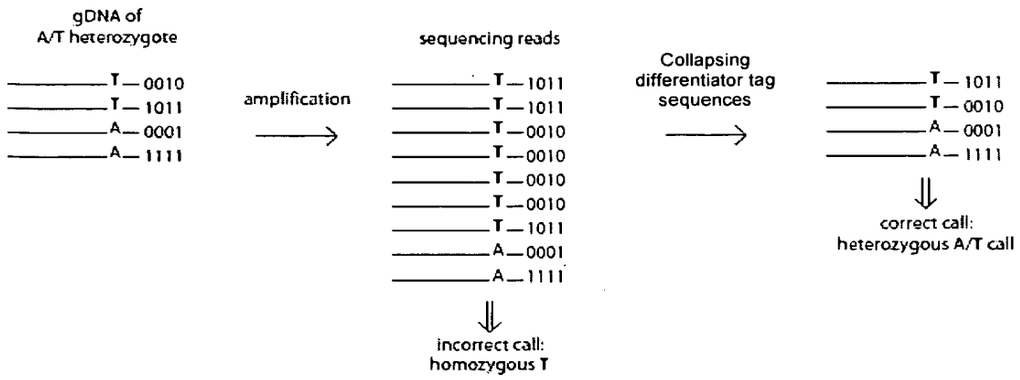
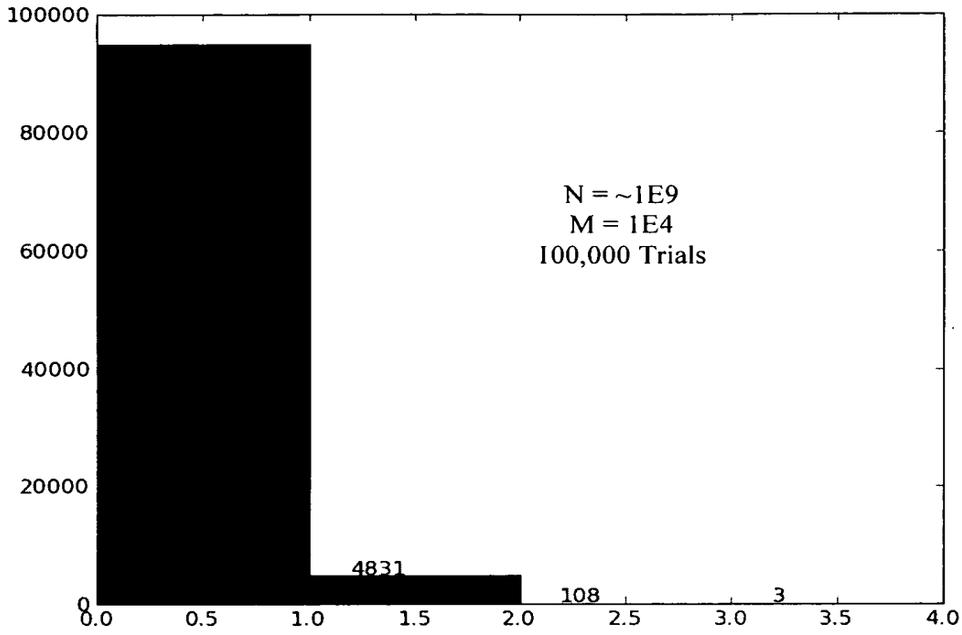

gDNA of
A/T heterozygote
T — 0010
T — 1011
A — 0001
A — 1111
amplification
sequencing reads
T — 1011
T — 1011
T — 0010
T — 0010
T — 0010
T — 0010
T — 1011
A — 0001
A — 1111
incorrect call:
homozygous T
Collapsing
differentiator tag
sequences
T — 1011
T — 0010
A — 0001
A — 1111
correct call:
heterozygous A/T call

FIGURE 6




100,000 Trials

FIGURE 7

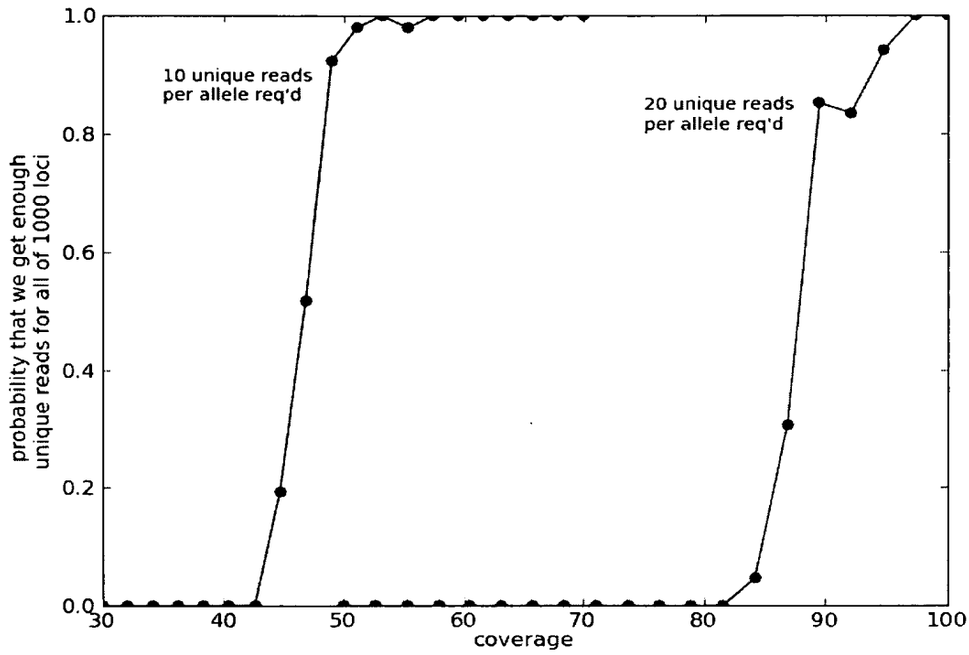

1.0
0.8
0.6
0.4
0.2
0.0
probability that we get enough unique reads for all of 1000 loci
10 unique reads per allele req'd
20 unique reads per allele req'd
30
40
50
60
70
80
90
100
coverage

Figure 8

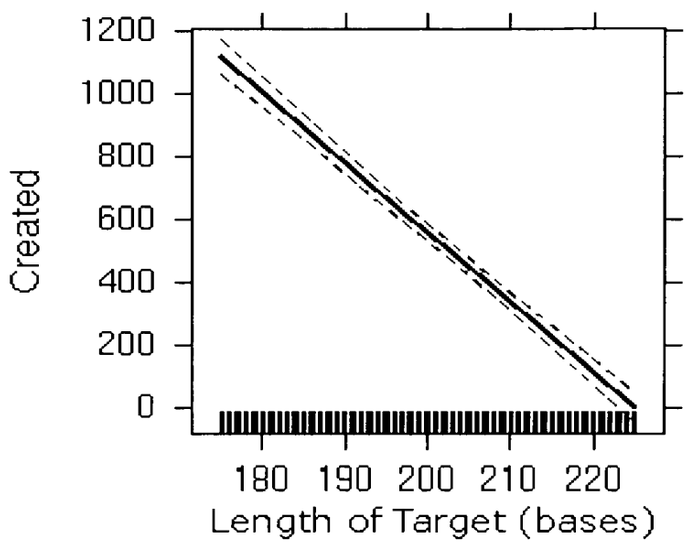

1200
1000
800
600
400
200
0
Created
180 190 200 210 220
Length of Target (bases)

Figure 9

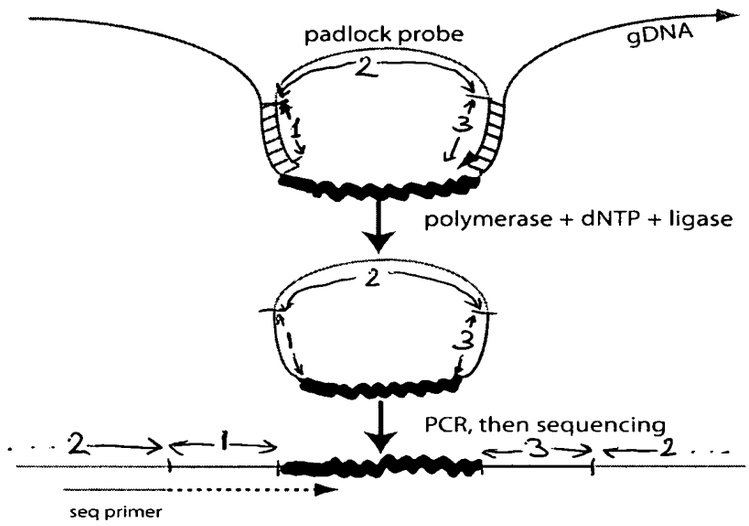

padlock probe
gDNA
2
1
3
polymerase + dNTP + ligase
2
1
3
PCR, then sequencing
2
1
3
2
seq primer

Figure 10

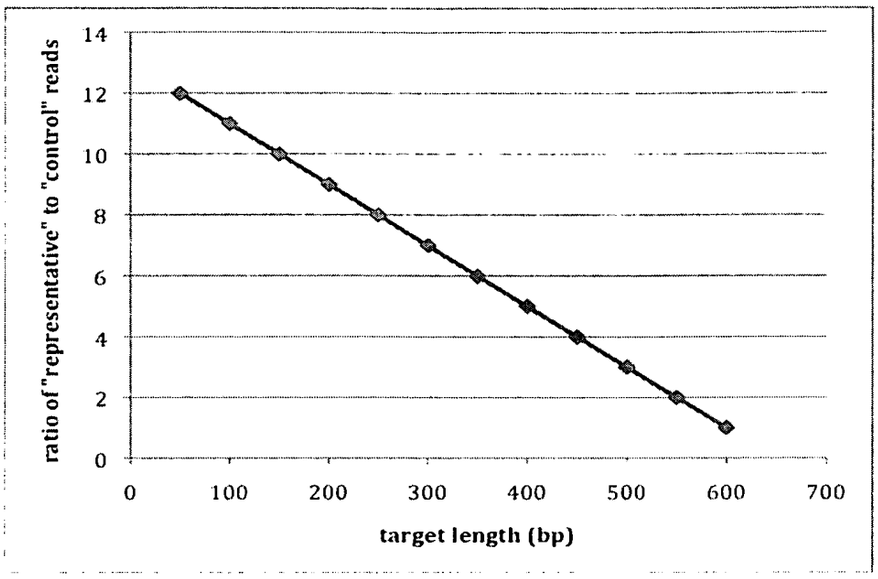

ratio of "representative" to "control" reads
14
12
10
8
6
4
2
0
0
100
200
300
400
500
600
700
target length (bp)

FIGURE 11A

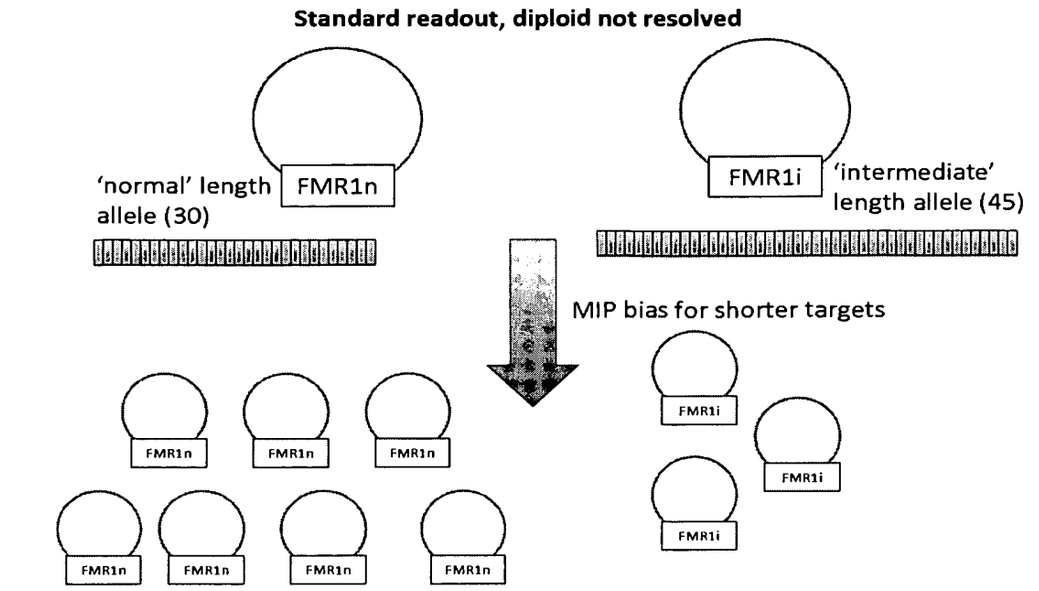

Standard readout, diploid not resolved
'normal' length allele (30)
FMR1n
FMR1i
'intermediate' length allele (45)
MIP bias for shorter targets
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1i
FMR1i
FMR1i
Readout: sum of alleles appears as 'long' end of normal length range, not intermediate het

FIGURE 11B

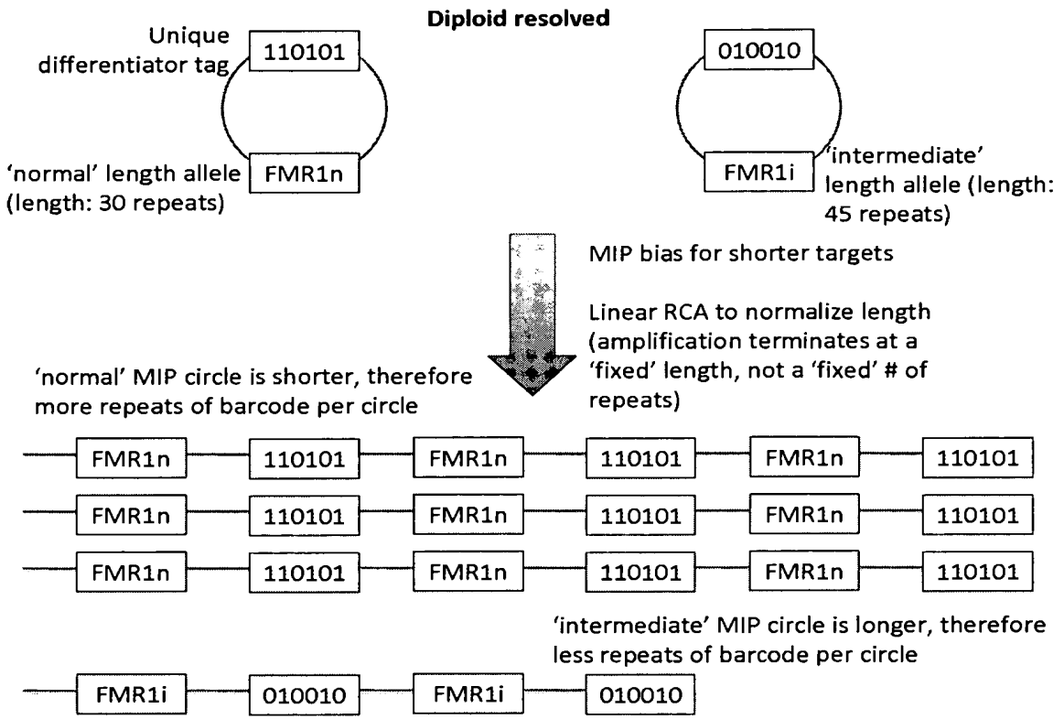

Diploid resolved
Unique differentiator tag
110101
'normal' length allele (length: 30 repeats)
FMR1n
010010
FMR1i
'intermediate' length allele (length: 45 repeats)
MIP bias for shorter targets
Linear RCA to normalize length (amplification terminates at a 'fixed' length, not a 'fixed' # of repeats)
'normal' MIP circle is shorter, therefore more repeats of barcode per circle
FMR1n 110101 FMR1n 110101 FMR1n 110101
FMR1n 110101 FMR1n 110101 FMR1n 110101
FMR1n 110101 FMR1n 110101 FMR1n 110101
'intermediate' MIP circle is longer, therefore less repeats of barcode per circle
FMR1i 010010 FMR1i 010010

FIGURE 11C

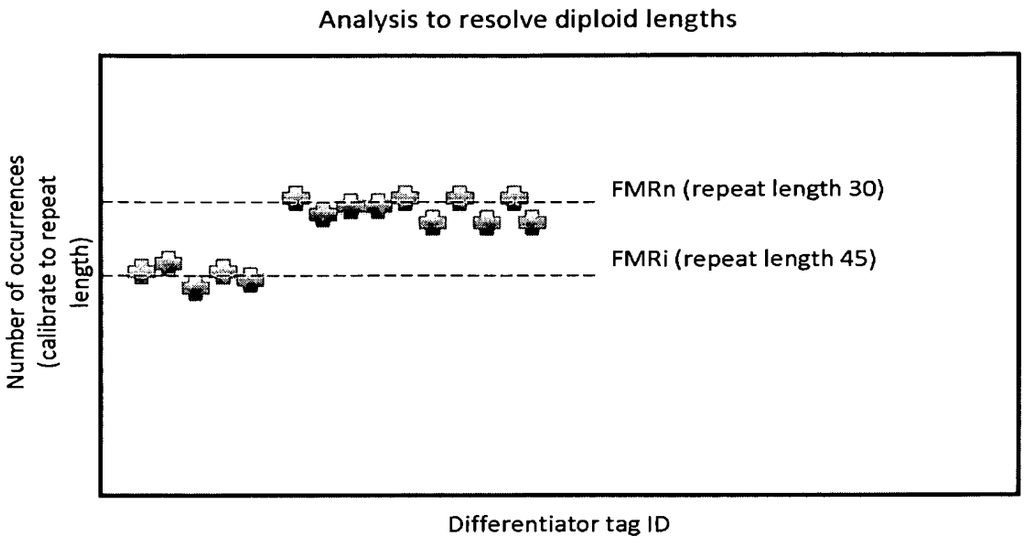

Analysis to resolve diploid lengths
Number of occurrences (calibrate to repeat length)
FMRn (repeat length 30)
FMRi (repeat length 45)
Differentiator tag ID

FIGURE 12

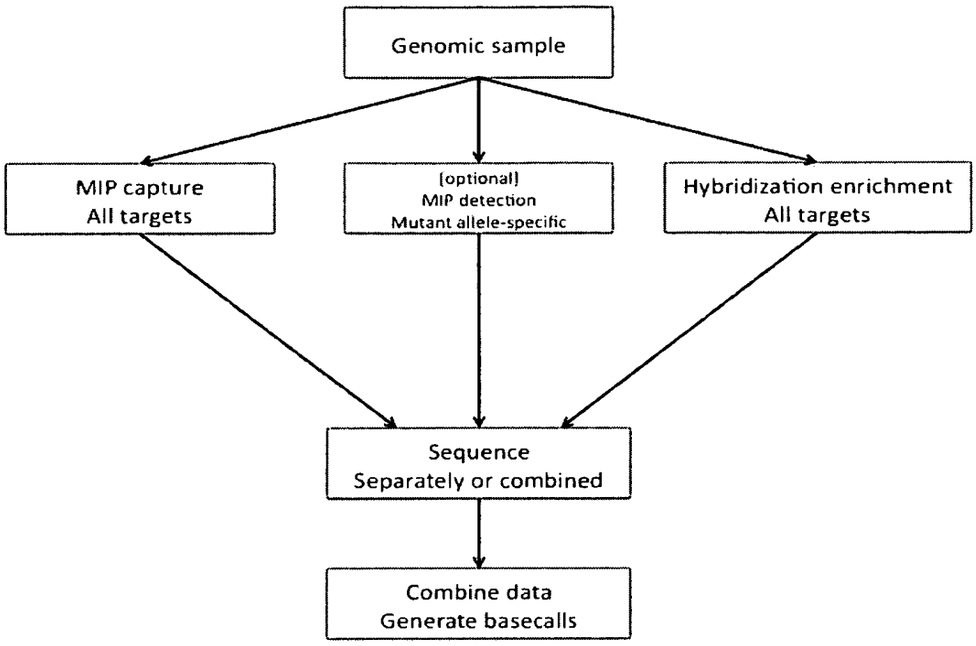

Genomic sample
MIP capture
All targets
(optional)
MIP detection
Mutant allele-specific
Hybridization enrichment
All targets
Sequence
Separately or combined
Combine data
Generate basecalls

FIGURE 13

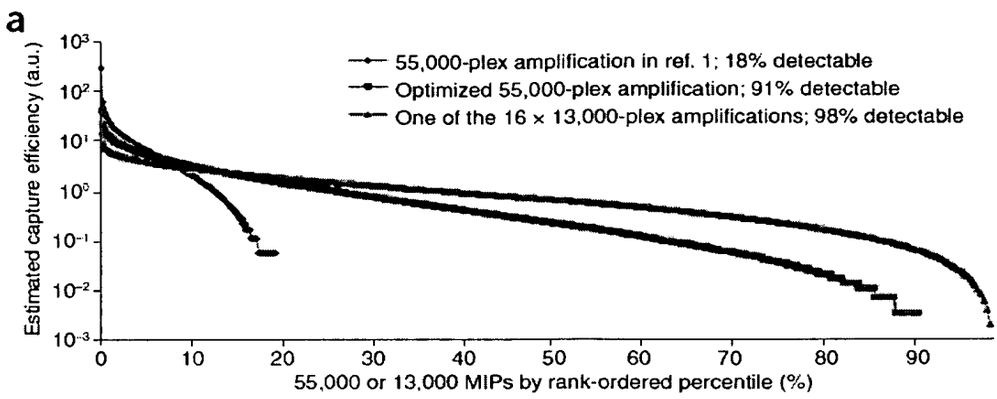
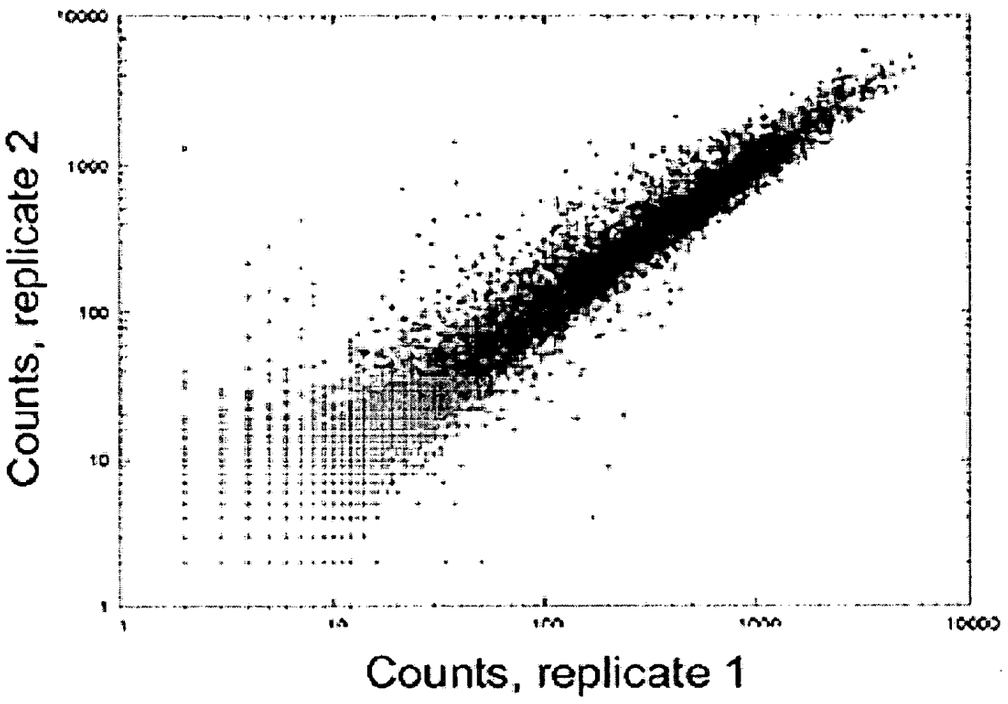

a
Estimated capture efficiency (a.u.)
$10^3$
$10^2$
$10^1$
$10^0$
$10^{-1}$
$10^{-2}$
$10^{-3}$
0
10
20
30
40
50
60
70
80
90
55,000 or 13,000 MIPs by rank-ordered percentile (%)
55,000-plex amplification in ref. 1; 18% detectable
Optimized 55,000-plex amplification; 91% detectable
One of the 16 × 13,000-plex amplifications; 98% detectable

FIGURE 14



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(71) Applicant *(for all designated States except US)*: **GOOD START GENETICS, INC.** [US/US]; 8 St. Mary's Street, Suite 621, Boston, MA 02215 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PORRECA, Gregory** [US/US]; 911 Garden Parkway, Ocean City, NJ 08226 (US). **LASERSON, Uri** [US/US]; 645 Beacon Street #215, Boston, MA 02215 (US). **LI, Jin, Billy** [CN/US]; 100 Station Landing, Unit 204, Medford, MA 02155 (US). **WASSMAN, E., Robert** [US/US]; 90 West Shore Drive, Marblehead, MA 01945 (US).

(74) Agent: **WALLER, Patrick, R., H.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

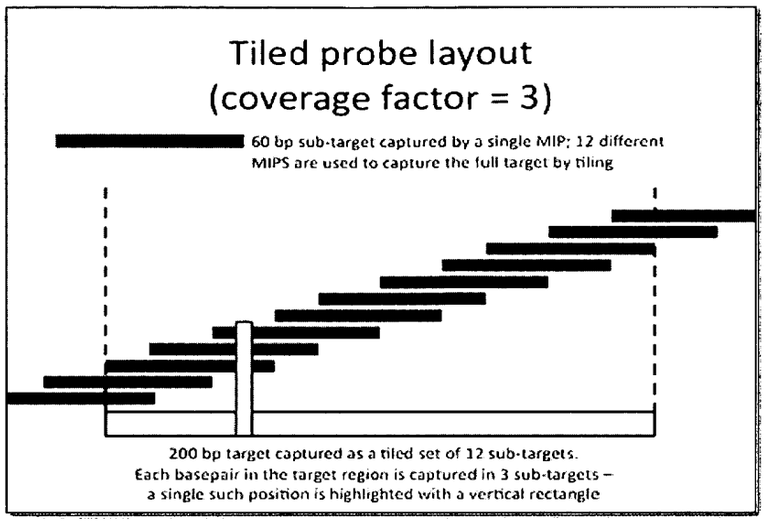
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*[Continued on next page]*

(54) Title: METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

**Figure 1**




(57) Abstract: Aspects of the invention relates to methods and compositions that are useful to reduce bias and increase the reproducibility of multiplex analysis of genetic loci. In some configurations, predetermined preparative steps and/or nucleic acid sequence analysis techniques are used in multiplex analyses for a plurality of genetic loci in a plurality of samples.

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— *with sequence listing part of description (Rule 5.2(a))*

# METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

## RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. §119(e) of U.S. provisional application serial number 61/174,470, filed April 30, 2009, U.S. provisional application serial number 61/178,923, filed May 15, 2009, U.S. provisional application serial number 61/179,358, filed May 18, 2009, and U.S. provisional application serial number 61/182,089, filed May 28, 2009, the entire contents of each of which are incorporated herein by reference.

## FIELD OF INVENTION

The invention relates to methods and compositions for determining genotypes in patient samples.

## BACKGROUND OF THE INVENTION

Information about the genotype of a subject is becoming more important and relevant for a range of healthcare decisions as the genetic basis for many diseases, disorders, and physiological characteristics is further elucidated. Medical advice is increasingly personalized, with individual decisions and recommendations being based on specific genetic information. Information about the type and number of alleles at one or more genetic loci impacts disease risk, prognosis, therapeutic options, and genetic counseling amongst other healthcare considerations.

For cost-effective and reliable medical and reproductive counseling on a large scale, it is important to be able correctly and unambiguously identify the allelic status for many different genetic loci in many subjects.

Numerous technologies have been developed for detecting and analyzing nucleic acid sequences from biological samples. These technologies can be used to genotype subjects and determine the allelic status of any locus of interest. However, they are not sufficiently robust and cost-effective to be scaled up for reliable high throughput analysis of many genetic loci in large numbers of patients. The frequency of incorrect or ambiguous calls is too high for current technology to manage large numbers of patient

samples without involving expensive and time-consuming steps to resolve uncertainties and provide confidence in the information output.

## SUMMARY OF THE INVENTION

Aspects of the invention relate to preparative and analytical methods and compositions for evaluating genotypes, and in particular, for determining the allelic identity (or identities in a diploid organism) of one or more genetic loci in a subject.

Aspects of the invention are based, in part, on the identification of different sources of ambiguity and error in genetic analyses, and, in part, on the identification of one or more approaches to avoid, reduce, recognize, and/or resolve these errors and ambiguities at different stages in a genetic analysis.

According to aspects of the invention, certain types of genetic information can be under-represented or over-represented in a genetic analysis due to a combination of stochastic variation and systematic bias in any of the preparative stages (e.g., capture, amplification, etc.), determining stages (e.g., allele-specific detection, sequencing, etc.), data interpretation stages (e.g., determining whether the assay information is sufficient to identify a subject as homozygous or heterozygous), and/or other stages.

According to aspects of the invention, error or ambiguity may be apparent in a genetic analysis, but not readily resolved without running additional samples or more expensive assays (*e.g.*, array-based assays may report no-calls due to noisy/low signal). According to further aspects of the invention, error or ambiguity may not be accounted for in a genetic analysis and incorrect base calls may be made even when the evidence for them is limited and/or not statistically significant (*e.g.*, next-generation sequencing technologies may report base calls even if the evidence for them is not statistically significant). According to further aspects of the invention error or ambiguity may be problematic for a multi-step genetic analysis because it is apparent but not readily resolved in one or more steps of the analysis and not apparent or accounted for in other steps of the analysis.

In some embodiments, sources of error and ambiguity in one or more steps can be addressed by capturing and/or interrogating each target locus of interest with one or more sets of overlapping probes that are designed to overcome any systematic bias or stochastic effects that may impact the complexity and/or fidelity of the genetic information that is generated.



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In some embodiments, sources of error and ambiguity in one or more steps can be addressed by capturing and/or interrogating each target locus of interest with at least one set of probes, wherein different probes are labeled with different identifiers that can be used to track the assay reactions and determine whether certain types of genetic information are under-represented or over-represented in the information that is generated.

In some embodiments, errors and ambiguities associated with the analysis of regions containing large numbers of sequence repeats are addressed by systematically analyzing frequencies of certain nucleic acids at particular stages in an assay (e.g., at a capture, sequencing, or detection stage). It should be appreciated that such techniques may be particularly useful in the context of a standardized protocol that is designed to allow many different loci to be evaluated in parallel without requiring different assay procedures for each locus. In some embodiments, the use of a single detection modality (*e.g.*, sequencing) to assay multiple types of genetic lesions (*e.g.*, point mutations, insertions/deletions, length polymorphisms) is advantageous in the clinical setting. In some embodiments of the invention, methods are provided that facilitate the use of multiple sample preparation steps in parallel, coupled with multiple analytical processes following sequence detection. Thus, in some embodiments of the invention, an improved workflow is provided that reduces error and uncertainty when simultaneously assaying different types of genetic lesions across multiple loci in multiple patients.

In some embodiments, aspects of the invention provide methods for overcoming preparative and/or analytical bias by combining two or more techniques, each having a different bias (e.g., a known bias towards under-representation or over-representation of one or more types of sequences), and using the resulting data to determine a genetic call for a subject with greater confidence.

It should be appreciated that in some embodiments, aspects of the invention relate to multiplex diagnostic methods. In some embodiments, multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel (e.g., one or more genetic loci from Table 1). In some embodiments, the genetic loci possess one or more polymorphisms (e.g., one or more polymorphisms from Table 2) the genotypes of which correspond to disease causing alleles. Accordingly, in some embodiments, the disclosure provides methods for assessing multiple heritable disorders in parallel. In some



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embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample) as aspects of the invention are not limited in this respect.

Other samples may include tumor tissue or circulating tumor cells. In some embodiments, a patient sample (*e.g.*, a tumor tissue or cell sample) is mosaic for one or more mutations of interest, and thus, may require higher sensitivity than is needed for a germline mutation analysis. In some embodiments, a sample comprises cells from a non-host organism (*e.g.*, bacterial or viral infections in a human subject) or a sample for environmental monitoring (*e.g.*, bacterial, viral, fungal composition of a soil, water, or air sample).

Accordingly, in some embodiments, aspects of the methods disclosed herein relate to genotyping a polymorphism of a target nucleic acid. In some embodiments, the genotyping may comprise determining that one or more alleles of the target nucleic acid are heterozygous or homozygous. In further embodiments, the genotyping may comprise determining the sequence of a polymorphism and comparing that sequence to a control sequence that is indicative of a disease risk. In some embodiments, the polymorphism is selected from a locus in Table 1 or Table 2. However, it should be appreciated that any locus associated with a disease or condition of interest may be used.

In some embodiments, a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject). In some embodiments, an assessment is provided to a couple, based on their respective genotypes at one or more genetic loci, of the risk of their having one or more children having a genotype associated with a disease or condition (e.g., a homozygous or heterozygous genotype associated with a disease or condition). In some embodiments, a subject or a couple may seek genetic or reproductive counseling in connection with a genotype determined according to embodiments of the invention. In some embodiments, genetic information from a tumor or circulating tumor cells is used to determine prognosis and guide selection of appropriate drugs/treatments.



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It should be appreciated that any of the methods or compositions described herein may be used in combination with any of the medical evaluations associated with one or more genetic loci as described herein.

In some embodiments, aspects of the invention provide effective methods for overcoming challenges associated with systematic errors (bias) and/or stochastic effects in multiplex genomic capture and/or analysis (including sequencing analysis). In some embodiments, aspects of the invention are useful to avoid, reduce and/or account for variability in one or more sampling and/or analytical steps. For example, in some embodiments, variability in target nucleic acid representation and unequal sampling of heterozygous alleles in pools of captured target nucleic acids can be overcome.

Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the detection of target nucleic acids in multiplex capture methods. In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of different sets of multiple probes (e.g., molecular inversion probes MIPs) that capture overlapping regions of a target nucleic acid to achieve a more uniform representation of the target nucleic acids in a capture pool compared with methods of the prior art. In other embodiments, methods reduce bias, or the risk of bias, associated with large scale parallel capture of genetic loci, e.g., for diagnostic purposes. In other embodiments, methods are provided for increasing reproducibility (e.g., by reducing the effect of polymorphisms on target nucleic acid capture) in the detection of a plurality of genetic loci in parallel. In further embodiments, methods are provided for reducing the effect of probe synthesis and/or probe amplification variability on the analysis of a plurality of genetic loci in parallel.

According to some aspects, methods of analyzing a plurality of genetic loci are provided. In some embodiments, the methods comprise contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion, isolating a plurality of nucleic acids each having a nucleic acid sequence of a different



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subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids.

In other embodiments, methods comprise contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the sequence of the 3' region of a different probe, isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids.

Aspects of the disclosure are based, in part, on the discovery of methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, namely high variability in the capture and amplification of nucleic acids and disproportionate representation of heterozygous alleles in sequencing libraries. Accordingly, in some embodiments, the disclosure provides methods that reduce errors associated with the variability in the capture and amplification of nucleic acids. In other embodiments, the methods improve allelic representation in sequencing libraries and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of differentiator tag sequences to uniquely tag individual nucleic acid molecules. In some embodiments, the differentiator tag sequence permit the detection of bias based on the occurrence of combinations of differentiator tag and target sequences observed in a sequencing reaction. In other embodiments, the methods reduce errors caused by bias, or the risk of bias, associated with the capture, amplification and sequencing of genetic loci, e.g., for diagnostic purposes.

Aspects of the invention relate to providing sequence tags (referred to as differentiator tags) that are useful to determine whether target nucleic acid sequences identified in an assay are from independently isolated target nucleic acids or from multiple copies of the same target nucleic acid molecule (e.g., due to bias in a preparative step, for example, amplification). This information can be used to help analyze a



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threshold number of independently isolated target nucleic acids from a biological sample in order to obtain sequence information that is reliable and can be used to make a genotype conclusion (e.g., call) with a desired degree of confidence. This information also can be used to detect bias in one or more nucleic acid preparative steps.

In some embodiments, the methods disclosed herein are useful for any application where reduction of bias, e.g., associated with genomic isolation, amplification, sequencing, is important. For example, detection of cancer mutations in a heterogeneous tissue sample, detection of mutations in maternally-circulating fetal DNA, and detection of mutations in cells isolated during a preimplantation genetic diagnostic procedure.

Accordingly, in some aspects, methods of genotyping a subject are provided. In some embodiments, the methods comprise determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject.

In some embodiments, the isolated nucleic acids are products of a circularization selection-based preparative method, e.g., molecular inversion probe capture products. In other embodiments, the isolated nucleic acids are products of an amplification-based preparative methods. In other embodiments, the isolated nucleic acids are products of hybridization-based preparative methods.

Circularization selection-based preparative methods selectively convert regions of interest (target nucleic acids) into a covalently-closed circular molecule which is then isolated typically by removal (usually enzymatic, e.g. with exonuclease) of any non-circularized linear nucleic acid. Oligonucleotide probes (e.g., molecular inversion probes) are designed which have ends that flank the region of interest (target nucleic acid) and, optionally, primer sites, e.g., sequencing primer sites. The probes are allowed to hybridize to the genomic target, and enzymes are used to first (optionally) fill in any gap between probe ends and second ligate the probe closed. Following circularization, any remaining (non-target) linear nucleic acid is typically removed, resulting in isolation



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(capture) of target nucleic acid. Circularization selection-based preparative methods include molecular inversion probe capture reactions and 'selector' capture reactions. In some embodiments, molecular inversion probe capture of a target nucleic acid is indicative of the presence of a polymorphism in the target nucleic acid.

In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using one or more oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or more of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and differentiator tag sequences. Amplification-based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel).

Hybridization-based preparative methods involve selectively immobilizing target nucleic acids for further manipulation. It is to be understood that one or more oligonucleotides (immobilization oligonucleotides), which comprise differentiator tag sequences, and which may be from 15 to 170 nucleotides in length, are used which hybridize along the length of a target region of a genetic locus to immobilize it. In some embodiments, immobilization oligonucleotides, are either immobilized before hybridization is performed (e.g., Roche/Nimblegen 'sequence capture'), or are prepared such that they include a moiety (e.g. biotin) which can be used to selectively immobilize the target nucleic acid after hybridization by binding to e.g., streptavidin-coated microbeads (e.g. Agilent 'SureSelect').

It should be appreciated that any of the circularization, amplification, and/or hybridization based methods described herein may be used in connection with one or more of the tiling/staggering, tagging, size-detection, and/or sensitivity enhancing algorithms described herein.

In some embodiments, the methods disclosed herein comprise determining the sequence of molecular inversion probe capture products, each comprising a molecular



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inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, and wherein the target nucleic acid is a captured genomic locus of a subject, and genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products.

In some embodiments, the methods disclosed herein comprise obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products. In certain embodiments, obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence. In specific embodiments, capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

In one embodiment, the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype. In some embodiments, the methods further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

According to some aspects, methods of analyzing a plurality of genetic loci are provided. In some embodiments, the methods comprise obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (e.g., a sequence that is complementary to the sequence of a nucleic acid that is used as a primer for sequencing or other extension reaction), amplifying the plurality of molecular



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inversion probe capture products, determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. In some embodiments, the methods further comprise genotyping target sequences in the plurality, wherein the genotyping comprises correcting for bias, if detected.

In some embodiments, the target nucleic acid is a gene (or portion thereof) selected from Table 1. In some embodiments, the genotyping comprises determining the sequence of a target nucleic acid (e.g., a polymorphic sequence) at one or more (both) alleles of a genome (a diploid genome) of a subject. In certain embodiments, the genotyping comprises determining the sequence of a target nucleic acid at both alleles of a diploid genome of a subject, wherein in the target nucleic acid comprises, or consists of, a sequence of Table 1, Table 2, or other locus of interest.

In some embodiments, aspects of the invention provide methods and compositions for identifying nucleic acid insertions or deletions in genomic regions of interest without determining the nucleotide sequences of these regions. Aspects of the invention are particularly useful for detecting nucleic acid insertions or deletions in genomic regions containing nucleic acid sequence repeats (e.g., di- or tri-nucleotide repeats). However, the invention is not limited to analyzing nucleic acid repeats and may be used to detect insertions or deletions in any target nucleic acid of interest. Aspects of the invention are particularly useful for analyzing multiple loci in a multiplex assay.

In some embodiments, aspects of the invention relate to determining whether an amount of target nucleic acid that is captured in a genomic capture assay is higher or lower than expected. In some embodiments, a statistically significant deviation from an expected amount (e.g., higher or lower) is indicative of the presence of a nucleic acid insertion or deletion in the genomic region of interest. In some embodiments, the amount is a number of nucleic acid molecules that are captured. In some embodiments, the amount is a number of independently captured nucleic acid molecules in a sample. It should be appreciated that the captured nucleic acids may be literally captured from a sample, or their sequences may be captured without actually capturing the original nucleic acids in the sample. For example, nucleic acid sequences may be captured in an



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assay that involves a template-based extension of nucleic acids having the region of interest, in the sample.

Aspects of the invention are based on the recognition that the efficiency of certain capture techniques is affected by the length of the nucleic acid being captured. Accordingly, an increase or decrease in the length of a target nucleic acid (e.g., due to an insertion or deletion of a repeated sequence) can alter the capture efficiency of that nucleic acid. In some embodiments, a difference in the capture efficiency (e.g., a statistically significant difference in the capture efficiency) of a target nucleic acid is indicative of an insertion or deletion in the target nucleic acid. It should be appreciated that the capture efficiency for a target nucleic acid may be evaluated based on an amount of captured nucleic acid (e.g., number of captured nucleic acid molecules) relative to a control amount (e.g., based on an amount of control nucleic acid that is captured). However, the invention is not limited in this respect and other techniques for evaluating capture efficiency also may be used.

According to aspects of the invention, evaluating the capture efficiency as opposed to determining the sequence of the entire repeat region reduces errors associated with sequencing through repeat regions. Repeat sequences often give rise to stutters or skips in sequencing reactions that make it very difficult to accurately determine the number of repeats in a target region without running multiple sequencing reactions under different conditions and carefully analyzing the results. Such procedures are cumbersome and not readily scalable in a manner that is consistent with high throughput analyses of target nucleic acids. In some embodiments, repeat regions may be longer than the length of the individual sequence read, making length determination on the basis of a single read impossible. For example, when using next-generation sequencing the repeat regions may be longer than the length of the individual sequence read, making length determination on the basis of a single read impossible. Accordingly, aspects of the invention are useful to increase the sensitivity of detecting insertions or deletions in target regions, particularly target regions containing repeated sequences.

In some embodiments, aspects of the invention relate to capturing genomic nucleic acid sequences using a molecular inversion probe (e.g., MIP or Padlock probe) technique, and determining whether the amount (e.g., number) of captured sequences is higher or lower than expected. In some embodiments, the amount (e.g., number) of captured sequences is compared to an amount (e.g., number) of sequences captured in a



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control assay. The control assay may involve analyzing a control sample that contains a nucleic acid from the same genetic locus having a known sequence length (e.g., a known number of nucleic acid repeats). However, a control may involve analyzing a second (e.g., different) genetic locus that is not expected to contain any insertions or deletions. The second genetic locus may be analyzed in the same sample as the locus being interrogated or in a different sample where its length has been previously determined. The second genetic locus may be a locus that is not characterized by the presence of nucleic acid repeats (and thus not expected to contain insertions or deletions of the repeat sequence).

In some embodiments, a target nucleic acid region that is being evaluated may be determined by the identity of the targeting arms of a probe that is designed to capture the target region (or sequence thereof). For example, the targeting arms of a MIP probe may be designed to be complementary (e.g., sufficiently complementary for selective hybridization and//or polymerase extension and/or ligation) to genomic regions flanking a target region suspected of containing an insertion or deletion. It should be appreciated that two targeting arms may be designed to be complementary (e.g., sufficiently complementary for selective hybridization and/or polymerase extension and/or ligation) to the two flanking regions that are immediately adjacent (e.g., immediately 5' and 3', respectively) to a region of a sequence repeat on one strand of a genomic nucleic acid. However, one or both targeting arms may be designed to hybridize several bases (e.g., 1-5, 5-10, 10-25, 25-50, or more) upstream or downstream from the repeat region in such a way that the captured sequence includes a region of unique genomic sequence that on one or both sides of the repeat region. This unique region can then be used to identify the captured target (e.g., based on sequence or hybridization information).

In some embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) different loci may be interrogated in parallel in a single assay (e.g., in a multiplex assay). In some embodiments, the ratio of captured nucleic acids for each locus may be used to determine whether a nucleic acid insertion or deletion is present in one locus relative to the other. For example, the ratio may be compared to a control ratio that is representative of the two loci when neither one has an insertion or deletion relative to control sequences (e.g., sequences that are normal or known to be associated with healthy phenotypes for those loci). However, the amount of captured nucleic acids may be compared to any suitable control as discussed herein.



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The locus of a captured sequence may be identified by determining a portion of unique sequence 5' and/or 3' to the repeat region in the target nucleic acid suspected of containing a deletion or insertion. This does not require sequencing the captured repeat region itself. However, some or all of the repeat region also could be sequenced as aspects of the invention are not limited in this respect.

Aspects of the invention may be combined with one or more sequence-based assays (e.g., SNP detection assays), for example in a multiplex format, to determine the genotype of one or more regions of a subject.

In some embodiments, methods of detecting a polymorphism in a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise evaluating the efficiency of capture at one or more loci and determining whether one or both alleles at that locus contain an insertion or deletion relative to a control locus (e.g., a locus indicative of a length of repeat sequence that is associated with a healthy phenotype).

Accordingly, aspects of the invention relate to methods for determining whether a target nucleic acid has an abnormal length by evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length. It should be appreciated that the term "abnormal" is a relative term based on a comparison to a "normal" length. In some embodiments, a normal length is a length that is associated with a normal (e.g., healthy or non-carrier phenotype). Accordingly, an abnormal length is a length that is either shorter or longer than the normal length. In some embodiments, the presence of an abnormal length is indicative of an increased risk that the locus is associated with a disease or a disease carrier phenotype. In some embodiments, the abnormal length is indicative that the subject is either has a disease or condition or is a carrier of a disease or condition (e.g., associated with the locus). However, it should be appreciated that the description of embodiments relating to detecting the presence of an abnormal length also support detecting the presence of a length that is different from an expected or control length.

In some embodiments, aspects of the invention relate to estimating the length of a target nucleic acid (e.g., of a sub-target region within a target nucleic acid). In some embodiments, aspects of the invention relate to methods for estimating the length of a



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target nucleic acid by contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid, wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid, wherein the first and second regions are on a common strand of the target nucleic acid, and wherein the nucleotide sequence of the target between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid; and capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes; and measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the sub-target nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. It should be appreciated that methods for estimating a nucleic acid length may involve comparing a capture efficiency for a target nucleic acid region to two or more reference efficiencies for known nucleic acid lengths in order to determine whether the target nucleic acid region is smaller, intermediate, or larger in size than the known control lengths. In some embodiments, a series of nucleic acids of known different lengths may be used to provide a calibration curve for evaluating the length of a target nucleic acid region of interest.

In some embodiments, the capture efficiency of a target region suspected of having a deletion or insertion is determined by comparing the capture efficiency to a reference indicative of a normal capture efficiency. In some embodiments, the capture efficiency is lower than the reference capture efficiency. In some embodiments, the subject is identified as having an insertion in the target region. In some embodiments, the capture efficiency is higher than the reference capture efficiency. In some embodiments, the subject is identified as having a deletion in the target region. In some embodiments, the subject is identified as being heterozygous for the insertion. In some embodiments, the subject is identified as being heterozygous for the deletion.

In some embodiments of any of the methods described herein (e.g., tiling/staggering, tagging, size-detection, and/or sensitivity enhancement) aspects of the invention relate to capturing a sub-target nucleic acid (or a sequence of a sub-target nucleic acid). In some embodiments, a molecular inversion probe technique is used. In some embodiments, a molecular inversion probe is a single linear strand of nucleic acid



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that comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid. In some embodiments, the first and second targeting arms are between about 10 and about 100 nucleotides long. In some embodiments, the first and second targeting arms are about 10-20, 20-30, 30-40, or 40-50 nucleotides long. In some embodiments, the first and second targeting arms are about 20 nucleotides long. In some embodiments, the first and second targeting arms have the same length. In some embodiments, the first and second targeting arms have different lengths. In some embodiments, each pair of first and second targeting arms in a set of probes has the same length. Accordingly, if one of the targeting arms is longer, the other one is correspondingly shorter. This allows for a quality control step in some embodiments to confirm that all captured probe/target sequence products have the same length after a multiplexed plurality of capture reactions. In some embodiments, a set of probes may be designed to have the same length if the intervening region is varied to accommodate any differences in the length of either one or both of the first and second targeting arms.

In some embodiments, the hybridization Tms of the first and second targeting arms are similar. In some embodiments, the hybridization Tms of the first and second targeting arms are within 2-5 °C of each other. In some embodiments, the hybridization Tms of the first and second targeting arms are identical. In some embodiments, the hybridization Tms of the first and second targeting arms are close to empirically-determined optima but not necessarily identical.

In some embodiments, the first and second targeting arms of a molecular inversion probe have different Tms. For example, the Tm of the first targeting arm (at the 5' end of the molecular inversion probe) may be higher than the Tm of the second targeting arm (at the 3' end of the molecular inversion probe). According to aspects of the invention, and without wishing to be bound by theory, a relatively high Tm for the first targeting arm may help avoid or prevent the first targeting arm from being displaced after hybridization by the extension product of the 3' end of the second targeting arm. It should be appreciated that a reference to the Tm of a targeting arm as used herein relates to the Tm of hybridization of the targeting arm to a nucleic acid having the



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complementary sequence (e.g., the region of the target nucleic acid that has a sequence that is complementary to the sequence of the targeting arm). It also should be appreciated that the Tms of the targeting arms described herein may be calculated using any appropriate method. For example, in some embodiments an experimental method (e.g., a gel shift assay, a hybridization assay, a melting curve analysis, for example in a PCR machine with a SYBR dye by stepping through a temperature ramp while monitoring signal level from an intercalating dye, for example, bound to a double-stranded DNA, etc.) may be used to determine one or more Tms empirically. In some embodiments, an optimal Tm may be determined by evaluating the number of products formed (e.g., for each of a plurality of MIP probes), and determining the optimal Tm as the center point in a histogram of Tm for all targeting arms. In some embodiments, a predictive algorithm may be used to determine a Tm theoretically. In some embodiments, a relatively simple predictive algorithm may be used based on the number of G/C and A/T base pairs when the sequence is hybridized to its target and/or the length of the hybridized product (e.g., for example, 64.9 + 41 * ([G+C] - 16.4) / (A + T + G + C), see for example, Wallace,R.B., Shaffer,J., Murphy,R.F., Bonner,J., Hirose,T., and Itakura,K. (1979) Nucleic Acids Res 6:3543-3557). In some embodiments, a more complex algorithm may be used to account for the effects of base stacking entropy and enthalpy, ion concentration, and primer concentration (see, for example, SantaLucia J (1998), Proc Natl Acad Sci USA, 95:1460-5). In some embodiments an algorithm may use modified parameters (e.g., nearest-neighbor parameters for basepair entropy/enthalpy values). It should be appreciated that any suitable algorithm may be used as aspects of the invention are not limited in this respect. However, it also should be appreciated that different methodologies may results in different calculated or predicted Tms for the same sequences. Accordingly, in some embodiments, the same empirical and/or theoretical method is used to determine the Tms of different sequences for a set of probes to avoid a negative impact of any systematic difference in the Tm determination or prediction when designing a set of probes with predetermined similarities or differences for different Tms.

In some embodiments, the Tm of the first targeting arm may be about 1 °C, about 2 °C, about 3 °C, about 4 °C, about 5 °C, or more than about 5 °C higher than the Tm of the second targeting arm. In some embodiments, each probe in a plurality of probes (e.g., each probe in a set of 5-10, each probe in a set of at least 10, each probe in a set of



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10-50, each probe in a set of 50-100, each probe in a set of 100-500, each probe in a set of 500-1,000, each probe in a set of 1,000-1,500, each probe in a set of 1,500-2,000, each probe in a set of 2,000-3,000, 3,000-5,000, 5,000-10,000 or each probe in a set of at least 5,000 different probes) has a unique first targeting arm (e.g., they all have different sequences) and a unique second targeting arm (e.g., they all have different sequences). In some embodiments, for at least 10% of the probes (e.g., at least 25%, 25%-50%, 50%-75%, 75%-90%, 90%-95% or over 95%, or all of the probes) the first targeting arm has a Tm for its complementary sequence that is higher (e.g., about 1 °C, about 2 °C, about 3 °C, about 4 °C, about 5 °C, or more than about 5 °C higher) than the Tm of the second targeting arm for its complementary sequence. In some embodiments, each of the first targeting arms have similar or identical Tms for their respective complementary sequences and each of the second targeting arms have similar or identical Tms for their respective complementary sequences (and the first targeting arms have higher Tms than the second targeting arms). For example, in some embodiments, the Tm of the first arm(s) may be about 58 °C and the Tm of the second arm(s) may be about 56 °C. In some embodiments, the Tm of the first arm(s) may be about 68 °C, and the Tm of the second arm(s) may be about 65 °C. It should be appreciated that in some embodiments the similarity (e.g., within a range of 1 °C, 2 °C, 3 °C, 4 °C, 5 °C) or identity of the Tms for the different targeting arms should be based either on empirical data for each arm or based on the same predictive algorithm for each arm (e.g., Wallace,R.B., Shaffer,J., Murphy,R.F., Bonner,J., Hirose,T., and Itakura,K. (1979) Nucleic Acids Res 6:3543-3557, SantaLucia J (1998), Proc Natl Acad Sci USA, 95:1460-5, or other algorithm).

In some embodiments, the Tm of the first targeting arm of a molecular inversion probe (at the 5' end of the molecular inversion probe) is selected to be sufficiently stable to prevent displacement of the first targeting arm from its complementary sequence on a target nucleic acid. In some embodiments, the Tm of the first targeting arm is 50-55 °C, at least 55 °C, 55-60 °C, at least 60 °C, 60-65 °C, at least 65 °C, at least 70 °C, at least 75 °C, or at least 80 °C. As discussed above, it should be appreciated that the for a particular targeting arm may be determined empirically or theoretically. Different theoretical models may be used to determine a Tm and it should be appreciated that the predicted Tm for a particular sequence may be different depending on the algorithm used for the prediction. In some embodiments, each probe in a plurality of probes (e.g., each probe in a set of 5-10, each probe in a set of at least 10, each probe in a set of 10-50, each



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probe in a set of 50-100, each probe in a set of 100-500, or each probe in a set of at least 500 different probes) has a different first targeting arm (e.g., different sequences) but each different first targeting arm has a similar or identical Tm for its complementary sequence on a target nucleic acid. It should be appreciated that in some embodiments the similarity (e.g., within a range of 1 C, 2 C, 3 C, 4 C, 5 C) or identity of the Tms for the different targeting arms should be based either on empirical data for each arm or based on the same predictive algorithm for each arm (e.g., Wallace,R.B., Shaffer,J., Murphy,R.F., Bonner,J., Hirose,T., and Itakura,K. (1979) Nucleic Acids Res 6:3543-3557, SantaLucia J (1998), Proc Natl Acad Sci USA, 95:1460-5, or other algorithm).

In some embodiments, the sub-target nucleic acid contains a nucleic acid repeat. In some embodiments, the nucleic acid repeat is a dinucleotide or trinucleotide repeat. In some embodiments, the sub-target nucleic acid contains 10-100 copies of the nucleic acid repeat in the absence of an abnormal increase or decrease in nucleic acid repeats. In some embodiments, the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat. In some embodiments, one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats. In some embodiments, one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats. In some embodiments, the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

In some embodiments, aspects of the invention relate to evaluating the length of a plurality of different target nucleic acids in a biological sample. In some embodiments, the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes. In some embodiments, each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends. In some embodiments, each different molecular inversion probe comprises the same primer-binding sequence.

In some embodiments, aspects of the invention relate to analyzing nucleic acid from a biological sample obtained from a subject. In some embodiments, the biological sample is a blood sample. In some embodiments, the biological sample is a tissue sample, specific cell population, tumor sample, circulating tumor cells, or environmental



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sample. In some embodiments, the biological sample is a single cell. In some embodiments, nucleic acids are analyzed in biological samples obtained from a plurality of different subjects. In some embodiments, nucleic acids from a biological sample are analyzed in multiplex reactions. It should be appreciated that a biological sample contains a plurality of copies of a genome derived from a plurality of cells in the sample. Accordingly, a sample may contain a plurality of independent copies of a target nucleic acid region of interest, the capture efficiency of which can be used to evaluate its size as described herein.

In some embodiments, aspects of the invention relate to evaluating a nucleic acid capture efficiency by determining an amount of target nucleic acid that is captured (e.g., an amount of sub-target nucleic acid sequences that are captured). In some embodiments, the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules (e.g., the amount of independently captured molecules that have the sequence of the sub-target region). In some embodiments, the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid. In some embodiments, the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid. In some embodiments, the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion. In some embodiments, the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction. As described herein, a number of independently captured nucleic acid sequences can be determined by contacting a nucleic acid sample with a preparation of a probe (e.g., a MIP probe as described herein). It should be appreciated that the preparation may comprise a plurality of copies of the same probe and accordingly a plurality of independent copies of the target region may be captured by different probe molecules. The number of probe molecules that actually capture a sequence can be evaluated by determining an amount or number of captured molecules using any suitable technique. This number is a reflection of both the number of target molecules in the sample and the efficiency of capture of those target molecules, which in turn is related to the size of the target molecules as described herein. Accordingly, the capture efficiency can be evaluated by controlling for the abundance of the target nucleic acid, for example by comparing the number or amount of captured target molecules to an appropriate control (e.g., a known size and



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amount of control nucleic acid, or a different locus that should be present in the same amount in the biological sample and is not expected to contain any insertions or deletions). It should be appreciated that other factors may affect the capture efficiency of a particular target nucleic acid region (e.g., the sequence of the region, the GC content, the presence of secondary structures, etc.). However, these factors also can be accounted for by using appropriate controls (e.g., known sequences having similar properties, the same sequences, other genomic sequences expected to be present in the biological sample at the same frequency, etc., or any combination thereof).

In some embodiments, aspects of the invention relate to identifying a subject as having an insertion or deletion in one or more alleles of a genetic locus if the capture efficiency for that genetic locus is statistically significantly different than a reference capture efficiency.

It should be appreciated that hybridization conditions used for any of the capture techniques described herein (e.g., MIP capture techniques) can be based on known hybridization buffers and conditions.

In some embodiments, the methods disclosed herein are useful for any application where the detection of deletions or insertions is important.

In some embodiments, aspects of the invention relate to basing a nucleic acid sequence analysis on results from two or more different nucleic acid preparatory techniques that have different systematic biases in the types of nucleic acids that they sample. According to the invention, different techniques have different sequence biases that are systematic and not simply due to stochastic effects during nucleic acid capture or amplification. Accordingly, the degree of oversampling required to overcome variations in nucleic acid preparation needs to be sufficient to overcome the biases (e.g., an oversampling of 2-5 fold, 5-10 fold, 5-15 fold, 15-20 fold, 20-30 fold, 30-50 fold, or intermediate to higher fold).

According to some embodiments, different techniques have different characteristic or systematic biases. For example, one technique may bias a sample analysis towards one particular allele at a genetic locus of interest, whereas a different technique would bias the sample analysis towards a different allele at the same locus. Accordingly, the same sample may be identified as being different depending on the type of technique that is used to prepare nucleic acid for sequence analysis. This effectively



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represents a sensitivity limitation, because each technique has different relative sensitivities for polymorphic sequences of interest.

According to aspects of the invention, the sensitivity of a nucleic acid analysis can be increased by combining the sequences from different nucleic acid preparative steps and using the combined sequence information for a diagnostic assay (e.g., for a making a call as to whether a subject is homozygous or heterozygous at a genetic locus of interest).

In some embodiments, the invention provides a method of increasing the sensitivity of a nucleic acid detection assay by obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample, obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample, assaying the sequences obtained in both first and second nucleic acid preparations, and using the sequence information from both first and second nucleic acid preparations to determine the genotype of the target nucleic acid in the biological sample, wherein the first and second preparative methods have different systematic sequence biases. In some embodiments, the first and second nucleic acid preparations are combined prior to performing a sequence assay. In some embodiments, separate sequence assays are performed on the first and second nucleic acid preparations and the sequence information from both assays are combined to determine the genotype of the target nucleic acid in the biological sample. In some embodiments, the first preparative method is an amplification-based, a hybridization-based, or a circular probe-based preparative method. In some embodiments, the second method is an amplification-based, a hybridization-based, or a circular probe-based preparative method. In some embodiments, the first and second methods are of different types (e.g., only one of them is an amplification-based, a hybridization-based, or a circular probe-based preparative method, and the other one is one or the other two types of method). Accordingly, in some embodiments the second preparative method is an amplification-based, a hybridization-based, or a circular probe-based preparative method, provided that the second method is different from the first method. However, in some embodiments, both methods may be of the same type, provided they are different methods (e.g., both are amplification based or hybridization-based, but are different types of amplification or hybridization methods, e.g., with different relative biases).



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In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using a pair of oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or both of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and identifier sequences (e.g., source sequences). PCR based methods may include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel).

Hybridization-based preparative may methods involve selectively immobilizing target nucleic acids for further manipulation. It is to be understood that one or more oligonucleotides (immobilization oligonucleotides), which in some embodiments may be from 10 to 200 nucleotides in length, are used which hybridize along the length of a target region of a genetic locus to immobilize it. In some embodiments, immobilization oligonucleotides are either immobilized before hybridization is performed (e.g., Roche/Nimblegen 'sequence capture'), or are prepared such that they include a moiety (e.g., biotin) which can be used to selectively immobilize the target nucleic acid after hybridization by binding to e.g., streptavidin-coated microbeads (e.g., Agilent 'SureSelect').

Circularization selection-based preparative methods selectively convert each region of interest into a covalently-closed circular molecule which is then isolated by removal (usually enzymatic, e.g., with exonuclease) of any non-circularized linear nucleic acid. Oligonucleotide probes are designed which have ends that flank the region of interest. The probes are allowed to hybridize to the genomic target, and enzymes are used to first (optionally) fill in any gap between probe ends and second ligate the probe closed. In some embodiments, following circularization, any remaining (non-target) linear nucleic acid can be removed, resulting in isolation (capture) of target nucleic acid. Circularization selection-based preparative methods include molecular inversion probe capture reactions and 'selector' capture reactions. However, other techniques may be



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used as aspects of the invention are not limited in this respect. In some embodiments, molecular inversion probe capture of a target nucleic acid is indicative of the presence of a polymorphism in the target nucleic acid.

A variety of methods may be used to evaluate and compare bias profiles of each preparative technique. Next-generation sequencing may be used to quantitatively measure the abundance of each isolated target nucleic acid obtained from a certain preparative method. This abundance may be compared to a control abundance value (e.g., a known starting abundance of the target nucleic acid) and/or with an abundance determined through the use of an alternative preparative method. For example, a set of target nucleic acids may be isolated by one or more of the three preparative methods; the target nucleic acid may be observed x times using the amplification technique, y times using the hybridization enrichment technique, and z times using the circularization selection technique. A pairwise correlation coefficient may be computed between each abundance value (e.g., x and y, x and z, and y and z) to assess bias in nucleic acid isolation between pairs of preparative methods. Since the mechanisms of isolation are different in each approach, the abundances will usually be different and largely uncorrelated with each other.

In some embodiments, the invention provides a method of obtaining a nucleic acid preparation that is representative of a target nucleic acid in a biological sample by obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample, obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample, and combining the first and second nucleic acid preparations to obtain a combined preparation that is representative of the target nucleic acid in the biological sample.

In some embodiments of any of the methods described herein, a third preparation of the target nucleic acid is obtained using a third preparative method that is different from the first and second preparative methods, wherein the first, second, and third preparative methods all have different systematic sequence biases. In some embodiments of any of the methods described herein, the different preparative methods are used for a plurality of different loci in the biological sample to increase the sensitivity of a multiplex nucleic acid analysis. In some embodiments, the target nucleic acid has a sequence of a gene selected from Table 1.



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However, it should be appreciated that a genotyping method of the invention may include several steps, each of which independently may involve one or more different preparative techniques described herein. In some embodiments, a nucleic acid preparation may be obtained using one or more (e.g., 2, 3, 4, 5, or more) different techniques described herein (e.g., amplification, hybridization capture, circular probe capture, etc., or any combination thereof) and the nucleic acid preparation may be analyzed using one or more different techniques (e.g., amplification, hybridization capture, circular probe capture, etc., or any combination thereof) that are selected independently of the techniques used for the initial preparation.

In some embodiments, aspects of the invention also provide compositions, kits, devices, and analytical methods for increasing the sensitivity of nucleic acid assays. Aspects of the invention are particularly useful for increasing the confidence level of genotyping analyses. However, aspects of the invention may be used in the context of any suitable nucleic acid analysis, for example, but not limited to, a nucleic acid analysis that is designed to determine whether more than one sequence variant is present in a sample.

In some embodiments, aspects of the invention relate to a plurality of nucleic acid probes (e.g., 10-50, 50-100, 100-250, 250-500, 500-1,000, 1,000-2,000, 2,000-5,000, 5,000-7,500, 7,500-10,000, or lower, higher, or intermediate number of different probes). In some embodiments, each probe or each of a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) has a different first targeting arm. In some embodiments, each probe or each probe of a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) has a different second targeting arm. In some embodiments, the first and second targeting arms are separated by the same intervening sequence. In some embodiments, the first and second targeting arms are complementary to target nucleic acid sequences that are separated by the same or a similar length (e.g., number of nucleic acids, for example, 0-25, 25-50, 50-100, 100-250, 250-500, 500-1,000, 1,000-2,500 or longer or intermediate number of nucleotides) on their respective target nucleic acids (e.g., genomic loci). In some embodiments, each probe or a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) includes a first primer binding sequence. In some embodiments, the primer binding sequence is the same (e.g., it can be used to prime sequencing or other extension reaction). In some embodiments, each probe or a subset of probes (e.g., 10-25%, 25-50%, 50-75%, 75-90%, or 90-99%) includes a unique



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identifier sequence tag (e.g., that is predetermined and can be used to distinguish each probe).

In some embodiments, the methods disclosed herein are useful for any application where sensitivity is important. For example, detection of cancer mutations in a heterogenous tissue sample, detection of mutations in maternally-circulating fetal DNA, and detection of mutations in cells isolated during a preimplantation genetic diagnostic procedure.

According to some aspects of the invention, methods of detecting a polymorphism in a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise obtaining a nucleic acid preparation using a preparative method (e.g., any of the preparative methods disclosed herein) on a biological sample, and performing a molecular inversion probe capture reaction on the nucleic acid preparation, wherein a molecular inversion probe capture (e.g., using a mutation-detection MIP) of a target nucleic acid of the nucleic acid preparation is indicative of the presence of a mutation (polymorphism) in the target nucleic acid, optionally wherein the polymorphism is selected from Table 2.

According to some aspects of the invention, methods of genotyping a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise obtaining a nucleic acid preparation using a preparative method on a biological sample, sequencing a target nucleic acid of the nucleic acid preparation, and performing a molecular inversion probe capture reaction on the biological sample, wherein a molecular inversion probe capture of the target nucleic acid in the biological sample is indicative of the presence of a polymorphism in the target nucleic acid, genotyping the target nucleic acid based on the results of the sequencing and the capture reaction.

In some embodiments of the methods disclosed herein, the target nucleic acid has a sequence of a gene selected from Table 1.

It should be appreciated that any one or more embodiments described herein may be used for evaluating multiple genetic markers in parallel. Accordingly, in some embodiments, aspects of the invention relate to determining the presence of one or more markers (e.g., one or more alleles) at multiple different genetic loci in parallel. Accordingly, the risk or presence of multiple heritable disorders may be evaluated in parallel. In some embodiments, the risk of having offspring with one or more heritable



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disorders may be evaluated. In some embodiments, an evaluation may be performed on a biological sample of a parent or a child (e.g., at a pre-implantation, prenatal, perinatal, or postnatal stage). In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table I or 2) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample. A patient or subject may be a human. However, aspects of the invention are not limited to humans and may be applied to other species (e.g., mammals, birds, reptiles, other vertebrates or invertebrates) as aspects of the invention are not limited in this respect. A subject or patient may be male or female. In some embodiments, in connection with reproductive genetic counseling, samples from a male and female member of a couple may be analyzed. In some embodiments, for example, in connection with an animal breeding program, samples from a plurality of male and female subjects may be analyzed to determine compatible or optimal breeding partners or strategies for particular traits or to avoid one or more diseases or conditions. Accordingly, reproductive risks may be determined and/or reproductive recommendations may be provided based on information derived from one or more embodiments of the invention.

However, it should be appreciated that aspects of the invention may be used in connection with any medical evaluation where the presence of one or more alleles at a genetic locus of interest is relevant to a medical determination (e.g., risk or detection of disease, disease prognosis, therapy selection, therapy monitoring, etc.). Further aspects of the invention may be used in connection with detection, in tumor tissue or circulating tumor cells, of mutations in cellular pathways that cause cancer or predict efficacy of treatment regimens, or with detection and identification of pathogenic organisms in the environment or a sample obtained from a subject, *e.g.*, a human subject.

These and other aspects of the invention are described in more detail in the following description and non-limiting examples and drawings.

## BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout;

FIG. 2 illustrates a non-limiting embodiment of a staggered probe layout;

FIG. 3 illustrates a non-limiting embodiment of an alternating staggered probe layout;



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FIGs. 4A, B, and C depict various non-limiting methods for combining differentiator tag sequence and target sequences (NNNN depicts a differentiator tag sequence);

FIG. 5 depicts a non-limiting method for genotyping based on target and differentiator tag sequences;

FIG. 6 depicts non-limiting results of a simulation of a MIP capture reaction;

FIG. 7 depicts a non-limiting graph of sequencing coverage;

FIG. 8 illustrates that shorter sequences are captured with higher efficiency that longer sequences using MIPs;

FIG. 9 illustrates a non-limiting scheme of padlock (MIP) capture of a region that includes both repetitive regions (thick wavy line) and the adjacent unique sequence (thick strait line);

FIG. 10 illustrates a non-limiting hypothetical relationship between target gap size and the relative number of reads of the repetitive region;

FIG. 11A depicts MIP capture of FMR1 repeat regions from a diploid genome;

FIG. 11B depicts preparative methods for biallelic resolution of FMR1 repeat region lengths in a diploid genome using MIP capture probes and unique differentiator tags;

FIG. 11C depicts an analysis of FMR1 repeat region lengths in a diploid genome;

FIG. 12 is a schematic of an embodiment of an algorithm of the invention;

FIG. 13 illustrates a non-limiting example of a graph of per-target abundance with MIP capture; and,

FIG. 14 shows a non-limiting a graph of correlation between two MIP capture reactions.

## DETAILED DESCRIPTION

Aspects of the invention relate to preparative and analytical methods and compositions for evaluating genotypes, and in particular, for determining the allelic identity (or identities in a diploid organism) of one or more genetic loci in a subject. Aspects of the invention are based, in part, on the identification of different sources of ambiguity and error in genetic analyses, and, in part, on the identification of one or more approaches to avoid, reduce, recognize, and/or resolve these errors and ambiguities at different stages in a genetic analysis. Aspects of the invention relate to methods and



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compositions for addressing bias and/or stochastic variation associated with one or more preparative and/or analytical steps of a nucleic acid evaluation technology. In some embodiments, preparative methods can be adapted to avoid or reduce the risk of bias skewing the results of a genetic analysis. In some embodiments, analytical methods can be adapted to recognize and correct for data variations that may give rise to misinterpretation (e.g., incorrect calls such as homozygous when the subject is actually heterozygous or heterozygous when the subject is actually homozygous). Methods of the invention may be used for any type of mutation, for example a single base change (e.g., insertion, deletion, transversion or transition, etc.), a multiple base insertion, deletion, duplication, inversion, and/or any other change or combination thereof.

In some embodiments, additional or alternative techniques may be used to address loci characterized by multiple repeats of a core sequence where the length of the repeat is longer than a typical sequencing read thereby making it difficult to determine whether a deletion or duplication of one or more core sequence units has occurred based solely on a sequence read.

In some embodiments, increased confidence in an assay result may be obtained by i) selecting two or more different preparative and/or analytical techniques that have different biases (e.g., known to have different biases), ii) evaluating a patient sample using the two or more different techniques, iii) comparing the results from the two or more different techniques, and/or iv) determining whether the results are consistent for the two or more different techniques. In some embodiments, if determining in step (iv) indicates that the results are consistent (e.g., the same) then increased confidence in the assay result is obtained. In other embodiments, if determining in step (iv) indicates that the results are inconsistent (e.g., that the results are ambiguous) then one or more additional preparative and/or analytical techniques, which have a different bias (e.g., known to have a different bias) compared with the two or more different preparative and/or analytical techniques selected in step (i), are used to evaluate the patient sample, and the results of the one or more additional preparative and/or analytical techniques are compared with the results from step (ii) to resolve the inconsistency.

In some embodiments, two or more independent samples may be obtained from a subject and independently analyzed. In some embodiments, two or more independent samples are obtained at approximately the same time point. In some embodiments, two or more independent samples are obtained at multiple different time points. In some



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embodiments, the use of two or more independent sample facilitates the elimination, normalization, and/or quantification of stochastic measurement noise. It is to be appreciated that two or more independent samples may be obtained in connection with any of the methods disclosed herein, including, for example, methods for pathogen profiling in a human or other animal subjects, monitoring tumor progression/regression, analyzing circulating tumor cells, analyzing fetal cells in maternal circulation, and analyzing/monitoring/profiling of environmental pathogens.

In some embodiments, one or more of the techniques described herein may be combined in a single assay protocol for evaluating multiple patient samples in parallel.

It should be appreciated that aspects of the invention may be useful for high throughput, cost-effective, yet reliable, genotyping of multiple patient samples (e.g., in parallel, for example in multiplex reactions). In some embodiments, aspects of the invention are useful to reduce the error frequency in a multiplex analysis. Certain embodiments may be particularly useful where multiple reactions (e.g., multiple loci and/or multiple patient samples) are being processed. For example, 10-25, 25-50, 50-75, 75-100 or more loci may be evaluated for each subject out of any number of subject samples that may be processed in parallel (e.g., 1-25, 25-50, 50-100, 100-500, 500-1,000, 1,000-2,500, 2,500-5,000 or more or intermediate numbers of patient samples). It should be appreciated that different embodiments of the invention may involve conducting two or more target capture reactions and/or two or more patient sample analyses in parallel in a single multiplex reaction. For example, in some embodiments a plurality of capture reactions (e.g., using different capture probes for different target loci) may be performed in a single multiplex reaction on a single patient sample. In some embodiments, a plurality of captured nucleic acids from each one of a plurality of patient samples may be combined in a single multiplex analysis reaction. In some embodiments, samples from different subjects are tagged with subject-specific (e.g., patient-specific) tags (e.g., unique sequence tags) so that the information from each product can be assigned to an identified subject. In some embodiments, each of the different capture probes used for each patient sample have a common patient-specific tag. In some embodiments, the capture probes do not have patient-specific tags, but the captured products from each subject may be amplified using one or a pair of amplification primers that are labeled with a patient-specific tag. Other techniques for associating a patient-specific tag with the captured product from a single patient sample may be used as aspects of the



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invention are not limited in this respect. It should be appreciated that patient-specific tags as used herein may refer to unique tags that are assigned to identified patients in a particular assay. The same tags may be used in a separate multiplex analysis with a different set of patient samples (e.g., from different patients) each of which is assigned one of the tags. In some embodiments, different sets of unique tags may be used in sequential (e.g., alternating) multiplex reactions in order to reduce the risk of contamination from one assay to the next and allow contamination to be detected on the basis of the presence of tags that are not expected to be present in a particular assay.

Embodiments of the invention may be used for any of a number of different settings: reproductive settings, disease screening, identifying subjects having cancer, identifying subjects having increased risk for a disease, stratifying a population of subjects according to one or more of a number of factors, for example responsiveness to a particular drug, lack or not of an adverse reaction (or risk therefore) to a particular drug, and/or providing information for medical records (e.g., homozygosity, heterozygosity at one or more loci). It should be appreciated that the invention is not limited to genomic analysis of patient samples. For example, aspects of the invention may be useful for high throughput genetic analysis of environment samples to detect pathogens.

In some embodiments, the methods disclosed herein are useful for diagnosis of one or more heritable disorders. In some embodiments, a heritable disorder that may be diagnosed with the methods disclosed herein is a genetic disorder that is prevalent in the Ashkenazi Jewish population. In some embodiments, the heritable disorders are selected from: 21-Hydroxylase-Defiocient Congenital Adrenal Hyperplasia; ABCC8-Related Hyperinsulinism; Alpha-Thalassemia, includes Constant Spring, & MR associated; Arylsulfatase A Deficiency-Metyachromatic Leukodystrophy; Biotinidase Deficiency-Holocarboxylase Synthetase Deficiency; Bloom's Syndrome; Canavan Disease; CFTR-Related Disorders-cystic fibrosis; Citrullinemia Type I; Combined MMA & Homocystinuria-dblC; Dystrophinopathies (DMD & BMD); Familial Dysautonomia; Fanconi Anemia-FANCC; Galactosemia-Classical: Galactokinase Defiency & Galactose Epimerase Deficiency; Gaucher Disease; GJB2-Related DFNB 1 Nonsyndromic Hearing Loss and Deafness; Glutaric acidemia Type 1; Hemoglobinopathies beta-chain disorders; Glycogen Storage Disease Type 1A; Maple Syrup Urine Disease; Types 1A, 1B, 2, 3; Medium Chain Acyl-Coenzyme A; Dehydrogenase Deficiency-MCADD;



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Methylmalonic Acidemia; Mucolipidosis IV; Nemaline Myopathy; Nieman-Pick Type A-Acid Sphingomyelinase Deficiency; Non-Ketotic Hyperglycinemia-Glycine Encephalopathy; Ornithine Transcarbamylase Deficiency; PKU Phenylalanine Hydroxylase Deficiency; Propionic Acidemia; Short Chain Acyl-CoA Dehydrogenase Deficiency-SCADD; Smith-Lemli-Opitz Syndrome; Spinal Muscular Atrophy (SMN1)-SMA; Tay Sachs-HexA Deficiency; Usher Synbdrome-Type I (Type IB, Type IC, Type ID, Type IF, Type IG) ; X-Linked Mental Retardation ARX-Related Disorders; X-Linked Mental Retardation with Cerebellar Cypoplasia and sistinctive Facial Appearance; X-Linked Mental Retardation; includes 9, 21, 30, 46, 58, 63, 88, 89; X-linked mental retardation: FM1-Related Disorders-FRXA, Fragile X MR; X-linked SMR: Renpenning Syndrome 1; Zellweger Spectrum disorders - Peroxisomal Bifunctional Enzyme Deficiencies including Zellweger, NALD, and/or infantile Refsums. However, all of these, subsets of these, other genes, or combinations thereof may be used.

According to some aspects, the disclosure relates to multiplex diagnostic methods. In some embodiments, multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel (e.g., a genetic locus of Table 1). In some embodiments, genetic loci possess one or more polymorphisms (e.g., a polymorphism of Table 2) the genotypes of which correspond to disease causing alleles. Accordingly, in some embodiments, the disclosure provides methods for assessing multiple heritable disorders in parallel.

In some embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample. A patient or subject may be a human. However, aspects of the invention are not limited to humans and may be applied to other species (e.g., mammals, birds, reptiles, other vertebrates or invertebrates) as aspects of the invention are not limited in this respect. A subject or patient may be male or female. In some embodiments, in connection with reproductive genetic counseling, samples from a male and female member of a couple may be analyzed. In some embodiments, for example, in connection with an animal breeding program, samples from a plurality of male and female subjects may be analyzed to



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determine compatible or optimal breeding partners or strategies for particular traits or to avoid one or more diseases or conditions.

However, it should be appreciated that any other diseases may be studied and/or risk factors for diseases or disorders including, but not limited to allergies, responsiveness to treatment, cancer tumor profiling for treatment and prognosis, monitoring and identification of patient infections, and monitoring of environmental pathogens.

*1. Reducing representational bias in multiplex amplification reactions:*

In some embodiments, aspects of the invention relate to methods that reduce bias and increase reproducibility in multiplex detection of genetic loci, e.g., for diagnostic purposes.

Molecular inversion probe technology is used to detect or amplify particular nucleic acid sequences in potentially complex mixtures. Use of molecular inversion probes has been demonstrated for detection of single nucleotide polymorphisms (Hardenbol et al. 2005 Genome Res 15:269-75) and for preparative amplification of large sets of exons (Porreca et al. 2007 Nat Methods 4:931-6, Krishnakumar et al. 2008 Proc Natl Acad Sci USA 105:9296-301). One of the main benefits of the method is in its capacity for a high degree of multiplexing, because generally thousands of targets may be captured in a single reaction containing thousands of probes. However, challenges associated with, for example, amplification efficiency (See, e.g., Turner EH, et al., Nat Methods. 2009 Apr 6:1-2.) have limited the practical utility of the method in research and diagnostic settings.

Aspects of the disclosure are based, in part, on the discovery of effective methods for overcoming challenges associated with systematic errors (bias) in multiplex genomic capture and sequencing methods, namely high variability in target nucleic acid representation and unequal sampling of heterozygous alleles in pools of captured target nucleic acids (e.g., isolated from a biological sample). Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the detection of target nucleic acids in multiplex capture methods. In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of different sets of



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multiple probes (e.g., molecular inversion probes MIPs) that capture overlapping regions of a target nucleic acid to achieve a more uniform representation of the target nucleic acids in a capture pool compared with methods of the prior art. In other embodiments, methods reduce bias, or the risk of bias, associated with large scale parallel capture of genetic loci, e.g., for diagnostic purposes. In other embodiments, methods are provided for increasing reproducibility (e.g., by reducing the effect of polymorphisms on target nucleic acid capture) in the detection of a plurality of genetic loci in parallel. In further embodiments, methods are provided for reducing the effect of probe synthesis and/or probe amplification variability on the analysis of a plurality of genetic loci in parallel.

In some aspects, the disclosure provides probe sets that comprise a plurality of different probes. As used herein, a 'probe' is a nucleic acid having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of a target nucleic acid or subregion thereof. An exemplary probe is a molecular inversion probe (MIP). A 'target nucleic acid' may be a genetic locus. Exemplary genetic loci are disclosed herein in Table 1 (RefSeqGene Column).

While probes have been typically designed to meet certain constraints (e.g. melting temperature, G/C content, etc.) known to partially affect capture/amplification efficiency (Ball et al (2009) Nat Biotech 27:361-8 AND Deng et al (2009) Nat Biotech 27:353-60), a set of constraints which is sufficient to ensure either largely uniform or highly reproducible capture/amplification efficiency has not previously been achieved. As disclosed herein, uniformity and reproducibility can be increased by designing multiple probes per target, such that each base in the target is captured by more than one probe. In some embodiments, the disclosure provides multiple MIPs per target to be captured, where each MIP in a set designed for a given target nucleic acid has a central region and a 5' region and 3' region ('targeting arms') which hybridize to (at least partially) different nucleic acids in the target nucleic acid (immediately flanking a subregion of the target nucleic acid). Thus, differences in efficiency between different targeting arms and fill-in sequences may be averaged across multiple MIPs for a single target, which results in more uniform and reproducible capture efficiency.

In some embodiments, the methods involve designing a single probe for each target (a target can be as small as a single base or as large as a kilobase or more of contiguous sequence).



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It may be preferable, in some cases, to design probes to capture molecules (e.g., target nucleic acids or subregions thereof) having lengths in the range of 1 - 200 bp (as used herein, a bp refers to a base pair on a double-stranded nucleic acid – however, where lengths are indicated in bps, it should be appreciated that single-stranded nucleic acids having the same number of bases, as opposed to base pairs, in length also are contemplated by the invention). However, probe design is not so limited. For example, probes can be designed to capture targets having lengths in the range of up to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000, or more bps, in some cases.

It is to be appreciated that the length of a capture molecule (e.g., a target nucleic acid or subregion thereof) is selected based upon multiple considerations. For example, where analysis of a target involves sequencing, e.g., with a next-generation sequencer, the target length should typically match the sequencing read-length so that shotgun library construction is not necessary. However, it should be appreciated that captured nucleic acids may be sequenced using any suitable sequencing technique as aspects of the invention are not limited in this respect.

It is also to be appreciated that some target nucleic acids are too large to be captured with one probe. Consequently, it may be necessary to capture multiple subregions of a target nucleic acid in order to analyze the full target.

In some embodiments, a subregion of a target nucleic acid is at least 1 bp. In other embodiments, a subregion of a target nucleic acid is at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp or more. In other embodiments, a subregion of a target nucleic acid has a length that is up to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more percent of a target nucleic acid length.

The skilled artisan will also appreciate that consideration is made, in the design of MIPs, for the relationship between probe length and target length. In some embodiments, MIPs are designed such that they are several hundred basepairs (e.g., up to 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp or more) longer than corresponding target (e.g., subregion of a target nucleic acid, target nucleic acid).

In some embodiments, lengths of subregions of a target nucleic acid may differ. For example, if a target nucleic acid contains regions for which probe hybridization is not possible or inefficient, it may be necessary to use probes that capture subregions of



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one or more different lengths in order to avoid hybridization with problematic nucleic acids and capture nucleic acids that encompass a complete target nucleic acid.

Aspects of the invention involve using multiple probes, e.g., MIPs, to amplify each target nucleic acid. In some embodiments, the set of probes for a given target can be designed to 'tile' across the target, capturing the target as a series of shorter sub-targets. In some embodiments, where a set of probes for a given target is designed to 'tile' across the target, some probes in the set capture flanking non-target sequence). Alternately, the set can be designed to 'stagger' the exact positions of the hybridization regions flanking the target, capturing the full target (and in some cases capturing flanking non-target sequence) with multiple probes having different targeting arms, obviating the need for tiling. The particular approach chosen will depend on the nature of the target set. For example, if small regions are to be captured, a staggered-end approach might be appropriate, whereas if longer regions are desired, tiling might be chosen. In all cases, the amount of bias-tolerance for probes targeting pathological loci can be adjusted ('dialed in') by changing the number of different MIPs used to capture a given molecule.

In some embodiments, the 'coverage factor', or number of probes used to capture a basepair in a molecule, is an important parameter to specify. Different numbers of probes per target are indicated depending on whether one is using the tiling approach (see, e.g., FIG. 1) or one of the staggered approaches (see, e.g., FIGs. 2 or 3).

FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout showing ten captured sub-targets tiled across a single target. Each position in the target is covered by three sub-targets such that MIP performance per base pair is averaged across three probes.

FIG. 2 illustrates a non-limiting embodiment of a staggered probe layout showing the targets captured by a set of three MIPs. Each MIP captures the full target, shown in black, plus (in some cases) additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes. Targeting arms land immediately adjacent to the black or gray regions shown. It should be appreciated that in some embodiments, the targeting arms (not shown) can be designed so that they do not overlap with each other.

FIG. 3 illustrates a non-limiting embodiment of an alternating staggered probe layout showing the targets captured by a set of three MIPs. Each MIP captures the full



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target, shown in black, plus (in some cases) additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes. Targeting arms land immediately adjacent to the black or gray regions shown.

It should be appreciated that for any of the layouts, the targeting arms on adjacent tiled or staggered probes may be designed to either overlap, not overlap, or overlap for only a subset of the probes.

In certain embodiments for any of the layouts, a coverage factor of about 3 to about 10 is used. However, the methods are not so limited and coverage factors of up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or more may be used. It is to be appreciated that the coverage factor selected may depend the probe layout being employed. For example, in the tiling approach, for a desired coverage factor, the number of probes per target is typically a function of target length, sub-target length, and spacing between adjacent sub-target start locations (step size). For example, for a desired coverage factor of 3, a 200 bp target with a start-site separation of 20 bp and sub-target length of 60 bp may be encompassed with 12 MIPs (FIG. 1). Thus, a specific coverage factor may be achieved by varying the number of probes per target nucleic acid and the length of the molecules captured. In the staggered approach, a fixed-length target nucleic acid is captured as several subregions or as 'super-targets', which are molecules comprising the target nucleic acid and additional flanking nucleic acids, which may be of varying lengths. For example, a target of 50 bp can be captured at a coverage factor of 3 with 3 probes in either a 'staggered' (FIG. 2) or 'alternating staggered' configuration (FIG. 3).

The coverage factor will be driven by the extent to which detection bias is tolerable. In some cases, where the bias tolerance is small, it may be desirable to target more subregions of target nucleic acid with, perhaps, higher coverage factors. In some embodiments, the coverage factor is up to 2, 3, 4, 5, 6, 7, 8, 9, 10 or more.

In some embodiments, when a tiled probe layout is used, when the target length is greater than 1 bp and when a step size (distance between the 5'-end of a target and the 5' end of its adjacent target) is less than the length of a target or subregion thereof, it is possible to compute probe number for a particular target based on target length (T), sub-target length (S), and coverage factor (C), such that probe number = T/(S/C) + (C-1).



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In some aspects, the disclosure provides methods to increase the uniformity of amplification efficiency when multiple molecules are amplified in parallel; methods to increase the reproducibility of amplification efficiency; methods to reduce the contribution of targeting probe variability to amplification efficiency; methods to reduce the effect on a given target nucleic acid of polymorphisms in probe hybridization regions; and/or methods to simplify downstream workflows when multiplex amplification by MIPs is used as a preparative step for analysis by nucleic acid sequencing.

Polymorphisms in the target nucleic acid under the regions flanking a target can interfere with hybridization, polymerase fill-in, and/or ligation. Furthermore, this may occur for only one allele, resulting in allelic drop-out, which ultimately decreases downstream sequencing accuracy. In some embodiments, using a set of MIPs having multiple hybridization sites for the capture of any given target, the probability of loss from polymorphism is substantially decreased because not all targeting arms in the set of MIPs will cover the location of the mutation.

Probes for MIP capture reactions may be synthesized on programmable microarrays because of the large number of sequences required. Because of the low synthesis yields of these methods, a subsequent amplification step is required to produce sufficient probe for the MIP amplification reaction. The combination of multiplex oligonucleotide synthesis and pooled amplification results in uneven synthesis error rates and representational biases. By synthesizing multiple probes for each target, variation from these sources may be averaged out because not all probes for a given target will have the same error rates and biases.

Multiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs, or preparatively, often for next-generation sequencing or other sequencing techniques. In the preparative setting, the output of an amplification reaction is generally the input to a shotgun library protocol, which then becomes the input to the sequencing platform. The shotgun library is necessary in part because next-generation sequencing yields reads significantly shorter than amplicons such as exons. In addition to the bias-reduction afforded by the multi-tiled approach described here, tiling also obviates the need for shotgun library preparation. Since the length of the capture molecule can be specified when the probes, e.g., MIPs, are designed, it can be chosen to



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match the readlength of the sequencer. In this way, reads can 'walk' across an exon by virtue of the start position of each capture molecule in the probe set for that exon.

*2. Reducing analytical errors associated with bias in nucleic acid preparations:*

In some embodiments, aspects of the invention relate to preparative steps in DNA sequencing-related technologies that reduce bias and increase the reliability and accuracy of downstream quantitative applications.

There are currently many genomics assays that utilize next-generation (polony-based) sequencing to generate data, including genome resequencing, RNA-seq for gene expression, bisulphite sequencing for methylation, and Immune-seq, among others. In order to make quantitative measurements (including genotype calling), these methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids. The majority of these techniques require a preparative step to construct a high-complexity library of DNA molecules that is representative of a sample of interest. This may include chemical or biochemical treatment of the DNA (e.g., bisulphite treatment), capture of a specific subset of the genome (e.g., padlock probe capture, solution hybridization), and a variety of amplification techniques (e.g., polymerase chain reaction, whole genome amplification, rolling circle amplification).

Systematic and random errors are common problems associated with genome amplification and sequencing library construction techniques. For example, genomic sequencing library may contain an over- or under-representation of particular sequences from a source genome as a result of errors (bias) in the library construction process. Such bias can be particularly problematic when it results in target sequences from a genome being absent or undetectable in the sequencing libraries. For example, an under-representation of particular allelic sequences (e.g., heterozygotic alleles) from a genome in a sequencing library can result in an apparent homozygous representation in a sequencing library. As most downstream sequencing library quantification techniques depend on stochastic counting processes, these problems have typically been addressed by sampling enough (over-sampling) to obtain a minimum number of observations necessary to make statistically significant decisions. However, the strategy of oversampling is generally limited to elimination of low-count Poisson noise, and the



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approach wastes resources and increases the expense required to perform such experiments. Moreover, oversampling can result in a reduced statistical confidence in certain conclusions (e.g., diagnostic calls) based on the data. Accordingly, new approaches are needed for overcoming bias in sequencing library preparatory methods.

Aspects of the disclosure are based, in part, on the discovery of methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, namely high variability in the capture and amplification of nucleic acids and disproportionate representation of heterozygous alleles in sequencing libraries. Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the capture and amplification of nucleic acids. In other embodiments, the methods improve allelic representation in sequencing libraries and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of differentiator tag sequences to uniquely tag individual nucleic acid molecules. In some embodiments, the differentiator tag sequence permits the detection of bias based on the frequency with which pairs of differentiator tag and target sequences are observed in a sequencing reaction. In other embodiments, the methods reduce errors caused by bias, or the risk of bias, associated with the capture, amplification and sequencing of genetic loci, e.g., for diagnostic purposes.

Aspects of the invention relate to associating unique sequence tags (referred to as differentiator tag sequences) with individual target molecules that are independently captured and/or analyzed (e.g., prior to amplification or other process that may introduce bias). These tags are useful to distinguish independent target molecules from each other thereby allowing an analysis to be based on a known number of individual target molecules. For example, if each of a plurality of target molecule sequences obtained in an assay is associated with a different differentiator tag, then the target sequences can be considered to be independent of each other and a genotype likelihood can be determined based on this information. In contrast, if each of the plurality of target molecule sequences obtained in the assay is associated with the same differentiator tag, then they probably all originated from the same target molecule due to over-representation (e.g., due to biased amplification) of this target molecule in the assay. This provides less information than the situation where each nucleic acid was associated with a different



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differentiator tag. In some embodiments, a threshold number of independently isolated molecules (e.g., unique combinations of differentiator tag and target sequences) is analyzed to determine the genotype of a subject.

In some embodiments, the invention relates to compositions comprising pools (libraries) of preparative nucleic acids that each comprise "differentiator tag sequences" for detecting and reducing the effects of bias, and for genotyping target nucleic acid sequences. As used herein, a "differentiator tag sequence" is a sequence of a nucleic acid (a preparative nucleic acid), which in the context of a plurality of different isolated nucleic acids, identifies a unique, independently isolated nucleic acid. Typically, differentiator tag sequences are used to identify the origin of a target nucleic acid at one or more stages of a nucleic acid preparative method. For example, in the context of a multiplex nucleic acid capture reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, target nucleic acid capture events. Also, in the context of a multiplex nucleic acid amplification reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, primary amplicons of a target nucleic acid, for example. Thus, combinations of target nucleic acid and differentiator tag sequence (target:differentiator tag sequences) of an isolated nucleic acid of a preparative method provide a basis for identifying unique, independently isolated target nucleic acids. FIG. 4A-C depict various non-limiting examples of methods for combining differentiator tag sequence and target sequences.

It will be apparent to the skilled artisan that differentiator tags may be synthesized using any one of a number of different methods known in the art. For example, differentiator tags may be synthesized by random nucleotide addition. Differentiator tag sequences are typically of a predefined length, which is selected to control the likelihood of producing unique target:differentiator tag sequences in a preparative reaction (e.g., amplification-based reaction, a circularization selection-based reaction, e.g., a MIP reaction). Differentiator tag sequences may be, up to 5, up to 6, up to 7 up to 8, up to 9, up to 10, up to 11, up to 12, up to 13, up to 14, up to 15, up to 16, up to 17, up to 18, up to 19, up to 20, up to 21, up to 22, up to 23, up to 24, up to 25, or more nucleotides in length. For purposes of genotyping, isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and observance of threshold numbers of



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unique combinations of target nucleic acid and differentiator tag sequences provide a certain statistical confidence in the genotype.

During a library preparation process, each nucleic acid molecule may be tagged with a unique differentiator tag sequence in a configuration that permits the differentiator tag sequence to be sequenced along with the target nucleic acid sequence of interest (the nucleic acid sequence for which the library is being prepared, e.g., a polymorphic sequence). The incorporation of the nucleic acid comprising a differentiator tag sequence at a particular step allows the detection and correction of biases in subsequent steps of the protocol.

A large library of unique differentiator tag sequences may be created by using degenerate, random-sequence polynucleotides of defined length. The differentiator tag sequences of the polynucleotides may be read at the final stage of the sequencing. The observations of the differentiator tag sequences may be used to detect and correct biases in the final sequencing read-out of the library. For example, the total possible number of differentiator tag sequences, which may be produced, e.g., randomly, is $4^N$, where N is the length of the differentiator tag sequence. Thus, it is to be understood that the length of the differentiator tag sequence may be adjusted such that the size of the population of MIPs having unique differentiator tag sequences is sufficient to produce a library of MIP capture products in which identical independent combinations of target nucleic acid and differentiator tag sequence are rare. As used herein combinations of target nucleic acid and differentiator tag sequences, may also be referred to as "target:differentiator tag sequences".

In the final readout of a sequencing process, each read may have an additional unique differentiator tag sequence. In some embodiments, when differentiator tag sequences are distributed randomly in a library, all the unique differentiator tag sequences will be observed about an equal number of times. Accordingly, the number of occurrences of a differentiator tag sequence may follow a Poisson distribution.

In some embodiments, overrepresentation of target:differentiator tag sequences in a pool of preparative nucleic acids (e.g., amplified MIP capture products) is indicative of bias in the preparative process (e.g., bias in the amplification process). For example, target:differentiator tag sequence combinations that are statistically overrepresented are indicative of bias in the protocol at one or more steps between the incorporation of the



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differentiator tag sequences into MIPs and the actual sequencing of the MIP capture products.

The number of reads of a given target:differentiator tag sequence may be indicative (may serve as a proxy) of the amount of that target sequence present in the originating sample. In some embodiments, the numbers of occurrence of sequences in the originating sample is the quantity of interest. For example, using the methods disclosed herein, the occurrence of differentiator tag sequences in a pool of MIPs may be predetermined (e.g., may be the same for all differentiator tag sequences). Accordingly, changes in the occurrence of differentiator tag sequences after amplification and sequencing may be indicative of bias in the protocol. Bias may be corrected to provide an accurate representation of the composition of the original MIP pool, e.g., for diagnostic purposes.

According to some aspects, a library of preparative nucleic acid molecules (e.g., MIPs, each nucleic acid in the library having a unique differentiator tag sequence, may be constructed such that the number of nucleic acid molecules in the library is significantly larger than the number prospective target nucleic acid molecules to be captured using the library. This ensures that products of the preparative methods include only unique target:differentiator tag sequence; e.g., in a MIP reaction the capture step would undersample the total population of unique differentiator tag sequences in the MIP library. For example, an experiment utilizing 1 μg of genomic DNA will contain about ~150,000 copies of a diploid genome. For a MIP library, each MIP in the library comprising a randomly produced 12-mer differentiator tag sequence (~1.6 million possible unique differentiator tag sequences), there would be more than 100 unique differentiator tag sequences per genomic copy. For a MIP library, each MIP in the library comprising a randomly produced 15-mer differentiator tag sequence (~1 billion possible unique differentiator tag sequences), there would be more than 7000 unique differentiator tag sequences per genomic copy. Therefore, the probability of the same differentiator tag sequence being incorporated multiple times is incredibly small. Thus, it is to be appreciated that the length of the differentiator tag sequence is to be selected based on the amount of target sequence in a MIP capture reaction and the desired probability for having multiple, independent occurrences of target:differentiator tag sequence combinations.



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FIG. 5 depicts a non-limiting method for genotyping based on target and differentiator tag sequences. Sequencing reads of target and differentiator tags sequences are collapsed to make diploid genotype calls. FIG. 6 depicts non-limiting results of a simulation of a MIP capture reaction in which MIP probes, each having a differentiator tag sequence of 15 nucleotides, are combined with 10000 target sequence copies (e.g., genome equivalents). In this simulated reaction, the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is 0.05. The Y axis reflects the number of observations. The X axis reflects the number of independent occurrences of target:differentiator tag combinations. FIG. 7 depicts a non-limiting graph of sequencing coverage, which can help ensure that alleles are sampled to sufficient depth (e.g., either 10x or 20x minimum sampling per allele, assuming 1000 targets). In this non-limiting example, the X axis is total per-target coverage required, and the Y axis is the probability that a given total coverage will result in at least 10x or 20x coverage for each allele.

The skilled artisan will appreciate that as part of a MIP library preparation process, adapters may be ligated onto the ends of the molecules of interest. Adapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites. In addition, barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007/0020640 A1 (McCloskey et al.)

The actual incorporation of the random differentiator tag sequences can be performed through various methods known in the art. For example, nucleic acids comprising differentiator tag sequences may be incorporated by ligation. This is a flexible method, because molecules having differentiator tag sequence can be ligated to any blunt-ended nucleic acids. The sequencing primers must be incorporated subsequently such that they sequence both the differentiator tag sequence and the target sequence. Alternatively, the sequencing adaptors can be synthesized with the random differentiator tag sequences at their 3' end (as degenerate bases), so that only one ligation must be performed. Another method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags. Another



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method to incorporate the differentiator tag sequences is to synthesize them into a padlock probe prior to performing a gene capture reaction. The differentiator tag sequence is incorporated 3' to the targeting arm but 5' to the amplification primer that will be used downstream in the protocol. Another method to incorporate the differentiator tag sequences is as a tag on a gene-specific or poly-dT reverse-transcription primer. This allows the differentiator tag sequence to be incorporated directly at the cDNA level.

In some embodiments, at the incorporation step, the distribution of differentiator tag sequences can be assumed to be uniform. In this case, bias in any part of the protocol would change the uniformity of this distribution, which can be observed after sequencing. This allows the differentiator tag sequence to be used in any preparative process where the ultimate output is sequencing of many molecules in parallel.

Differentiator tag sequences may be incorporated into probes (e.g., MIPs) of a plurality when they are synthesized on-chip in parallel, such that degeneracy of the incorporated nucleotides is sufficient to ensure near-uniform distribution in the plurality of probes. It is to be appreciated that amplification of a pool of unique differentiator tag sequences may itself introduce bias in the initial pool. However, in most practical cases, the scale of synthesis (e.g., by column synthesis, chip based synthesis, etc.) is large enough that amplification of an initial pool of differentiator tag sequences is not necessary. By avoiding amplification or selection steps on the pool of unique differentiator tag sequences, potential bias may be minimized.

One example of the use of the differentiator tag sequences is in genome re-sequencing. Considering that the raw accuracy of most next-generation sequencing instruments is relatively low, it is crucial to oversample the genomic loci of interest. Furthermore, since there are two alleles at every locus, it is important to sample enough to ensure that both alleles have been observed a sufficient number of times to determine with a sufficient degree of statistical confidence whether the sample is homozygous or heterozygous. Indeed, the sequencing is performed to sample the composition of molecules in the originating sample. However, after multiple reads have been collected for a given locus, it is possible that due to bias (e.g., caused by PCR amplification steps), a large fraction of the reads are derived from a single originating molecule. This would skew the population of target sequences observed, and would affect the outcome of the genotype call. For example, it is possible that a locus that is heterozygous is called as



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homozygous, because there are only a few observations of the second allele out of many observations of that locus. However, if information is available on differentiator tag sequences, this situation could be averted, because the over-represented allele would be seen to also have an over-represented differentiator tag sequence (i.e., the sequences with the overrepresented differentiator tag sequence all originated from the same single molecule). Therefore, the sequences and corresponding distribution of differentiator tag sequences can be used as an additional input to the genotype-calling algorithm to significantly improve the accuracy and confidence of the genotype calls.

In some aspects, the disclosure provides methods for analyzing a plurality of target sequences which are genetic loci or portions of genetic loci (e.g., a genetic locus of Table 1). The genetic loci may be analyzed by sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs). Exemplary polymorphisms are disclosed in Table 2. The skilled artisan will appreciate that other polymorphisms are known in the art and may be identified, for example, by querying the Entrez Single Nucleotide Polymorphism database, for example, by searching with a GeneID from Table 1.

**Table 1: Target Nucleic Acids**

| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| CYP21A2 | 1589 | cytochrome P450, family 21, subfamily A, polypeptide 2 | CAH1; CPS1; CA21H; CYP21; CYP21B; P450c21B; MGC150536; MGC150537; CYP21A2 | 201910 | NG_008337.1 | 6p21.3 |
| ABCC8 | 6833 | ATP-binding cassette, sub-family C (CFTR/MRP), member 8 | HI; SUR; HHF1; MRP8; PHHI; SUR1; ABC36; HRINS; TNDM2; ABCC8 | 600509 | NG_008867.1 | 11p15.1 |
| ATRX | 546 | alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) | SHS; XH2; XNP; ATR2; SFMI; RAD54; MRXHF1; RAD54L; ZNF-HX; MGC2094; ATRX | 300032 | NG_008838.1 | Xq13.1-q21.1 |
| ARSA | 410 | arylsulfatase A | MLD; ARSA | 607574 | NG_009260.1 | 22q13.31-qter; 22q13.33 |
| PSAP | 5660 | Prosaposin | GLBA; SAP1; FLJ00245; MGC110993; PSAP | 176801 | NG_008835.1 | 10q21-q22 |
| BTD | 686 | Biotinidase | BTD | 609019 | NG_008019.1 | 3p25 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| HLCS | 3141 | holocarboxylase synthetase (biotin-(proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)) ligase) | HCS; HLCS | 609018 | NC_000021.7 | 21q22.1; 21q22.13 |
| BLM | 641 | Bloom syndrome, RecQ helicase-like | BS; RECQ2; RECQL2; RECQL3; MGC126616; MGC131618; MGC131620; BLM | 604610 | NG_007272.1 | 15q26.1 |
| ASPA | 443 | aspartoacylase (Canavan disease) | ASP; ACY2; ASPA | 608034 | NG_008399.1 | 17pter-p13 |
| CFTR | 1080 | cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) | CF; MRP7; ABC35; ABCC7; CFTR/MRP; TNR-CFTR; dJ760C5.1; CFTR | 602421 | NC_000007.12 | 7q31.2 |
| ASS1 | 445 | argininosuccinate synthetase 1 | ASS; CTLN1; ASS1 | 603470 | NG_011542.1 | 9q34.1 |
| MMACHC | 25974 | methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria | cblC; FLJ25671; DKFZp564I122; RP11-291L19.3; MMACHC | 609831 | NC_000001.9 | 1p34.1 |
| IKBKAP | 8518 | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein | FD; DYS; ELP1; IKAP; IKI3; TOT1; FLJ12497; DKFZp781H1425; IKBKAP | 603722 | NG_008788.1 | 9q31 |
| FANCC | 2176 | Fanconi anemia, complementation group C | FA3; FAC; FACC; FLJ14675; FANCC | 227645 | NG_011707.1 | 9q22.3 |
| GALK1 | 2584 | galactokinase 1 | GK1; GALK; GALK1 | 604313 | NG_008079.1 | 17q24 |
| GALT | 2592 | galactose-1-phosphate uridylyltransferase | GALT | 606999 | NC_000009.10 | 9p13 |
| GALE | 2582 | UDP-galactose-4-epimerase | SDR1E1; FLJ95174; FLJ97302; GALE | 606953 | NG_007068.1 | 1p36-p35 |
| GBA | 2629 | glucosidase, beta; acid (includes glucosylceramidase) | GCB; GBA1; GLUC; GBA | 606463 | NG_009783.1 | 1q21 |
| GJB2 | 2706 | gap junction protein, beta 2, 26kDa | HID; KID; PPK; CX26; DFNA3; DFNB1; NSRD1; DFNA3A; DFNB1A; GJB2 | 121011 | NG_008358.1 | 13q11-q12 |
| GCDH | 2639 | glutaryl-Coenzyme A dehydrogenase | GCD; ACAD5; GCDH | 608801 | NG_009292.1 | 19p13.2 |
| G6PC | 2538 | glucose-6-phosphatase, catalytic subunit | G6PT; GSD1; GSD1a; MGC163350; G6PC | 232200 | NG_011808.1 | 17q21 |
| HBB | 3043 | hemoglobin, beta | CD113t-C; beta-globin; HBB | 141900 | NG_000007.3 | 11p15.5 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| BCKDHA | 593 | branched chain keto acid dehydrogenase E1, alpha polypeptide | MSU; MSUD1; OVD1A; BCKDE1A; FLJ45695; BCKDHA | 608348 | NC_000019.8 | 19q13.1-q13.2 |
| BCKDHB | 594 | branched chain keto acid dehydrogenase E1, beta polypeptide | E1B; FLJ17880; dJ279A18.1; BCKDHB | 248611 | NG_009775.1 | 6q13-q15 |
| DBT | 1629 | dihydrolipoamide branched chain transacylase E2 | E2; E2B; BCATE2; MGC9061; DBT | 248610 | NG_011852.1 | 1p31 |
| DLD | 1738 | dihydrolipoamide dehydrogenase | E3; LAD; DLDH; GCSL; PHE3; DLD | 238331 | NG_008045.1 | 7q31-q32 |
| ACADM | 34 | acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain | MCAD; ACAD1; MCADH; FLJ18227; FLJ93013; FLJ99884; ACADM | 607008 | NG_007045.1 | 1p31 |
| MMAA | 166785 | methylmalonic aciduria (cobalamin deficiency) cblA type | cblA; MGC120010; MGC120011; MGC120012; MGC120013; MMAA | 607481 | NG_007536.1 | 4q31.22 |
| MMAB | 326625 | methylmalonic aciduria (cobalamin deficiency) cblB type | ATR; cblB; MGC20496; MMAB | 607568 | NG_007096.1 | 12q24 |
| MUT | 4594 | methylmalonyl Coenzyme A mutase | MCM; MUT | 609058 | NG_007100.1 | 6p12.3 |
| MCOLN1 | 57192 | mucolipin 1 | ML4; MLIV; MST080; TRPML1; MSTP080; TRP-ML1; TRPM-L1; MCOLN1 | 605248 | NC_000019.8 | 19p13.3-p13.2 |
| ACTA1 | 58 | actin, alpha 1, skeletal muscle | ACTA; ASMA; CFTD; MPFD; NEM1; NEM2; NEM3; CFTD1; CFTDM; ACTA1 | 102610 | NG_006672.1 | 1q42.13 |
| TPM3 | 7170 | tropomyosin 3 | TM3; TRK; NEM1; TM-5; TM30; TM30nm; TPMsk3; hscp30; MGC3261; FLJ41118; MGC14582; MGC72094; OK/SW-cl.5; TPM3 | 191030 | NG_008621.1 | 1q21.2 |
| TNNT1 | 7138 | troponin T type 1 (skeletal, slow) | ANM; TNT; STNT; TNTS; FLJ98147; MGC104241; TNNT1 | 191041 | NG_011829.1 | 19q13.4 |
| NEB | 4703 | nebulin | NEM2; NEB177D; FLJ11505; FLJ36536; FLJ39568; FLJ39584; DKFZp686C1456; NEB | 161650 | NG_009382.1 | 2q22 |
| SMPD1 | 6609 | sphingomyelin phosphodiesterase 1, acid lysosomal | ASM; NPD; SMPD1 | 607608 | NG_011780.1 | 11p15.4-p15.1 |
| GLDC | 2731 | glycine dehydrogenase (decarboxylating) | GCE; NKH; GCSP; HYGN1; MGC138198; MGC138200; GLDC | 238300 | NC_000009.10 | 9p22 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| GCSH | 2653 | glycine cleavage system protein H (aminomethyl carrier) | GCE; NKH; GCSH | 238330 | NC_000016.8 | 16q23.2 |
| AMT | 275 | aminomethyltransferase | GCE; NKH; GCST; AMT | 238310 | NC_000003.10 | 3p21.2-p21.1 |
| OTC | 5009 | ornithine carbamoyltransferase | OCTD; MGC129967; MGC129968; MGC138856; OTC | 300461 | NG_008471.1 | Xp21.1 |
| PAH | 5053 | phenylalanine hydroxylase | PH; PKU; PKU1; PAH | 612349 | NG_008690.1 | 12q22-q24.2 |
| DHPR | 5860 | quinoid dihydropteridine reductase | DHPR; PKU2; SDR33C1; FLJ42391; QDPR | 612676 | NG_008763.1 | 4p15.31 |
| PTS | 5805 | 6-pyruvoyltetrahydropterin synthase | PTPS; FLJ97081; PTS | 261640 | NG_008743.1 | 11q22.3-q23.3 |
| PCCA | 5095 | propionyl Coenzyme A carboxylase, alpha polypeptide | PCCA | 232000 | NG_008768.1 | 13q32 |
| PCCB | 5096 | propionyl Coenzyme A carboxylase, beta polypeptide | DKFZp451E113; PCCB | 232050 | NG_008939.1 | 3q21-q22 |
| ACADS | 35 | acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain | SCAD; ACAD3; ACADS | 606885 | NG_007991.1 | 12q22-qter |
| DHCR7 | 1717 | 7-dehydrocholesterol reductase | SLOS; DHCR7 | 602858 | NC_000011.8 | 11q13.2-q13.5 |
| SMN1 | 6606 | survival of motor neuron 1, telomeric | SMA; SMN; SMA1; SMA2; SMA3; SMA4; SMA@; SMNT; BCD541; T-BCD541; SMN1 | 600354 | NG_008691.1 | 5q13 |
| HEXA | 3073 | hexosaminidase A (alpha polypeptide) | TSD; MGC99608; HEXA | 606869 | NG_009017.1 | 15q23-q24 |
| MYO7A | 4647 | myosin VIIA | DFNB2; MYU7A; NSRD2; USH1B; DFNA11; MYOVIIA; MYO7A | 276903 | NG_009086.1 | 11q13.5 |
| USH1C | 10083 | Usher syndrome 1C (autosomal recessive, severe) | PDZ73; AIE-75; DFNB18; PDZ-45; PDZ-73; NY-CO-37; NY-CO-38; ush1cpst; PDZ-73/NY-CO-38; USH1C | 605242 | NC_000011.8 | 11p15.1-p14 |
| CDH23 | 64072 | cadherin-like 23 | USH1D; DFNB12; FLJ00233; FLJ36499; KIAA1774; KIAA1812; MGC102761; DKFZp434P2350; CDH23 | 605516 | NG_008835.1 | 10q21-q22 |
| PCDH15 | 65217 | protocadherin 15 | USH1F; DFNB23; DKFZp667A1711; PCDH15 | 605514 | NG_009191.1 | 10q21.1 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| SANS | 124590 | Usher syndrome 1G (autosomal recessive) | SANS; ANKS4A; FLJ33924; USH1G | 607696 | NG_007882.1 | 17q25.1 |
| ARX | 170302 | aristaless related homeobox | ISSX; PRTS; MRX29; MRX32; MRX33; MRX36; MRX38; MRX43; MRX54; MRX76; MRX87; MRXS1; ARX | 300382 | NG_008281.1 | Xp21 |
| OPHN1 | 4983 | oligophrenin 1 | OPN1; MRX60; OPHN1 | 300127 | NG_008960.1 | Xq12 |
| JARID1C | 8242 | lysine (K)-specific demethylase 5C | MRXJ; SMCX; MRXSJ; XE169; JARID1C; DXS1272E; KDM5C | 314690 | NG_008085.1 | Xp11.22-p11.21 |
| FTSJ1 | 24140 | FtsJ homolog 1 (E. coli) | JM23; MRX9; SPB1; TRM7; CDLIV; MRX44; FTSJ1 | 300499 | NG_008879.1 | Xp11.23 |
| SLC6A8 | 6535 | solute carrier family 6 (neurotransmitter transporter, creatine), member 8 | CRT; CT1; CRTR; MGC87396; SLC6A8 | 300036 | NC_000023.9 | Xq28 |
| DLG3 | 1741 | discs, large homolog 3 (Drosophila) | MRX; MRX90; NEDLG; NE-Dlg; SAP102; SAP-102; KIAA1232; DLG3 | 300189 | NC_000023.9 | Xq13.1 |
| TM4SF2 | 7102 | tetraspanin 7 | A15; MXS1; CD231; MRX58; CCG-B7; TM4SF2; TALLA-1; TM4SF2b; DXS1692E; TSPAN7 | 300096 | NG_009160.1 | Xp11.4 |
| ZNF41 | 7592 | zinc finger protein 41 | MRX89; MGC8941; ZNF41 | 314995 | NG_008238.1 | Xp11.23 |
| FACL4 | 2182 | acyl-CoA synthetase long-chain family member 4 | ACS4; FACL4; LACS4; MRX63; MRX68; ACSL4 | 300157 | NG_008053.1 | Xq22.3-q23 |
| PQBP1 | 10084 | polyglutamine binding protein 1 | SHS; MRX55; MRXS3; MRXS8; NPW38; RENS1; PQBP1 | 300463 | NC_000023.9 | Xp11.23 |
| PEX1 | 5189 | peroxisomal biogenesis factor 1 | ZWS1; PEX1 | 602136 | NG_008341.1 | 7q21.2 |
| PXMP3 | 5828 | peroxisomal membrane protein 3, 35kDa | PAF1; PEX2; PMP3; PAF-1; PMP35; RNF72; PXMP3 | 170993 | NG_008371.1 | 8q21.1 |
| PEX6 | 5190 | peroxisomal biogenesis factor 6 | PAF2; PAF-2; PXAAA1; PEX6 | 601498 | NG_008370.1 | 6p21.1 |
| PEX10 | 5192 | peroxisomal biogenesis factor 10 | NALD; RNF69; MGC1998; PEX10 | 602859 | NG_008342.1 | 1p36.32 |
| PEX12 | 5193 | peroxisomal biogenesis factor 12 | PAF-3; PEX12 | 601758 | NG_008447.1 | 17q12 |
| PEX5 | 5830 | peroxisomal biogenesis factor 5 | PXR1; PTS1R; PTS1-BP; FLJ50634; FLJ50721; FLJ51948; PEX5 | 600414 | NG_008448.1 | 12p13.31 |
| PEX26 | 55670 | peroxisomal biogenesis factor 26 | FLJ20695; PEX26M1T; Pex26pM1T; PEX26 | 608666 | NG_008339.1 | 22q11.21 |



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The mutations listed in Table 2 are documented polymorphisms in several disease-associated genes (CFTR is mutated in cystic fibrosis, GBA is mutated in Gaucher disease, ASPA is mutated in Canavan disease, HEXA is mutated in Tay Sachs disease). The polymorphisms are of several types: insertion/deletion polymorphisms which will cause frameshifts (and thus generally interrupt protein function) unless the insertion/deletion length is a multiple of 3bp, and substitutions which can alter the amino acid sequence of the protein and in some cases cause complete inactivation by introduction of a stop codon.

**Table 2: Non-limiting examples of polymorphisms**

| Gene name | GeneID | SNP ID | Mutation | SEQ ID NO: |
|---|---|---|---|---|
| CFTR | 1080 | rs63500661 | TCACATCACCAAGTTAAAAAAAAAAA[A/G]G GGGCGGGGGGGCAGAATGAAAATT | 1 |
| CFTR | 1080 | rs63107760 | AAACAAGGATGAATTAAGTTTTTTTT[-/T]AAAAAAGAAACATTTGGTAAGGGGA | 2 |
| CFTR | 1080 | rs62469443 | ATCACCAAGTTAAAAAAAAAAAAGGG[A/G]C GGGGGGGCAGAATGAAAATTGCAT | 3 |
| CFTR | 1080 | rs62469442 | CTATTGAACCAGAACCAAACAGGAAT[A/G]C CATAGCATTTTGTAAACTAAACTG | 4 |
| CFTR | 1080 | rs62469441 | CAGGAGTTCAAGACCAGCCTACTAAA[A/C]C ACACACACACACACACACACACAC | 5 |
| CFTR | 1080 | rs62469439 | GATTAAATAATAGTGTTTATGTACCC[C/G]GC TTATAGGAGAAGAGGGTGTGTGT | 6 |
| CFTR | 1080 | rs62469438 | ATTGTTATCTTTTCATATAAGGTAAC[A/T]GA GGCCCAGAGAGATTAAATAACAT | 7 |
| CFTR | 1080 | rs62469437 | TAATTTTAATTAAGTAAATTTAATTG[A/G]TA GATAAATAAGTAGATAAAAAATA | 8 |
| CFTR | 1080 | rs62469436 | GTATAAAAAAAAAAAAAAAAAAAGTT[A/T]G AATGTTTTCTTGCATTCAGAGCCT | 9 |
| CFTR | 1080 | rs62469435 | ATACTAAAAATTTAAAGTTCTCTTGC[A/G]AT ATATTTTCTTAATATCTTACATC | 10 |
| CFTR | 1080 | rs62469434 | TGCTGGGATTACAGGCGTGAGCCACC[A/G]C GCCTGGCCTGATGGGACATATTTT | 11 |
| CFTR | 1080 | rs62469433 | CTACAATATAAGTATAGTATTGCAAA[A/C]CC ATCAGGAAGGGTGTTAACTATTT | 12 |
| CFTR | 1080 | rs61763210 | GTTGTCTCCAAACTTTTTTTCAGGTG[-/AGA]AGGTGGCCAACCGAGCTTCGGAAAG | 13 |
| CFTR | 1080 | rs61720488 | TTTTTTCATAAAAGATTATATAAAGG[A/C]TA TTGCTTTTGAATCACAAACACTA | 14 |
| CFTR | 1080 | rs61481156 | ATCTAGTGAGCAGTCAGGAAAGAGAA[C/T]T TCCAGATCCTGGAAATCAGGGTTA | 15 |
| CFTR | 1080 | rs61443875 | TAGAGTATAAAAAAAAAAAAAAAAA[-/A]GTTTGAATGTTTTCTTGCATTCAGA | 16 |



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| | | | | |
|---|---|---|---|---|
| CFTR | 1080 | rs61312222 | TGCAAATGCCAACTATCAAAGATATT[C/G]GAGTATACTGTCAATAAACTTCATA | 17 |
| CFTR | 1080 | rs61159372 | TCCTCAACAGTTAGAAACAATATTTT[C/G]AGTGATTTCCCATGCCAACTTTACT | 18 |
| CFTR | 1080 | rs61094145 | TTTTTGGTATTGTTGTTAAATAAGTG[A/G]GAATTCAATACAGTATAATGTCTGT | 19 |
| CFTR | 1080 | rs61086387 | CTTGAAATCGGATATATATATATATA[-/TGTATATATATATATATATATATATATATACATATATATATATA]GTATTATCCCTGTTTTCACAGTTTT | 20 |
| CFTR | 1080 | rs60996744 | AGAGGGGCTGTGAAGGACACCAAGGA[A/G]GAGACTAAGAGCCAGGAGGGAAAAC | 21 |
| CFTR | 1080 | rs60960860 | TAGAGTTTATTAGCTTTTACTACTCT[A/G]CTTAGTTACTTTGTGTTACAGAATA | 22 |
| CFTR | 1080 | rs60923902 | ACTAGTGATGATGAGCTTCTTTTCAT[-/AT]GTTTGTTGGCTGCATAAATGTCTTC | 23 |
| CFTR | 1080 | rs60912824 | GCAGAGAAAAGAGGGGCTGTGAAGGA[C/G]ACCAAGGAGGAGACTAAGAGCCAGG | 24 |
| CFTR | 1080 | rs60887846 | TTCAGAGGTCTACCACTGGTGCATAC[G/T]CTAATCACAGTGTCGAAAATTTTAC | 25 |
| CFTR | 1080 | rs60793174 | AAGAAAGAGCAAAAGAGGGCAAACTT[C/T]TCATACATTTTTGATGTCGAAACCA | 26 |
| CFTR | 1080 | rs60788575 | CCTAAAGTTTAAAAAGAAAAAAAAAA[-/A]GGAAGAAGGAATTAAAAATCCAAAG | 27 |
| CFTR | 1080 | rs60760741 | GTGTGTGTGTGTATATATATATATAT[A/T]TATATATTTTTTTTTTCCTGAGCCA | 28 |
| CFTR | 1080 | rs60456599 | AAACTGTTGATGTTTTCATTTATTTA[C/G]ATCATTGGAAAACTTTAGATTCTAG | 29 |
| CFTR | 1080 | rs60363249 | TTTATCCATTCTTAACCAGAACAGAC[A/G]TTTTTTCAGAGCTGGTCCAGGAAAA | 30 |
| CFTR | 1080 | rs60355115 | TTGAAATCGGATATATATATATATAT[A/G]TATATATATATATATATATATATAT | 31 |
| CFTR | 1080 | rs60308689 | TAGTTTTTTATTTCCTCATATTATTT[-/T]CAGTGGCTTTTTCTTCCACATCTTT | 32 |
| CFTR | 1080 | rs60271242 | ACATAGTTCTCAGTGGTACAACTACA[A/G]GTGATTTCTCTTTTCTTATTTCTGG | 33 |
| CFTR | 1080 | rs60010318 | AGAGCAATGGCATCCCTTGTCTTGTG[C/T]TATACAGGATGCAGCAATTTATAGG | 34 |
| CFTR | 1080 | rs59961323 | TTCTGTCTACATAAGATGTCATACTA[A/G]ATTATCTTTTCCAGCATGCATTCAG | 35 |
| CFTR | 1080 | rs59961270 | CAGGGTGGCATGTTAGGCAGTGCTTA[A/G]AATAAATGAGTTGGTTATACAAGTA | 36 |
| CFTR | 1080 | rs59837506 | AGGACACACACACACACACACACACA[-/CA]TGCACACACATTTAAATAGATGCAT | 37 |
| CFTR | 1080 | rs59572090 | TAAAAAATTGGTATAATGAAATTGCA[C/T]TTGTAGTCTTTGGACATTTAAATCC | 38 |
| CFTR | 1080 | rs59548252 | TTTCAATACTTAAGAGGTACGCAGAG[A/G]AAAGAGGGGCTGTGAAGGACACCAA | 39 |
| CFTR | 1080 | rs59519859 | CAGCAATGAATATTTTGAGGCTGAGG[C/T]GCTGAGGGGTAAAATTGCAGCCTGG | 40 |
| CFTR | 1080 | rs59509837 | TTATGGTTTATATTTTTGTGTCTTCT[-/CTTT]AACACATCTTTTCTAGCAGAATTCA | 41 |



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| | | | | |
|---|---|---|---|---|
| CFTR | 1080 | rs59417037 | GTATTTTAGTTTTTTTTTTTTGTTTG[-/T]TTTGTTTTGTTTTGTTTTGTTTTTG | 42 |
| CFTR | 1080 | rs59159458 | TGGGTGACTCCATTTTTACTTTTAGT[C/T]TGGTCTGTTGAGGCCTCGTGAGAGA | 43 |
| CFTR | 1080 | rs59048119 | TATTTTCATGTATTTTAGTTTTTTTTT[-/TTTT]GTTTGTTTTGTTTTGTTTTGTTTTG | 44 |
| CFTR | 1080 | rs58970500 | GTGTGTGTGTATATATATATATATAT[A/T]TATATTTTTTTTTTCCTGAGCCAAA | 45 |
| CFTR | 1080 | rs58942292 | AACCTATTAGCATGTCTGGCAGAAAA[-/A]TAGATACTTAATAAATTTCTTAAAT | 46 |
| CFTR | 1080 | rs58917054 | GAGGCTTAGACAGTTTAAGTAACTCA[A/G]GCATGGTTACACAACTAGCTAGGGC | 47 |
| CFTR | 1080 | rs58837484 | GTGTGAGTATTATGAGACCATATGTT[A/G]GGAGATTTTATTTGGTATTGAGGAT | 48 |
| CFTR | 1080 | rs58829491 | GAAACCCCACCCCTTCTATAGTTTTC[C/T]CTTTAATATTTACAATGGAACCATT | 49 |
| CFTR | 1080 | rs58805195 | CATATATATATAGTGTGTGTGTGTGT[A/G]TATATATATATATATATATTTTT | 50 |
| GBA | 2629 | rs60866785 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC | 51 |
| GBA | 2629 | rs60239603 | GGCAGGTAATATCTAGTACCTTACTT[A/T]TATTTCCTGAGCACATTCTACATTT | 52 |
| GBA | 2629 | rs56310840 | GGCCAGGAATGGGAGTGCTTAGGTGC[A/G]GAGGTGGCACTGTTCCCGCAGCTGC | 53 |
| GBA | 2629 | rs41264927 | TGAAAACTCCATCCCCTCAGGGTCAT[C/T]AGATGAAGAGAAGACCACAGGGGTT | 54 |
| GBA | 2629 | rs41264925 | TGTAGGTAAGGGTCACATGTGGGAGA[C/G]GCAGCTGTGGGTAGGTCAGCCCTGT | 55 |
| GBA | 2629 | rs36024691 | CCAAGAAGGCGCCATTACACTCCAGC[-/C]TGGGCGACAGGGCGAGACTCCCTCA | 56 |
| GBA | 2629 | rs36024092 | TGCCACACCCAGCTAATTTGTGTGTG[-/G]TATGTGTGTGTATGTATGTGTGTGT | 57 |
| GBA | 2629 | rs35682967 | GTTCCTCCAGTAATTTTTTTTTTTTT[-/T]GGTTTTGAGACAGAGTCTTGCCCTG | 58 |
| GBA | 2629 | rs35033592 | ATCATGCCCAGATAATTTTTTTTTTT[-/T]GTATTTTAGTAGACACAGGGTTTCA | 59 |
| GBA | 2629 | rs34732744 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC | 60 |
| GBA | 2629 | rs34620635 | CCTGTGAGGGGCACATTCCTTAGTAG[-/C]TAAGGAGTTGGGGGTGTGAAGATCC | 61 |
| GBA | 2629 | rs34302637 | ACAGGCTACTGGCTGGGCCCAGGCAA[-/A]GGGGGCCTTGGCAGGAAAAGTTCCT | 62 |
| GBA | 2629 | rs33949225 | GCGAGAGAGAGAGAGAGAGAGAGAGG[-/AG]AGCCGGCGCGAGAACTACGCATGCG | 63 |
| GBA | 2629 | rs28678003 | AAGAAGAAAAATAAAAAGAAAGTGGG[C/T]CAGACCGAGAGAACAGGAAGCCTGA | 64 |
| GBA | 2629 | rs28559737 | AAGGACAAAGGCAAAGAGACAAAGGC[G/T]CAACACTGGGGGTCCCCAGAGAGTG | 65 |
| GBA | 2629 | rs28373017 | TACCTAGTCACTTCCTGCCTCCATGG[C/T]GCAAAAGGGGATGGGTGTGCCTCTT | 66 |
| GBA | 2629 | rs12752133 | CTCTTCCGAGGTTCCACCCTGAACAC[C/T]TTCCTGCTCCCTCGTGGTGTAGAGT | 67 |



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| | | | | |
|---|---|---|---|---|
| GBA | 2629 | rs12747811 | TTCTGACTGGCAACCAGCCCCACTCT[C/T]TG GGAGCCCTCAGGAATGAACTTGC | 68 |
| GBA | 2629 | rs12743554 | gctcagcctcccaggctggagtgcag[A/T]ggcgcgatctcggctcac cgcaacc | 69 |
| GBA | 2629 | rs12041778 | CATGAACCACATCAAATGAGATTTAG[C/T]GG GAGTGGCACACACAGTCATGACC | 70 |
| GBA | 2629 | rs12034326 | AAGCAGCCCTGGGGAGTCGGGGCGGG[A/G]C CTGGATTGGAAAAGAGACGGTCAC | 71 |
| GBA | 2629 | rs11558184 | CTCCAAGTTCTGGGAGCAGAGTGTGC[A/G]G CTAGGCTCCTGGGATCGAGGGATG | 72 |
| GBA | 2629 | rs11430678 | GTTCCTCCAGTAAttttttttttt[- /G/T]gttttgagacagagtcttgccctgt | 73 |
| GBA | 2629 | rs11264345 | CTAGTACCTTACTTCCCTCAAGTTCA[A/T]TC ATCTCACAGATATTTCCTGAGCA | 74 |
| GBA | 2629 | rs10908459 | aattagccgtgcgtggtggcgggtgc[C/T]tgtaatcccacgtacttgg gaggct | 75 |
| GBA | 2629 | rs10796940 | CCATGGCCAGCCGGGGAGGGGACGGG[A/C]A CACACAGACCCACACAGAGACTCA | 76 |
| GBA | 2629 | rs10668496 | agcgagagagagagagagagagagag[- /AG]gagCCGGCGCGAGAACTACGCATGC | 77 |
| GBA | 2629 | rs7416991 | CGTAGCAGTTAGCAGATGATAGGCGG[C/G/T] GAAATCTTATTTCACAGGGCATTAA | 78 |
| GBA | 2629 | rs4024049 | CTGGCCCTGGTGACAGTGGGGCTGTG[C/T]GT GGGGCCAGAGCCTTCTCAGAGGT | 79 |
| GBA | 2629 | rs4024048 | CAGATACTGGCCCTGGTGACAGTGGG[A/G]C TGTGCGTGGGGCCAGAGCCTTCTC | 80 |
| GBA | 2629 | rs4024047 | GACAGATACTGGCCCTGGTGACAGTG[G/T]G GCTGTGCGTGGGGCCAGAGCCTTC | 81 |
| GBA | 2629 | rs3841430 | GGCTCctctctctctctctctctc[- /TC]gctcgctctctcgctctctcgctct | 82 |
| GBA | 2629 | rs3754485 | GTTTCAGACCAGCCTGGCCAACATAG[C/T]GA AACCCCATCTCTACTAAAAATAA | 83 |
| GBA | 2629 | rs3205619 | AGTGGGCGATTGGATGGAGCTGAGTA[C/T]G GGGCCCATCCAGGCTAATCACACC | 84 |
| GBA | 2629 | rs2990227 | CCGGGCTCCGTGAATGTTTGTCACAT[C/G]TC TGAAGAACGTATGAATTACATAA | 85 |
| GBA | 2629 | rs2990226 | GAATCCCAACCCCGACGCTCGTCGCC[C/G]G GCTCCGTGAATGTTTGTCACATGT | 86 |
| GBA | 2629 | rs2990225 | GCGAATCCCAACCCCGACGCTCGTCG[C/T]CG GGCTCCGTGAATGTTTGTCACAT | 87 |
| GBA | 2629 | rs2990224 | TGGGCAGAAGTCAGGGTCCAAAGAAA[G/T]G GCAAAGAAAAGTGTcagtggctca | 88 |
| ASPA | 443 | rs63751297 | TAAGAAAGACGTTTTTGATTTTTTTC[A/G]GA CTTCTCTGGCTCCACTACCCTGC | 89 |
| ASPA | 443 | rs62071301 | CTGATTCCTGGCCAGGAGCGGTGGCT[C/T]AC GCCTGTAATCCCAGCGCTTTGGG | 90 |
| ASPA | 443 | rs62071300 | TAAAAATGCTGATTCCTGGCCAGGAG[C/T]GG TGGCTCACGCCTGTAATCCCAGC | 91 |
| ASPA | 443 | rs62071299 | TTTAAAAATGCTGATTCCTGGCCAGG[A/C]GC GGTGGCTCACGCCTGTAATCCCA | 92 |
| ASPA | 443 | rs62071297 | CAAGACCTGTCAAAGATCTGAGAAAT[A/T]TT ACCCGACTTACAAGCTAACCATT | 93 |



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| | | | | |
|---|---|---|---|---|
| ASPA | 443 | rs61697033 | ACTGTAATAAGTGCTGTAAAAGAAAT[A/G]C ACAAAATAATATAGCAGAGGGTAT | 94 |
| ASPA | 443 | rs60743592 | CTTGAGGTCAGGAGTTCAAGACCAGT[C/T]TG GGCAACATGGGGAAAACCTTGTC | 95 |
| ASPA | 443 | rs60666840 | AGGTTGCAGTGAGCCGAGATCATGCC[A/G]TT GCACTCCAGCCGGGGCAACAAAA | 96 |
| ASPA | 443 | rs60147514 | ACAAGTGTCTTGAAATTATCTGTGAT[C/T]TG CTATAGAGCAATACTTTTGTAAA | 97 |
| ASPA | 443 | rs59930743 | GTGGGTATATGCAGCTCTATGCACTA[C/T]CT GCTCATTTATTTGGTAAATCTAA | 98 |
| ASPA | 443 | rs59690349 | TGTGTGTGTGTGCGTGTGTGTGTGTG[- /TGTGTGTG]ATCATAAGAGTGGCTGCAGCAA ACT | 99 |
| ASPA | 443 | rs59676360 | AGTCTGGAGTGCAATGGTGCAATCTC[A/G]GC TCACTGCAGCCTCCACCTCCGGG | 100 |
| ASPA | 443 | rs59335404 | CTCCTAATGGATATTTCCTAAATTTT[G/T]CTG AACAGAATTTAACTTGAGCTGG | 101 |
| ASPA | 443 | rs58879097 | ATTTAAAAATGGATTTCTAGAAAAAC[A/G]AT CACATACTTGAATATTTTAGCAA | 102 |
| ASPA | 443 | rs58686774 | CTATAAATGGGTAGCATGAGGGATTC[A/G]A GGAGGTGGCTGAAAGAAGCACGTA | 103 |
| ASPA | 443 | rs57511162 | AAGAAACCAAGCATAGTAGAGTGTTA[A/G]A AAACCAAAGCAACTAAACAACTGT | 104 |
| ASPA | 443 | rs55859596 | CGGGGCTCAGAACTTGTAACAGAAAA[A/T]T AAAATATACTCCACTCAAGGGAAT | 105 |
| ASPA | 443 | rs55742972 | TACTACACTTCACGGATACTGTACTT[- /GTACTT]TTTTTCCAAATTGAAGGTTTTTGGC | 106 |
| ASPA | 443 | rs55640436 | TTGTTTTTGTTTTTGTTTTTGTTTTT[- /GTTTTTGTTTTT]TGAGATGGAGTCTCGCTCT GTCGCC | 107 |
| ASPA | 443 | rs36225687 | TTTGCCTTACTACACTTCACGGATAC[- /TGTACT]TGTACTTTTTTTCCAAATTGAAGGT | 108 |
| ASPA | 443 | rs36051310 | GAGGTGGCTGAAAGAAGCACGTATCC[- /C]TGATGGCATGGTTGCGGGTTATATG | 109 |
| ASPA | 443 | rs36034906 | GAGAAAAGCAGTTCCTGGAACACCCC[- /C]ACCCCTTAACCCCTTATCTCTGCTT | 110 |
| ASPA | 443 | rs36033666 | TTACATATGTATACATGTGCCATGTT[- /T]GGTGTGCCGCACCCATTAACTCGTC | 111 |
| ASPA | 443 | rs35730123 | CTTTTTCCAGATTTTTTTTTTTTTTT[- /T]GAGACAGAGTTTCACTCTTGTTGCC | 112 |
| ASPA | 443 | rs35629100 | TTTGGAAATCTTAAGCTTTTATTTGG[- /G]TGTCACAGAGAAACAGGATCTGTAT | 113 |
| ASPA | 443 | rs35614631 | TACTTTAAGTTTTAGGGTACATGTGC[- /A]CCATGTGCAGGTTTGTTACATATGT | 114 |
| ASPA | 443 | rs35225782 | ATTCATGACCAGCCACATAAATGCAC[- /A]GTATTACTTCGCAAGCATGCCAATG | 115 |
| ASPA | 443 | rs35178659 | GTGCACTAGAATTAGCTAAAGTGGGG[- /G]AAAAAAAGATGCATTTGATGGTCTA | 116 |
| ASPA | 443 | rs35095578 | AACCTCCACCTCCCAGGTTCAAGAGA[- /A]TTCTCCTGCCTCAGCCTCCCAAGTA | 117 |
| ASPA | 443 | rs35002210 | CCTCCCTGTGATCCGAAGTAGCAGAC[A/G]TA CTTAACTTCCATGGTGGATTGTT | 118 |



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| | | | | |
|---|---|---|---|---|
| ASPA | 443 | rs34744839 | AAAACATTATTATATCTAGAAAAAAA[-/A]TGTATCTTAACCATTGTGGGAAGTG | 119 |
| ASPA | 443 | rs34680506 | TTGAAGGTAAAATCATAGGGAGTTGG[-/G]AGCTGTCCTCTTGCGCTGAATCAGT | 120 |
| ASPA | 443 | rs34365618 | ACTTGTGGCCTTTTTGGAGAGGTTAG[-/CA]ACTCTGAAAACTCTGTCCCTGGACC | 121 |
| ASPA | 443 | rs34275920 | GAAGGAGAAAAAGAGAGGAAATAAGT[-/T]AAAATAATAAACACAATTAATAAAG | 122 |
| ASPA | 443 | rs34109510 | TGTATACATGTGCCATGTTGGTGTGC[C/T]GCACCCATTAACTCGTCATTTAGCA | 123 |
| ASPA | 443 | rs34054576 | TCACCTGTCACCTCCTATAGAACTTT[-/C]CCCTGACCCTCCTCTATAGCATTAA | 124 |
| ASPA | 443 | rs34015272 | ATAAATGATCATCATTCACAGTAGGG[-/G]TTTTGTTTTGTTTTTTTTCTGGAAA | 125 |
| ASPA | 443 | rs34002091 | ACAGACATATCTACAAACACACTTTT[-/T]CACATATTTGTGTAAGTCATTTATG | 126 |
| ASPA | 443 | rs28940574 | AAAGACAACTAAACTAACGCTCAATG[A/C]AAAAAGTATTCGCTGCTGTTTACAT | 127 |
| ASPA | 443 | rs28940279 | TACCGTGTACCCCGTGTTTGTGAATG[A/C]GGCCGCATATTACGAAAAGAAAGAA | 128 |
| ASPA | 443 | rs17850703 | CAGGGCTGGAGGTAAAACCATTTATT[A/G]CTAACCCCAGAGCAGTGAAGAAGTG | 129 |
| ASPA | 443 | rs17222495 | TTCTTCATTGCCTATTGAAGAGAGAG[C/T]GGAATGCTTTGGTTGCCAGATATGG | 130 |
| ASPA | 443 | rs17175228 | CACAAGATCTCATTACTCAGGAGCTG[C/T]CCAAGTGTCTAATGTACTTAGTTAA | 131 |
| ASPA | 443 | rs16953074 | TTCTGTGTAACATTTCATTTAAGCAA[A/G]GGATTCGGCAAATCAAAAATTGTCA | 132 |
| ASPA | 443 | rs16953070 | TAAAACGTATTGAAGGTATTATTGAC[G/T]CTGTTGAAGCAAAGAGAACAAAACA | 133 |
| HEXA | 3073 | rs62022858 | ATCTGCTCTTCCAGTTGGATGACAAG[C/T]CTTGCTGTCTAACACCTGCTGCAGA | 134 |
| HEXA | 3073 | rs62022857 | CCATTTTTTGTTGTATTTTTTTTTTC[C/T]TGAATACTTTTTATCGCAGTTGGTT | 135 |
| HEXA | 3073 | rs62017872 | CCCTGTCTCTAAAAGAAAAAAAAAAA[A/G]AAAAAAAAAGAAAACAAAACCCAA | 136 |
| HEXA | 3073 | rs62017871 | AGTGGCTCCAAAAAGGTCATGGAACC[C/T]CTTGAGGATGATGCAAATTGACTCT | 137 |
| HEXA | 3073 | rs61662730 | TAAAGTTACTTTTCTTTTATTGACTT[C/T]CCCTTATTTTTTAACCTTATGCTTT | 138 |
| HEXA | 3073 | rs61329913 | CAGAGTTAAAAAAAAAAAAAAAAAA[-/A]GGAAGTAGCAGCAACAGCTTGGAAA | 139 |
| HEXA | 3073 | rs60920713 | GTTGCCCAGGGTTGAGTGCAGAGGCA[C/T]ATCTTGGCTCACAGCAACCTCTGCC | 140 |
| HEXA | 3073 | rs60783213 | AAGGCTTTTTTTTTTTTTTTTTTTT[-/TTTT]GAGACAGAGTCTTGCTGTGTCACCC | 141 |
| HEXA | 3073 | rs60644867 | GCCTACATTCTGCAAAGAGGAGGGAA[C/G]ATTCACAGCTCCATACTTGAACCCT | 142 |
| HEXA | 3073 | rs60288568 | CCAAAGGAGAATAGCTCTAGGGGAGG[C/G]AGGTGGATGAGTATGCATGGGGGAG | 143 |
| HEXA | 3073 | rs59888548 | GACTCCATCTCAAAAAAAAAAAAAA[-/A]TGCAGTCTAATGGCAGAATTAGACT | 144 |



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|---|---|---|---|---|
| HEXA | 3073 | rs59733856 | TTATTTATTTATTTATTTATTTTTGA[A/G]ACA GGGTCTCTGTTGTCCAGGCTGG | 145 |
| HEXA | 3073 | rs59427837 | TTTTGAGGCAGGGTCTCACTCTGTTG[C/T]CC AGGGTTGAGTGCAGAGGCACATC | 146 |
| HEXA | 3073 | rs59171976 | CGCCTTGCGAAGGCCCCACAGCTTGC[C/T]TG TGACAAACGTTCATAGGCAAATG | 147 |
| HEXA | 3073 | rs58706602 | GGAGGTCTGTACAAAGCACCACCTAC[C/T]TC ATGGGTCAGTTTCCACAGCAGAA | 148 |
| HEXA | 3073 | rs58696963 | GAATCTTATAATTCACTGTGTACCTC[- /CCTC]TGTTTCATATTTTCGCAATTGAACT | 149 |
| HEXA | 3073 | rs58610850 | AACATAGTATCTAATATAGCTTTACA[C/T]CC AAAGCCAAAATATGAATACACTG | 150 |
| HEXA | 3073 | rs58016062 | TTGTTTTGTTTTGTTTGGGGGGGGGG[- /G]TTGTTTTTCTGAGAGGGAGTCTTGC | 151 |
| HEXA | 3073 | rs57733983 | CATACCAAAGGGCAGCTGGAGGGATA[C/T]A GACGGAAGTCATGTGGAGAGTGAA | 152 |
| HEXA | 3073 | rs57476645 | CAGGTGTGAGCCACCACGACCACCAA[A/T]T TAGCTCTTTTTACTCCTTCCCTTC | 153 |
| HEXA | 3073 | rs56870003 | AGTGGTAGCTGATTTTGCTTCTGGAT[A/C]CT TTGCCACCTTCCCACTCTTTAAT | 154 |
| HEXA | 3073 | rs56338339 | AAAGACCTGTTTCTTAAAAAAAAAAA[- /AGAAAAAAAAAAA]GAAAGAAAAGAAAAG AAAAAAACAG | 155 |
| HEXA | 3073 | rs55995352 | TAAAAAATCTTTCAATGAGGAGATGT[C/T]CC CAGAGCAAGACAGCTGTAGGATG | 156 |
| HEXA | 3073 | rs55860138 | AAAAGAAAAAAAAAAAAAAAAAAAA[- /A]GAAAACAAAACCCAAACCCATAAAG | 157 |
| HEXA | 3073 | rs55743646 | CCTGTCTCTAAAAGAAAAAAAAAAAA[A/G]A AAAAAAAAGAAAACAAAACCCAAA | 158 |
| HEXA | 3073 | rs55665666 | GTTATCATAGAAAAATATCACACTCT[- /GT]CTGTATCCCCACTTCCAGAAACTGT | 159 |
| HEXA | 3073 | rs36106892 | CAGGAGCTCATAGAATTACATACAAT[- /C]TTTTTTTTTTTTTTTTTGAGACAGCG | 160 |
| HEXA | 3073 | rs36091525 | TTGAGAATCTTATAATTCACTGTGTA[- /CCTC]CCTCTGTTTCATATTTTCGCAATTG | 161 |
| HEXA | 3073 | rs35949555 | CCACTACCACAGTGCCTAGAGAACAA[C/T]A TGTGTTTAATAATATTTAAATAAT | 162 |
| HEXA | 3073 | rs35827424 | CCCTGTCTCTAAAAGAAAAAAAAAAA[- /A]AAAAAAAAAAGAAAACAAAACCCAA | 163 |
| HEXA | 3073 | rs35729578 | CCATTATATCATTCATTTCCCACTCA[- /T]TTTCTTCATTCCAACCAAGATATAT | 164 |
| HEXA | 3073 | rs35649102 | TCCGTCTCAAAAAAAAAAAAAAAAAG[- /A]GAAAGGAATTATTCTCATGTATACA | 165 |
| HEXA | 3073 | rs35118677 | CTGGGGCAGTTAAAAAGAAAAACAAA[- /C]CCCTGGTCCCTGCCCTTGAGGAGAT | 166 |
| HEXA | 3073 | rs35005352 | CTCCAGGGTCCCATTCCAGGACCACA[- /C]GCCTGCTACCTCTGCAGCTCACTCA | 167 |
| HEXA | 3073 | rs34736306 | GGATTGACATATACCAGTTAGACGGA[- /T]TTTTTTTTTCCATAAACCAGGCTCA | 168 |
| HEXA | 3073 | rs34607939 | ACAAATAATTACTACATATCTACAAC[A/G]TT CCAGATACAGAAGAAATGGCCAA | 169 |



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| | | | | |
|---|---|---|---|---|
| HEXA | 3073 | rs34496117 | TAAACACACTTGAAACATCATATAAA[-/ATG]ATATTACTACAAGACTTAACCGTAA | 170 |
| HEXA | 3073 | rs34300017 | ACACAGGTAATCCATGTTTATTATAG[-/A]AAAATGCCACATTACTCTTTATTGA | 171 |
| HEXA | 3073 | rs34206496 | AGTTATCATAGAAAAATATCACACTC[-/TG]TCTGTATCCCCACTTCCAGAAACTG | 172 |
| HEXA | 3073 | rs34110830 | AATGAACTTACAGGAAGGTAATATAT[-/G]GGAAATAAACATCTTATTGAATTTA | 173 |
| HEXA | 3073 | rs34093438 | GGACCCCTGAAAGGCACAAGACACCC[-/T]TTCAGGTTCACACTTCCTGAAAGCT | 174 |
| HEXA | 3073 | rs34085965 | CCACCAATCACCAGAGCCTTCTGCTC[A/G]GGGGTACCTGAGGGAAAACAAGCAA | 175 |
| HEXA | 3073 | rs34004907 | AAAGACTGAAAAAACATTCATAACTA[-/T]TTTTCTTGTTATCCTCGGAAATGTC | 176 |
| HEXA | 3073 | rs28942072 | TATCTTCATCTTGGAGGAGATGAGGT[C/T]GATTTCACCTGCTGGAAGTCCAACC | 177 |
| HEXA | 3073 | rs28942071 | TTGCCTATGAACGTTTGTCACACTTC[C/T]GCTGTGAGTTGCTGAGGCGAGGTGT | 178 |
| HEXA | 3073 | rs28941771 | GCTTGCTGTTGGATACATCTCGCCAT[C/T]ACCTGCCACTCTCTAGCATCCTGGA | 179 |
| HEXA | 3073 | rs28941770 | CCGGGGCTTGCTGTTGGATACATCTC[G/T]CCATTACCTGCCACTCTCTAGCATC | 180 |

*3. Nucleic acid target length evaluation:*

In some embodiments, aspects of the invention relate to methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats.

Genomic regions that contain nucleic acid sequence repeats are often the site of genetic instability due to the amplification or contraction of the number of sequence repeats (e.g., the insertion or deletion of one or more units of the repeated sequence). Instability in the length of genomic regions that contain high numbers of repeat sequences has been associated with a number of hereditary and non hereditary diseases and conditions.

For example, "Fragile X syndrome, or Martin-Bell syndrome, is a genetic syndrome which results in a spectrum of characteristic physical, intellectual, emotional and behavioral features which range from severe to mild in manifestation. The syndrome is associated with the expansion of a single trinucleotide gene sequence (CGG) on the X chromosome, and results in a failure to express the FMR-1 protein which is required for normal neural development. There are four generally accepted forms of Fragile X syndrome which relate to the length of the repeated CGG sequence; Normal (29-31 CGG



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repeats), Premutation (55-200 CGG repeats), Full Mutation (more than 200 CGG repeats), and Intermediate or Gray Zone Alleles (40 - 60 repeats)."

Other examples include cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26). The are currently many sequencing-based assays for determining the number of nucleic acid sequence repeats at a particular locus and identifying the presence of nucleic acid insertions or deletions. However, such techniques are not useful in a high throughput multiplex analysis where the entire length of a region may not be sequenced.

In contrast, in some embodiments, aspects of the invention relate to detecting the presence of an insertion or deletion at a genomic locus without requiring the locus to be sequenced (or without requiring the entire locus to be sequenced). Aspects of the invention are particularly useful for detecting an insertion or deletion in a nucleic acid region that contains high levels of sequence repeats. The presence of sequence repeats at a genetic locus is often associated with relatively high levels of polymorphism in a population due to insertions or deletions of one or more of the sequence repeats at the locus. The polymorphisms can be associated with diseases or predisposition to diseases (e.g., certain polymorphic alleles are recessive alleles associated with a disease or condition). However, the presence of sequence repeats often complicates the analysis of a genetic locus and increases the risk of errors when using sequencing techniques to determine the precise sequence and number of repeats at that locus.

In some embodiments, aspects of the invention relate to determining the size of a genetic locus by evaluating the capture frequency of a portion of that locus suspected of containing an insertion or deletion (e.g., due to the presence of sequence repeats) using a nucleic acid capture technique (e.g., a nucleic acid sequence capture technique based on molecular inversion probe technology). According to aspects of the invention, a statistically significant difference in capture efficiency for a genetic locus of interest in different biological samples (e.g., from different subjects) is indicative of different relative lengths in those samples. It should be appreciated that the length differences may be at one or both alleles of the genetic locus. Accordingly, aspects of the invention may be used to identify polymorphisms regardless of whether biological samples being interrogated at heterozygous or homozygous for the polymorphisms. According to



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aspects of the invention, subjects that contain one or more loci with an insertion or deletion can be identified by analyzing capture efficiencies for nucleic acids obtained from one or more biological samples using appropriate controls (e.g., capture efficiencies for known nucleic acid sizes, capture efficiencies for other regions that are not suspected of containing an insertion or deletion in the biological sample(s), or predetermined reference capture efficiencies, or any combination thereof. However, it should be appreciated that aspects of the invention are not limited by the nature or presence of the control. In some embodiments, if a statistically significant variation in capture efficiency is detected, a subject may be identified as being at risk for a disease or condition associated with insertions or deletions at that genetic locus. In some embodiments, the subject may be analyzed in greater detail in order to determine the precise nature of the insertion or deletion and whether the subject is heterozygous or homozygous for one or more insertions or deletions. For example, gel electrophoresis of an amplification (e.g., PCR) product of the locus, or Southern blotting, or any combination thereof can be used as an orthogonal approach to verify the length of the locus. In some embodiments, a more exhaustive and detailed sequence analysis of the locus can be performed to identify the number and types of insertions and deletions. However, other techniques may be used to further analyze a locus identified as having an abnormal length according to aspects of the invention.

Accordingly, aspects of the invention relate to detecting abnormal nucleic acid lengths in genomic regions of interest. In some embodiments, the invention aims to estimate the size of genomic regions that are hard to be accessed, such as repetitive elements. However, it should be appreciated that methods of the invention do not require that the precise length be estimated. In some embodiments, it is sufficient to determine that one or more alleles with abnormal lengths are present at a locus of interest (e.g., based on the detection of abnormal capture efficiencies).

In a non-limiting example, fragile X can be used to illustrate aspects of the invention where the size of trinucleotide repeats (genotype) is linked to a symptom (phenotype). However, it should be appreciated that fragile X is a non-limiting example and similar analyses may be performed for other genetic loci (e.g., independently or simultaneously in multiplex analyses).

Use of molecular inversion probes (MIPs) has been demonstrated for detection of single nucleotide polymorphisms (Hardenbol et al. 2005 Genome Res 15:269-75) and for



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preparative amplification of large sets of exons (Porreca et al. 2007 Nat Methods 4:931-6, Krishnakumar et al. 2008 Proc Natl Acad Sci USA 105:9296-301). In both cases, oligonucleotide probes are designed which have ends ('targeting arms') that hybridize up-stream and down-stream of the locus that is to be amplified.

In some embodiments, aspects of the invention are based on the recognition that the effect of length on probe capturing efficiency can be used in the context of an assay (e.g., a high throughput and/or multiplex assay) to allow the length of sequences to be determined without requiring sequencing of the entire region being evaluated. This is particularly useful for repeat regions that are prone to changes in size. As illustrated in FIG. 8, which is reproduced from Deng et al., Nature Biotech. 27:353-60, (see Supplemental Fig. 1G of Deng et al.,) illustrates that shorter sequences are captured with higher efficiency that longer sequences using MIPs. The statistical package R and its *effects* module were used for this analysis. A linear model was used, and each individual factor was assumed to be independent. The dashed lines represent a 95% confidence interval. Shorter target sequences were captured with higher efficiency than long target sequences ($p < 2x10^{-16}$). However, the use of this differential capture efficiency for systematic sequence length analysis was not previously recognized.

In some embodiments, following probe hybridization, polymerase fill-in and ligation reactions are performed to convert the hybridized probe to a covalently-closed, circular molecule containing the desired target. PCR or rolling circle amplification plus exonuclease digestion of non-circularized material is performed to isolate and amplify the circular targets from the starting nucleic acid pool. Since one of the main benefits of the method is the potential for a high degree of multiplexing, generally thousands of targets are captured in a single reaction containing thousands of probes.

According to aspects of the invention, repetitive regions are surrounded by non-repetitive unique sequences, which can be used to amplify the repeat-containing regions using, for example, PCR or padlock (MIP)-based method.

In addition to the repetitive regions, a probe (e.g., a MIP or padlock probe) can be designed to include at least a sequence that is sufficient to be uniquely identified in the genome (or target pool). After the probe is circularized and amplified, the amplicon can be end-sequenced so that the unique sequence can be identified and served as the "representative" of the repetitive region as illustrated in FIG. 9. FIG. 9 illustrates a non-limiting scheme of padlock (MIP) capture of a region that includes both repetitive



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regions (thick wavy line) and the adjacent unique sequence (thick strait line). The regions of the probe are indicated with the targeting arms shown as regions "1" and "3." An intervening region that may be, or include, a sequencing primer binding site is shown as "2." After the padlock is circularized and amplified, it can be end-sequenced to obtain the sequence of the unique sequence, which represents the repetitive region of interest. Although capturing efficiency is overall negatively correlated with target length, different probe sequences may have unique features. Therefore, multiple probes could be designed and tested so that an optimal one is chosen to be sensitive enough to differentiate repetitive sizes of roughly 0-150bp, 150-600bp, and beyond, which represent normal, premutation and full mutation of fragile X syndrome, respectively. However, it should be appreciated that other probe sizes and sequences can be designed, and optionally optimized, to distinguish a range of repeat region size differences (e.g., length differences of about 3-30 bases, about 30-60 bases, about 60-90 bases, about 90-120 bases, about 120-150 bases, about 150-300 bases, about 300-600 bases, about 600-900 bases, or any intermediate or longer length difference). It should be appreciated that a length difference may be an increase in size or a decrease in size.

In some embodiments, an initial determination of an unexpected capture frequency is indicative of the presence of size difference. In some embodiments, an increase in capture frequency is indicative of a deletion. In some embodiments, a decrease in capture frequency is indicative of an insertion. However, it should be appreciated that depending on specific sequence parameters and the relative sizes of the capture probes, the target region, and the deletions or insertions, a change in capture frequency can be associated with either an increase or decrease in target region length. In some embodiments, the precise nature of the change can be determined using one or more additional techniques as described herein.

Accordingly, in some aspects a MIP probe includes a linear nucleic acid strand that contains two hybridization sequences or targeting arms, one at each end of the linear probe, wherein each of the hybridization sequences is complementary to a separate sequence on a the same strand of a target nucleic acid, and wherein these sequences on the target nucleic acid flank the two ends of the target nucleic acid sequence of interest. It should be appreciated that upon hybridization, the two ends of the probe are inverted with respect to each other in the sense that both 5' and 3' ends of the probe hybridize to



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the same strand to separate regions flanking the target region (as illustrated in FIG. 9 for example).

In some embodiments, the hybridization sequences are between about 10-100 nucleotides long, for example between about 10-30, about 30-60, about 60-90, or about 20, about 30, about 40, or about 50 nucleotides long. However, other lengths may be used depending on the application. In some embodiments, the hybridization Tms of both targeting arms of a probe are designed or selected to be similar. In some embodiments, the hybridization Tms of the targeting arms of a plurality of probes designed to capture different target regions are selected or designed to be similar so that they can be used together in a multiplex reaction. Accordingly, a typical size of a MIP probe prior to fill-in is about 60-80 nucleotides long. However, other sizes can be used depending on the sizes of the targeting arms and any other sequences (e.g., primer binding or tag sequences) that are present in the MIP probe. In some embodiments, MIP probes are designed to avoid sequence-dependent secondary structures. In some embodiments, MIP probes are designed such that the targeting arms do not overlap with known polymorphic regions. In some embodiments, targeting arms that can be used for capturing the repeat region of the Fragile X locus can have the following sequences or complementary to these sequences depending on the strand that is captured.

left: CTCCGTTTCGGTTTCACTTC (SEQ ID NO: 181)

right: ATCTTCTCTTCAGCCCTGCT (SEQ ID NO: 182)

The typical captured size using these targeting arms is about 100 nucleotides in length (e.g., about 30 repeats of a tri-nucleotide repeat).

In some embodiments, the number of reads obtained for the "representative" of the repetitive region is not informative to estimate the target length because it is dependent on the total number of reads obtained. To overcome this, it is useful to include one or more probes that target other "control" regions where no or minimal polymorphism exists among populations. Because of the systematic consistency of capturing efficiency (see, e.g., FIG. 9), the ratio of reads obtained for the repetitive "representative" to reads obtained for the control region(s) will be tuned using DNA with defined numbers of repeats. Ultimately, the ratio can serve as a measure of the repeat length as illustrated in FIG. 10. FIG. 10 illustrates a non-limiting hypothetical relationship between target gap size and the relative number of reads of the repetitive



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region, which is measured by the ratio of the repeat "representative" reads vs. the "control" region reads. The unit of y-axis is arbitrary.

In some embodiments, to better tell targets with similar size range apart, the whole repetitive region can be sequenced by making a shotgun library (e.g., by making a shotgun library from a captured sequence, for example a sequence captured using a MIP probe). The longer the repeat is, the more short reads of repeats will be obtained. Therefore, the target length will contribute twice to the relative number of "repetitive" reads, which will gain better resolution of differentiating targets. In some embodiments, the expectation is that the number of reads from any given repeat will be a direct function of the number of repeats present. However, in some embodiments, a Poisson sampling-induced spread may need to be considered and in some embodiments may be sufficiently large to limit the resolution.

When a precise measurement of the length of both alleles from a diploid sample is desired, further manipulations may be required. This is because the capture efficiency measured will actually be the average efficiency of the two alleles. To effectively achieve separate measurements for each allele, barcodes (e.g., sequence tags) can be used that allow the efficiency of individual capture events (from individual genomic loci) to be followed. FIG. 11A-C shows the approach. For a given locus, MIPs are synthesized to contain one of a large number differentiator tags in their backbone such that the probability of any two MIPs in a reaction having the same differentiator tag sequence is low. MIP capture is performed on the sample; the reaction will be biased for shorter target lengths, and therefore the reaction product will be comprised of more 'short' circles than 'long' circles. Each circle should bear a unique differentiator tag sequence. Then, linear RCA (lRCA) is performed on the circles. In the lRCA reaction, circles are converted into long, linear concatemers of themselves. The lRCA reaction for a given circle stops when the concatemer has reached a 'fixed' length (based on the processivity/error rate of the polymerase). Concatemers derived from smaller circles will therefore contain more copies of the differentiator tag, and concatemers derived from larger circles will contain fewer copies of the differentiator tag. The number of each differentiator tag sequence is counted, for example, by next-generation sequencing. When number of occurrences is plotted against differentiator tag ID, the data will naturally cluster into two groups reflecting the lengths of the two alleles in the diploid sample. The allele lengths can therefore be read directly off this graph, after absolute



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length calibration using known standards. In some embodiments, a sequencing technique (e.g., a next-generation sequencing technique) is used to sequence part of one or more captured targets (e.g., or amplicons thereof) and the sequences are used to count the number of different barcodes that are present. Accordingly, in some embodiments, aspects of the invention relate to a highly-multiplexed qPCR reaction.

Other non-limiting examples of loci at which insertions or deletions or repeat sequences may be associated with a disease or condition are provided in Tables 3 and 4. It should be appreciated that the presence of an abnormal length at any one or more of these loci may be evaluated according to aspects of the invention. In some embodiments, two or more of these loci or other loci may be evaluated in a single multiplex reaction using different probes designed to hybridize under the same reaction conditions to different target nucleic acid in a biological sample.

Table 3.

**Polyglutamine (PolyQ) Diseases**

| Type | Gene | Normal/wildtype | Pathogenic |
|---|---|---|---|
| DRPLA (Dentatorubropallidoluysian atrophy) | ATN1 or DRPLA | 6 - 35 | 49 - 88 |
| HD (Huntington's disease) | HTT (Huntingtin) | 10 - 35 | 35+ |
| SBMA (Spinobulbar muscular atrophy or Kennedy disease) | Androgen receptor on the X chromosome. | 9 - 36 | 38 - 62 |
| SCA1 (Spinocerebellar ataxia Type 1) | ATXN1 | 6 - 35 | 49 - 88 |
| SCA2 (Spinocerebellar ataxia Type 2) | ATXN2 | 14 - 32 | 33 - 77 |
| SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease) | ATXN3 | 12 - 40 | 55 - 86 |
| SCA6 (Spinocerebellar ataxia Type 6) | CACNA1A | 4 - 18 | 21 - 30 |
| SCA7 (Spinocerebellar ataxia Type 7) | ATXN7 | 7 - 17 | 38 - 120 |
| SCA17 (Spinocerebellar ataxia Type 17) | TBP | 25 - 42 | 47 - 63 |

Table 4.



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**Non-Polyglutamine Diseases**

| Type | Gene | Codon | Normal/wildtype | Pathogenic |
|---|---|---|---|---|
| FRAXA (Fragile X syndrome) | *FMR1*, on the X-chromosome | **CGG** | 6 - 53 | 230+ |
| FXTAS (Fragile X-associated tremor/ ataxia syndrome) | *FMR1*, on the X-chromosome | **CGG** | 6 - 53 | 55-200 |
| FRAXE (Fragile XE mental retardation) | AFF2 or FMR2, on the X-chromosome | **GCC** | 6 - 35 | 200+ |
| FRDA (Friedreich's ataxia) | FXN or X25, (frataxin) | **GAA** | 7 - 34 | 100+ |
| DM (Myotonic dystrophy) | DMPK | **CTG** | 5 - 37 | 50+ |
| SCA8 (Spinocerebellar ataxia Type 8) | OSCA or SCA8 | **CTG** | 16 - 37 | 110 – 250 |
| SCA12 (Spinocerebellar ataxia Type 12) | PPP2R2B or SCA12 | **CAG** On 5' end | 7 - 28 | 66 - 78 |

The following examples illustrate aspects and embodiments of the invention and are not intended to be limiting or restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

*4. Increasing detection sensitivity:*

In some embodiments, aspects of the invention relate to methods for increasing the sensitivity of nucleic acid detection assays.

There are currently many genomic assays that utilize next-generation (e.g., polony-based) sequencing to generate data, including genome resequencing, RNA-seq for gene expression, bisulphite sequencing for methylation, and Immune-seq, among others. In order to make quantitative measurements (including genotype calling), these



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methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids. The majority of these techniques require a preparative step to construct a high-complexity library of DNA molecules that is representative of a sample of interest. Current assays use one of several alternative nucleic acid preparative techniques (e.g., amplification, for example PCR-based amplification; sequence-specific capture, for example, using immobilized capture probes; or target capture into a circularized probe followed by a sequence analysis step. In order to reduce errors associated with the unpredictability (stochastic nature) of nucleic acid isolation and sequence analysis techniques, current methods involve oversampling a target nucleic acid preparation in order to increase the likelihood that all sequences that are present in the original nucleic acid sample will be represented in the final sequence data. For example, a genomic sequencing library may contain an over- or under-representation of particular sequences from a source nucleic acid sample (e.g., genome preparation) as a result of stochastic variations in the library construction process. Such variations can be particularly problematic when they result in target sequences from a genome being absent or undetectable in a sequencing library. For example, an under-representation of particular allelic sequences (e.g., heterozygotic alleles) from a genome in a sequencing library can result in an apparent homozygous representation in a sequencing library.

In contrast, aspects of the invention relate to basing a nucleic acid sequence analysis on results from two or more different nucleic acid preparatory techniques that have different systematic biases in the types of nucleic acids that they sample rather than simply oversampling the target nucleic acid. According to some embodiments, different techniques have different sequence biases that are systematic and not simply due to stochastic effects during nucleic acid capture or amplification. Accordingly, in some embodiments, the degree of oversampling required to overcome variations in nucleic acid preparation needs to be sufficient to overcome the biases. In some embodiments, the invention provides methods that reduce the need for oversampling by combining nucleic acid and/or sequence results obtained from two or more different nucleic acid preparative techniques that have different biases.

According to the invention, different techniques have different characteristic or systematic biases. For example, one technique may bias a sample analysis towards one



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particular allele at a genetic locus of interest, whereas a different technique would bias the sample analysis towards a different allele at the same locus. Accordingly, the same sample may be identified as being different depending on the type of technique that is used to prepare nucleic acid for sequence analysis. This effectively represents a sensitivity issue, because each technique has a different relative sensitivities for polymorphic sequences of interest.

According to aspects of the invention, the sensitivity of a nucleic acid analysis can be increased by combining the sequences from different nucleic acid preparative steps and using the combined sequence information for a diagnostic assay (e.g., for a making a call as to whether a subject is homozygous or heterozygous at a genetic locus of interest).

Currently, the ability of DNA sequencing to detect mutations is limited by the ability of the upstream sample isolation (e.g., by amplification, immobilization enrichment, circularization capture, etc.) methods to reliably isolate the locus of interest. If one wishes to make heterozygote base-calls for a diploid genome (e.g. a human sample presented for molecular diagnostic sequencing), it is important in some embodiments that the isolation method produces near- or perfectly- uniform amounts of the two alleles to be sequenced (at least sufficiently uniform to be "called" unambiguously as a heterozygote or a homozygote for a locus of interest).

Sample preparative methods may fall into three classes: 1) single- or several-target amplification (e.g., uniplex PCR, 'multiplex' PCR), 2) multi-target hybridization enrichment (e.g., Agilent SureSelect 'hybrid capture' [Gnirke et al 2009, *Nature methods* 27:182-9], Roche/Nimblegen 'sequence capture' [Hodges et al 2007, *Nature genetics* 39:1522-7], and 3) multi-target circularization selection (e.g. molecular inversion probes or padlock probes, [Porreca et al 2007, *Nature methods* 4:931-6, Turner et al 2009, *Nature methods* 6:315-6], 'selectors' [Dahl et al 2005, *Nucleic acids research* 33:e71]). Each of these methods can result in a pool of isolated product that does not adequately represent the input abundance distribution. For example, the two alleles at a heterozygous position can become skewed far from their input 50:50 ratio to something that results in a missed basecall during downstream sequencing. For example, if the ratio was skewed from 50:50 to 10:90, and the sample was sequenced to 10X average coverage, there is a high probability that one of the two alleles would not be observed once in the ten sequencing reads. This would reduce the sensitivity of the sequencing



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method by converting a heterozygous position to homozygous (where potentially the 'mutant' allele was the one not observed). In some embodiments, a skewed ratio is a particular issue that decreases the sensitivity of detecting mutations present in a heterogeneous tumor tissue. For example, if only 10% of the cells analyzed in a heterogeneous sample harbored a heterozygous mutation, the mutation would be expected to be present in 5% of sequence reads, not 50%. In this scenario, the need for robust, sensitive detection may be even more acute.

The methods disclosed herein are based, in part, on the discovery that certain classes of isolation methods have different modes of bias. The disclosure provide methods for increasing the sensitivity of the downstream sequencing by using a combination of multiple isolation methods (e.g., one or more from at least two of the classes disclosed herein) for a sample. This is particularly important in molecular diagnostics where high sensitivity is required to minimize the chances of 'missing' a disease-associated mutation. For example, given a nominal false-negative error rate of $1x10^{-3}$ for sequencing following circularization selection, and a false-negative error rate of $1x10^{-3}$ for sequencing following hybridization enrichment, one can achieve a final false-negative rate of $1x10^{-6}$ by performing both techniques on the sample (assuming failures in each method are fully independent). For a recessive disease with carrier frequency of 0.1, caused by a single fully-penetrant mutant allele, the number of missed carrier diagnoses would decrease from 1000 per million patients tested to 1 per million patients tested. Furthermore, if the testing was used in the context of prenatal carrier screening, the number of affected children born as a result of missing the carrier call in one parent would decrease from 25 per million to 25 per billion born.

Additionally, the disclosure provides combinations of preparative methods to effectively increase sequencing coverage in regions containing disease-associated alleles. Since heterozygote error rate is largely tied to both deviations from 50:50 allele representation, and in the case of next-generation DNA sequencing deviations from average abundance (such that less abundant isolated targets are more likely to be undersampled at one or both alleles), selectively increasing coverage in these regions will also selectively increase sensitivity. Furthermore, MIPs that detect presence or absence of specific known disease-associated mutations can be used to increase sensitivity selectively. In some embodiments, these MIPs would have a targeting arm whose 3' –most region is complementary to the expected mutation, and has a fill-in



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length of 0 or more bp. Thus, the MIP will form only if the mutation is present, and its presence will be detected by sequencing.

Additionally, algorithms disclosed herein may be used to determine base identity with varying levels of stringency depending on whether the given position has any known disease-associated alleles. Stringency can be reduced in such positions by decreasing the minimum number of observed mutant reads necessary to make a consensus base-call. This will effectively increase sensitivity for mutant allele detection at the cost of decreased specificity.

An embodiment of the invention combines MIPs plus hybridization enrichment, plus optionally extra MIPs targeted to specific known, common disease-associated loci, e.g., to detect the presence of a polymorphism in a target nucleic acid. A non-limiting example is illustrated in FIG. 12 that illustrates a schematic using MIPs plus hybridization enrichment, plus optionally extra MIPs targeted to specific known, common disease-associated loci, e.g., to detect the presence of a polymorphism in a target nucleic acid.

FIGs. 13 and 14 illustrate different capture efficiencies for MIP-based captures. FIG. 13 shows a graph of per-target abundance with MIP capture. In this graph, bias largely drives the heterozygote error rate, since targets which are less abundant here are less likely to be covered in sufficient depth during sequencing to adequately sample both alleles. This is from Turner et al 2009, Nature methods 6:315-6. Hybridization enrichment results in a qualitatively similar abundance distribution, but the abundance of a given target is likely not correlated between the two methods. FIG. 14 shows a graph of correlation between two MIP capture reactions from Ball et al 2009, Nature biotechnology 27:361-8. Each point represents the target abundance in replicate 1 and replicate 2. Pearson correlation r=0.956. This indicates that MIP capture reproducibly biases targets to specific abundances. Hybridization enrichment is similarly correlated from one capture to the next.

According to aspects of the invention, such biases can be detected or overcome by systematically combining different capture and/or analytical techniques in an assay that interrogates a plurality of loci in a plurality of subject samples.

Accordingly, it should be appreciated that in any of the embodiments described herein (e.g., tiling/staggering, tagging, size-detection, sensitivity enhancing algorithms,



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or any combination thereof), aspects of the invention involve preparing genomic nucleic acid and/or contacting them with one or more different probes (e.g., capture probes, hybridization probes, MIPs, others etc.). In some embodiments, the amount of genomic nucleic acid used per subject ranges from 1 ng to 10 micrograms (e.g., 500 ng to 5 micrograms). However, higher or lower amounts (e.g., less than 1 ng, more than 10 micrograms, 10-50 micrograms, 50-100 micrograms or more) may be used. In some embodiments, for each locus of interest, the amount of probe used per assay may be optimized for a particular application. In some embodiments, the ratio (molar ratio, for example measured as a concentration ratio) of probe to genome equivalent (e.g., haploid or diploid genome equivalent, for example for each allele or for both alleles of a nucleic acid target or locus of interest) ranges from 1/100, 1/10, 1/1, 10/1, 100/1, 1000/1. However, lower, higher, or intermediate ratios may be used.

In some embodiments, the amount of target nucleic acid and probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios. In some embodiments, in order to normalize genomic DNA and probe, the genomic DNA concentration is read using a standard spectrophotometer or by fluorescence (e.g., using a fluorescent intercalating dye). The probe concentration may be determined experimentally or using information specified by the probe manufacturer.

Similarly, once a locus has been captured (e.g., on a MIP or other probe or in another form), it may be amplified and/or sequenced in a reaction involving one or more primers. The amount of primer added for each reaction can range from 0.1 pmol to 1 nmol, 0.15 pmol to 1.5 nmol (for example around 1.5 pmol). However, other amounts (e.g., lower, higher, or intermediate amounts) may be used.

In some embodiments, it should be appreciated that one or more intervening sequences (e.g., sequence between the first and second targeting arms on a MIP capture probe), identifier or tag sequences, or other probe sequences that are not designed to hybridize to a target sequence (e.g., a genomic target sequence) should be designed to avoid excessive complementarity (to avoid cross-hybridization) to target sequences or other sequences (e.g., other genomic sequences) that may be in a biological sample. For example, these sequences may be designed have a sufficient number of mismatches with any genomic sequence (e.g., at least 5, 10, 15, or more mismatches out of 30 bases) or as having a Tm (e.g., a mismatch Tm) that is lower (e.g., at least 5, 10, 15, 20, or more degrees C lower) than the hybridization reaction temperature.



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It should be appreciated that a targeting arm as used herein may be designed to hybridize (e.g., be complementary) to either strand of a genetic locus of interest if the nucleic acid being analyzed is DNA (e.g., genomic DNA). However, in the context of MIP probes, whichever strand is selected for one targeting arm will be used for the other one. However, in the context of RNA analysis, it should be appreciated that a targeting arm should be designed to hybridize to the transcribed RNA. It also should be appreciated that MIP probes referred to herein as "capturing" a target sequence are actually capturing it by template-based synthesis rather than by capturing the actual target molecule (other than for example in the initial stage when the arms hybridize to it or in the sense that the target molecule can remain bound to the extended MIP product until it is denatured or otherwise removed).

It should be appreciated that in some embodiments a targeting arm may include a sequence that is complementary to one allele or mutation (e.g., a SNP or other polymorphism, a mutation, etc.) so that the probe will preferentially hybridize (and capture) target nucleic acids having that allele or mutation. However, in many embodiments, each targeting arm is designed to hybridize (e.g., be complementary) to a sequence that is not polymorphic in the subjects of a population that is being evaluated. This allows target sequences to be captured and/or sequenced for all alleles and then the differences between subjects (e.g., calls of heterozygous or homozygous for one or more loci) can be based on the sequence information and/or the frequency as described herein.

It should be appreciated that sequence tags (also referred to as barcodes) may be designed to be unique in that they do not appear at other positions within a probe or a family of probes and they also do not appear within the sequences being targeted. Thus they can be used to uniquely identify (e.g., by sequencing or hybridization properties) particular probes having other characteristics (e.g., for particular subjects and/or for particular loci).

It also should be appreciated that in some embodiments probes or regions of probes or other nucleic acids are described herein as comprising or including certain sequences or sequence characteristics (e.g., length, other properties, etc.). However, it should be appreciated that in some embodiments, any of the probes or regions of probes or other nucleic acids consist of those regions (e.g., arms, central regions, tags, primer sites, etc., or any combination thereof) of consist of those sequences or have sequences with characteristics that consist of one or more characteristics (e.g., length, or other



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properties, etc.) as described herein in the context of any of the embodiments (e.g., for tiled or staggered probes, tagged probes, length detection, sensitivity enhancing algorithms or any combination thereof).

It should be appreciated that probes, primers, and other nucleic acids designed or used herein may be synthetic, natural, or a combination thereof. Accordingly, as used herein, the term "nucleic acid" refers to multiple linked nucleotides (i.e., molecules comprising a sugar (e.g., ribose or deoxyribose) linked to an exchangeable organic base, which is either a pyrimidine (e.g., cytosine (C), thymidine (T) or uracil (U)) or a purine (e.g., adenine (A) or guanine (G)). "Nucleic acid" and "nucleic acid molecule" may be used interchangeably and refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms shall also include polynucleosides (i.e., a polynucleotide minus a phosphate) and any other organic base containing nucleic acid. The organic bases include adenine, uracil, guanine, thymine, cytosine and inosine. Unless otherwise stated, nucleic acids may be single or double stranded. The nucleic acid may be naturally or non-naturally occurring. Nucleic acids can be obtained from natural sources, or can be synthesized using a nucleic acid synthesizer (i.e., synthetic). Harvest and isolation of nucleic acids are routinely performed in the art and suitable methods can be found in standard molecular biology textbooks. (See, for example, Maniatis' Handbook of Molecular Biology.) The nucleic acid may be DNA or RNA, such as genomic DNA, mitochondrial DNA, mRNA, cDNA, rRNA, miRNA, or a combination thereof. Non-naturally occurring nucleic acids such as bacterial artificial chromosomes (BACs) and yeast artificial chromosomes (YACs) can also be used.

The invention also contemplates the use of nucleic acid derivatives. As will be described herein, the use of certain nucleic acid derivatives may increase the stability of the nucleic acids of the invention by preventing their digestion, particularly when they are exposed to biological samples that may contain nucleases. As used herein, a nucleic acid derivative is a non-naturally occurring nucleic acid or a unit thereof. Nucleic acid derivatives may contain non-naturally occurring elements such as non-naturally occurring nucleotides and non-naturally occurring backbone linkages.

Nucleic acid derivatives may contain backbone modifications such as but not limited to phosphorothioate linkages, phosphodiester modified nucleic acids, phosphorothiolate modifications, combinations of phosphodiester and phosphorothioate nucleic acid, methylphosphonate, alkylphosphonates, phosphate esters,



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alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, carboxymethyl esters, methylphosphorothioate, phosphorodithioate, p-ethoxy, and combinations thereof. The backbone composition of the nucleic acids may be homogeneous or heterogeneous.

Nucleic acid derivatives may contain substitutions or modifications in the sugars and/or bases. For example, they include nucleic acids having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position (e.g., an 2'-O-alkylated ribose group). Nucleic acid derivatives may include non-ribose sugars such as arabinose. Nucleic acid derivatives may contain substituted purines and pyrimidines such as C-5 propyne modified bases, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, 2-thiouracil and pseudoisocytosine. In some embodiments, substitution(s) may include one or more substitutions/modifications in the sugars/bases, groups attached to the base, including biotin, fluorescent groups (fluorescein, cyanine, rhodamine, etc), chemically-reactive groups including carboxyl, NHS, thiol, etc., or any combination thereof.

A nucleic acid may be a peptide nucleic acid (PNA), locked nucleic acid (LNA), DNA, RNA, or co-nucleic acids of the same such as DNA-LNA co-nucleic acids. PNA are DNA analogs having their phosphate backbone replaced with 2-aminoethyl glycine residues linked to nucleotide bases through glycine amino nitrogen and methylenecarbonyl linkers. PNA can bind to both DNA and RNA targets by Watson-Crick base pairing, and in so doing form stronger hybrids than would be possible with DNA or RNA based oligonucleotides in some cases.

PNA are synthesized from monomers connected by a peptide bond (Nielsen, P.E. et al. Peptide Nucleic Acids, Protocols and Applications, Norfolk: Horizon Scientific Press, p. 1-19 (1999)). They can be built with standard solid phase peptide synthesis technology. PNA chemistry and synthesis allows for inclusion of amino acids and polypeptide sequences in the PNA design. For example, lysine residues can be used to introduce positive charges in the PNA backbone. All chemical approaches available for the modifications of amino acid side chains are directly applicable to PNA. Several types of PNA designs exist, and these include single strand PNA (ssPNA), bisPNA and pseudocomplementary PNA (pcPNA).



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The structure of PNA/DNA complex depends on the particular PNA and its sequence. ssPNA binds to single stranded DNA (ssDNA) preferably in antiparallel orientation (i.e., with the N-terminus of the ssPNA aligned with the 3' terminus of the ssDNA) and with a Watson-Crick pairing. PNA also can bind to DNA with a Hoogsteen base pairing, and thereby forms triplexes with double stranded DNA (dsDNA) (Wittung, P. et al., *Biochemistry* 36:7973 (1997)).

A locked nucleic acid (LNA) is a modified RNA nucleotide. An LNA form hybrids with DNA, which are at least as stable as PNA/DNA hybrids (Braasch, D.A. et al., *Chem & Biol.* 8(1):1-7(2001)). Therefore, LNA can be used just as PNA molecules would be. LNA binding efficiency can be increased in some embodiments by adding positive charges to it. LNAs have been reported to have increased binding affinity inherently.

Commercial nucleic acid synthesizers and standard phosphoramidite chemistry are used to make LNAs. Therefore, production of mixed LNA/DNA sequences is as simple as that of mixed PNA/peptide sequences. The stabilization effect of LNA monomers is not an additive effect. The monomer influences conformation of sugar rings of neighboring deoxynucleotides shifting them to more stable configurations (Nielsen, P.E. et al. Peptide Nucleic Acids, Protocols and Applications, Norfolk: Horizon Scientific Press, p. 1-19 (1999)). Also, lesser number of LNA residues in the sequence dramatically improves accuracy of the synthesis. Most of biochemical approaches for nucleic acid conjugations are applicable to LNA/DNA constructs.

These and other aspects of the invention are illustrated by the following non-limiting examples.

## EXAMPLES

The following examples illustrate non-limiting embodiments of the invention.

**Example 1: Design a set of capture probes for a human target exon**

All targets are captured as a set of partially-overlapping subtargets. For example, in the tiling approach, a 200 bp target exon might be captured as a set of 12 subtargets,



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each 60bp in length (FIG. 1). Each subtarget is chosen such that it partially overlaps two or three other targets.

In some embodiments, all probes are composed of three regions: 1) a 20 bp 'targeting arm' comprised of sequence which hybridizes immediately upstream from the sub-target, 2) a 30 bp 'constant region' comprised of sequence used as a pair of amplification priming sites, and 3) a second 20 bp 'targeting arm' comprised of sequence which hybridizes immediately downstream from the sub-target. Targeting arm sequences will be different for each capture probe in a set, while constant region sequence will be the same for all probes in the set, allowing all captured targets to be amplified with a single set of primers. Targeting arm sequences should be designed such that any given pair of 20 bp sequences is unique in the target genome (to prevent spurious capture of undesired sites). Additionally, melting temperatures should be matched for all probes in the set such that hybridization efficiency is uniform for all probes at a constant temperature (e.g., 60 C). Targeting arm sequences should be computationally screened to ensure they do not form strong secondary structure that would impair their ability to basepair with the genomic target.

***Hybridize capture probes to human genomic sample***

Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

***Convert hybridized probes into covalently-closed circular products containing subtargets***

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)



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- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

***Purify circularized probe / subtarget products from un-reacted probes and genomic DNA***

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubte at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

***Amplify circular material by PCR using primers specific to the 'constant region' of the probes***

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3') (SEQ ID NO: 183)
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3') (SEQ ID NO: 184)
- 
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

1. 95C for 5 min



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2. 95C for 30 sec
3. 58C for 60 sec
4. 72C for 60 sec
5. goto 2, N more times

***Prepare a shotgun next-generation sequencing library for analysis***

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

**Example 2: Use of differentiator tag sequences to detect and correct bias in a MIP-capture reaction of a set of exon targets**

The first step in performing the detection/correction is to determine how many differentiator tag sequences are necessary for the given sample. In this example, 1000 genomic targets corresponding to 1000 exons were captured. Since the differentiator tag sequence is part of the probe, it will measure/report biases that occur from the earliest protocol steps. Also, being located in the backbone, the differentiator tag sequence can easily be sequenced from a separate priming site, and therefore not impact the total achievable read-length for the target sequence. MIP probes are synthesized using standard column-based oligonucleotide synthesis by any number of vendors (e.g. IDT), and differentiator tag sequences are introduced as 'degenerate' positions in the backbone. Each degenerate position increases the total number of differentiator tag sequences synthesized by a factor of 4, so a 10 nt degenerate region implies a differentiator tag sequence complexity of ~1e6 species.

*Hybridize capture probes to human genomic sample*



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Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

*Convert hybridized probes into covalently-closed circular products containing subtargets*

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

*Purify circularized probe / subtarget products from un-reacted probes and genomic DNA*

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubate at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

*Amplify circular material by PCR using primers specific to the 'constant region' of the probes*

Prepare PCR mixture:



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- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3') (SEQ ID NO: 183)
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3') (SEQ ID NO: 184)
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

6. 95C for 5 min
7. 95C for 30 sec
8. 58C for 60 sec
9. 72C for 60 sec
10. goto 2, N more times

*Prepare a shotgun next-generation sequencing library for analysis*

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in lenth.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.



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*Perform sequencing of library according to manufacturer's directions (e.g. Illumina,ABI, etc), reading both the target sequence and the differentiator tag sequence.*

*Analyze data by correcting for any biases detected by quantitation of differentiator tag sequence abundance*

Construct a table of target:differentiator tag abundances from the read data, e.g.:

| Target ID | Differentiator tag sequence ID | Count |
|---|---|---|
| 1 | 3547 | 1 |
| 2 | 4762 | 1 |
| 1 | 9637 | 1 |
| 1 | 1078 | 5 |
| 3 | 4762 | 1 |
| 1 | 2984 | 1 |

All 'count' entries should be '1', since any particular target:differentiator tag mapping will not occur more than once by chance, and therefore will only be observed if bias was present somewhere in the sample preparation process. For any target:differentiator tag combination observed more than once, all such reads are 'collapsed' into a single read before consensus basecalls are determined. This will cancel the effect of bias on consensus basecall accuracy. FIG. 5 depicts a method for making diploid genotype calls in which repeat target:differentiator tag combination are collapsed.

**Example 3: Differentiator tag sequence design for MIP capture reactions**

For a set of targets, the number of differentiator tag sequences necessary to be confident (within some statistical bounds) that a certain differentiator tag sequence will not be observed more than once by chance in combination with a certain target sequence was determined. The total number of unique differentiator tag sequences for a certain differentiator tag sequence length is determined as $4^{(\text{Length in nucleotides of the differentiator tag sequence})}$. For a molecular inversion probe capture reaction that uses MIP probes having differentiator tag sequences, the probability of performing the capture reaction and capturing one or more copies of a target sequence having the same differentiator tag sequence is calculated as: p = 1 - [N! / (N-M)!] / [N^M], wherein N is the total number



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of possible unique differentiator tag sequences and M is the number of target sequence copies in the capture reaction. Thus, by varying the differentiator tag sequence length it is possible to perform a MIP capture reaction in which the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is set at a predetermined probability value.

For example, for a differentiator tag sequence of 15 nucleotides in length, there are 1,073,741,824 possible differentiator tag sequences. A MIP capture reaction in which MIP probes, each having a differentiator tag sequence of 15 nucleotides, are combined with 10000 target sequence copies (e.g., genome equivalents), the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is 0.05. In this example, the MIP reaction will produce very few (usually 0, but occasionally 1 or more) targets where multiple copies are tagged with the same differentiator tag sequence. FIG. 6 depicts results of a simulation for 100000 capture reactions having 15 nucleotide differentiator tag sequences and 10000 target sequences.

**Example 4: Assessment of the probability for obtaining enough sequencing reads to make accurate base-calls at multiple independent loci, as a function of sequencing coverage.**

Monte Carlo simulations were performed to determine sequencing coverage requirements. The simulations assume 10000 genomic copies of a given locus (target) half mom alleles and half dad alleles. The simulations further assume 1% efficiency of capture for the MIP reaction. The simulation samples from a capture mix 100 times without replacement to create a set of 100 capture products. The simulation then samples from the set of 100 capture products with replacement (assuming unbiased amplification) to generate 'reads' from either mom or dad. The number of reads sampled depends on the coverage. The number of independent reads from both mom and dad necessary to make a high-quality base-call (assumed to be 10 or 20 reads) were then determined. The process was repeated 1000 times for each coverage level, and the fraction of times that enough reads from both parents were successfully obtained was determined. This fraction was raised to the power 1000, assuming we have 1000 independent loci that must obtain successful base-calls, plotted (See FIG. 7). Result show that roughly 50x coverage is required to capture each allele >= 10x with > 0.95 probability.



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**Example 5: MIP capture of 'target' locus and 'control' loci**

In some embodiments, to accurately quantify the efficiency of target locus capture, at least three sets of control loci are captured in parallel that have *a priori* been shown to serve as proxies for various lengths of target locus. For example, if the target locus is expected to have a length between 50 and 1000 bp, then sets of control loci having lengths of 50, 250, and 1000 bp could be captured (e.g. 20 loci per set should provide adequate protection from outliers), and their abundance digitally measured by sequencing. These loci should be chosen such that minimal variation in efficiency between samples and on multiple runs of the same sample is observed (and are therefore 'efficiency invariant'). These will serve as 'reference' points that define the shape of the curve of abundance-vs-length. Determining the length of the target is then simply a matter of 'reading' the length from the appropriate point on the calibration curve.

In some embodiments, the statistical confidence one has in the estimate of target length from this method is driven largely by three factors: 1) reproducibility / variation of the abundance data used to generate the calibration curve; 2) goodness of fit of the regression to the 'control' datapoints; 3) reproducibility of abundance data for the target locus being measured. Statistical bounds on 1) and 2) will be known in advance, having been measured during development of the assay. Additionally, statistical bounds on 3) will be known in general in advance, since assay development should include adequate population sampling and measure of technical reproducibility. Standard statistical methods should be used to combine these three measures into a single *P* value for any given experimental measure of target abundance.

In some embodiments, given the set of calibration observations, and a linear regression fit to that data, the regression can be used to predict the length value for n observations of the target locus whose length is unknown. First, choose an acceptable range for the confidence interval of the length estimate. For example, in the case of distinguishing "normal" (87-93bp) from "premutation" (165-600bp) potential cases of Fragile X, the goal is to measure length to sufficient precision to distinguish 93 bp from 165 bp. The predicted response value, computed when n observations is substituted into the equation for the regressed line, will have arbitrary precision. However, if for example a 95% confidence level is desired, that 95% confidence interval must be sufficiently short that it does not overlap both the "normal" and "premutation" length



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ranges. Continuing the example, if one calculates a length of 190 from n=400 MIP observations, and based on the regression from calibration data, the 95% confidence interval is 190 +/- 20 bp, one can conclude the sample represents a "premutation" length with 95% certainty. Conversely, if the calibration data were less robust, error estimates of the regression would be higher, leading to larger confidence intervals on the predicted response value. In some embodiments, if the 95% CI were calculated as 190 +/- 100 bp from n=400, one could not determine whether the predicted response value corresponds to a "normal" or "premutation" length.

In some embodiments, the confidence interval for a predicted response is calculated as:

*The estimate for the response* $\hat{y}$ *is identical to the estimate for the mean of the response:* $\hat{y} = b_0 + b_1 x^*$. *The confidence interval for the predicted value is given by* $\hat{y} \pm t^* s_{\hat{y}}$, *where* $\hat{y}$ *is the fitted value corresponding to* $x^*$. *The value* $t^*$ *is the upper (1 - C)/2 critical value for the t(n - 2) distribution.*

In some embodiments, a technique for analyzing a locus of interest can involve the following steps.

***Convert hybridized probes into covalently-closed circular products containing subtargets***

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

***Purify circularized probe / subtarget products from un-reacted probes and genomic DNA***

Prepare exonuclease reaction mixture:



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- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubte at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

***Amplify circular material by PCR using primers specific to the 'constant region' of the probes***

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA-Ilmn (platform-specific amplification sequence plus 'circle constant region'-specific sequence)
- 1.5 ul 10 uM CP-2-RA-Ilmn (platform-specific amplification sequence plus 'circle constant region'-specific sequence)
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

11. 95C for 5 min
12. 95C for 30 sec
13. 58C for 60 sec
14. 72C for 60 sec
15. goto 2, N more times

***Perform sequencing (e.g., next-generation sequencing) on sample for digital quantitation according to manufacturer's instructions (e.g., Illumina, ABI)***

**Example 6: MIP-capture reaction of a set of exon target nucleic acids**



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MIP probes are synthesized using standard column-based oligonucleotide synthesis by any number of vendors (e.g. IDT).

*Hybridize capture probes to human genomic sample*

Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

*Convert hybridized probes into covalently-closed circular products containing target nucleic acids*

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

*Purify circularized probe/target nucleic acid products from un-reacted probes and genomic DNA*

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubate at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

*Amplify circular material by PCR using primers specific to the 'constant region' of the probes*

Prepare PCR mixture:



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- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3') (SEQ ID NO: 183)
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3') (SEQ ID NO: 184)
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

16. 95C for 5 min
17. 95C for 30 sec
18. 58C for 60 sec
19. 72C for 60 sec
20. goto 2, N more times

*Prepare a shotgun next-generation sequencing library for analysis*

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

*Perform sequencing of library according to manufacturer's directions (e.g. Illumina,ABI, etc), reading the target sequence to determine abundance of the target nucleic acid.*



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**Example 7: Use of MIPs, hybridization, and mutation-detection MIPs to genotype a set of 1000 targets**

MIPs, hybridization, and mutation-detection MIPs are used to genotype a set of 1000 targets. The protocol permits detection of any of 50 specific known point mutations

First, separate MIP, hybridization, and mutation-detection MIP reactions are performed on a biological sample. A MIP capture reaction is performed essentially as described in Turner et al 2009, Nature methods 6:315-6. A set of MIPs is designed such that each probe in the set flanks one of the 1000 targets. Separately, a hybridization enrichment reaction is performed using the Agilent SureSelect procedure. Prior to selection, the genomic DNA to be enriched is converted into a shotgun sequencing library using Illumina's 'Fragment Library' kit and protocol. Agilent's web interface is used to design a set of probes which will hybridize to the target nucleic acids. Separately, a set of probes are designed (mutation-detection MIPs) which will form MIPs only if mutations (e.g., specific polymorphisms) are present. Each mutation-detection MIP has a 3'-most base identity that is specific for a single known mutation. A reaction with this set of mutation-detection MIPs is performed to selectively detect the presence of any mutant alleles.

Once all three reactions have been performed, the two MIP reactions are combined (e.g., at potentially non-equimolar ratios to further increase sensitivity of mutation detection) into a single tube, and run as one sample on the next-generation DNA sequencing instrument. The hybridization-enriched reaction is run as a separate sample on the next-generation DNA sequencing instrument. Reads from each 'sample' are combined by a software algorithm which forms a consensus diploid genotype at each position in the target set by evaluating the total coverage at each position, the origin of each read in that total coverage, the quality score of each individual read, and the presence (or absence) of any reads derived from mutation-specific MIPs overlapping the region.

It should be appreciated that the preceding examples are non-limiting and aspects of the invention may be implemented as described herein using alternative techniques and/or protocols that are available to one or ordinary skill in the art.



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It will be clear that the methods may be practiced other than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present disclosure are possible in light of the above teachings and, therefore, are within the scope of the claims. Preferred features of each aspect of the disclosure are as for each of the other aspects mutatis mutandis. The documents including patents, patent applications, journal articles, or other disclosures mentioned herein are hereby incorporated by reference in their entirety. In the event of conflict, the disclosure of present application controls, other than in the event of clear error.



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## CLAIMS

What is claimed is:

1. A method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set,
wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion,
isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and
analyzing the isolated nucleic acids.

2. The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion.

3. The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.

4. The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5. A method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set,
wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe,



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isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

6. The method of claim 1 or 5, wherein the target nucleic acids are selected from Table 1.

7. The method of claim 1 or 5, wherein the analyzing comprises genotyping a polymorphism of the target nucleic acid.

8. The method of claim 7, wherein the polymorphism is selected from Table 2.

9. A method of genotyping a subject, the method comprising:

determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence,

wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject.

10. The method of claim 9., wherein the isolated nucleic acids are molecular inversion probe capture products.

11. A method of genotyping a subject, the method comprising:

determining the sequence of molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, and wherein the target nucleic acid is a captured genomic locus of a subject, and



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genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products.

12. A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject,

amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

14. The method of claim 12 or 13, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

15. The method of any one of claims 12-14, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. The method of any one of claims 12-14, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.



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17. A method of analyzing a plurality of genetic loci, the method comprising:

obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence,

amplifying the plurality of molecular inversion probe capture products,

determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and

if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination.

18. The method of claim 17, further comprising genotyping target sequences in the plurality, wherein the genotyping comprises correcting for bias, if detected.

19. The method of any of claims 9-18, wherein the target nucleic acid sequence is

a sequence of a gene selected from Table 1.

20. A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. The method of claim 20-21, wherein the capture efficiency is lower than the reference capture efficiency.



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23. The method of claim 22, wherein the subject is identified as having an insertion in the target region.

24. The method of claim 20-21, wherein the capture efficiency is higher than the reference capture efficiency.

25. The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.

27. The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. The method of any prior claim, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

30. The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31. The method of claim 30, wherein the first and second targeting arms are about 10-20, 20-30, 30-40, or 40-50 nucleotides long.



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32. The method of claim 31, wherein the first and second targeting arms are about 20 nucleotides long.

33. The method of any one of claims 29-31, wherein the first and second targeting arms have the same length.

34. The method of any one of claims 29-33, wherein the hybridization Tms of the first and second targeting arms are similar.

35. The method of claim 34, wherein the hybridization Tms of the first and second targeting arms are within 2-5 C of each other.

36. The method of claim 34, wherein the hybridization Tms of the first and second targeting arms are identical.

37. The method of any one of claims 28-36, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. The method of claim 37, wherein the sub-target nucleic acid contains 10-100 copies of the nucleic acid repeat in the absence of an abnormal increase or decrease in nucleic acid repeats.

40. The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. The method of any one of claims 29-36, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.



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42. The method of any one of claims 29-36, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.

43. The method of any one of claims 29-36, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. The method of any of the prior claims, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48. The method of any prior claim, wherein the biological sample is a blood sample.

49. The method of any prior claim, wherein the biological sample is a tissue sample.

50. The method of any prior claim, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.



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52. The method of claim 50-51, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.

53. The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.

55. The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. The method of any prior claim, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57. A method for estimating the length of a target nucleic acid, the method comprising:

(i) contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid,

wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid,

wherein the first and second regions are on a common strand of the target nucleic acid, and

wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid; and

(ii) capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes; and



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(iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the sub-target nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid.

58. A method of increasing the sensitivity of a nucleic acid detection assay, the method comprising:

obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample,

obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample,

assaying the sequences obtained in both first and second nucleic acid preparations,

using the sequence information from both first and second nucleic acid preparations to determine the genotype of the target nucleic acid in the biological sample, wherein the first and second preparative methods have different systematic sequence biases.

59. The method of claim 58, wherein the first and second nucleic acid preparations are combined prior to performing a sequence assay.

60. The method of claim 58, wherein separate sequence assays are performed on the first and second nucleic acid preparations and the sequence information from both assays are combined to determine the genotype of the target nucleic acid in the biological sample.

61. The method of claim 58, wherein the first preparative method is a PCR-based, a hybridization-based, or a circular probe-based preparative method.

62. The method of claim 61, wherein the second preparative method is a PCR-based, a hybridization-based, or a circular probe-based preparative method, provided that the second method is different from the first method.



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63. A method of obtaining a nucleic acid preparation that is representative of the nucleic acid in a biological sample, the method comprising

obtaining a first nucleic acid preparation using a first preparative method on a biological sample,

obtaining a second nucleic acid preparation using a second preparative method on the biological sample, and

combining the first and second nucleic acid preparations to obtain a combined preparation that is representative of nucleic acid in the biological sample.

64. The method of claim 1 or 6, further comprising performing a third nucleic acid preparation using a third preparative method that is different from the first and second preparative methods, wherein the first, second, and third preparative methods all have different systematic sequence biases.

65. The method of claim any of the prior claims, wherein the different preparative methods are used for a plurality of different loci in the biological sample to increase the sensitivity of a multiplex nucleic acid analysis.

66. A method of genotyping a nucleic acid in a biological sample, the method comprising:

obtaining a nucleic acid preparation using a preparative method on a biological sample,

sequencing a target nucleic acid of the nucleic acid preparation,

and performing a molecular inversion probe capture reaction on the biological sample, wherein a molecular inversion probe capture of the target nucleic acid in the biological sample is indicative of the presence of a polymorphism in the target nucleic acid,

genotyping the target nucleic acid based on the results of the sequencing and the capture reaction.

67. The method of any of the prior claims, wherein the target nucleic acid has a sequence of a gene selected from Table 1.



Petitioner Illumina, Inc., EX1002, p. 347

**Figure 1**

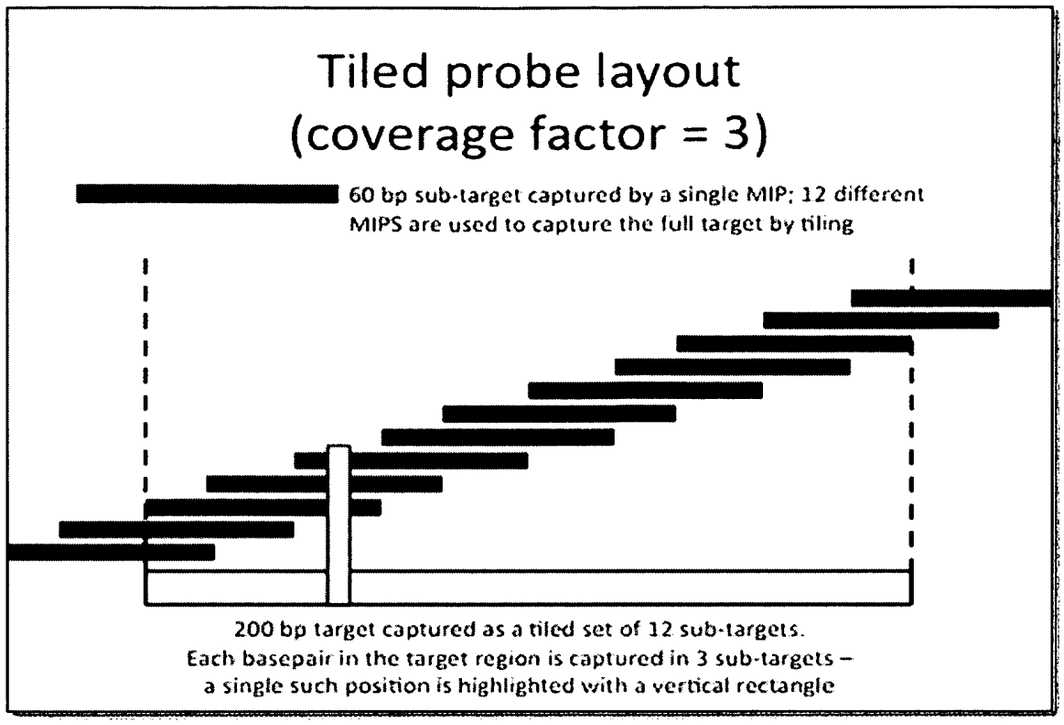

Tiled probe layout
(coverage factor = 3)
60 bp sub-target captured by a single MIP; 12 different MIPS are used to capture the full target by tiling
200 bp target captured as a tiled set of 12 sub-targets.
Each basepair in the target region is captured in 3 sub-targets –
a single such position is highlighted with a vertical rectangle

**Figure 2**

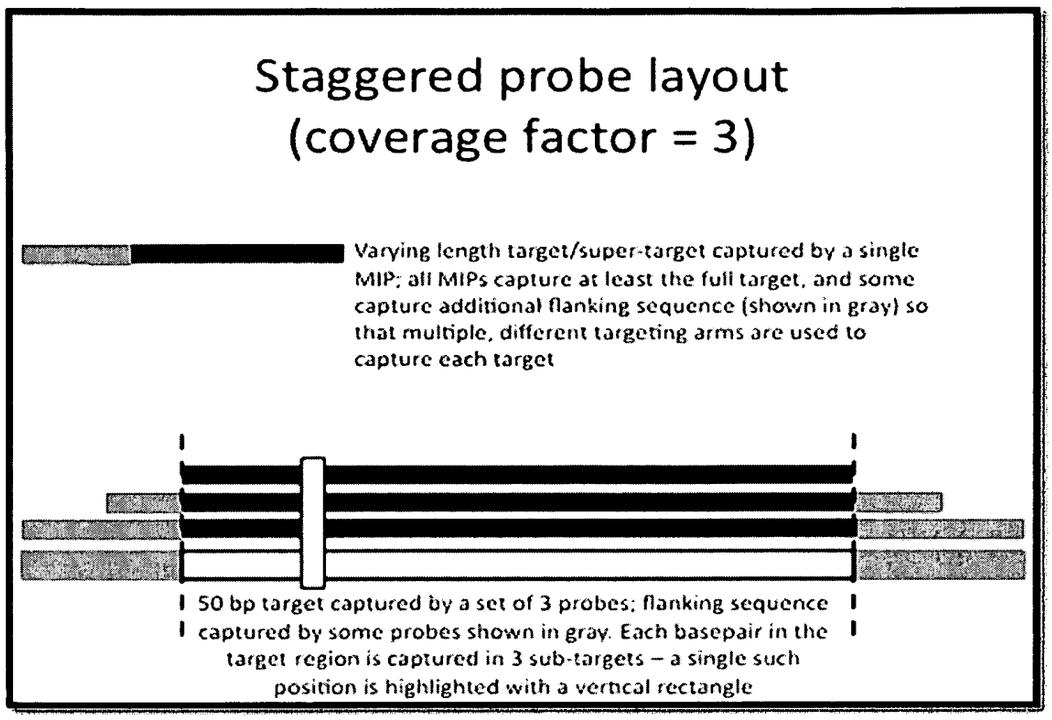

Staggered probe layout
(coverage factor = 3)
Varying length target/super-target captured by a single MIP; all MIPs capture at least the full target, and some capture additional flanking sequence (shown in gray) so that multiple, different targeting arms are used to capture each target
50 bp target captured by a set of 3 probes; flanking sequence captured by some probes shown in gray. Each basepair in the target region is captured in 3 sub-targets – a single such position is highlighted with a vertical rectangle

**Figure 3**

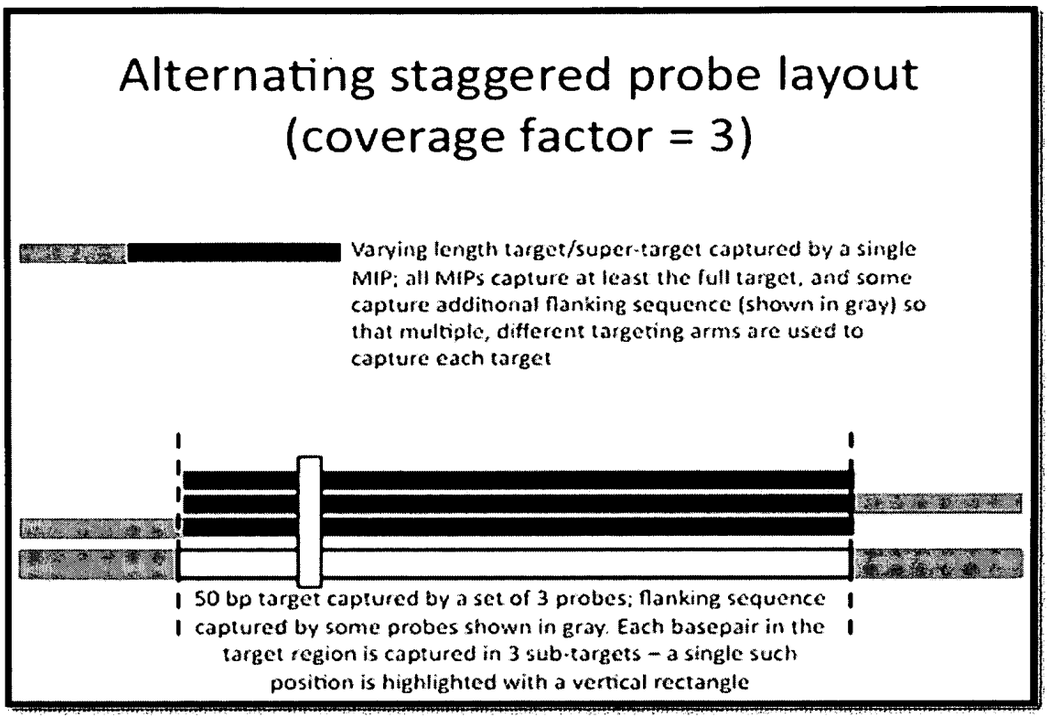

Alternating staggered probe layout
(coverage factor = 3)
Varying length target/super-target captured by a single MIP; all MIPs capture at least the full target, and some capture additional flanking sequence (shown in gray) so that multiple, different targeting arms are used to capture each target
50 bp target captured by a set of 3 probes; flanking sequence captured by some probes shown in gray. Each basepair in the target region is captured in 3 sub-targets – a single such position is highlighted with a vertical rectangle

FIGURE 4

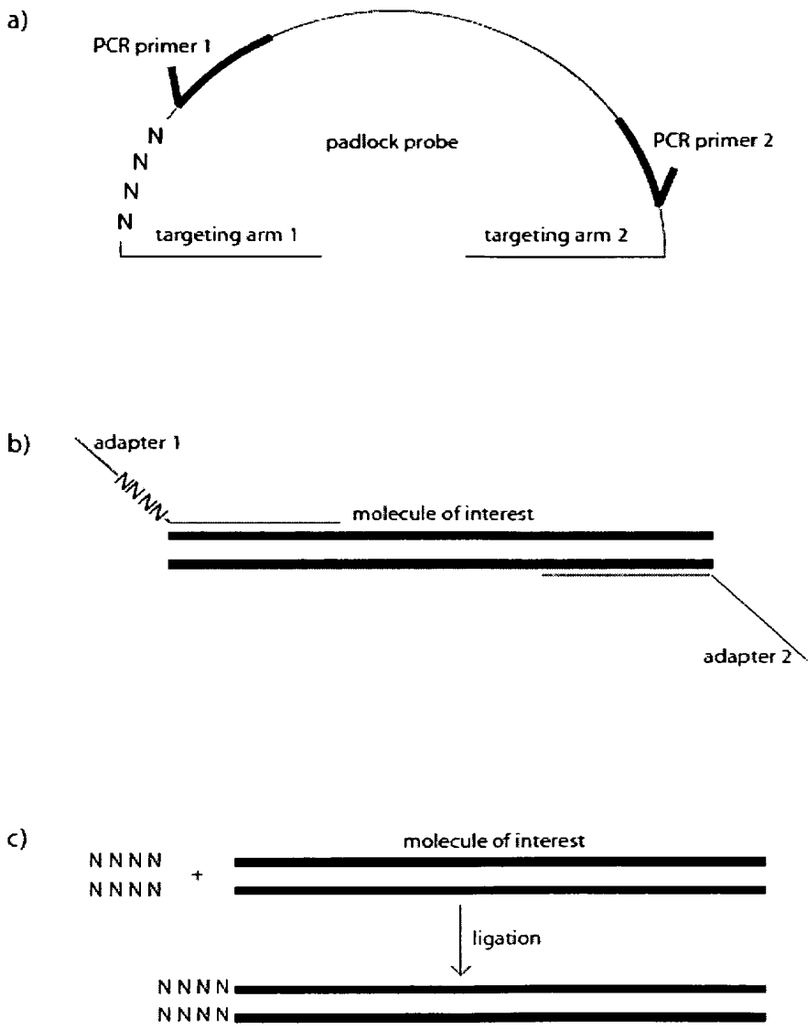

a)
PCR primer 1
N
N
N
N
padlock probe
PCR primer 2
targeting arm 1
targeting arm 2
b)
adapter 1
NNNN
molecule of interest
adapter 2
c)
NNNN
NNNN
+
molecule of interest
ligation
NNNN
NNNN

FIGURE 5

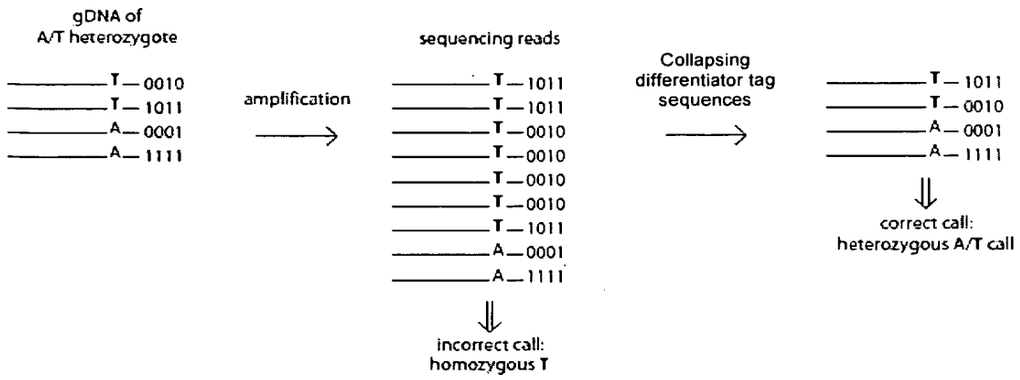
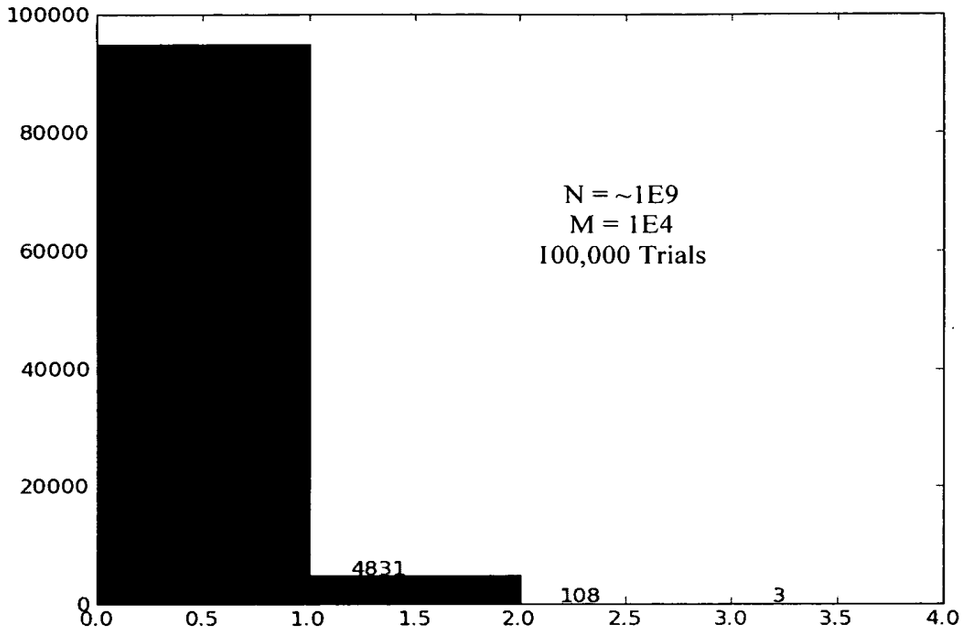

gDNA of
A/T heterozygote
T — 0010
T — 1011
A — 0001
A — 1111
amplification
sequencing reads
T — 1011
T — 1011
T — 0010
T — 0010
T — 0010
T — 0010
T — 1011
A — 0001
A — 1111
incorrect call:
homozygous T
Collapsing
differentiator tag
sequences
T — 1011
T — 0010
A — 0001
A — 1111
correct call:
heterozygous A/T call

FIGURE 6




100,000 Trials

FIGURE 7

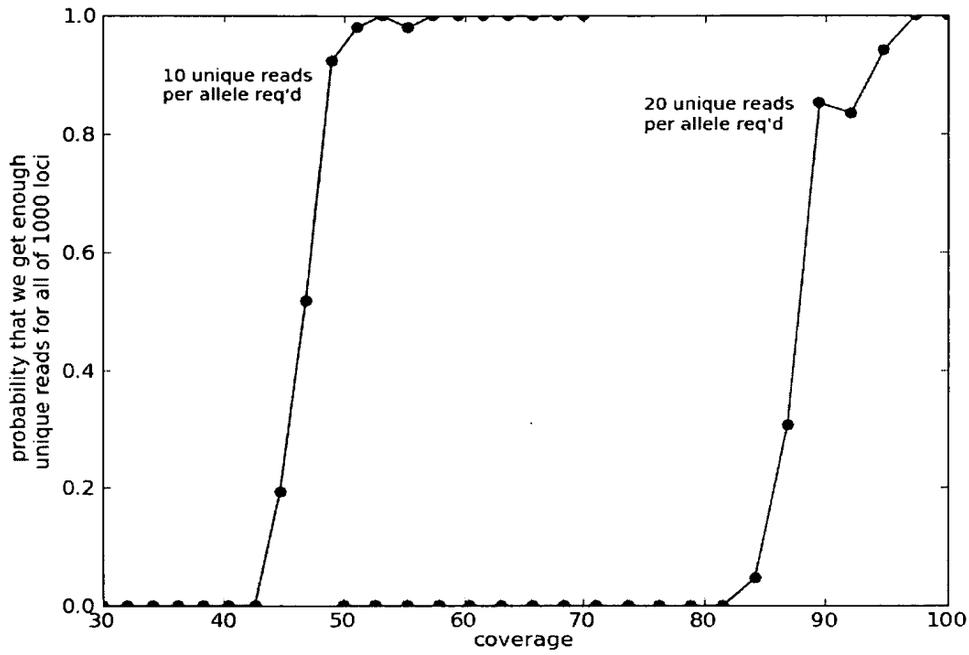

1.0
0.8
0.6
0.4
0.2
0.0
probability that we get enough unique reads for all of 1000 loci
10 unique reads per allele req'd
20 unique reads per allele req'd
30
40
50
60
70
80
90
100
coverage

Figure 8

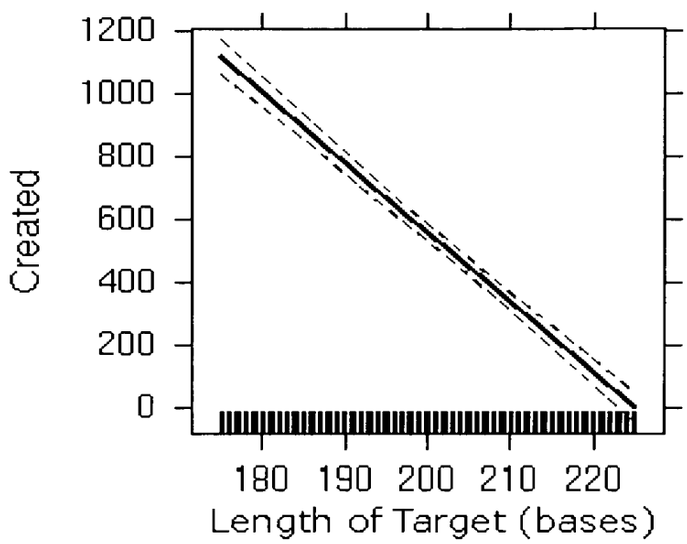

1200
1000
800
600
400
200
0
Created
180 190 200 210 220
Length of Target (bases)

Figure 9

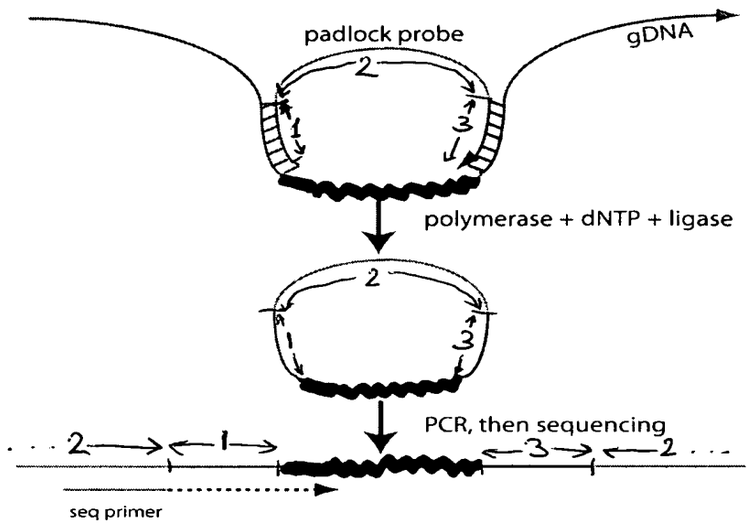

padlock probe
gDNA
2
1
3
polymerase + dNTP + ligase
2
1
3
PCR, then sequencing
2
1
3
2
seq primer

Figure 10

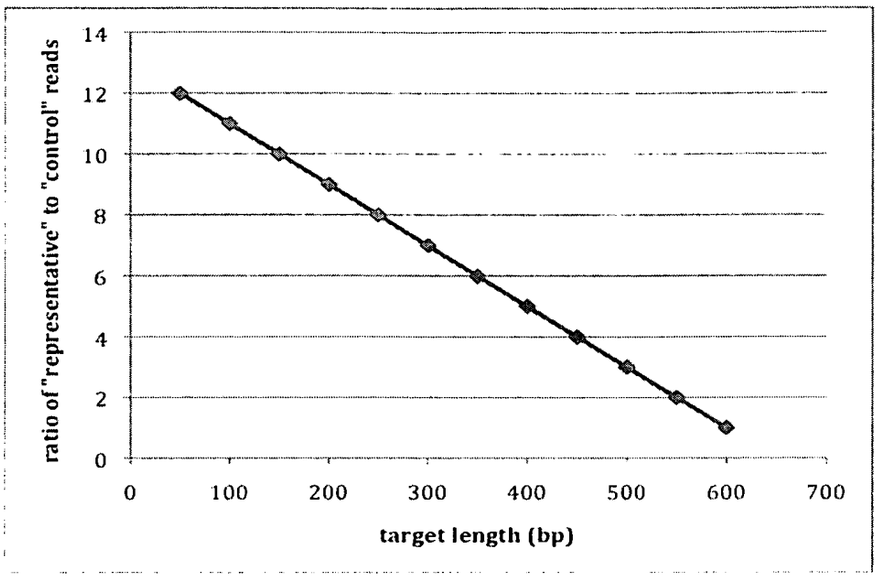

ratio of "representative" to "control" reads
14
12
10
8
6
4
2
0
0
100
200
300
400
500
600
700
target length (bp)

FIGURE 11A

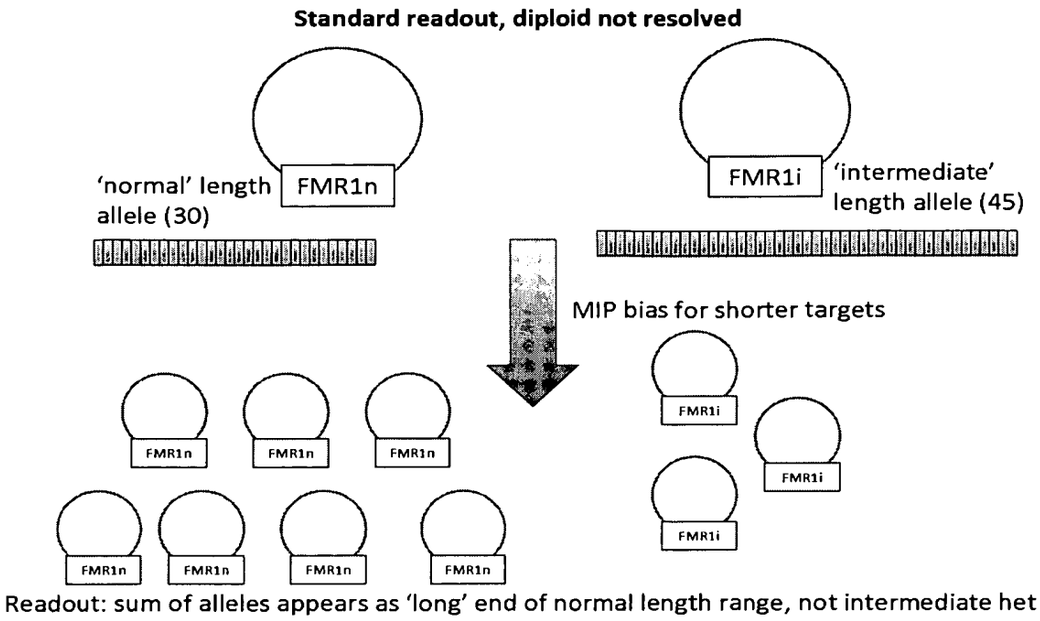

Standard readout, diploid not resolved
'normal' length allele (30)
FMR1n
FMR1i
'intermediate' length allele (45)
MIP bias for shorter targets
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1i
FMR1i
FMR1i
Readout: sum of alleles appears as 'long' end of normal length range, not intermediate het

FIGURE 11B

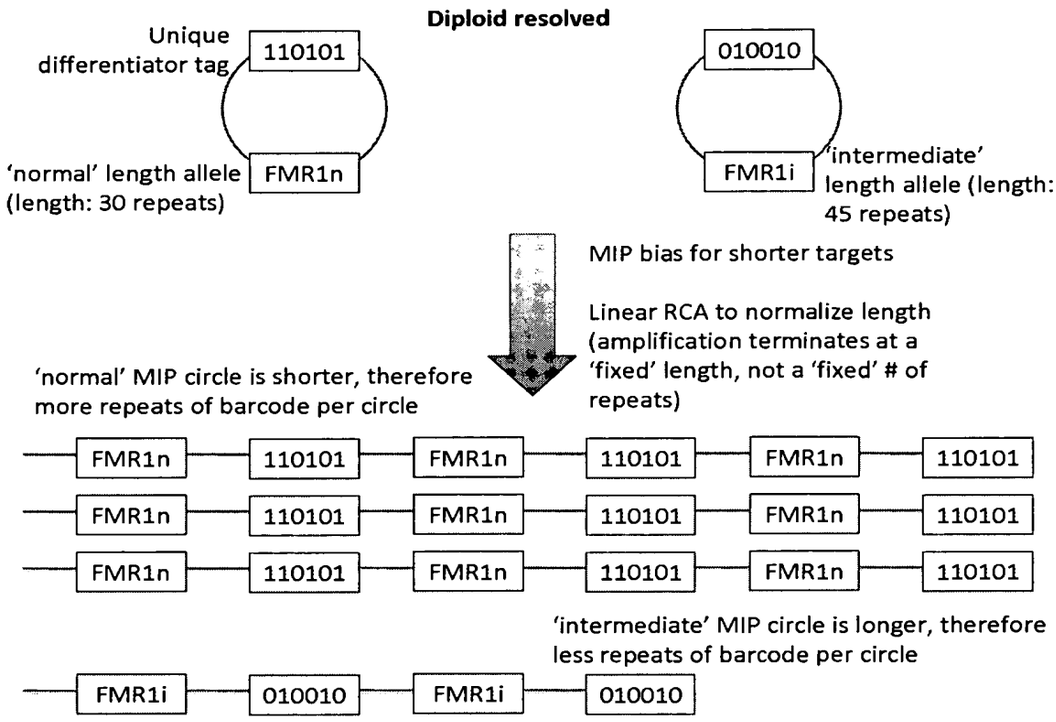

Diploid resolved
Unique differentiator tag
110101
'normal' length allele (length: 30 repeats)
FMR1n
010010
FMR1i
'intermediate' length allele (length: 45 repeats)
MIP bias for shorter targets
Linear RCA to normalize length (amplification terminates at a 'fixed' length, not a 'fixed' # of repeats)
'normal' MIP circle is shorter, therefore more repeats of barcode per circle
FMR1n 110101 FMR1n 110101 FMR1n 110101
FMR1n 110101 FMR1n 110101 FMR1n 110101
FMR1n 110101 FMR1n 110101 FMR1n 110101
'intermediate' MIP circle is longer, therefore less repeats of barcode per circle
FMR1i 010010 FMR1i 010010

FIGURE 11C

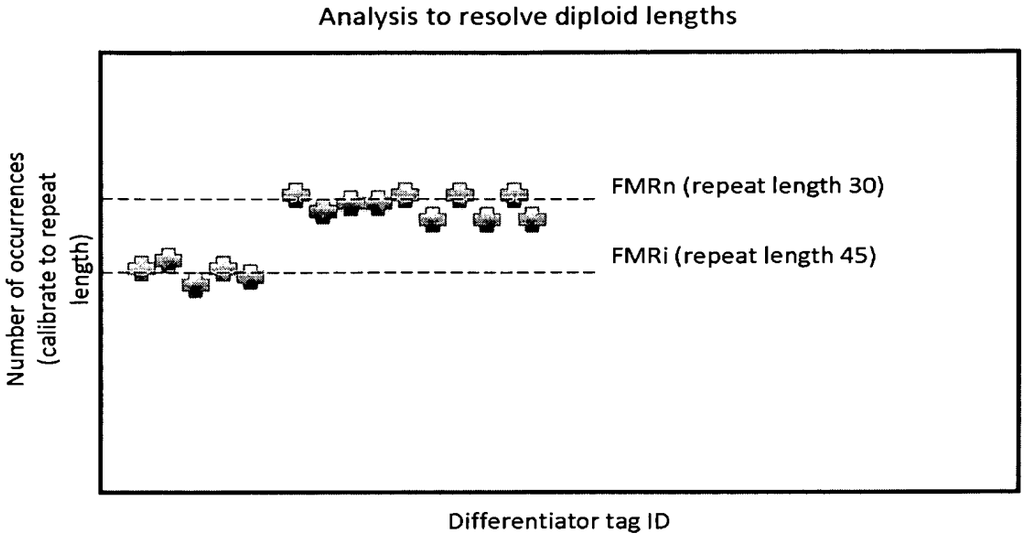

Analysis to resolve diploid lengths
Number of occurrences (calibrate to repeat length)
FMRn (repeat length 30)
FMRi (repeat length 45)
Differentiator tag ID

FIGURE 12

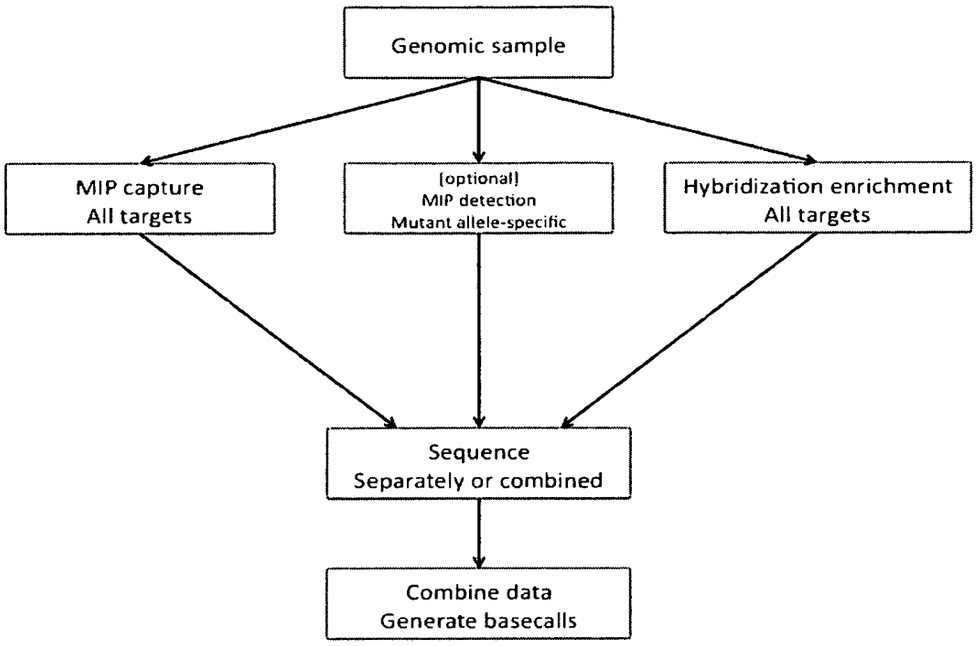

Genomic sample
MIP capture
All targets
(optional)
MIP detection
Mutant allele-specific
Hybridization enrichment
All targets
Sequence
Separately or combined
Combine data
Generate basecalls

FIGURE 13

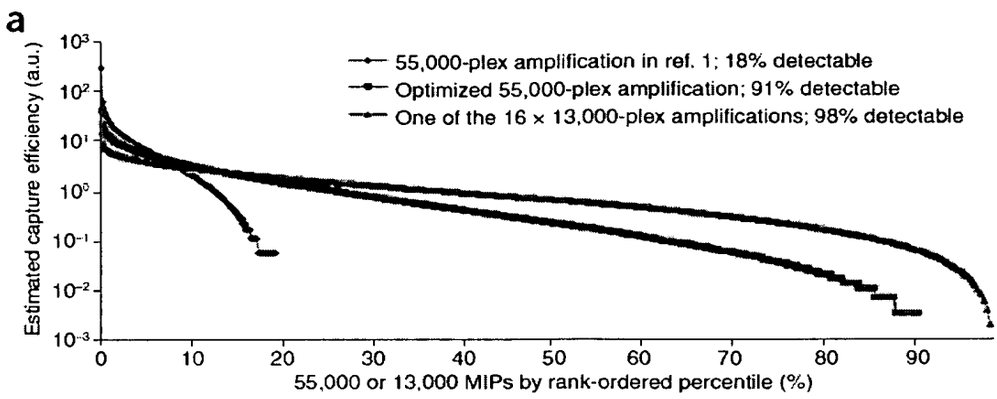
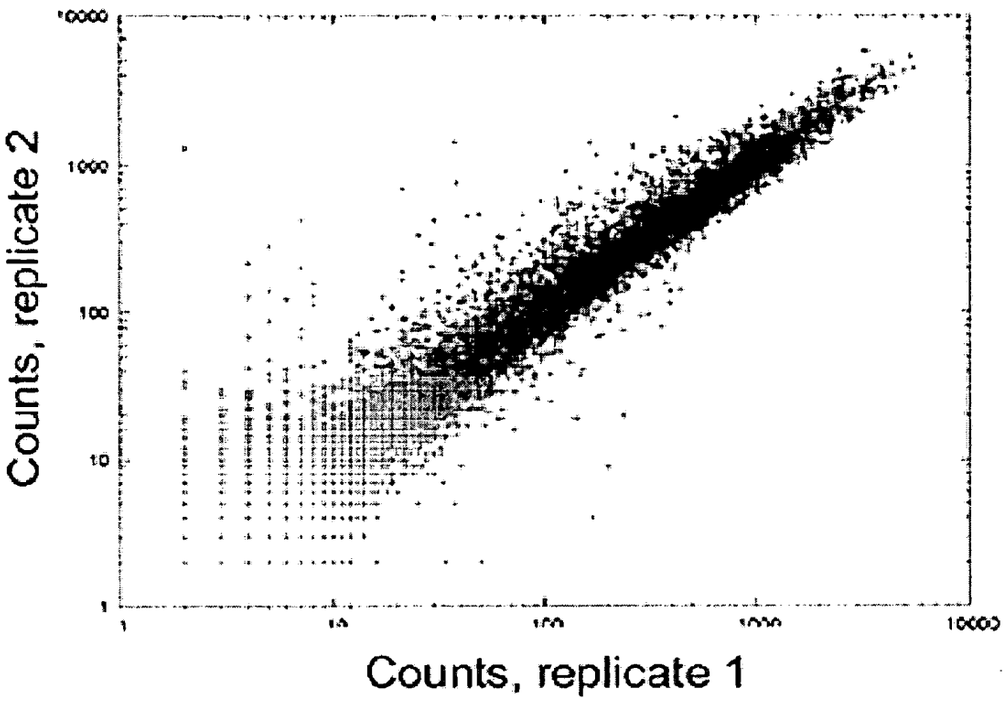

a
Estimated capture efficiency (a.u.)
$10^3$
$10^2$
$10^1$
$10^0$
$10^{-1}$
$10^{-2}$
$10^{-3}$
0
10
20
30
40
50
60
70
80
90
55,000 or 13,000 MIPs by rank-ordered percentile (%)
55,000-plex amplification in ref. 1; 18% detectable
Optimized 55,000-plex amplification; 91% detectable
One of the 16 × 13,000-plex amplifications; 98% detectable

FIGURE 14



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| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | GSGE-002/01US 29258/20 |
|---|---|---|
| | Application Number | |
| Title of Invention | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | |

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

## Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

## Applicant Information:

**Applicant 1** Remove

| Applicant Authority | ◉ Inventor | ○ Legal Representative under 35 U.S.C. 117 | ○ Party of Interest under 35 U.S.C. 118 |
|---|---|---|---|

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Gregory | | Porreca | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service

| City | Cambridge | State/Province | MA | Country of Residence [i] | US |
|---|---|---|---|---|---|

**Citizenship under 37 CFR 1.41(b)** [i] US

**Mailing Address of Applicant:**

| Address 1 | 57 Reservoir Street |
|---|---|
| Address 2 | |

| City | Cambridge | State/Province | MA |
|---|---|---|---|
| Postal Code | 02138 | Country [i] | US |

**Applicant 2** Remove

| Applicant Authority | ◉ Inventor | ○ Legal Representative under 35 U.S.C. 117 | ○ Party of Interest under 35 U.S.C. 118 |
|---|---|---|---|

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Uri | | Laserson | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service

| City | Boston | State/Province | MA | Country of Residence [i] | US |
|---|---|---|---|---|---|

**Citizenship under 37 CFR 1.41(b)** [i] US

**Mailing Address of Applicant:**

| Address 1 | 645 Beacon Street |
|---|---|
| Address 2 | #215 |

| City | Boston | State/Province | MA |
|---|---|---|---|
| Postal Code | 02215 | Country [i] | US |

**Applicant 3** Remove

| Applicant Authority | ◉ Inventor | ○ Legal Representative under 35 U.S.C. 117 | ○ Party of Interest under 35 U.S.C. 118 |
|---|---|---|---|

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Jin | Billy | Li | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service

| City | Medford | State/Province | MA | Country of Residence [i] | US |
|---|---|---|---|---|---|

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | GSGE-002/01US 29258/20 |
|---|---|---|
| | Application Number | |
| Title of Invention | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | |

| | | | | | |
|---|---|---|---|---|---|
| **Citizenship under 37 CFR 1.41(b)** i | CN | | | | |
| **Mailing Address of Applicant:** | | | | | |
| **Address 1** | 100 Station Landing | | | | |
| **Address 2** | Unit 204 | | | | |
| **City** | Medford | **State/Province** | MA | | |
| **Postal Code** | 02155 | **Country**i | US | | |

**Applicant 4** Remove

**Applicant Authority** (•) Inventor ( ) Legal Representative under 35 U.S.C. 117 ( ) Party of Interest under 35 U.S.C. 118

| **Prefix** | **Given Name** | **Middle Name** | **Family Name** | **Suffix** |
|---|---|---|---|---|
| | E. | Robert | Wassman | |

**Residence Information (Select One)** (•) US Residency ( ) Non US Residency ( ) Active US Military Service

| | | | | | |
|---|---|---|---|---|---|
| **City** | Marblehead | **State/Province** | MA | **Country of Residence** i | US |
| **Citizenship under 37 CFR 1.41(b)** i | US | | | | |
| **Mailing Address of Applicant:** | | | | | |
| **Address 1** | 90 West Shore Drive | | | | |
| **Address 2** | | | | | |
| **City** | Marblehead | **State/Province** | MA | | |
| **Postal Code** | 01945 | **Country**i | US | | |

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

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**For further information see 37 CFR 1.33(a).**

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| | | | |
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| **Customer Number** | 21710 | | |
| **Email Address** | ip@brownrudnick.com | Add Email | Remove Email |
| **Email Address** | aschoen@brownrudnick.com | Add Email | Remove Email |
| **Email Address** | tmeyers@brownrudnick.com | | Remove Email |

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | GSGE-002/01US 29258/20 |
|---|---|---|
| | Application Number | |
| Title of Invention | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | |

## Application Information:

| | | | |
|---|---|---|---|
| **Title of the Invention** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |
| **Attorney Docket Number** | GSGE-002/01US 29258/20 | **Small Entity Status Claimed** ☒ | |
| **Application Type** | Nonprovisional | | |
| **Subject Matter** | Utility | | |
| **Suggested Class (if any)** | | **Sub Class (if any)** | |
| **Suggested Technology Center (if any)** | | | |
| **Total Number of Drawing Sheets (if any)** | 16 | **Suggested Figure for Publication (if any)** | 1 |

## Publication Information:

| | |
|---|---|
| ☐ | Request Early Publication (Fee required at time of Request 37 CFR 1.219) |
| ☐ | **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S. C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing. |

## Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
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|---|---|---|---|
| Please Select One: | ◉ Customer Number | ○ US Patent Practitioner | ○ Limited Recognition (37 CFR 11.9) |
| Customer Number | 21710 | | |

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This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

| | | | |
|---|---|---|---|
| Prior Application Status | Pending | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
| | a 371 of international | PCT/US10/01293 | 2010-04-30 |
| Prior Application Status | Expired | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
| PCT/US10/01293 | non provisional of | 61174470 | 2009-04-30 |
| Prior Application Status | Expired | | Remove |

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | GSGE-002/01US 29258/20 |
|---|---|---|
| | Application Number | |
| Title of Invention | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | |

| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
|---|---|---|---|
| | non provisional of | 61178923 | 2009-05-15 |
| Prior Application Status | Expired | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
| | non provisional of | 61179358 | 2009-05-18 |
| Prior Application Status | Expired | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
| | non provisional of | 61182089 | 2009-05-28 |
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This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

Remove

| Application Number | Country [i] | Parent Filing Date (YYYY-MM-DD) | Priority Claimed |
|---|---|---|---|
| | | | ○ Yes ◉ No |

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

## Assignee Information:

Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of Title 37 of the CFR to have an assignment recorded in the Office.

**Assignee 1** Remove

| | | | |
|---|---|---|---|
| If the Assignee is an Organization check here. ☒ | | | |
| Organization Name | Good Start Genetics, Inc. | | |
| **Mailing Address Information:** | | | |
| **Address 1** | 237 Putnam Avenue | | |
| Address 2 | | | |
| **City** | Cambridge | **State/Province** | MA |
| **Country** [i] | US | Postal Code | 02139 |
| Phone Number | | Fax Number | |
| Email Address | | | |

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## Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | GSGE-002/01US 29258/20 |
|---|---|---|
| | Application Number | |
| Title of Invention | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | |

| **Signature** | /s/Thomas Meyers/ | | | Date (YYYY-MM-DD) | 2011-10-28 |
|---|---|---|---|---|---|
| First Name | Thomas | Last Name | Meyers | Registration Number | 36989 |

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# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | |
| **Filing Date:** | |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Filer:** | Thomas C. Meyers/Franziska Salcedo |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |

Filed as Small Entity

## U.S. National Stage under 35 USC 371 Filing Fees

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| Basic National Stage Fee | 2631 | 1 | 190 | 190 |
| Natl Stage Search Fee - U.S. was the ISA | 2641 | 1 | 60 | 60 |
| Natl Stage Exam Fee - all other cases | 2633 | 1 | 125 | 125 |
| **Pages:** | | | | |
| Natl Stage Appl Sz fee per 50 pgs >100 | 2681 | 1 | 155 | 155 |
| **Claims:** | | | | |
| Claims in excess of 20 | 2615 | 19 | 30 | 570 |
| **Miscellaneous-Filing:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| Oath/decl > 30 mo. from priority date | 2617 | 1 | 65 | 65 |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **1165** |

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**APPLICATION NUMBER:** ***61/178,923***
**FILING DATE:** ***May 15, 2009***
**RELATED PCT APPLICATION NUMBER:** ***PCT/US10/01293***

**THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS** ***US61/178,923***

Certified by

David W. Kappos

Under Secretary of Commerce
for Intellectual Property
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PTO/SB/16 (12-08)
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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

**Express Mail Label No.** _____

| INVENTOR(S) | | |
|---|---|---|
| Given Name (first and middle [if any] ) | Family Name or Surname | Residence (City and either State or Foreign Country) |
| Gregory | Porreca | Ocean City, New Jersey |
| Robert | Wassman | Marblehead, Massachusetts |
| | | |
| | | |
| | | |

*Additional inventors are being named on the* _____ *separately numbered sheets attached hereto.*

**TITLE OF THE INVENTION (500 characters max):**

METHODS AND COMPOSITIONS FOR ENHANCING THE SENSITIVITY OF NUCLEIC ACID ANALYSIS

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

[X] The address corresponding to Customer Number: 23628

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Address

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| City | State | Zip |
| Country | Telephone | Email |

**ENCLOSED APPLICATION PARTS (*check all that apply*)**

[X] Application Data Sheet. See 37 CFR 1.76 [ ] CD(s), Number of CDs _____

[X] Drawing(s) *Number of Sheets* 3 [ ] Other (specify) _____

[X] Specification (e.g. description of the invention) *Number of Pages* 31

Fees Due: Filing Fee of $220 ($110 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

**METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT**

[X] Applicant claims small entity status. See 37 CFR 1.27.

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**1666113.1**

***PROVISIONAL APPLICATION COVER SHEET***
***Page 2 of 2***

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| | | | |
|---|---|---|---|
| SIGNATURE | /Daniel W. Young/ | Date | May 15, 2009 |
| TYPED or PRINTED NAME | Daniel W. Young, Ph.D. | REGISTRATION NO. *(if appropriate)* | 62,868 |
| TELEPHONE | 617.646.8000 | Docket Number: | G0814.70003US00 |

**Application Data Sheet**

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**Applicant Information**

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Status:: | Full Capacity |
| Given Name:: | Gregory |
| Family Name:: | Porreca |
| City of Residence:: | Ocean City |
| State or Province of Residence:: | NJ |
| Country of Residence:: | US |
| Street of mailing address:: | 911 Gardens Parkway |
| City of mailing address:: | Ocean City |

| | |
|---|---|
| State or Province of mailing address:: | NJ |
| Postal or Zip Code of mailing address:: | 08226 |

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Status:: | Full Capacity |
| Given Name:: | Robert |
| Family Name:: | Wassman |
| City of Residence:: | Marblehead |
| State or Province of Residence:: | MA |
| Country of Residence:: | US |
| Street of mailing address:: | 90 West Shore Drive |
| City of mailing address:: | Marblehead |
| State or Province of mailing address:: | MA |
| Postal or Zip Code of mailing address:: | 01945 |

**Correspondence Information**

| | |
|---|---|
| Correspondence Customer Number:: | 23628 |

**Representative Information**

| | |
|---|---|
| Representative Customer Number:: | 23628 |

**Domestic Priority Information**

**Foreign Priority Information**

**Assignee Information**

| | |
|---|---|
| Assignee Name:: | Good Start Genetics, Inc. |
| Street of Mailing Address:: | 8 St. Mary's Street, Suite 621 |
| City of Mailing Address:: | Boston |
| State or Province of Mailing Address:: | Massachusetts |
| Country of Mailing Address:: | United States |
| Postal or Zip Code of Mailing Address:: | 02215 |





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# METHODS AND COMPOSITIONS FOR ENHANCING THE SENSITIVITY OF NUCLEIC ACID ANALYSIS

## Field of the Invention

The invention relates to methods for increasing the sensitivity of nucleic acid detection assays.

## Background of the Invention

There are currently many genomic assays that utilize next-generation (polony-based) sequencing to generate data, including genome resequencing, RNA-seq for gene expression, bisulphite sequencing for methylation, and Immune-seq, among others. In order to make quantitative measurements (including genotype calling), these methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids. The majority of these techniques require a preparative step to construct a high-complexity library of DNA molecules that is representative of a sample of interest. Current assays use one of several alternative nucleic acid preparative techniques (e.g., amplification, for example PCR-based amplification; sequence-specific capture, for example, using immobilized capture probes; or target capture into a circularized probe followed by a sequence analysis step. In order to reduce errors associated with the unpredictability (stochastic nature) of nucleic acid isolation and sequence analysis techniques, current methods involve oversampling a target nucleic acid preparation in order to increase the likelihood that all sequences that are present in the original nucleic acid sample will be represented in the final sequence data. For example, a genomic sequencing library may contain an over- or under-representation of particular sequences from a source nucleic acid sample (e.g., genome preparation) as a result of stochastic variations in the library construction process. Such variations can be particularly problematic when they result in target sequences from a genome being absent or undetectable in a sequencing library. For example, an under-representation of particular allelic sequences (e.g., heterozygotic alleles) from a genome in a sequencing library can result in an apparent homozygous representation in a sequencing library. As most downstream sequencing library quantification techniques depend on stochastic counting processes, these problems have typically been addressed by sampling enough (over-sampling) to obtain a minimum number



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of observations necessary to make statistically significant decisions. However, the strategy of oversampling is generally limited to elimination of low-count Poisson noise, and the approach wastes resources and increases the expense required to perform such experiments. Moreover, oversampling can result in a reduced statistical confidence in certain conclusions (e.g., diagnostic calls) based on the data. Accordingly, new approaches are needed for overcoming variations in sequencing library preparatory methods.

## Brief Summary of the Invention

Aspects of the invention relate to basing a nucleic acid sequence analysis on results from two or more different nucleic acid preparatory techniques that have different systematic biases in the types of nucleic acids that they sample. According to the invention, different techniques have different sequence biases that are systematic and not simply due to stochastic effects during nucleic acid capture or amplification. Accordingly, the degree of oversampling required to overcome variations in nucleic acid preparation needs to be sufficient to overcome the biases.

According to the invention, different techniques have different characteristic or systematic biases. For example, one technique may bias a sample analysis towards one particular allele at a genetic locus of interest, whereas a different technique would bias the sample analysis towards a different allele at the same locus. Accordingly, the same sample may be identified as being different depending on the type of technique that is used to prepare nucleic acid for sequence analysis. This effectively represents a sensitivity issue, because each technique has different relative sensitivities for polymorphic sequences of interest.

According to aspects of the invention, the sensitivity of a nucleic acid analysis can be increased by combining the sequences from different nucleic acid preparative steps and using the combined sequence information for a diagnostic assay (e.g., for a making a call as to whether a subject is homozygous or heterozygous at a genetic locus of interest).

In some embodiments, the invention provides a method of increasing the sensitivity of a nucleic acid detection assay by obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample, obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample, assaying the sequences obtained in both first and second nucleic acid preparations, and using the sequence information from both first and second nucleic acid preparations to determine the genotype of the target nucleic acid in the biological sample, wherein the first and second preparative



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methods have different systematic sequence biases. In some embodiments, the first and second nucleic acid preparations are combined prior to performing a sequence assay. In some embodiments, separate sequence assays are performed on the first and second nucleic acid preparations and the sequence information from both assays are combined to determine the genotype of the target nucleic acid in the biological sample. In some embodiments, the first preparative method is an amplification-based, a hybridization-based, or a circular probe-based preparative method. In some embodiments, the second method is an amplification-based, a hybridization-based, or a circular probe-based preparative method. In some embodiments, the first and second methods are of different types (e.g., only one of them is an amplification-based, a hybridization-based, or a circular probe-based preparative method, and the other one is one or the other two types of method). Accordingly, in some embodiments the second preparative method is an amplification-based, a hybridization-based, or a circular probe-based preparative method, provided that the second method is different from the first method. However, in some embodiments, both methods may be of the same type, provided they are different methods (e.g., both are amplification based or hybridization-based, but are different types of amplification or hybridization methods, e.g., with different relative biases).

In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using a pair of oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or both of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and identifier sequences (e.g., source sequences). PCR based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel).

Hybridization-based preparative methods involve selectively immobilizing target nucleic acids for further manipulation. It is to be understood that one or more oligonucleotides (immobilization oligonucleotides), which in some embodiments may be from 15 to 170 nucleotides in length, are used which hybridize along the length of a target region of a genetic locus to immobilize it. In some embodiments, immobilization oligonucleotides are either immobilized before hybridization is performed (e.g.,



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Roche/Nimblegen 'sequence capture'), or are prepared such that they include a moiety (e.g. biotin) which can be used to selectively immobilize the target nucleic acid after hybridization by binding to e.g., streptavidin-coated microbeads (e.g. Agilent 'SureSelect').

Circularization selection-based preparative methods selectively convert each region of interest into a covalently-closed circular molecule which is then isolated by removal (usually enzymatic, e.g. with exonuclease) of any non-circularized linear nucleic acid. Oligonucleotide probes are designed which have ends that flank the region of interest. The probes are allowed to hybridize to the genomic target, and enzymes are used to first (optionally) fill in any gap between probe ends and second ligate the probe closed. Following circularization, any remaining (non-target) linear nucleic acid is removed, resulting in isolation (capture) of target nucleic acid. Circularization selection-based preparative methods include molecular inversion probe capture reactions and 'selector' capture reactions. In some embodiments, molecular inversion probe capture of a target nucleic acid is indicative of the presence of a polymorphism in the target nucleic acid.

A variety of methods may be used to evaluate and compare bias profiles of each preparative technique. Next-generation sequencing may be used to quantitatively measure the abundance of each isolated target nucleic acid obtained from a certain preparative method. This abundance may be compared to a control abundance value (e.g., a known starting abundance of the target nucleic acid) and/or with an abundance determined through the use of an alternative preparative method. For example, a set of target nucleic acids may be isolated by one or more of the three preparative methods; the target nucleic acid may be observed x times using the amplification technique, y times using the hybridization enrichment technique, and z times using the circularization selection technique. A pairwise correlation coefficient may be computed between each abundance value (e.g. x and y, x and z, and y and z) to assess bias in nucleic acid isolation between pairs of preparative methods. Since the mechanisms of isolation are different in each approach, the abundances will usually be different and largely uncorrelated with each other.

In some embodiments, the invention provides a method of obtaining a nucleic acid preparation that is representative of a target nucleic acid in a biological sample by obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample, obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample, and combining the first and second nucleic acid preparations to obtain a combined preparation that is representative of the target nucleic acid in the biological sample.



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In some embodiments of any of the methods described herein, a third preparation of the target nucleic acid is obtained using a third preparative method that is different from the first and second preparative methods, wherein the first, second, and third preparative methods all have different systematic sequence biases. In some embodiments of any of the methods described herein, the different preparative methods are used for a plurality of different loci in the biological sample to increase the sensitivity of a multiplex nucleic acid analysis. In some embodiments, the target nucleic acid has a sequence of a gene selected from Table 1.

However, it should be appreciated that a genotyping method of the invention may include several steps, each of which independently may involve one or more different preparative techniques described herein. In some embodiments, a nucleic acid preparation may be obtained using one or more (e.g., 2, 3, 4, 5, or more) different techniques described herein (e.g., amplification, hybridization capture, circular probe capture, etc., or any combination thereof) and the nucleic acid preparation may be analyzed using one or more different techniques (e.g., amplification, hybridization capture, circular probe capture, etc., or any combination thereof) that are selected independently of the techniques used for the initial preparation.

Aspects of the invention also provide compositions, kits, devices, and analytical methods for increasing the sensitivity of nucleic acid assays. Aspects of the invention are particularly useful for increasing the confidence level of genotyping analyses. However, aspects of the invention may be used in the context of any suitable nucleic acid analysis, for example, but not limited to, a nucleic acid analysis that is designed to determine whether more than one sequence variant is present in a sample.

In some embodiments, a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject). In some embodiments, an assessment is provided to a couple, based on their respective genotypes at one or more genetic loci, of the risk of their having one or more children having a genotype associated with a disease or condition (e.g., a homozygous or heterozygous genotype associated with a disease or condition). In some embodiments, a subject or a couple may seek genetic or reproductive counseling in connection with a genotype determined according to embodiments of the invention. The methods disclosed herein are useful for any application where sensitivity is important. For example, detection of cancer mutations in a heterogenous tissue sample, detection of mutations in maternally-circulating fetal DNA, and detection of mutations in cells isolated during a preimplantation genetic diagnostic procedure.



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According to some aspects of the invention, methods of detecting a polymorphism in a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise obtaining a nucleic acid preparation using a preparative method (e.g., any of the preparative methods disclosed herein) on a biological sample, and performing a molecular inversion probe capture reaction on the nucleic acid preparation, wherein a molecular inversion probe capture (e.g., using a mutation-detection MIP) of a target nucleic acid of the nucleic acid preparation is indicative of the presence of a mutation (polymorphism) in the target nucleic acid, optionally wherein the polymorphism is selected from Table 2.

According to some aspects of the invention, methods of genotyping a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise obtaining a nucleic acid preparation using a preparative method on a biological sample, sequencing a target nucleic acid of the nucleic acid preparation, and performing a molecular inversion probe capture reaction on the biological sample, wherein a molecular inversion probe capture of the target nucleic acid in the biological sample is indicative of the presence of a polymorphism in the target nucleic acid, genotyping the target nucleic acid based on the results of the sequencing and the capture reaction.

In some embodiments of the methods disclosed herein, the target nucleic acid has a sequence of a gene selected from Table 1.

## Brief Description of the Drawings

Figure 1 is a schematic of an embodiment of the invention showing MIPs plus hybridization enrichment, plus optionally extra MIPs targeted to specific known, common disease-associated loci, e.g., to detect the presence of a polymorphism in a target nucleic acid.

Figure 2 is a graph of per-target abundance with MIP capture. In this graph, bias largely drives the heterozygote error rate, since targets which are less abundant here are less likely to be covered in sufficient depth during sequencing to adequately sample both alleles. This is from Turner et al 2009, Nature methods 6:315-6. Hybridization enrichment results in a qualitatively similar abundance distribution, but the abundance of a given target is likely not correlated between the two methods.

Figure 3 is a graph of correlation between two MIP capture reactions from Ball et al 2009, Nature biotechnology 27:361-8. Each point represents the target abundance in replicate 1 and replicate 2. Pearson correlation r=0.956. This indicates that MIP capture



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reproducibly biases targets to specific abundances. Hybridization enrichment is similarly correlated from one capture to the next.

## Detailed Description of the Invention

Aspects of the invention relate to basing a nucleic acid sequence analysis on results from two or more different nucleic acid preparatory techniques that have different systematic biases in the types of nucleic acids that they sample. According to the invention, different techniques have different sequence biases that are systematic and not simply due to stochastic effects during nucleic acid capture or amplification. Accordingly, in some embodiments, the degree of oversampling required to overcome variations in nucleic acid preparation needs to be sufficient to overcome the biases. In some embodiments, the invention provides methods that reduce the need for oversampling by combining nucleic acid and/or sequence results obtained from two or more different nucleic acid preparative techniques that have different biases.

According to the invention, different techniques have different characteristic or systematic biases. For example, one technique may bias a sample analysis towards one particular allele at a genetic locus of interest, whereas a different technique would bias the sample analysis towards a different allele at the same locus. Accordingly, the same sample may be identified as being different depending on the type of technique that is used to prepare nucleic acid for sequence analysis. This effectively represents a sensitivity issue, because each technique has a different relative sensitivities for polymorphic sequences of interest.

According to aspects of the invention, the sensitivity of a nucleic acid analysis can be increased by combining the sequences from different nucleic acid preparative steps and using the combined sequence information for a diagnostic assay (e.g., for a making a call as to whether a subject is homozygous or heterozygous at a genetic locus of interest).

Currently, the ability of DNA sequencing to detect mutations is limited by the ability of the upstream sample isolation (e.g., by amplification, immobilization enrichment, circularization capture, etc.) methods to reliably isolate the locus of interest. If one wishes to make heterozygote base-calls for a diploid genome (e.g. a human sample presented for molecular diagnostic sequencing), it is imperative that the isolation method produces near- or perfectly- uniform amounts of the two alleles to be sequenced.

Sample preparative methods may fall into three classes: 1) single- or several- target amplification (e.g. uniplex PCR, 'multiplex' PCR), 2) multi-target hybridization enrichment



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(e.g. Agilent SureSelect 'hybrid capture' [Gnirke et al 2009, *Nature methods* 27:182-9], Roche/Nimblegen 'sequence capture' [Hodges et al 2007, *Nature genetics* 39:1522-7], and 3) multi-target circularization selection (e.g. molecular inversion probes or padlock probes, [Porreca et al 2007, *Nature methods* 4:931-6, Turner et al 2009, *Nature methods* 6:315-6], 'selectors' [Dahl et al 2005, *Nucleic acids research* 33:e71]). Each of these methods can result in a pool of isolated product that does not adequately represent the input abundance distribution. For example, the two alleles at a heterozygous position can become skewed far from their input 50:50 ratio to something that results in a missed basecall during downstream sequencing. For example, if the ratio was skewed from 50:50 to 10:90, and the sample was sequenced to 10X average coverage, there is a high probability that one of the two alleles would not be observed once in the ten sequencing reads. This would reduce the sensitivity of the sequencing method by converting a heterozygous position to homozygous (where potentially the 'mutant' allele was the one not observed).

The methods disclosed herein are based, in part, on the discovery that certain classes of isolation methods have different modes of bias. The disclosure provide methods for increasing the sensitivity of the downstream sequencing by using a combination of multiple isolation methods (e.g., one or more from at least two of the classes disclosed herein) for a sample. This is particularly important in molecular diagnostics where high sensitivity is required to minimize the chances of 'missing' a disease-associated mutation. For example, given a nominal false-negative error rate of $1x10^{-3}$ for sequencing following circularization selection, and a false-negative error rate of $1x10^{-3}$ for sequencing following hybridization enrichment, one can achieve a final false-negative rate of $1x10^{-6}$ by performing both techniques on the sample (assuming failures in each method are fully independent). For a recessive disease with carrier frequency of 0.1, caused by a single fully-penetrant mutant allele, the number of missed carrier diagnoses would decrease from 1000 per million patients tested to 1 per million patients tested. Furthermore, if the testing was used in the context of prenatal carrier screening, the number of affected children born as a result of missing the carrier call in one parent would decrease from 25 per million to 25 per billion born.

Additionally, the disclosure provides combinations of preparative methods to effectively increase sequencing coverage in regions containing disease-associated alleles. Since heterozygote error rate is largely tied to both deviations from 50:50 allele representation, and in the case of next-generation DNA sequencing deviations from average abundance (such that less abundant isolated targets are more likely to be undersampled at one or both alleles), selectively increasing coverage in these regions will also selectively increase



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sensitivity. Furthermore, MIPs that detect presence or absence of specific known disease-associated mutations can be used to increase sensitivity selectively. These MIPs would have a targeting arm whose 3' –most region is complementary to the expected mutation, and has a fill-in length of 0 or more bp. Thus, the MIP will form only if the mutation is present, and its presence will be detected by sequencing.

Additionally, algorithms disclosed herein may be used to determine base identity with varying levels of stringency depending on whether the given position has any known disease-associated alleles. Stringency can be reduced in such positions by decreasing the minimum number of observed mutant reads necessary to make a consensus base-call. This will effectively increase sensitivity for mutant allele detection at the cost of decreased specificity.

In some aspects, the disclosure provides methods for analyzing a plurality of target sequences which are genetic loci or portions of genetic loci (e.g., a genetic locus of Table 1). The genetic loci may be analyzed by sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs). Exemplary polymorphisms are disclosed in Table 2. The skilled artisan will appreciate that other polymorphisms are known in the art and may be identified, for example, by querying the Entrez Single Nucleotide Polymorphism database, for example, by searching with a GeneID from Table 1.

The mutations listed in Table 2 are documented polymorphisms in several disease-associated genes (CFTR is mutated in cystic fibrosis, GBA is mutated in Gaucher disease, ASPA is mutated in Canavan disease, HEXA is mutated in Tay Sachs disease). The polymorphisms are of several types: insertion/deletion polymorphisms which will cause frameshifts (and thus generally interrupt protein function) unless the insertion/deletion length is a multiple of 3bp, and substitutions which can alter the amino acid sequence of the protein and in some cases cause complete inactivation by introduction of a stop codon.

The methods disclosed here are also useful for diagnosis of one or more heritable disorders. In some embodiments, a heritable disorder that may be diagnosed with the methods disclosed herein is a genetic disorder that is prevalent in the Ashkenazi Jewish population. In some embodiments, the heritable disorders are selected from: 21-Hydroxylase-Defiocient Congenital Adrenal Hyperplasia; ABCC8-Related Hyperinsulinism; Alpha-Thalassemia, includes Constant Spring, & MR associated; Arylsulfatase A Deficiency-Metyachromatic Leukodystrophy; Biotinidase Deficiency-Holocarboxylase Synthetase Deficiency; Bloom's Syndrome; Canavan Disease; CFTR-Related Disorders-cystic fibrosis; Citrullinemia Type I; Combined MMA & Homocystinuria-dblC; Dystrophinopathies (DMD & BMD); Familial Dysautonomia; Fanconi Anemia-FANCC; Galactosemia-Classical:



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Galactokinase Defiency & Galactose Epimerase Deficiency; Gaucher Disease; GJB2-Related DFNB 1 Nonsyndromic Hearing Loss and Deafness; Glutaric acidemia Type 1; Hemoglobinopathies beta-chain disorders; Glycogen Storage Disease Type 1A; Maple Syrup Urine Disease; Types 1A, 1B, 2, 3; Medium Chain Acyl-Coenzyme A; Dehydrogenase Deficiency-MCADD; Methylmalonic Acidemia; Mucolipidosis IV; Nemaline Myopathy; Nieman-Pick Type A-Acid Sphingomyelinase Deficiency; Non-Ketotic Hyperglycinemia-Glycine Encephalopathy; Ornithine Transcarbamylase Deficiency; PKU Phenylalanine Hydroxylase Deficiency; Propionic Acidemia; Short Chain Acyl-CoA Dehydrogenase Deficiency-SCADD; Smith-Lemli-Opitz Syndrome; Spinal Muscular Atrophy (SMN1)-SMA; Tay Sachs-HexA Deficiency; Usher Synbdrome-Type I (Type IB, Type IC, Type ID, Type IF, Type IG) ; X-Linked Mental Retardation ARX-Related Disorders; X-Linked Mental Retardation with Cerebellar Cypoplasia and sistinctive Facial Appearance; X-Linked Mental Retardation; includes 9, 21, 30, 46, 58, 63, 88, 89; X-linked mental retardation: FM1-Related Disorders-FRXA, Fragile X MR; X-linked SMR: Renpenning Syndrome 1; Zellweger Spectrum disorders - Peroxisomal Bifunctional Enzyme Deficiencies including Zellweger, NALD, infantile Refsums.

**Table 1: Target Nucleic Acids**

| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| CYP21A2 | 1589 | cytochrome P450, family 21, subfamily A, polypeptide 2 | CAH1; CPS1; CA21H; CYP21; CYP21B; P450c21B; MGC150536; MGC150537; CYP21A2 | 201910 | NG_008337.1 | 6p21.3 |
| ABCC8 | 6833 | ATP-binding cassette, sub-family C (CFTR/MRP), member 8 | HI; SUR; HHF1; MRP8; PHHI; SUR1; ABC36; HRINS; TNDM2; ABCC8 | 600509 | NG_008867.1 | 11p15.1 |
| ATRX | 546 | alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) | SHS; XH2; XNP; ATR2; SFM1; RAD54; MRXHF1; RAD54L; ZNF-HX; MGC2094; ATRX | 300032 | NG_008838.1 | Xq13.1-q21.1 |
| ARSA | 410 | arylsulfatase A | MLD; ARSA | 607574 | NG_009260.1 | 22q13.31-qter; 22q13.33 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| PSAP | 5660 | Prosaposin | GLBA; SAP1; FLJ00245; MGC110993; PSAP | 176801 | NG_008835.1 | 10q21-q22 |
| BTD | 686 | Biotinidase | BTD | 609019 | NG_008019.1 | 3p25 |
| HLCS | 3141 | holocarboxylase synthetase (biotin-(proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)) ligase) | HCS; HLCS | 609018 | NC_000021.7 | 21q22.1; 21q22.13 |
| BLM | 641 | Bloom syndrome, RecQ helicase-like | BS; RECQ2; RECQL2; RECQL3; MGC126616; MGC131618; MGC131620; BLM | 604610 | NG_007272.1 | 15q26.1 |
| ASPA | 443 | aspartoacylase (Canavan disease) | ASP; ACY2; ASPA | 608034 | NG_008399.1 | 17pter-p13 |
| CFTR | 1080 | cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) | CF; MRP7; ABC35; ABCC7; CFTR/MRP; TNR-CFTR; dJ760C5.1; CFTR | 602421 | NC_000007.12 | 7q31.2 |
| ASS1 | 445 | argininosuccinate synthetase 1 | ASS; CTLN1; ASS1 | 603470 | NG_011542.1 | 9q34.1 |
| MMACHC | 25974 | methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria | cblC; FLJ25671; DKFZp564I122; RP11-291L19.3; MMACHC | 609831 | NC_000001.9 | 1p34.1 |
| IKBKAP | 8518 | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein | FD; DYS; ELP1; IKAP; IKI3; TOT1; FLJ12497; DKFZp781H1425; IKBKAP | 603722 | NG_008788.1 | 9q31 |
| FANCC | 2176 | Fanconi anemia, complementation group C | FA3; FAC; FACC; FLJ14675; FANCC | 227645 | NG_011707.1 | 9q22.3 |
| GALK1 | 2584 | galactokinase 1 | GK1; GALK; GALK1 | 604313 | NG_008079.1 | 17q24 |
| GALT | 2592 | galactose-1-phosphate uridylyltransferase | GALT | 606999 | NC_000009.10 | 9p13 |
| GALE | 2582 | UDP-galactose-4-epimerase | SDR1E1; FLJ95174; FLJ97302; GALE | 606953 | NG_007068.1 | 1p36-p35 |
| GBA | 2629 | glucosidase, beta; acid (includes | GCB; GBA1; GLUC; GBA | 606463 | NG_009783.1 | 1q21 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| | | glucosylceramidase) | | | | |
| GJB2 | 2706 | gap junction protein, beta 2, 26kDa | HID; KID; PPK; CX26; DFNA3; DFNB1; NSRD1; DFNA3A; DFNB1A; GJB2 | 121011 | NG_008358.1 | 13q11-q12 |
| GCDH | 2639 | glutaryl-Coenzyme A dehydrogenase | GCD; ACAD5; GCDH | 608801 | NG_009292.1 | 19p13.2 |
| G6PC | 2538 | glucose-6-phosphatase, catalytic subunit | G6PT; GSD1; GSD1a; MGC163350; G6PC | 232200 | NG_011808.1 | 17q21 |
| HBB | 3043 | hemoglobin, beta | CD113t-C; beta-globin; HBB | 141900 | NG_000007.3 | 11p15.5 |
| BCKDHA | 593 | branched chain keto acid dehydrogenase E1, alpha polypeptide | MSU; MSUD1; OVD1A; BCKDE1A; FLJ45695; BCKDHA | 608348 | NC_000019.8 | 19q13.1-q13.2 |
| BCKDHB | 594 | branched chain keto acid dehydrogenase E1, beta polypeptide | E1B; FLJ17880; dJ279A18.1; BCKDHB | 248611 | NG_009775.1 | 6q13-q15 |
| DBT | 1629 | dihydrolipoamide branched chain transacylase E2 | E2; E2B; BCATE2; MGC9061; DBT | 248610 | NG_011852.1 | 1p31 |
| DLD | 1738 | dihydrolipoamide dehydrogenase | E3; LAD; DLDH; GCSL; PHE3; DLD | 238331 | NG_008045.1 | 7q31-q32 |
| ACADM | 34 | acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain | MCAD; ACAD1; MCADH; FLJ18227; FLJ93013; FLJ99884; ACADM | 607008 | NG_007045.1 | 1p31 |
| MMAA | 166785 | methylmalonic aciduria (cobalamin deficiency) cblA type | cblA; MGC120010; MGC120011; MGC120012; MGC120013; MMAA | 607481 | NG_007536.1 | 4q31.22 |
| MMAB | 326625 | methylmalonic aciduria (cobalamin deficiency) cblB type | ATR; cblB; MGC20496; MMAB | 607568 | NG_007096.1 | 12q24 |
| MUT | 4594 | methylmalonyl Coenzyme A mutase | MCM; MUT | 609058 | NG_007100.1 | 6p12.3 |
| MCOLN1 | 57192 | mucolipin 1 | ML4; MLIV; MST080; TRPML1; MSTP080; TRP-ML1; TRPM-L1; MCOLN1 | 605248 | NC_000019.8 | 19p13.3-p13.2 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| ACTA1 | 58 | actin, alpha 1, skeletal muscle | ACTA; ASMA; CFTD; MPFD; NEM1; NEM2; NEM3; CFTD1; CFTDM; ACTA1 | 102610 | NG_006672.1 | 1q42.13 |
| TPM3 | 7170 | tropomyosin 3 | TM3; TRK; NEM1; TM-5; TM30; TM30nm; TPMsk3; hscp30; MGC3261; FLJ41118; MGC14582; MGC72094; OK/SW-cl.5; TPM3 | 191030 | NG_008621.1 | 1q21.2 |
| TNNT1 | 7138 | troponin T type 1 (skeletal, slow) | ANM; TNT; STNT; TNTS; FLJ98147; MGC104241; TNNT1 | 191041 | NG_011829.1 | 19q13.4 |
| NEB | 4703 | nebulin | NEM2; NEB177D; FLJ11505; FLJ36536; FLJ39568; FLJ39584; DKFZp686C1456; NEB | 161650 | NG_009382.1 | 2q22 |
| SMPD1 | 6609 | sphingomyelin phosphodiesterase 1, acid lysosomal | ASM; NPD; SMPD1 | 607608 | NG_011780.1 | 11p15.4-p15.1 |
| GLDC | 2731 | glycine dehydrogenase (decarboxylating) | GCE; NKH; GCSP; HYGN1; MGC138198; MGC138200; GLDC | 238300 | NC_000009.10 | 9p22 |
| GCSH | 2653 | glycine cleavage system protein H (aminomethyl carrier) | GCE; NKH; GCSH | 238330 | NC_000016.8 | 16q23.2 |
| AMT | 275 | aminomethyltransferase | GCE; NKH; GCST; AMT | 238310 | NC_000003.10 | 3p21.2-p21.1 |
| OTC | 5009 | ornithine carbamoyltransferase | OCTD; MGC129967; MGC129968; MGC138856; OTC | 300461 | NG_008471.1 | Xp21.1 |
| PAH | 5053 | phenylalanine hydroxylase | PH; PKU; PKU1; PAH | 612349 | NG_008690.1 | 12q22-q24.2 |
| DHPR | 5860 | quinoid dihydropteridine reductase | DHPR; PKU2; SDR33C1; FLJ42391; QDPR | 612676 | NG_008763.1 | 4p15.31 |
| PTS | 5805 | 6-pyruvoyltetrahydropterin synthase | PTPS; FLJ97081; PTS | 261640 | NG_008743.1 | 11q22.3-q23.3 |
| PCCA | 5095 | propionyl Coenzyme A carboxylase, alpha polypeptide | PCCA | 232000 | NG_008768.1 | 13q32 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| PCCB | 5096 | propionyl Coenzyme A carboxylase, beta polypeptide | DKFZp451E113; PCCB | 232050 | NG_008939.1 | 3q21-q22 |
| ACADS | 35 | acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain | SCAD; ACAD3; ACADS | 606885 | NG_007991.1 | 12q22-qter |
| DHCR7 | 1717 | 7-dehydrocholesterol reductase | SLOS; DHCR7 | 602858 | NC_000011.8 | 11q13.2-q13.5 |
| SMN1 | 6606 | survival of motor neuron 1, telomeric | SMA; SMN; SMA1; SMA2; SMA3; SMA4; SMA@; SMNT; BCD541; T-BCD541; SMN1 | 600354 | NG_008691.1 | 5q13 |
| HEXA | 3073 | hexosaminidase A (alpha polypeptide) | TSD; MGC99608; HEXA | 606869 | NG_009017.1 | 15q23-q24 |
| MYO7A | 4647 | myosin VIIA | DFNB2; MYU7A; NSRD2; USH1B; DFNA11; MYOVIIA; MYO7A | 276903 | NG_009086.1 | 11q13.5 |
| USH1C | 10083 | Usher syndrome 1C (autosomal recessive, severe) | PDZ73; AIE-75; DFNB18; PDZ-45; PDZ-73; NY-CO-37; NY-CO-38; ush1cpst; PDZ-73/NY-CO-38; USH1C | 605242 | NC_000011.8 | 11p15.1-p14 |
| CDH23 | 64072 | cadherin-like 23 | USH1D; DFNB12; FLJ00233; FLJ36499; KIAA1774; KIAA1812; MGC102761; DKFZp434P2350; CDH23 | 605516 | NG_008835.1 | 10q21-q22 |
| PCDH15 | 65217 | protocadherin 15 | USH1F; DFNB23; DKFZp667A1711; PCDH15 | 605514 | NG_009191.1 | 10q21.1 |
| SANS | 124590 | Usher syndrome 1G (autosomal recessive) | SANS; ANKS4A; FLJ33924; USH1G | 607696 | NG_007882.1 | 17q25.1 |
| ARX | 170302 | aristaless related homeobox | ISSX; PRTS; MRX29; MRX32; MRX33; MRX36; MRX38; MRX43; MRX54; MRX76; MRX87; MRXS1; ARX | 300382 | NG_008281.1 | Xp21 |
| OPHN1 | 4983 | oligophrenin 1 | OPN1; MRX60; OPHN1 | 300127 | NG_008960.1 | Xq12 |
| JARID1C | 8242 | lysine (K)-specific demethylase 5C | MRXJ; SMCX; MRXSJ; XE169; JARID1C; | 314690 | NG_008085.1 | Xp11.22-p11.21 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| | | | DXS1272E; KDM5C | | | |
| FTSJ1 | 24140 | FtsJ homolog 1 (E. coli) | JM23; MRX9; SPB1; TRM7; CDLIV; MRX44; FTSJ1 | 300499 | NG_008879.1 | Xp11.23 |
| SLC6A8 | 6535 | solute carrier family 6 (neurotransmitter transporter, creatine), member 8 | CRT; CT1; CRTR; MGC87396; SLC6A8 | 300036 | NC_000023.9 | Xq28 |
| DLG3 | 1741 | discs, large homolog 3 (Drosophila) | MRX; MRX90; NEDLG; NE-Dlg; SAP102; SAP-102; KIAA1232; DLG3 | 300189 | NC_000023.9 | Xq13.1 |
| TM4SF2 | 7102 | tetraspanin 7 | A15; MXS1; CD231; MRX58; CCG-B7; TM4SF2; TALLA-1; TM4SF2b; DXS1692E; TSPAN7 | 300096 | NG_009160.1 | Xp11.4 |
| ZNF41 | 7592 | zinc finger protein 41 | MRX89; MGC8941; ZNF41 | 314995 | NG_008238.1 | Xp11.23 |
| FACL4 | 2182 | acyl-CoA synthetase long-chain family member 4 | ACS4; FACL4; LACS4; MRX63; MRX68; ACSL4 | 300157 | NG_008053.1 | Xq22.3-q23 |
| PQBP1 | 10084 | polyglutamine binding protein 1 | SHS; MRX55; MRXS3; MRXS8; NPW38; RENS1; PQBP1 | 300463 | NC_000023.9 | Xp11.23 |
| PEX1 | 5189 | peroxisomal biogenesis factor 1 | ZWS1; PEX1 | 602136 | NG_008341.1 | 7q21.2 |
| PXMP3 | 5828 | peroxisomal membrane protein 3, 35kDa | PAF1; PEX2; PMP3; PAF-1; PMP35; RNF72; PXMP3 | 170993 | NG_008371.1 | 8q21.1 |
| PEX6 | 5190 | peroxisomal biogenesis factor 6 | PAF2; PAF-2; PXAAA1; PEX6 | 601498 | NG_008370.1 | 6p21.1 |
| PEX10 | 5192 | peroxisomal biogenesis factor 10 | NALD; RNF69; MGC1998; PEX10 | 602859 | NG_008342.1 | 1p36.32 |
| PEX12 | 5193 | peroxisomal biogenesis factor 12 | PAF-3; PEX12 | 601758 | NG_008447.1 | 17q12 |
| PEX5 | 5830 | peroxisomal biogenesis factor 5 | PXR1; PTS1R; PTS1-BP; FLJ50634; FLJ50721; FLJ51948; PEX5 | 600414 | NG_008448.1 | 12p13.31 |
| PEX26 | 55670 | peroxisomal biogenesis factor 26 | FLJ20695; PEX26M1T; Pex26pM1T; PEX26 | 608666 | NG_008339.1 | 22q11.21 |



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**Table 2: Polymorphisms**

| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs63500661 | TCACATCACCAAGTTAAAAAAAAAAA[A/G]GGGGCGGGGGGGCAGAATGAAAATT |
| CFTR | 1080 | rs63107760 | AAACAAGGATGAATTAAGTTTTTTTT[-/T]AAAAAAGAAACATTTGGTAAGGGGA |
| CFTR | 1080 | rs62469443 | ATCACCAAGTTAAAAAAAAAAAAGGG[A/G]CGGGGGGGCAGAATGAAAATTGCAT |
| CFTR | 1080 | rs62469442 | CTATTGAACCAGAACCAAACAGGAAT[A/G]CCATAGCATTTTGTAAACTAAACTG |
| CFTR | 1080 | rs62469441 | CAGGAGTTCAAGACCAGCCTACTAAA[A/C]CACACACACACACACACACACAC |
| CFTR | 1080 | rs62469439 | GATTAAATAATAGTGTTTATGTACCC[C/G]GCTTATAGGAGAAGAGGGTGTGTGT |
| CFTR | 1080 | rs62469438 | ATTGTTATCTTTTCATATAAGGTAAC[A/T]GAGGCCCAGAGAGATTAAATAACAT |
| CFTR | 1080 | rs62469437 | TAATTTTAATTAAGTAAATTTAATTG[A/G]TAGATAAATAAGTAGATAAAAAATA |
| CFTR | 1080 | rs62469436 | GTATAAAAAAAAAAAAAAAAAAAGTT[A/T]GAATGTTTTCTTGCATTCAGAGCCT |
| CFTR | 1080 | rs62469435 | ATACTAAAAATTTAAAGTTCTCTTGC[A/G]ATATATTTTCTTAATATCTTACATC |
| CFTR | 1080 | rs62469434 | TGCTGGGATTACAGGCGTGAGCCACC[A/G]CGCCTGGCCTGATGGGACATATTTT |
| CFTR | 1080 | rs62469433 | CTACAATATAAGTATAGTATTGCAAA[A/C]CCATCAGGAAGGGTGTTAACTATTT |
| CFTR | 1080 | rs61763210 | GTTGTCTCCAAACTTTTTTTCAGGTG[-/AGA]AGGTGGCCAACCGAGCTTCGGAAAG |
| CFTR | 1080 | rs61720488 | TTTTTTCATAAAAGATTATATAAAGG[A/C]TATTGCTTTTGAATCACAAACACTA |
| CFTR | 1080 | rs61481156 | ATCTAGTGAGCAGTCAGGAAAGAGAA[C/T]TTCCAGATCCTGGAAATCAGGGTTA |
| CFTR | 1080 | rs61443875 | TAGAGTATAAAAAAAAAAAAAAAAA[-/A]GTTTGAATGTTTTCTTGCATTCAGA |
| CFTR | 1080 | rs61312222 | TGCAAATGCCAACTATCAAAGATATT[C/G]GAGTATACTGTCAATAAACTTCATA |
| CFTR | 1080 | rs61159372 | TCCTCAACAGTTAGAAACAATATTTT[C/G]AGTGATTTCCCATGCCAACTTTACT |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs61094145 | TTTTTGGTATTGTTGTTAAATAAGTG[A/G]GAATTCAATACAGTATAATG TCTGT |
| CFTR | 1080 | rs61086387 | CTTGAAATCGGATATATATATATATA[- /TGTATATATATATATATATATATATATATATATACATATATATATATA]GTA TTATCCCTGTTTTCACAGTTTT |
| CFTR | 1080 | rs60996744 | AGAGGGGCTGTGAAGGACACCAAGGA[A/G]GAGACTAAGAGCCAGGAG GGAAAAC |
| CFTR | 1080 | rs60960860 | TAGAGTTTATTAGCTTTTACTACTCT[A/G]CTTAGTTACTTTGTGTTACAG AATA |
| CFTR | 1080 | rs60923902 | ACTAGTGATGATGAGCTTCTTTTCAT[- /AT]GTTTGTTGGCTGCATAAATGTCTTC |
| CFTR | 1080 | rs60912824 | GCAGAGAAAAGAGGGGCTGTGAAGGA[C/G]ACCAAGGAGGAGACTAAG AGCCAGG |
| CFTR | 1080 | rs60887846 | TTCAGAGGTCTACCACTGGTGCATAC[G/T]CTAATCACAGTGTCGAAAAT TTTAC |
| CFTR | 1080 | rs60793174 | AAGAAAGAGCAAAAGAGGGCAAACTT[C/T]TCATACATTTTTGATGTCG AAACCA |
| CFTR | 1080 | rs60788575 | CCTAAAGTTTAAAAAGAAAAAAAAAA[- /A]GGAAGAAGGAATTAAAAATCCAAAG |
| CFTR | 1080 | rs60760741 | GTGTGTGTGTGTATATATATATATAT[A/T]TATATATTTTTTTTTTCCTGA GCCA |
| CFTR | 1080 | rs60456599 | AAACTGTTGATGTTTTCATTTATTTA[C/G]ATCATTGGAAAACTTTAGATT CTAG |
| CFTR | 1080 | rs60363249 | TTTATCCATTCTTAACCAGAACAGAC[A/G]TTTTTTCAGAGCTGGTCCAG GAAAA |
| CFTR | 1080 | rs60355115 | TTGAAATCGGATATATATATATATAT[A/G]TATATATATATATATATATA TATAT |
| CFTR | 1080 | rs60308689 | TAGTTTTTTATTTCCTCATATTATTT[- /T]CAGTGGCTTTTTCTTCCACATCTTT |
| CFTR | 1080 | rs60271242 | ACATAGTTCTCAGTGGTACAACTACA[A/G]GTGATTTCTCTTTTCTTATTT CTGG |
| CFTR | 1080 | rs60010318 | AGAGCAATGGCATCCCTTGTCTTGTG[C/T]TATACAGGATGCAGCAATTT ATAGG |
| CFTR | 1080 | rs59961323 | TTCTGTCTACATAAGATGTCATACTA[A/G]ATTATCTTTTCCAGCATGCAT TCAG |
| CFTR | 1080 | rs59961270 | CAGGGTGGCATGTTAGGCAGTGCTTA[A/G]AATAAATGAGTTGGTTATA CAAGTA |
| CFTR | 1080 | rs59837506 | AGGACACACACACACACACACACACA[- /CA]TGCACACACATTTAAATAGATGCAT |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs59572090 | TAAAAAATTGGTATAATGAAATTGCA[C/T]TTGTAGTCTTTGGACATTTAAATCC |
| CFTR | 1080 | rs59548252 | TTTCAATACTTAAGAGGTACGCAGAG[A/G]AAAGAGGGGCTGTGAAGGACACCAA |
| CFTR | 1080 | rs59519859 | CAGCAATGAATATTTTGAGGCTGAGG[C/T]GCTGAGGGGTAAAATTGCAGCCTGG |
| CFTR | 1080 | rs59509837 | TTATGGTTTATATTTTTGTGTCTTCT[-/CTTT]AACACATCTTTTCTAGCAGAATTCA |
| CFTR | 1080 | rs59417037 | GTATTTTAGTTTTTTTTTTTTTGTTTG[-/T]TTTGTTTTGTTTTGTTTTGTTTTTG |
| CFTR | 1080 | rs59159458 | TGGGTGACTCCATTTTTACTTTTAGT[C/T]TGGTCTGTTGAGGCCTCGTGAGAGA |
| CFTR | 1080 | rs59048119 | TATTTTCATGTATTTTAGTTTTTTTT[-/TTTT]GTTTGTTTTGTTTTGTTTTGTTTTG |
| CFTR | 1080 | rs58970500 | GTGTGTGTGTATATATATATATATAT[A/T]TATATTTTTTTTTTCCTGAGCCAAA |
| CFTR | 1080 | rs58942292 | AACCTATTAGCATGTCTGGCAGAAAA[-/A]TAGATACTTAATAAATTTCTTAAAT |
| CFTR | 1080 | rs58917054 | GAGGCTTAGACAGTTTAAGTAACTCA[A/G]GCATGGTTACACAACTAGCTAGGGC |
| CFTR | 1080 | rs58837484 | GTGTGAGTATTATGAGACCATATGTT[A/G]GGAGATTTTATTTGGTATTGAGGAT |
| CFTR | 1080 | rs58829491 | GAAACCCCACCCCTTCTATAGTTTTC[C/T]CTTTAATATTTACAATGGAACCATT |
| CFTR | 1080 | rs58805195 | CATATATATATAGTGTGTGTGTGTGT[A/G]TATATATATATATATATATATTTTT |
| GBA | 2629 | rs60866785 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs60239603 | GGCAGGTAATATCTAGTACCTTACTT[A/T]TATTTCCTGAGCACATTCTACATTT |
| GBA | 2629 | rs56310840 | GGCCAGGAATGGGAGTGCTTAGGTGC[A/G]GAGGTGGCACTGTTCCCGCAGCTGC |
| GBA | 2629 | rs41264927 | TGAAAACTCCATCCCCTCAGGGTCAT[C/T]AGATGAAGAGAAGACCACAGGGGTT |
| GBA | 2629 | rs41264925 | TGTAGGTAAGGGTCACATGTGGGAGA[C/G]GCAGCTGTGGGTAGGTCAGCCCTGT |
| GBA | 2629 | rs36024691 | CCAAGAAGGCGCCATTACACTCCAGC[-/C]TGGGCGACAGGGCGAGACTCCCTCA |
| GBA | 2629 | rs36024092 | TGCCACACCCAGCTAATTTGTGTGTG[- |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | /G]TATGTGTGTGTATGTATGTGTGTGT |
| GBA | 2629 | rs35682967 | GTTCCTCCAGTAATTTTTTTTTTTTT[-/T]GGTTTTGAGACAGAGTCTTGCCCTG |
| GBA | 2629 | rs35033592 | ATCATGCCCAGATAATTTTTTTTTTTT[-/T]GTATTTTAGTAGACACAGGGTTTCA |
| GBA | 2629 | rs34732744 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs34620635 | CCTGTGAGGGGCACATTCCTTAGTAG[-/C]TAAGGAGTTGGGGGTGTGAAGATCC |
| GBA | 2629 | rs34302637 | ACAGGCTACTGGCTGGGCCCAGGCAA[-/A]GGGGGCCTTGGCAGGAAAAGTTCCT |
| GBA | 2629 | rs33949225 | GCGAGAGAGAGAGAGAGAGAGAGAGG[-/AG]AGCCGGCGCGAGAACTACGCATGCG |
| GBA | 2629 | rs28678003 | AAGAAGAAAAATAAAAAGAAAGTGGG[C/T]CAGACCGAGAGAACAGGAAGCCTGA |
| GBA | 2629 | rs28559737 | AAGGACAAAGGCAAAGAGACAAAGGC[G/T]CAACACTGGGGGTCCCCAGAGAGTG |
| GBA | 2629 | rs28373017 | TACCTAGTCACTTCCTGCCTCCATGG[C/T]GCAAAAGGGGATGGGTGTGCCTCTT |
| GBA | 2629 | rs12752133 | CTCTTCCGAGGTTCCACCCTGAACAC[C/T]TTCCTGCTCCCTCGTGGTGTAGAGT |
| GBA | 2629 | rs12747811 | TTCTGACTGGCAACCAGCCCCACTCT[C/T]TGGGAGCCCTCAGGAATGAACTTGC |
| GBA | 2629 | rs12743554 | gctcagcctcccaggctggagtgcag[A/T]ggcgcgatctcggctcaccgcaacc |
| GBA | 2629 | rs12041778 | CATGAACCACATCAAATGAGATTTAG[C/T]GGGAGTGGCACACACAGTCATGACC |
| GBA | 2629 | rs12034326 | AAGCAGCCCTGGGGAGTCGGGGCGGG[A/G]CCTGGATTGGAAAAGAGACGGTCAC |
| GBA | 2629 | rs11558184 | CTCCAAGTTCTGGGAGCAGAGTGTGC[A/G]GCTAGGCTCCTGGGATCGAGGGATG |
| GBA | 2629 | rs11430678 | GTTCCTCCAGTAAttttttttttttt[-/G/T]gttttgagacagagtcttgccctgt |
| GBA | 2629 | rs11264345 | CTAGTACCTTACTTCCCTCAAGTTCA[A/T]TCATCTCACAGATATTTCCTGAGCA |
| GBA | 2629 | rs10908459 | aattagccgtgcgtggtggcgggtgc[C/T]tgtaatcccacgtacttgggaggct |
| GBA | 2629 | rs10796940 | CCATGGCCAGCCGGGGAGGGGACGGG[A/C]ACACACAGACCCACACAGAGACTCA |
| GBA | 2629 | rs10668496 | agcgagagagagagagagagagagag[-/AG]gagCCGGCGCGAGAACTACGCATGC |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| GBA | 2629 | rs7416991 | CGTAGCAGTTAGCAGATGATAGGCGG[C/G/T]GAAATCTTATTTCACAGG GCATTAA |
| GBA | 2629 | rs4024049 | CTGGCCCTGGTGACAGTGGGGCTGTG[C/T]GTGGGGCCAGAGCCTTCTCA GAGGT |
| GBA | 2629 | rs4024048 | CAGATACTGGCCCTGGTGACAGTGGG[A/G]CTGTGCGTGGGGCCAGAGC CTTCTC |
| GBA | 2629 | rs4024047 | GACAGATACTGGCCCTGGTGACAGTG[G/T]GGCTGTGCGTGGGGCCAGA GCCTTC |
| GBA | 2629 | rs3841430 | GGCTCctctctctctctctctctc[-/TC]gctcgctctctcgctctctcgctct |
| GBA | 2629 | rs3754485 | GTTTCAGACCAGCCTGGCCAACATAG[C/T]GAAACCCCATCTCTACTAAA AATAA |
| GBA | 2629 | rs3205619 | AGTGGGCGATTGGATGGAGCTGAGTA[C/T]GGGGCCCATCCAGGCTAAT CACACC |
| GBA | 2629 | rs2990227 | CCGGGCTCCGTGAATGTTTGTCACAT[C/G]TCTGAAGAACGTATGAATTA CATAA |
| GBA | 2629 | rs2990226 | GAATCCCAACCCCGACGCTCGTCGCC[C/G]GGCTCCGTGAATGTTTGTCA CATGT |
| GBA | 2629 | rs2990225 | GCGAATCCCAACCCCGACGCTCGTCG[C/T]CGGGCTCCGTGAATGTTTGT CACAT |
| GBA | 2629 | rs2990224 | TGGGCAGAAGTCAGGGTCCAAAGAAA[G/T]GGCAAAGAAAAGTGTcagtgg ctca |
| ASPA | 443 | rs63751297 | TAAGAAAGACGTTTTTGATTTTTTTC[A/G]GACTTCTCTGGCTCCACTACC CTGC |
| ASPA | 443 | rs62071301 | CTGATTCCTGGCCAGGAGCGGTGGCT[C/T]ACGCCTGTAATCCCAGCGCT TTGGG |
| ASPA | 443 | rs62071300 | TAAAAATGCTGATTCCTGGCCAGGAG[C/T]GGTGGCTCACGCCTGTAATC CCAGC |
| ASPA | 443 | rs62071299 | TTTAAAAATGCTGATTCCTGGCCAGG[A/C]GCGGTGGCTCACGCCTGTAA TCCCA |
| ASPA | 443 | rs62071297 | CAAGACCTGTCAAAGATCTGAGAAAT[A/T]TTACCCGACTTACAAGCTA ACCATT |
| ASPA | 443 | rs61697033 | ACTGTAATAAGTGCTGTAAAAGAAAT[A/G]CACAAAATAATATAGCAGA GGGTAT |
| ASPA | 443 | rs60743592 | CTTGAGGTCAGGAGTTCAAGACCAGT[C/T]TGGGCAACATGGGGAAAAC CTTGTC |
| ASPA | 443 | rs60666840 | AGGTTGCAGTGAGCCGAGATCATGCC[A/G]TTGCACTCCAGCCGGGGCA ACAAAA |
| ASPA | 443 | rs60147514 | ACAAGTGTCTTGAAATTATCTGTGAT[C/T]TGCTATAGAGCAATACTTTT GTAAA |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| ASPA | 443 | rs59930743 | GTGGGTATATGCAGCTCTATGCACTA[C/T]CTGCTCATTTATTTGGTAAATCTAA |
| ASPA | 443 | rs59690349 | TGTGTGTGTGTGCGTGTGTGTGTGTG[-/TGTGTGTG]ATCATAAGAGTGGCTGCAGCAAACT |
| ASPA | 443 | rs59676360 | AGTCTGGAGTGCAATGGTGCAATCTC[A/G]GCTCACTGCAGCCTCCACCTCCGGG |
| ASPA | 443 | rs59335404 | CTCCTAATGGATATTTCCTAAATTTT[G/T]CTGAACAGAATTTAACTTGAGCTGG |
| ASPA | 443 | rs58879097 | ATTTAAAAATGGATTTCTAGAAAAAC[A/G]ATCACATACTTGAATATTTTAGCAA |
| ASPA | 443 | rs58686774 | CTATAAATGGGTAGCATGAGGGATTC[A/G]AGGAGGTGGCTGAAAGAAGCACGTA |
| ASPA | 443 | rs57511162 | AAGAAACCAAGCATAGTAGAGTGTTA[A/G]AAAACCAAAGCAACTAAACAACTGT |
| ASPA | 443 | rs55859596 | CGGGGCTCAGAACTTGTAACAGAAAA[A/T]TAAAATATACTCCACTCAAGGGAAT |
| ASPA | 443 | rs55742972 | TACTACACTTCACGGATACTGTACTT[-/GTACTT]TTTTTCCAAATTGAAGGTTTTTGGC |
| ASPA | 443 | rs55640436 | TTGTTTTTGTTTTTGTTTTTGTTTTT[-/GTTTTTGTTTTT]TGAGATGGAGTCTCGCTCTGTCGCC |
| ASPA | 443 | rs36225687 | TTTGCCTTACTACACTTCACGGATAC[-/TGTACT]TGTACTTTTTTTCCAAATTGAAGGT |
| ASPA | 443 | rs36051310 | GAGGTGGCTGAAAGAAGCACGTATCC[-/C]TGATGGCATGGTTGCGGGTTATATG |
| ASPA | 443 | rs36034906 | GAGAAAAGCAGTTCCTGGAACACCCC[-/C]ACCCCTTAACCCCTTATCTCTGCTT |
| ASPA | 443 | rs36033666 | TTACATATGTATACATGTGCCATGTT[-/T]GGTGTGCCGCACCCATTAACTCGTC |
| ASPA | 443 | rs35730123 | CTTTTTCCAGATTTTTTTTTTTTTTT[-/T]GAGACAGAGTTTCACTCTTGTTGCC |
| ASPA | 443 | rs35629100 | TTTGGAAATCTTAAGCTTTTATTTGG[-/G]TGTCACAGAGAAACAGGATCTGTAT |
| ASPA | 443 | rs35614631 | TACTTTAAGTTTTAGGGTACATGTGC[-/A]CCATGTGCAGGTTTGTTACATATGT |
| ASPA | 443 | rs35225782 | ATTCATGACCAGCCACATAAATGCAC[-/A]GTATTACTTCGCAAGCATGCCAATG |
| ASPA | 443 | rs35178659 | GTGCACTAGAATTAGCTAAAGTGGGG[-/G]AAAAAAAGATGCATTTGATGGTCTA |
| ASPA | 443 | rs35095578 | AACCTCCACCTCCCAGGTTCAAGAGA[- |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | /A]TTCTCCTGCCTCAGCCTCCCAAGTA |
| ASPA | 443 | rs35002210 | CCTCCCTGTGATCCGAAGTAGCAGAC[A/G]TACTTAACTTCCATGGTGGATTGTT |
| ASPA | 443 | rs34744839 | AAAACATTATTATATCTAGAAAAAAA[-/A]TGTATCTTAACCATTGTGGGAAGTG |
| ASPA | 443 | rs34680506 | TTGAAGGTAAAATCATAGGGAGTTGG[-/G]AGCTGTCCTCTTGCGCTGAATCAGT |
| ASPA | 443 | rs34365618 | ACTTGTGGCCTTTTTGGAGAGGTTAG[-/CA]ACTCTGAAAACTCTGTCCCTGGACC |
| ASPA | 443 | rs34275920 | GAAGGAGAAAAAGAGAGGAAATAAGT[-/T]AAAATAATAAACACAATTAATAAAG |
| ASPA | 443 | rs34109510 | TGTATACATGTGCCATGTTGGTGTGC[C/T]GCACCCATTAACTCGTCATTTAGCA |
| ASPA | 443 | rs34054576 | TCACCTGTCACCTCCTATAGAACTTT[-/C]CCCTGACCCTCCTCTATAGCATTAA |
| ASPA | 443 | rs34015272 | ATAAATGATCATCATTCACAGTAGGG[-/G]TTTTGTTTTGTTTTTTTTCTGGAAA |
| ASPA | 443 | rs34002091 | ACAGACATATCTACAAACACACTTTT[-/T]CACATATTTGTGTAAGTCATTTATG |
| ASPA | 443 | rs28940574 | AAAGACAACTAAACTAACGCTCAATG[A/C]AAAAAGTATTCGCTGCTGTTTACAT |
| ASPA | 443 | rs28940279 | TACCGTGTACCCCGTGTTTGTGAATG[A/C]GGCCGCATATTACGAAAAGAAAGAA |
| ASPA | 443 | rs17850703 | CAGGGCTGGAGGTAAAACCATTTATT[A/G]CTAACCCCAGAGCAGTGAAGAAGTG |
| ASPA | 443 | rs17222495 | TTCTTCATTGCCTATTGAAGAGAGAG[C/T]GGAATGCTTTGGTTGCCAGATATGG |
| ASPA | 443 | rs17175228 | CACAAGATCTCATTACTCAGGAGCTG[C/T]CCAAGTGTCTAATGTACTTAGTTAA |
| ASPA | 443 | rs16953074 | TTCTGTGTAACATTTCATTTAAGCAA[A/G]GGATTCGGCAAATCAAAAATTGTCA |
| ASPA | 443 | rs16953070 | TAAAACGTATTGAAGGTATTATTGAC[G/T]CTGTTGAAGCAAAGAGAACAAAACA |
| HEXA | 3073 | rs62022858 | ATCTGCTCTTCCAGTTGGATGACAAG[C/T]CTTGCTGTCTAACACCTGCTGCAGA |
| HEXA | 3073 | rs62022857 | CCATTTTTTGTTGTATTTTTTTTTTC[C/T]TGAATACTTTTTATCGCAGTTGGTT |
| HEXA | 3073 | rs62017872 | CCCTGTCTCTAAAAGAAAAAAAAAAA[A/G]AAAAAAAAAAGAAAACAAAACCCAA |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| HEXA | 3073 | rs62017871 | AGTGGCTCCAAAAAGGTCATGGAACC[C/T]CTTGAGGATGATGCAAATT GACTCT |
| HEXA | 3073 | rs61662730 | TAAAGTTACTTTTCTTTTATTGACTT[C/T]CCCTTATTTTTTAACCTTATGC TTT |
| HEXA | 3073 | rs61329913 | CAGAGTTAAAAAAAAAAAAAAAAAAA[-/A]GGAAGTAGCAGCAACAGCTTGGAAA |
| HEXA | 3073 | rs60920713 | GTTGCCCAGGGTTGAGTGCAGAGGCA[C/T]ATCTTGGCTCACAGCAACCT CTGCC |
| HEXA | 3073 | rs60783213 | AAGGCTTTTTTTTTTTTTTTTTTTTT[-/TTTT]GAGACAGAGTCTTGCTGTGTCACCC |
| HEXA | 3073 | rs60644867 | GCCTACATTCTGCAAAGAGGAGGGAA[C/G]ATTCACAGCTCCATACTTG AACCCT |
| HEXA | 3073 | rs60288568 | CCAAAGGAGAATAGCTCTAGGGGAGG[C/G]AGGTGGATGAGTATGCATG GGGGAG |
| HEXA | 3073 | rs59888548 | GACTCCATCTCAAAAAAAAAAAAAAA[-/A]TGCAGTCTAATGGCAGAATTAGACT |
| HEXA | 3073 | rs59733856 | TTATTTATTTATTTATTTATTTTTGA[A/G]ACAGGGTCTCTGTTGTCCAGG CTGG |
| HEXA | 3073 | rs59427837 | TTTTGAGGCAGGGTCTCACTCTGTTG[C/T]CCAGGGTTGAGTGCAGAGGC ACATC |
| HEXA | 3073 | rs59171976 | CGCCTTGCGAAGGCCCCACAGCTTGC[C/T]TGTGACAAACGTTCATAGGC AAATG |
| HEXA | 3073 | rs58706602 | GGAGGTCTGTACAAAGCACCACCTAC[C/T]TCATGGGTCAGTTTCCACAG CAGAA |
| HEXA | 3073 | rs58696963 | GAATCTTATAATTCACTGTGTACCTC[-/CCTC]TGTTTCATATTTTCGCAATTGAACT |
| HEXA | 3073 | rs58610850 | AACATAGTATCTAATATAGCTTTACA[C/T]CCAAAGCCAAAATATGAATA CACTG |
| HEXA | 3073 | rs58016062 | TTGTTTTGTTTTGTTTGGGGGGGGGG[-/G]TTGTTTTTCTGAGAGGGAGTCTTGC |
| HEXA | 3073 | rs57733983 | CATACCAAAGGGCAGCTGGAGGGATA[C/T]AGACGGAAGTCATGTGGAG AGTGAA |
| HEXA | 3073 | rs57476645 | CAGGTGTGAGCCACCACGACCACCAA[A/T]TTAGCTCTTTTTACTCCTTC CCTTC |
| HEXA | 3073 | rs56870003 | AGTGGTAGCTGATTTTGCTTCTGGAT[A/C]CTTTGCCACCTTCCCACTCTT TAAT |
| HEXA | 3073 | rs56338339 | AAAGACCTGTTTCTTAAAAAAAAAAA[-/AGAAAAAAAAAAA]GAAAGAAAAGAAAAGAAAAAAACAG |
| HEXA | 3073 | rs55995352 | TAAAAAATCTTTCAATGAGGAGATGT[C/T]CCCAGAGCAAGACAGCTGT |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | AGGATG |
| HEXA | 3073 | rs55860138 | AAAAGAAAAAAAAAAAAAAAAAAAA[-/A]GAAAACAAAACCCAAACCCATAAAG |
| HEXA | 3073 | rs55743646 | CCTGTCTCTAAAAGAAAAAAAAAAAA[A/G]AAAAAAAAAGAAAACAAAACCCAAA |
| HEXA | 3073 | rs55665666 | GTTATCATAGAAAAATATCACACTCT[-/GT]CTGTATCCCCACTTCCAGAAACTGT |
| HEXA | 3073 | rs36106892 | CAGGAGCTCATAGAATTACATACAAT[-/C]TTTTTTTTTTTTTTTTGAGACAGCG |
| HEXA | 3073 | rs36091525 | TTGAGAATCTTATAATTCACTGTGTA[-/CCTC]CCTCTGTTTCATATTTTCGCAATTG |
| HEXA | 3073 | rs35949555 | CCACTACCACAGTGCCTAGAGAACAA[C/T]ATGTGTTTAATAATATTTAAATAAT |
| HEXA | 3073 | rs35827424 | CCCTGTCTCTAAAAGAAAAAAAAAAA[-/A]AAAAAAAAAAGAAAACAAAACCCAA |
| HEXA | 3073 | rs35729578 | CCATTATATCATTCATTTCCCACTCA[-/T]TTTCTTCATTCCAACCAAGATATAT |
| HEXA | 3073 | rs35649102 | TCCGTCTCAAAAAAAAAAAAAAAAAG[-/A]GAAAGGAATTATTCTCATGTATACA |
| HEXA | 3073 | rs35118677 | CTGGGGCAGTTAAAAAGAAAAACAAA[-/C]CCCTGGTCCCTGCCCTTGAGGAGAT |
| HEXA | 3073 | rs35005352 | CTCCAGGGTCCCATTCCAGGACCACA[-/C]GCCTGCTACCTCTGCAGCTCACTCA |
| HEXA | 3073 | rs34736306 | GGATTGACATATACCAGTTAGACGGA[-/T]TTTTTTTTTCCATAAACCAGGCTCA |
| HEXA | 3073 | rs34607939 | ACAAATAATTACTACATATCTACAAC[A/G]TTCCAGATACAGAAGAAATGGCCAA |
| HEXA | 3073 | rs34496117 | TAAACACACTTGAAACATCATATAAA[-/ATG]ATATTACTACAAGACTTAACCGTAA |
| HEXA | 3073 | rs34300017 | ACACAGGTAATCCATGTTTATTATAG[-/A]AAAATGCCACATTACTCTTTATTGA |
| HEXA | 3073 | rs34206496 | AGTTATCATAGAAAAATATCACACTC[-/TG]TCTGTATCCCCACTTCCAGAAACTG |
| HEXA | 3073 | rs34110830 | AATGAACTTACAGGAAGGTAATATAT[-/G]GGAAATAAACATCTTATTGAATTTA |
| HEXA | 3073 | rs34093438 | GGACCCCTGAAAGGCACAAGACACCC[-/T]TTCAGGTTCACACTTCCTGAAAGCT |
| HEXA | 3073 | rs34085965 | CCACCAATCACCAGAGCCTTCTGCTC[A/G]GGGGTACCTGAGGGAAAACAAGCAA |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| HEXA | 3073 | rs34004907 | AAAGACTGAAAAAACATTCATAACTA[-/T]TTTTCTTGTTATCCTCGGAAATGTC |
| HEXA | 3073 | rs28942072 | TATCTTCATCTTGGAGGAGATGAGGT[C/T]GATTTCACCTGCTGGAAGTCCAACC |
| HEXA | 3073 | rs28942071 | TTGCCTATGAACGTTTGTCACACTTC[C/T]GCTGTGAGTTGCTGAGGCGAGGTGT |
| HEXA | 3073 | rs28941771 | GCTTGCTGTTGGATACATCTCGCCAT[C/T]ACCTGCCACTCTCTAGCATCCTGGA |
| HEXA | 3073 | rs28941770 | CCGGGGCTTGCTGTTGGATACATCTC[G/T]CCATTACCTGCCACTCTCTAGCATC |

Examples

**Example 1: MIP-capture reaction of a set of exon target nucleic acids**

MIP probes are synthesized using standard column-based oligonucleotide synthesis by any number of vendors (e.g. IDT).

*Hybridize capture probes to human genomic sample*

Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

*Convert hybridized probes into covalently-closed circular products containing target nucleic acids*

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O



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Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

*Purify circularized probe/target nucleic acid products from un-reacted probes and genomic DNA*

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubate at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

*Amplify circular material by PCR using primers specific to the 'constant region' of the probes*

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3')
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3')
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

1. 95C for 5 min
2. 95C for 30 sec
3. 58C for 60 sec
4. 72C for 60 sec
5. goto 2, N more times

*Prepare a shotgun next-generation sequencing library for analysis*

- Purify desired amplicon population from non-specific amplification products by gel extraction.



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- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

*Perform sequencing of library according to manufacturer's directions (e.g. Illumina,ABI, etc), reading the target sequence to determine abundance of the target nucleic acid.*

**Example 2: Use of MIPs, hybridization, and mutation-detection MIPs to genotype a set of 1000 targets**

MIPs, hybridization, and mutation-detection MIPs are used to genotype a set of 1000 targets. The protocol permits detection of any of 50 specific known point mutations

First, separate MIP, hybridization, and mutation-detection MIP reactions are performed on a biological sample. A MIP capture reaction is performed essentially as described in Turner et al 2009, Nature methods 6:315-6. A set of MIPs is designed such that each probe in the set flanks one of the 1000 targets. Separately, a hybridization enrichment reaction is performed using the Agilent SureSelect procedure. Prior to selection, the genomic DNA to be enriched is converted into a shotgun sequencing library using Illumina's 'Fragment Library' kit and protocol. Agilent's web interface is used to design a set of probes which will hybridize to the target nucleic acids. Separately, a set of probes are designed (mutation-detection MIPs) which will form MIPs only if mutations (e.g., specific polymorphisms) are present. Each mutation-detection MIP has a 3'-most base identity that is specific for a single known mutation. A reaction with this set of mutation-detection MIPs is performed to selectively detect the presence of any mutant alleles.

Once all three reactions have been performed, the two MIP reactions are combined (e.g., at potentially non-equimolar ratios to further increase sensitivity of mutation detection) into a single tube, and run as one sample on the next-generation DNA sequencing instrument. The hybridization-enriched reaction is run as a separate sample on the next-generation DNA sequencing instrument. Reads from each 'sample' are combined by a software algorithm which forms a consensus diploid genotype at each position in the target set by evaluating the total coverage at each position, the origin of each read in that total coverage, the quality score



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of each individual read, and the presence (or absence) of any reads derived from mutation-specific MIPs overlapping the region.

It will be clear that the methods may be practiced other than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present disclosure are possible in light of the above teachings and, therefore, are within the scope of the claims. Preferred features of each aspect of the disclosure are as for each of the other aspects mutatis mutandis. The documents including patents, patent applications, journal articles, or other disclosures mentioned herein are hereby incorporated by reference in their entirety. In the event of conflict, the disclosure of present application controls, other than in the event of clear error.

What is claimed is:



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Claims

1. A method of increasing the sensitivity of a nucleic acid detection assay, the method comprising:

obtaining a first preparation of a target nucleic acid using a first preparative method on a biological sample,

obtaining a second preparation of a target nucleic acid using a second preparative method on the biological sample,

assaying the sequences obtained in both first and second nucleic acid preparations,

using the sequence information from both first and second nucleic acid preparations to determine the genotype of the target nucleic acid in the biological sample, wherein the first and second preparative methods have different systematic sequence biases.

2. The method of claim 1, wherein the first and second nucleic acid preparations are combined prior to performing a sequence assay.

3. The method of claim 1, wherein separate sequence assays are performed on the first and second nucleic acid preparations and the sequence information from both assays are combined to determine the genotype of the target nucleic acid in the biological sample.

4. The method of claim 1, wherein the first preparative method is a PCR-based, a hybridization-based, or a circular probe-based preparative method.

5. The method of claim 4, wherein the second preparative method is a PCR-based, a hybridization-based, or a circular probe-based preparative method, provided that the second method is different from the first method.

6. A method of obtaining a nucleic acid preparation that is representative of the nucleic acid in a biological sample, the method comprising

obtaining a first nucleic acid preparation using a first preparative method on a biological sample,

obtaining a second nucleic acid preparation using a second preparative method on the biological sample, and



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combining the first and second nucleic acid preparations to obtain a combined preparation that is representative of nucleic acid in the biological sample.

7. The method of claim 1 or 6, further comprising performing a third nucleic acid preparation using a third preparative method that is different from the first and second preparative methods, wherein the first, second, and third preparative methods all have different systematic sequence biases.

8. The method of claim any of the prior claims, wherein the different preparative methods are used for a plurality of different loci in the biological sample to increase the sensitivity of a multiplex nucleic acid analysis.

9. A method of genotyping a nucleic acid in a biological sample, the method comprising:

obtaining a nucleic acid preparation using a preparative method on a biological sample,

sequencing a target nucleic acid of the nucleic acid preparation,

and performing a molecular inversion probe capture reaction on the biological sample, wherein a molecular inversion probe capture of the target nucleic acid in the biological sample is indicative of the presence of a polymorphism in the target nucleic acid,

genotyping the target nucleic acid based on the results of the sequencing and the capture reaction.

10. The method of any of the prior claims, wherein the target nucleic acid has a sequence of a gene selected from Table 1.



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**Abstract**

The invention relates to methods and compositions for increasing the representativeness and/or sensitivity of nucleic acid analyses by combining results from preparative techniques that have different systematic biases. Aspects of the invention are particularly useful for increasing the confidence of genotype determinations made in multiplex analyses.



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| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 5346897 |
| **Application Number:** | 61178923 |
| **International Application Number:** | |
| **Confirmation Number:** | 7567 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR ENHANCING THE SENSITIVITY OF NUCLEIC ACID ANALYSIS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 23628 |
| **Filer:** | Daniel W. Young/Danielle Calder |
| **Filer Authorized By:** | Daniel W. Young |
| **Attorney Docket Number:** | G0814.70003US00 |
| **Receipt Date:** | 15-MAY-2009 |
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## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Provisional Cover Sheet (SB16) | G081470003US00-TRN-DWY.pdf | 45027<br>f02dfbde496042cebd394698e8200d37dec2cba5 | no | 2 |
| **Warnings:** | | | | | |
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| **Information:** | | | | | |
| 2 | Fee Worksheet (PTO-875) | G081470003US00-FEE-DWY.pdf | 44523<br>c427890ef76bc2c6ac5168369242f25ab9781991 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Application Data Sheet | G081470003US00-ADS-DWY.pdf | 17549<br>a192dbdc0e459da4a8451d3206be470ab42418cb | no | 3 |
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| 4 | | G081470003US00-PROAPL-DWY.pdf | 177366<br>59c315b76ee3eebe2997a0a39317e7c1adc9fc53 | yes | 31 |

**Multipart Description/PDF files in .zip description**

| Document Description | Start | End |
|---|---|---|
| Specification | 1 | 28 |
| Claims | 29 | 30 |
| Abstract | 31 | 31 |

| | | | | | |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Appendix to the Specification | G081470003US00-DRW-DWY.pdf | 149816<br>f269116f89ff9aa9ffbe3c77f74100c1dba035b8 | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Fee Worksheet (PTO-875) | fee-info.pdf | 29700<br>21bad25314cf2074bd6f2273c9161253449c14d0 | no | 2 |
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**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

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**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

FIGURE 1

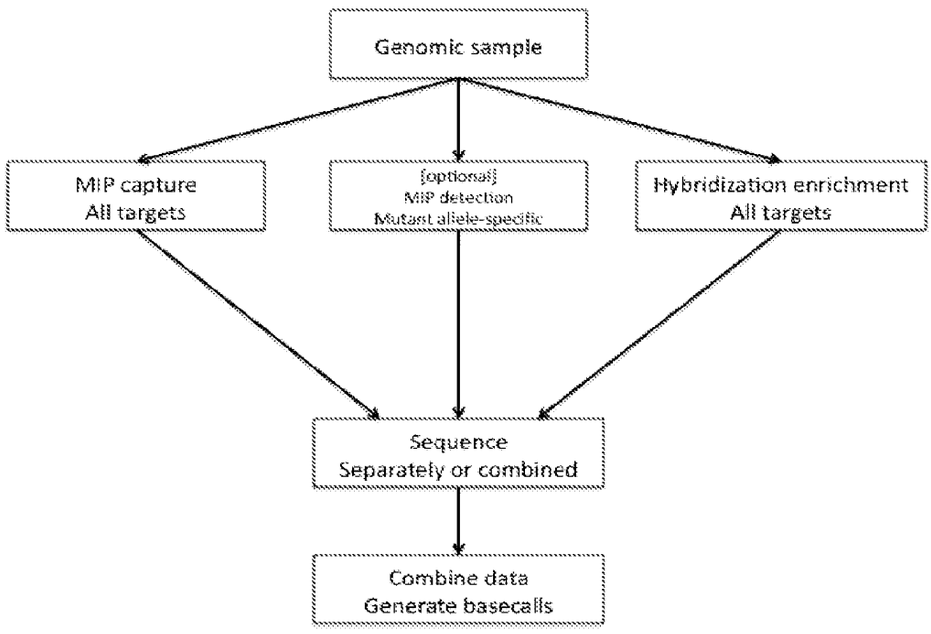

Genomic sample
MIP capture
All targets
[optional]
MIP detection
Mutant allele-specific
Hybridization enrichment
All targets
Sequence
Separately or combined
Combine data
Generate basecalls

FIGURE 2

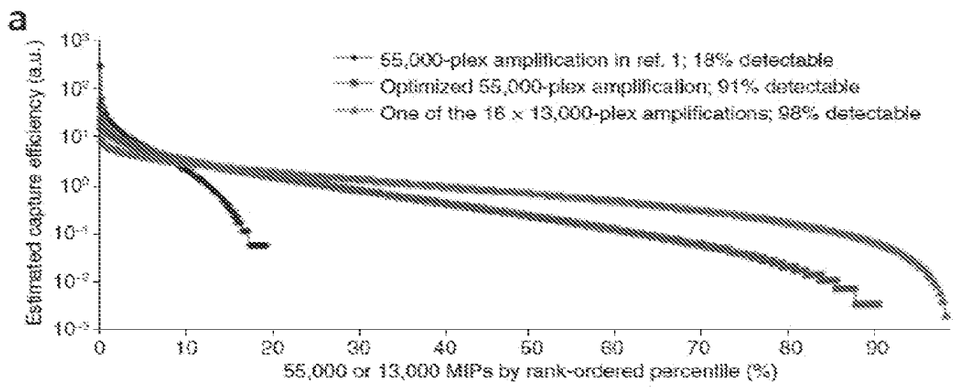
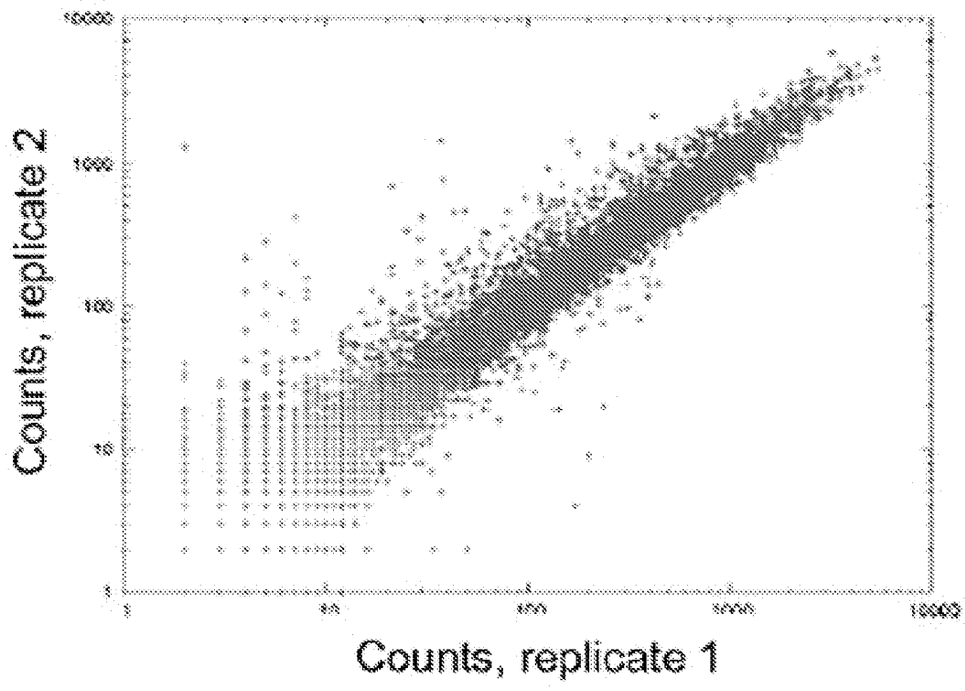

a
Estimated capture efficiency (a.u.)
55,000-plex amplification in ref. 1; 18% detectable
Optimized 55,000-plex amplification; 91% detectable
One of the 16 x 13,000-plex amplifications; 98% detectable
55,000 or 13,000 MIPs by rank-ordered percentile (%)

FIGURE 3



# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US2010/001293

International filing date: 30 April 2010 (30.04.2010)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 61/174,470
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World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

260605

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**APPLICATION NUMBER:** ***61/174,470***
**FILING DATE:** ***April 30, 2009***
**RELATED PCT APPLICATION NUMBER:** ***PCT/US10/01293***

**THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS** ***US61/174,470***

UNITED STATES PATENT AND TRADEMARK OFFICE · DEPARTMENT OF COMMERCE

Certified by

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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

***VIA EFS-web e-Filed: 4/30/2009***

| INVENTOR(S) | | |
|---|---|---|
| Given Name (first and middle [if any] ) | Family Name or Surname | Residence (City and either State or Foreign Country) |
| Greg | Porreca | Ocean City, New Jersey 08226 |
| Uri | Laserson | Boston, Massachusetts 02215 |
| Jin Billy | Li | Medford, Massachusetts 02155 |
| | | |
| | | |

*Additional inventors are being named on the _____ separately numbered sheets attached hereto.*

**TITLE OF THE INVENTION (500 characters max):**

METHOD FOR REDUCING REPRESENTATIONAL BIAS IN MULTIPLEX AMPLIFICATION OF NUCLEIC ACIDS

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

[x] The address corresponding to Customer Number: 23628

**OR**

[ ] Firm or Individual Name

Address

| | | |
|---|---|---|
| City | State | Zip |
| Country | Telephone | Email |

**ENCLOSED APPLICATION PARTS (*check all that apply*)**

[x] Application Data Sheet. See 37 CFR 1.76 [ ] CD(s), Number of CDs _____

[x] Drawing(s) *Number of Sheets* 3 [ ] Other (specify) _____

[x] Specification (e.g. description of the invention) *Number of Pages* 28

Fees Due: Filing Fee of $220 ($110 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

**METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT**

[x] Applicant claims small entity status. See 37 CFR 1.27.

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**1654549.1**

***PROVISIONAL APPLICATION COVER SHEET***
***Page 2 of 2***

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[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

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| | | | |
|---|---|---|---|
| SIGNATURE | /Patrick R.H. Waller/ | Date | April 30, 2009 |
| TYPED or PRINTED NAME | Patrick R.H. Waller | REGISTRATION NO. *(if appropriate)* | 41,418 |
| TELEPHONE | 617.646.8000 | Docket Number: | G0814.70000US00 |

***VIA EFS-web***
***e-Filed: 04/30/2009***

**Application Data Sheet**

**Application Information**

| | |
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| Sequence submission:: | None |
| Computer Readable Form (CRF):: | No |
| Title:: | METHOD FOR REDUCING REPRESENTATIONAL BIAS IN MULTIPLEX AMPLIFICATION OF NUCLEIC ACIDS |
| Attorney Docket Number:: | G0814.70000US00 |
| Request for Early Publication:: | No |
| Request for Non-Publication:: | No |
| Small Entity:: | Yes |
| Petition included:: | No |
| Secrecy Order in Parent Appl.:: | No |

**Applicant Information**

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | United States |
| Status:: | Full Capacity |
| Given Name:: | Greg |
| Family Name:: | Porreca |
| City of Residence:: | Ocean City |
| State or Province of Residence:: | NJ |
| Country of Residence:: | US |
| Street of Mailing Address:: | 911 Gardens Parkway |



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| | |
|---|---|
| City of Mailing Address:: | Ocean City |
| State or Province of Mailing Address:: | NJ |
| Postal or Zip Code of Mailing Address:: | 08226 |

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | United States |
| Status:: | Full Capacity |
| Given Name:: | Uri |
| Family Name:: | Laserson |
| City of Residence:: | Boston |
| State or Province of Residence:: | MA |
| Country of Residence:: | United States |
| Street of Mailing Address:: | 645 Beacon Street, #215 |
| City of Mailing Address:: | Boston |
| State or Province of Mailing Address:: | MA |
| Postal or Zip Code of Mailing Address:: | 02215 |

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | People's Republic of China |
| Status:: | Full Capacity |
| Given Name:: | Jin |
| Middle Name:: | Billy |
| Family Name:: | Li |
| City of Residence:: | Medford |
| State or Province of Residence:: | Massachusetts |
| Country of Residence:: | United States |
| Street of Mailing Address:: | 100 Station Landing, Unit 204 |

| | |
|---|---|
| City of Mailing Address:: | Medford |
| State or Province of Mailing Address:: | Massachusetts |
| Postal or Zip Code of Mailing Address:: | 02155 |

**Correspondence Information**

| | |
|---|---|
| Correspondence Customer Number:: | 23628 |

**Representative Information**

| | |
|---|---|
| Representative Customer Number:: | 23628 |

**Domestic Priority Information**

**Foreign Priority Information**

**Assignee Information**

| | |
|---|---|
| Assignee Name:: | Good Start Genetics, Inc. |
| Street of Mailing Address:: | 312 Marlborough St., #4 |
| City of Mailing Address:: | Boston |
| State or Province of Mailing Address:: | Massachusetts |
| Country of Mailing Address:: | United States |
| Postal or Zip Code of Mailing Address:: | 02116 |

METHOD FOR REDUCING REPRESENTATIONAL BIAS IN MULTIPLEX AMPLIFICATION OF NUCLEIC ACIDS

## Field of Invention

The invention relates to methods that reduce bias and increase reproducibility in multiplex detection of genetic loci, e.g., for diagnostic purposes.

## Background of the Invention

Molecular inversion probe technology is used to detect or amplify particular nucleic acid sequences in potentially complex mixtures. Use of molecular inversion probes has been demonstrated for detection of single nucleotide polymorphisms (Hardenbol et al. 2005 Genome Res 15:269-75) and for preparative amplification of large sets of exons (Porreca et al. 2007 Nat Methods 4:931-6, Krishnakumar et al. 2008 Proc Natl Acad Sci USA 105:9296-301). One of the main benefits of the method is in its capacity for a high degree of multiplexing, because generally thousands of targets may be captured in a single reaction containing thousands of probes. However, challenges associated with, for example, amplification efficiency (See, e.g., Turner EH, et al., Nat Methods. 2009 Apr 6:1-2.) have limited the practical utility of the method in research and diagnostic settings.

## Summary of the Invention

Aspects of the disclosure are based, in part, on the discovery of effective methods for overcoming challenges associated with systematic errors (bias) in multiplex genomic capture and sequencing methods, namely high variability in target nucleic acid representation and unequal sampling of heterozygous alleles in pools of captured target nucleic acids. Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the detection of target nucleic acids in multiplex capture methods. In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of different sets of multiple probes (e.g., molecular inversion probes MIPs) that capture overlapping regions of a target nucleic acid to achieve a more uniform representation of the target nucleic acids in a capture pool compared with methods of the



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prior art. In other embodiments, methods reduce bias, or the risk of bias, associated with large scale parallel capture of genetic loci, e.g., for diagnostic purposes. In other embodiments, methods are provided for increasing reproducibility (e.g., by reducing the effect of polymorphisms on target nucleic acid capture) in the detection of a plurality of genetic loci in parallel. In further embodiments, methods are provided for reducing the effect of probe synthesis and/or probe amplification variability on the analysis of a plurality of genetic loci in parallel.

According to some aspects, the disclosure relates to multiplex diagnostic methods. In some embodiments, multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel (e.g., a genetic locus of Table 1). In some embodiments, the genetic loci possess one or more polymorphisms (e.g., a polymorphism of Table 2) the genotypes of which correspond to disease causing alleles. Accordingly, in some embodiments, the disclosure provides methods for assessing multiple heritable disorders in parallel. In some embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample.

According to some aspects, methods of analyzing a plurality of genetic loci are provided. In some embodiments, the methods comprise contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion, isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids.

In other embodiments, methods comprise contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are



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complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the sequence of the 3' region of a different probe, isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids.

In some embodiments of the methods disclosed herein the target nucleic acids are selected from Table 1.

According to some aspects of the methods disclosed herein, the analyzing comprises genotyping a polymorphism of a target nucleic acid. In some embodiments, the genotyping may comprise determining that alleles of the target nucleic acid are heterozygous or homozygous. In further embodiments, the genotyping may comprise determining the sequence of a polymorphism and comparing that sequence to a control sequence that is indicative of a disease risk. In some embodiments, the polymorphism is selected from Table 2.

In some embodiments, a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject). In some embodiments, an assessment is provided to a couple, based on their respective genotypes at one or more genetic loci, of the risk of their having one or more children having a genotype associated with a disease or condition (e.g., a homozygous or heterozygous genotype associated with a disease or condition). In some embodiments, a subject or a couple may seek genetic or reproductive counseling in connection with a genotype determined according to embodiments of the invention.

## BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 depicts a tiled probe layout, showing twelve captured sub-targets (also referred to herein as subregions of a target nucleic acid) tiled across a single target. Each position in the target is covered by three sub-targets such that MIP capture performance per basepair is averaged across three probes.

Fig. 2 depicts a staggered probe layout, showing the targets captured by a set of three MIPs. Each MIP captures the full target, shown in black, plus (in some cases)



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additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes.

Fig. 3 depicts an alternating staggered probe layout, showing the targets captured by a set of three MIPs. Each MIP captures the full target, shown in black, plus (in some cases) additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes.

## DETAILED DESCRIPTION

Aspects of the disclosure are based, in part, on the discovery of effective methods for overcoming challenges associated with systematic errors (bias) in multiplex genomic capture and sequencing methods, namely high variability in target nucleic acid representation and unequal sampling of heterozygous alleles in pools of captured target nucleic acids (e.g., isolated from a biological sample). Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the detection of target nucleic acids in multiplex capture methods. In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of different sets of multiple probes (e.g., molecular inversion probes MIPs) that capture overlapping regions of a target nucleic acid to achieve a more uniform representation of the target nucleic acids in a capture pool compared with methods of the prior art. In other embodiments, methods reduce bias, or the risk of bias, associated with large scale parallel capture of genetic loci, e.g., for diagnostic purposes. In other embodiments, methods are provided for increasing reproducibility (e.g., by reducing the effect of polymorphisms on target nucleic acid capture) in the detection of a plurality of genetic loci in parallel. In further embodiments, methods are provided for reducing the effect of probe synthesis and/or probe amplification variability on the analysis of a plurality of genetic loci in parallel.

According to some aspects, the disclosure relates to multiplex diagnostic methods. In some embodiments, multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel (e.g., a genetic locus of Table 1). In some



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embodiments, genetic loci possess one or more polymorphisms (e.g., a polymorphism of Table 2) the genotypes of which correspond to disease causing alleles. Accordingly, in some embodiments, the disclosure provides methods for assessing multiple heritable disorders in parallel. In some embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample. A patient or subject may be a human. However, aspects of the invention are not limited to humans and may be applied to other species (e.g., mammals, birds, reptiles, other vertebrates or invertebrates) as aspects of the invention are not limited in this respect. A subject or patient may be male or female. In some embodiments, in connection with reproductive genetic counseling, samples from a male and female member of a couple may be analyzed. In some embodiments, for example, in connection with an animal breeding program, samples from a plurality of male and female subjects may be analyzed to determine compatible or optimal breeding partners or strategies for particular traits or to avoid one or more diseases or conditions.

In some aspects, the disclosure provides probe sets that comprise a plurality of different probes. As used herein, a 'probe' is a nucleic acid having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of a target nucleic acid or subregion thereof. An exemplary probe is a molecular inversion probe (MIP). A 'target nucleic acid' may be a genetic locus. Exemplary genetic loci are disclosed herein in Table 1 (RefSeqGene Column).

While probes have been typically designed to meet certain constraints (e.g. melting temperature, G/C content, etc.) known to partially affect capture/amplification efficiency (Ball et al (2009) Nat Biotech 27:361-8 AND Deng et al (2009) Nat Biotech 27:353-60), a set of constraints which is sufficient to ensure either largely uniform or highly reproducible capture/amplification efficiency has not previously been achieved. As disclosed herein, uniformity and reproducibility can be increased by designing multiple probes per target, such that each base in the target is captured by more than one probe. In some embodiments, the disclosure provides multiple MIPs per target to be captured, where each MIP in a set designed for a given target nucleic acid has a central



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region and a 5' region and 3' region ('targeting arms') which hybridize to (at least partially) different nucleic acids in the target nucleic acid (immediately flanking a subregion of the target nucleic acid). Thus, differences in efficiency between different targeting arms and fill-in sequences may be averaged across multiple MIPs for a single target, which results in more uniform and reproducible capture efficiency.

In some embodiments, the methods involve designing a single probe for each target (a target can be as small as a single base or as large as a kilobase or more of contiguous sequence).

It may be preferable, in some cases, to design probes to capture molecules (e.g., target nucleic acids or subregions thereof) having lengths in the range of 1 - 200 bp (as used herein, a bp refers to a base pair on a double-stranded nucleic acid – however, where lengths are indicated in bps, it should be appreciated that single-stranded nucleic acids having the same number of bases, as opposed to base pairs, in length also are contemplated by the invention). However, probe design is not so limited. For example, probes can be designed to capture targets having lengths in the range of up to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000, or more bps, in some cases.

It is to be appreciated that the length of a capture molecule (e.g., a target nucleic acid or subregion thereof) is selected based upon multiple considerations. For example, where analysis of a target involves sequencing, e.g., with a next-generation sequencer, the target length should typically match the sequencing read-length so that shotgun library construction is not necessary. However, it should be appreciated that captured nucleic acids may be sequenced using any suitable sequencing technique as aspects of the invention are not limited in this respect.

It is also to be appreciated that some target nucleic acids are too large to be captured with one probe. Consequently, it may be necessary to capture multiple subregions of a target nucleic acid in order to analyze the full target.

In some embodiments, a subregion of a target nucleic acid is at least 1 bp. In other embodiments, a subregion of a target nucleic acid is at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp or more. In other embodiments, a subregion of a target nucleic acid has a length that is up to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more percent of a target nucleic acid length.



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The skilled artisan will also appreciate that consideration is made, in the design of MIPs, for the relationship between probe length and target length. In some embodiments, MIPs are designed such that they are several hundred basepairs (e.g., up to 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp or more) longer than corresponding target (e.g., subregion of a target nucleic acid, target nucleic acid).

In some embodiments, lengths of subregions of a target nucleic acid may differ. For example, if a target nucleic acid contains regions for which probe hybridization is not possible or inefficient, it may be necessary to use probes that capture subregions of one or more different lengths in order to avoid hybridization with problematic nucleic acids and capture nucleic acids that encompass a complete target nucleic acid.

Aspects of the invention involve using multiple probes, e.g., MIPs, to amplify each target nucleic acid. In some embodiments, the set of probes for a given target can be designed to 'tile' across the target, capturing the target as a series of shorter sub-targets. Alternately, the set can be designed to 'stagger' the exact positions of the hybridization regions flanking the target, capturing the full target (and in some cases capturing flanking non-target sequence) with multiple probes having different targeting arms, obviating the need for tiling. The particular approach chosen will depend on the nature of the target set. For example, if small regions are to be captured, a staggered-end approach might be appropriate, whereas if longer regions are desired, tiling might be chosen. In all cases, the amount of bias-tolerance for probes targeting pathological loci can be adjusted ('dialed in') by changing the number of different MIPs used to capture a given molecule.

In some embodiments, the 'coverage factor', or number of probes used to capture a basepair in a molecule, is an important parameter to specify. Different numbers of probes per target are indicated depending on whether one is using the tiling approach (Figure 1) or one of the staggered approaches (Figures 2, 3). In certain embodiments, a coverage factor of about 3 to about 10 is used. However, the methods are not so limited and coverage factors of up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or more may be used. It is to be appreciated that the coverage factor selected may depend the probe layout being employed. For example, in the tiling approach, for a desired coverage factor, the number of probes per target is typically a function of target length, sub-target length, and spacing between adjacent sub-target start locations (step size). For example, for a



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desired coverage factor of 3, a 200 bp target with a start-site separation of 20 bp and sub-target length of 60 bp may be encompassed with 12 MIPs (Figure 1). Thus, a specific coverage factor may be achieved by varying the number of probes per target nucleic acid and the length of the molecules captured. In the staggered approach, a fixed-length target nucleic acid is captured as several subregions or as 'super-targets', which are molecules comprising the target nucleic acid and additional flanking nucleic acids, which may be of varying lengths. For example, a target of 50 bp can be captured at a coverage factor of 3 with 3 probes in either a 'staggered' (Figure 2) or 'alternating staggered' configuration (Figure 3).

The coverage factor will be driven by the extent to which detection bias is tolerable. In some cases, where the bias tolerance is small, it may be desirable to target more subregions of target nucleic acid with, perhaps, higher coverage factors. In some embodiments, the coverage factor is up to 2, 3, 4, 5, 6, 7, 8, 9, 10 or more.

In some embodiments, when a tiled probe layout is used, when the target length is greater than 1 bp and when a step size (distance between the 5'-end of a target and the 5' end of its adjacent target) is less than the length of a target or subregion thereof, it is possible to compute probe number for a particular target based on target length (T), sub-target length (S), and coverage factor (C), such that probe number = T/(S/C) + (C-1).

In some aspects, the disclosure provides methods to increase the uniformity of amplification efficiency when multiple molecules are amplified in parallel; methods to increase the reproducibility of amplification efficiency; methods to reduce the contribution of targeting probe variability to amplification efficiency; methods to reduce the effect on a given target nucleic acid of polymorphisms in probe hybridization regions; and/or methods to simplify downstream workflows when multiplex amplification by MIPs is used as a preparative step for analysis by nucleic acid sequencing.

Polymorphisms in the target nucleic acid under the regions flanking a target can interfere with hybridization, polymerase fill-in, and/or ligation. Furthermore, this may occur for only one allele, resulting in allelic drop-out, which ultimately decreases downstream sequencing accuracy. In some embodiments, using a set of MIPs having multiple hybridization sites for the capture of any given target, the probability of loss



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from polymorphism is substantially decreased because not all targeting arms in the set of MIPs will cover the location of the mutation.

Probes for MIP capture reactions may be synthesized on programmable microarrays because of the large number of sequences required. Because of the low synthesis yields of these methods, a subsequent amplification step is required to produce sufficient probe for the MIP amplification reaction. The combination of multiplex oligonucleotide synthesis and pooled amplification results in uneven synthesis error rates and representational biases. By synthesizing multiple probes for each target, variation from these sources may be averaged out because not all probes for a given target will have the same error rates and biases.

Multiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs, or preparatively, often for next-generation sequencing or other sequencing techniques. In the preparative setting, the output of an amplification reaction is generally the input to a shotgun library protocol, which then becomes the input to the sequencing platform. The shotgun library is necessary in part because next-generation sequencing yields reads significantly shorter than amplicons such as exons. In addition to the bias-reduction afforded by the multi-tiled approach described here, tiling also obviates the need for shotgun library preparation. Since the length of the capture molecule can be specified when the probes, e.g., MIPs, are designed, it can be chosen to match the readlength of the sequencer. In this way, reads can 'walk' across an exon by virtue of the start position of each capture molecule in the probe set for that exon.

In some aspects, the disclosure provides methods for analyzing a plurality of genetic loci (e.g., a genetic locus of Table 1). The genetic loci may be analyzed by sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs). Exemplary polymorphisms are disclosed in Table 2. The skilled artisan will appreciate that other polymorphisms are known in the art and may be identified, for example, by querying the Entrez Single Nucleotide Polymorphism database, for example, by searching with a GeneID from Table 1.

The mutations listed in Table 2 are documented polymorphisms in several disease-associated genes (CFTR is mutated in cystic fibrosis, GBA is mutated in Gaucher disease, ASPA is mutated in Canavan disease, HEXA is mutated in Tay Sachs disease). The polymorphisms are of several types: insertion/deletion polymorphisms



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which will cause frameshifts (and thus generally interrupt protein function) unless the insertion/deletion length is a multiple of 3bp, and substitutions which can alter the amino acid sequence of the protein and in some cases cause complete inactivation by introduction of a stop codon.

The methods disclosed here are also useful for diagnosis of one or more heritable disorders. In some embodiments, a heritable disorder that may be diagnosed with the methods disclosed herein is a genetic disorder that is prevalent in the Ashkenazi Jewish population. In some embodiments, the heritable disorders are selected from: 21-Hydroxylase-Defiocient Congenital Adrenal Hyperplasia; ABCC8-Related Hyperinsulinism; Alpha-Thalassemia, includes Constant Spring, & MR associated; Arylsulfatase A Deficiency-Metyachromatic Leukodystrophy; Biotinidase Deficiency-Holocarboxylase Synthetase Deficiency; Bloom's Syndrome; Canavan Disease; CFTR-Related Disorders-cystic fibrosis; Citrullinemia Type I; Combined MMA & Homocystinuria-dblC; Dystrophinopathies (DMD & BMD); Familial Dysautonomia; Fanconi Anemia-FANCC; Galactosemia-Classical: Galactokinase Defiency & Galactose Epimerase Deficiency; Gaucher Disease; GJB2-Related DFNB 1 Nonsyndromic Hearing Loss and Deafness; Glutaric acidemia Type 1; Hemoglobinopathies beta-chain disorders; Glycogen Storage Disease Type 1A; Maple Syrup Urine Disease; Types 1A, 1B, 2, 3; Medium Chain Acyl-Coenzyme A; Dehydrogenase Deficiency-MCADD; Methylmalonic Acidemia; Mucolipidosis IV; Nemaline Myopathy; Nieman-Pick Type A-Acid Sphingomyelinase Deficiency; Non-Ketotic Hyperglycinemia-Glycine Encephalopathy; Ornithine Transcarbamylase Deficiency; PKU Phenylalanine Hydroxylase Deficiency; Propionic Acidemia; Short Chain Acyl-CoA Dehydrogenase Deficiency-SCADD; Smith-Lemli-Opitz Syndrome; Spinal Muscular Atrophy (SMN1)-SMA; Tay Sachs-HexA Deficiency; Usher Synbdrome-Type I (Type IB, Type IC, Type ID, Type IF, Type IG) ; X-Linked Mental Retardation ARX-Related Disorders; X-Linked Mental Retardation with Cerebellar Cypoplasia and sistinctive Facial Appearance; X-Linked Mental Retardation; includes 9, 21, 30, 46, 58, 63, 88, 89; X-linked mental retardation: FM1-Related Disorders-FRXA, Fragile X MR; X-linked SMR: Renpenning Syndrome 1; Zellweger Spectrum disorders - Peroxisomal Bifunctional Enzyme Deficiencies including Zellweger, NALD, infantile Refsums.



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**Table 1: Target Nucleic Acids**

| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| CYP21A2 | 1589 | cytochrome P450, family 21, subfamily A, polypeptide 2 | CAH1; CPS1; CA21H; CYP21; CYP21B; P450c21B; MGC150536; MGC150537; CYP21A2 | 201910 | NG_008337.1 | 6p21.3 |
| ABCC8 | 6833 | ATP-binding cassette, sub-family C (CFTR/MRP), member 8 | HI; SUR; HHF1; MRP8; PHHI; SUR1; ABC36; HRINS; TNDM2; ABCC8 | 600509 | NG_008867.1 | 11p15.1 |
| ATRX | 546 | alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) | SHS; XH2; XNP; ATR2; SFM1; RAD54; MRXHF1; RAD54L; ZNF-HX; MGC2094; ATRX | 300032 | NG_008838.1 | Xq13.1-q21.1 |
| ARSA | 410 | arylsulfatase A | MLD; ARSA | 607574 | NG_009260.1 | 22q13.31-qter; 22q13.33 |
| PSAP | 5660 | prosaposin | GLBA; SAP1; FLJ00245; MGC110993; PSAP | 176801 | NG_008835.1 | 10q21-q22 |
| BTD | 686 | biotinidase | BTD | 609019 | NG_008019.1 | 3p25 |
| HLCS | 3141 | holocarboxylase synthetase (biotin-(proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)) ligase) | HCS; HLCS | 609018 | NC_000021.7 | 21q22.1; 21q22.13 |
| BLM | 641 | Bloom syndrome, RecQ helicase-like | BS; RECQ2; RECQL2; RECQL3; MGC126616; MGC131618; MGC131620; BLM | 604610 | NG_007272.1 | 15q26.1 |
| ASPA | 443 | aspartoacylase (Canavan disease) | ASP; ACY2; ASPA | 608034 | NG_008399.1 | 17pter-p13 |
| CFTR | 1080 | cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) | CF; MRP7; ABC35; ABCC7; CFTR/MRP; TNR-CFTR; dJ760C5.1; CFTR | 602421 | NC_000007.12 | 7q31.2 |
| ASS1 | 445 | argininosuccinate synthetase 1 | ASS; CTLN1; ASS1 | 603470 | NG_011542.1 | 9q34.1 |
| MMACHC | 25974 | methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria | cblC; FLJ25671; DKFZp564I122; RP11-291L19.3; MMACHC | 609831 | NC_000001.9 | 1p34.1 |
| IKBKAP | 8518 | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein | FD; DYS; ELP1; IKAP; IKI3; TOT1; FLJ12497; DKFZp781H1425; IKBKAP | 603722 | NG_008788.1 | 9q31 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| FANCC | 2176 | Fanconi anemia, complementation group C | FA3; FAC; FACC; FLJ14675; FANCC | 227645 | NG_011707.1 | 9q22.3 |
| GALK1 | 2584 | galactokinase 1 | GK1; GALK; GALK1 | 604313 | NG_008079.1 | 17q24 |
| GALT | 2592 | galactose-1-phosphate uridylyltransferase | GALT | 606999 | NC_000009.10 | 9p13 |
| GALE | 2582 | UDP-galactose-4-epimerase | SDR1E1; FLJ95174; FLJ97302; GALE | 606953 | NG_007068.1 | 1p36-p35 |
| GBA | 2629 | glucosidase, beta; acid (includes glucosylceramidase) | GCB; GBA1; GLUC; GBA | 606463 | NG_009783.1 | 1q21 |
| GJB2 | 2706 | gap junction protein, beta 2, 26kDa | HID; KID; PPK; CX26; DFNA3; DFNB1; NSRD1; DFNA3A; DFNB1A; GJB2 | 121011 | NG_008358.1 | 13q11-q12 |
| GCDH | 2639 | glutaryl-Coenzyme A dehydrogenase | GCD; ACAD5; GCDH | 608801 | NG_009292.1 | 19p13.2 |
| G6PC | 2538 | glucose-6-phosphatase, catalytic subunit | G6PT; GSD1; GSD1a; MGC163350; G6PC | 232200 | NG_011808.1 | 17q21 |
| HBB | 3043 | hemoglobin, beta | CD113t-C; beta-globin; HBB | 141900 | NG_000007.3 | 11p15.5 |
| BCKDHA | 593 | branched chain keto acid dehydrogenase E1, alpha polypeptide | MSU; MSUD1; OVD1A; BCKDE1A; FLJ45695; BCKDHA | 608348 | NC_000019.8 | 19q13.1-q13.2 |
| BCKDHB | 594 | branched chain keto acid dehydrogenase E1, beta polypeptide | E1B; FLJ17880; dJ279A18.1; BCKDHB | 248611 | NG_009775.1 | 6q13-q15 |
| DBT | 1629 | dihydrolipoamide branched chain transacylase E2 | E2; E2B; BCATE2; MGC9061; DBT | 248610 | NG_011852.1 | 1p31 |
| DLD | 1738 | dihydrolipoamide dehydrogenase | E3; LAD; DLDH; GCSL; PHE3; DLD | 238331 | NG_008045.1 | 7q31-q32 |
| ACADM | 34 | acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain | MCAD; ACAD1; MCADH; FLJ18227; FLJ93013; FLJ99884; ACADM | 607008 | NG_007045.1 | 1p31 |
| MMAA | 166785 | methylmalonic aciduria (cobalamin deficiency) cblA type | cblA; MGC120010; MGC120011; MGC120012; MGC120013; MMAA | 607481 | NG_007536.1 | 4q31.22 |
| MMAB | 326625 | methylmalonic aciduria (cobalamin deficiency) cblB type | ATR; cblB; MGC20496; MMAB | 607568 | NG_007096.1 | 12q24 |
| MUT | 4594 | methylmalonyl Coenzyme A mutase | MCM; MUT | 609058 | NG_007100.1 | 6p12.3 |
| MCOLN1 | 57192 | mucolipin 1 | ML4; MLIV; MST080; TRPML1; MSTP080; TRP-ML1; TRPM-L1; MCOLN1 | 605248 | NC_000019.8 | 19p13.3-p13.2 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| ACTA1 | 58 | actin, alpha 1, skeletal muscle | ACTA; ASMA; CFTD; MPFD; NEM1; NEM2; NEM3; CFTD1; CFTDM; ACTA1 | 102610 | NG_006672.1 | 1q42.13 |
| TPM3 | 7170 | tropomyosin 3 | TM3; TRK; NEM1; TM-5; TM30; TM30nm; TPMsk3; hscp30; MGC3261; FLJ41118; MGC14582; MGC72094; OK/SW-cl.5; TPM3 | 191030 | NG_008621.1 | 1q21.2 |
| TNNT1 | 7138 | troponin T type 1 (skeletal, slow) | ANM; TNT; STNT; TNTS; FLJ98147; MGC104241; TNNT1 | 191041 | NG_011829.1 | 19q13.4 |
| NEB | 4703 | nebulin | NEM2; NEB177D; FLJ11505; FLJ36536; FLJ39568; FLJ39584; DKFZp686C1456; NEB | 161650 | NG_009382.1 | 2q22 |
| SMPD1 | 6609 | sphingomyelin phosphodiesterase 1, acid lysosomal | ASM; NPD; SMPD1 | 607608 | NG_011780.1 | 11p15.4-p15.1 |
| GLDC | 2731 | glycine dehydrogenase (decarboxylating) | GCE; NKH; GCSP; HYGN1; MGC138198; MGC138200; GLDC | 238300 | NC_000009.10 | 9p22 |
| GCSH | 2653 | glycine cleavage system protein H (aminomethyl carrier) | GCE; NKH; GCSH | 238330 | NC_000016.8 | 16q23.2 |
| AMT | 275 | aminomethyltransferase | GCE; NKH; GCST; AMT | 238310 | NC_000003.10 | 3p21.2-p21.1 |
| OTC | 5009 | ornithine carbamoyltransferase | OCTD; MGC129967; MGC129968; MGC138856; OTC | 300461 | NG_008471.1 | Xp21.1 |
| PAH | 5053 | phenylalanine hydroxylase | PH; PKU; PKU1; PAH | 612349 | NG_008690.1 | 12q22-q24.2 |
| DHPR | 5860 | quinoid dihydropteridine reductase | DHPR; PKU2; SDR33C1; FLJ42391; QDPR | 612676 | NG_008763.1 | 4p15.31 |
| PTS | 5805 | 6-pyruvoyltetrahydropterin synthase | PTPS; FLJ97081; PTS | 261640 | NG_008743.1 | 11q22.3-q23.3 |
| PCCA | 5095 | propionyl Coenzyme A carboxylase, alpha polypeptide | PCCA | 232000 | NG_008768.1 | 13q32 |
| PCCB | 5096 | propionyl Coenzyme A carboxylase, beta polypeptide | DKFZp451E113; PCCB | 232050 | NG_008939.1 | 3q21-q22 |
| ACADS | 35 | acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain | SCAD; ACAD3; ACADS | 606885 | NG_007991.1 | 12q22-qter |
| DHCR7 | 1717 | 7-dehydrocholesterol reductase | SLOS; DHCR7 | 602858 | NC_000011.8 | 11q13.2-q13.5 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| SMN1 | 6606 | survival of motor neuron 1, telomeric | SMA; SMN; SMA1; SMA2; SMA3; SMA4; SMA@; SMNT; BCD541; T-BCD541; SMN1 | 600354 | NG_008691.1 | 5q13 |
| HEXA | 3073 | hexosaminidase A (alpha polypeptide) | TSD; MGC99608; HEXA | 606869 | NG_009017.1 | 15q23-q24 |
| MYO7A | 4647 | myosin VIIA | DFNB2; MYU7A; NSRD2; USH1B; DFNA11; MYOVIIA; MYO7A | 276903 | NG_009086.1 | 11q13.5 |
| USH1C | 10083 | Usher syndrome 1C (autosomal recessive, severe) | PDZ73; AIE-75; DFNB18; PDZ-45; PDZ-73; NY-CO-37; NY-CO-38; ush1cpst; PDZ-73/NY-CO-38; USH1C | 605242 | NC_000011.8 | 11p15.1-p14 |
| CDH23 | 64072 | cadherin-like 23 | USH1D; DFNB12; FLJ00233; FLJ36499; KIAA1774; KIAA1812; MGC102761; DKFZp434P2350; CDH23 | 605516 | NG_008835.1 | 10q21-q22 |
| PCDH15 | 65217 | protocadherin 15 | USH1F; DFNB23; DKFZp667A1711; PCDH15 | 605514 | NG_009191.1 | 10q21.1 |
| SANS | 124590 | Usher syndrome 1G (autosomal recessive) | SANS; ANKS4A; FLJ33924; USH1G | 607696 | NG_007882.1 | 17q25.1 |
| ARX | 170302 | aristaless related homeobox | ISSX; PRTS; MRX29; MRX32; MRX33; MRX36; MRX38; MRX43; MRX54; MRX76; MRX87; MRXS1; ARX | 300382 | NG_008281.1 | Xp21 |
| OPHN1 | 4983 | oligophrenin 1 | OPN1; MRX60; OPHN1 | 300127 | NG_008960.1 | Xq12 |
| JARID1C | 8242 | lysine (K)-specific demethylase 5C | MRXJ; SMCX; MRXSJ; XE169; JARID1C; DXS1272E; KDM5C | 314690 | NG_008085.1 | Xp11.22-p11.21 |
| FTSJ1 | 24140 | FtsJ homolog 1 (E. coli) | JM23; MRX9; SPB1; TRM7; CDLIV; MRX44; FTSJ1 | 300499 | NG_008879.1 | Xp11.23 |
| SLC6A8 | 6535 | solute carrier family 6 (neurotransmitter transporter, creatine), member 8 | CRT; CT1; CRTR; MGC87396; SLC6A8 | 300036 | NC_000023.9 | Xq28 |
| DLG3 | 1741 | discs, large homolog 3 (Drosophila) | MRX; MRX90; NEDLG; NE-Dlg; SAP102; SAP-102; KIAA1232; DLG3 | 300189 | NC_000023.9 | Xq13.1 |
| TM4SF2 | 7102 | tetraspanin 7 | A15; MXS1; CD231; MRX58; CCG-B7; TM4SF2; TALLA-1; TM4SF2b; DXS1692E; TSPAN7 | 300096 | NG_009160.1 | Xp11.4 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| ZNF41 | 7592 | zinc finger protein 41 | MRX89; MGC8941; ZNF41 | 314995 | NG_008238.1 | Xp11.23 |
| FACL4 | 2182 | acyl-CoA synthetase long-chain family member 4 | ACS4; FACL4; LACS4; MRX63; MRX68; ACSL4 | 300157 | NG_008053.1 | Xq22.3-q23 |
| PQBP1 | 10084 | polyglutamine binding protein 1 | SHS; MRX55; MRXS3; MRXS8; NPW38; RENS1; PQBP1 | 300463 | NC_000023.9 | Xp11.23 |
| PEX1 | 5189 | peroxisomal biogenesis factor 1 | ZWS1; PEX1 | 602136 | NG_008341.1 | 7q21.2 |
| PXMP3 | 5828 | peroxisomal membrane protein 3, 35kDa | PAF1; PEX2; PMP3; PAF-1; PMP35; RNF72; PXMP3 | 170993 | NG_008371.1 | 8q21.1 |
| PEX6 | 5190 | peroxisomal biogenesis factor 6 | PAF2; PAF-2; PXAAA1; PEX6 | 601498 | NG_008370.1 | 6p21.1 |
| PEX10 | 5192 | peroxisomal biogenesis factor 10 | NALD; RNF69; MGC1998; PEX10 | 602859 | NG_008342.1 | 1p36.32 |
| PEX12 | 5193 | peroxisomal biogenesis factor 12 | PAF-3; PEX12 | 601758 | NG_008447.1 | 17q12 |
| PEX5 | 5830 | peroxisomal biogenesis factor 5 | PXR1; PTS1R; PTS1-BP; FLJ50634; FLJ50721; FLJ51948; PEX5 | 600414 | NG_008448.1 | 12p13.31 |
| PEX26 | 55670 | peroxisomal biogenesis factor 26 | FLJ20695; PEX26M1T; Pex26pM1T; PEX26 | 608666 | NG_008339.1 | 22q11.21 |

**Table 2: Polymorphisms**

| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs63500661 | TCACATCACCAAGTTAAAAAAAAAAA[A/G]GGGGCGGGGGGGCAGAATGAAAATT |
| CFTR | 1080 | rs63107760 | AAACAAGGATGAATTAAGTTTTTTTT[-/T]AAAAAAGAAACATTTGGTAAGGGGA |
| CFTR | 1080 | rs62469443 | ATCACCAAGTTAAAAAAAAAAAGGG[A/G]CGGGGGGGCAGAATGAAAATTGCAT |
| CFTR | 1080 | rs62469442 | CTATTGAACCAGAACCAAACAGGAAT[A/G]CCATAGCATTTTGTAAACTAAACTG |
| CFTR | 1080 | rs62469441 | CAGGAGTTCAAGACCAGCCTACTAAA[A/C]CACACACACACACACACACACAC |
| CFTR | 1080 | rs62469439 | GATTAAATAATAGTGTTTATGTACCC[C/G]GCTTATAGGAGAAGAGGGTGTGTGT |
| CFTR | 1080 | rs62469438 | ATTGTTATCTTTTCATATAAGGTAAC[A/T]GAGGCCCAGAGAGATTAAATAACAT |
| CFTR | 1080 | rs62469437 | TAATTTTAATTAAGTAAATTTAATTG[A/G]TAGATAAATAAGTAGATAAAAAATA |
| CFTR | 1080 | rs62469436 | GTATAAAAAAAAAAAAAAAAAAAGTT[A/T]GAATGTTTTCTTGCATTCAGAGCCT |
| CFTR | 1080 | rs62469435 | ATACTAAAAATTTAAAGTTCTCTTGC[A/G]ATATATTTTCTTAATATCTTAC |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | ATC |
| CFTR | 1080 | rs62469434 | TGCTGGGATTACAGGCGTGAGCCACC[A/G]CGCCTGGCCTGATGGGACAT ATTTT |
| CFTR | 1080 | rs62469433 | CTACAATATAAGTATAGTATTGCAAA[A/C]CCATCAGGAAGGGTGTTAACT ATTT |
| CFTR | 1080 | rs61763210 | GTTGTCTCCAAACTTTTTTTCAGGTG[-/AGA]AGGTGGCCAACCGAGCTTCGGAAAG |
| CFTR | 1080 | rs61720488 | TTTTTTCATAAAAGATTATATAAAGG[A/C]TATTGCTTTTGAATCACAAAC ACTA |
| CFTR | 1080 | rs61481156 | ATCTAGTGAGCAGTCAGGAAAGAGAA[C/T]TTCCAGATCCTGGAAATCAG GGTTA |
| CFTR | 1080 | rs61443875 | TAGAGTATAAAAAAAAAAAAAAAAAA[-/A]GTTTGAATGTTTTCTTGCATTCAGA |
| CFTR | 1080 | rs61312222 | TGCAAATGCCAACTATCAAAGATATT[C/G]GAGTATACTGTCAATAAACTT CATA |
| CFTR | 1080 | rs61159372 | TCCTCAACAGTTAGAAACAATATTTT[C/G]AGTGATTTCCCATGCCAACTTT ACT |
| CFTR | 1080 | rs61094145 | TTTTTGGTATTGTTGTTAAATAAGTG[A/G]GAATTCAATACAGTATAATGT CTGT |
| CFTR | 1080 | rs61086387 | CTTGAAATCGGATATATATATATATA[-/TGTATATATATATATATATATATATATATACATATATATATATA]GTAT TATCCCTGTTTTCACAGTTTT |
| CFTR | 1080 | rs60996744 | AGAGGGGCTGTGAAGGACACCAAGGA[A/G]GAGACTAAGAGCCAGGAGG GAAAAC |
| CFTR | 1080 | rs60960860 | TAGAGTTTATTAGCTTTTACTACTCT[A/G]CTTAGTTACTTTGTGTTACAGA ATA |
| CFTR | 1080 | rs60923902 | ACTAGTGATGATGAGCTTCTTTTCAT[-/AT]GTTTGTTGGCTGCATAAATGTCTTC |
| CFTR | 1080 | rs60912824 | GCAGAGAAAAGAGGGGCTGTGAAGGA[C/G]ACCAAGGAGGAGACTAAGA GCCAGG |
| CFTR | 1080 | rs60887846 | TTCAGAGGTCTACCACTGGTGCATAC[G/T]CTAATCACAGTGTCGAAAATT TTAC |
| CFTR | 1080 | rs60793174 | AAGAAAGAGCAAAAGAGGGCAAACTT[C/T]TCATACATTTTTGATGTCGA AACCA |
| CFTR | 1080 | rs60788575 | CCTAAAGTTTAAAAAGAAAAAAAAAA[-/A]GGAAGAAGGAATTAAAAATCCAAAG |
| CFTR | 1080 | rs60760741 | GTGTGTGTGTGTATATATATATATAT[A/T]TATATATTTTTTTTTTCCTGAGC CA |
| CFTR | 1080 | rs60456599 | AAACTGTTGATGTTTTCATTTATTTA[C/G]ATCATTGGAAAACTTTAGATTC TAG |
| CFTR | 1080 | rs60363249 | TTTATCCATTCTTAACCAGAACAGAC[A/G]TTTTTTCAGAGCTGGTCCAGG AAAA |
| CFTR | 1080 | rs60355115 | TTGAAATCGGATATATATATATATAT[A/G]TATATATATATATATATATATAT ATAT |
| CFTR | 1080 | rs60308689 | TAGTTTTTTATTTCCTCATATTATTT[-/T]CAGTGGCTTTTTCTTCCACATCTTT |
| CFTR | 1080 | rs60271242 | ACATAGTTCTCAGTGGTACAACTACA[A/G]GTGATTTCTCTTTTCTTATTTC TGG |
| CFTR | 1080 | rs60010318 | AGAGCAATGGCATCCCTTGTCTTGTG[C/T]TATACAGGATGCAGCAATTTA TAGG |
| CFTR | 1080 | rs59961323 | TTCTGTCTACATAAGATGTCATACTA[A/G]ATTATCTTTTCCAGCATGCATT CAG |
| CFTR | 1080 | rs59961270 | CAGGGTGGCATGTTAGGCAGTGCTTA[A/G]AATAAATGAGTTGGTTATACA AGTA |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs59837506 | AGGACACACACACACACACACACA[-/CA]TGCACACACATTTAAATAGATGCAT |
| CFTR | 1080 | rs59572090 | TAAAAAATTGGTATAATGAAATTGCA[C/T]TTGTAGTCTTTGGACATTTAAATCC |
| CFTR | 1080 | rs59548252 | TTTCAATACTTAAGAGGTACGCAGAG[A/G]AAAGAGGGGCTGTGAAGGACACCAA |
| CFTR | 1080 | rs59519859 | CAGCAATGAATATTTTGAGGCTGAGG[C/T]GCTGAGGGGTAAAATTGCAGCCTGG |
| CFTR | 1080 | rs59509837 | TTATGGTTTATATTTTTGTGTCTTCT[-/CTTT]AACACATCTTTTCTAGCAGAATTCA |
| CFTR | 1080 | rs59417037 | GTATTTTAGTTTTTTTTTTTTGTTTG[-/T]TTTGTTTTGTTTTGTTTTGTTTTTG |
| CFTR | 1080 | rs59159458 | TGGGTGACTCCATTTTTACTTTTAGT[C/T]TGGTCTGTTGAGGCCTCGTGAGAGA |
| CFTR | 1080 | rs59048119 | TATTTTCATGTATTTTAGTTTTTTTTT[-/TTTT]GTTTGTTTTGTTTTGTTTTGTTTTG |
| CFTR | 1080 | rs58970500 | GTGTGTGTGTATATATATATATATAT[A/T]TATATTTTTTTTTTCCTGAGCCAAA |
| CFTR | 1080 | rs58942292 | AACCTATTAGCATGTCTGGCAGAAAA[-/A]TAGATACTTAATAAATTTCTTAAAT |
| CFTR | 1080 | rs58917054 | GAGGCTTAGACAGTTTAAGTAACTCA[A/G]GCATGGTTACACAACTAGCTAGGGC |
| CFTR | 1080 | rs58837484 | GTGTGAGTATTATGAGACCATATGTT[A/G]GGAGATTTTATTTGGTATTGAGGAT |
| CFTR | 1080 | rs58829491 | GAAACCCCACCCCTTCTATAGTTTTC[C/T]CTTTAATATTTACAATGGAACCATT |
| CFTR | 1080 | rs58805195 | CATATATATATAGTGTGTGTGTGTGT[A/G]TATATATATATATATATATATTTTT |
| GBA | 2629 | rs60866785 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs60239603 | GGCAGGTAATATCTAGTACCTTACTT[A/T]TATTTCCTGAGCACATTCTACATTT |
| GBA | 2629 | rs56310840 | GGCCAGGAATGGGAGTGCTTAGGTGC[A/G]GAGGTGGCACTGTTCCCGCAGCTGC |
| GBA | 2629 | rs41264927 | TGAAAACTCCATCCCCTCAGGGTCAT[C/T]AGATGAAGAGAAGACCACAGGGGTT |
| GBA | 2629 | rs41264925 | TGTAGGTAAGGGTCACATGTGGGAGA[C/G]GCAGCTGTGGGTAGGTCAGCCCTGT |
| GBA | 2629 | rs36024691 | CCAAGAAGGCGCCATTACACTCCAGC[-/C]TGGGCGACAGGGCGAGACTCCCTCA |
| GBA | 2629 | rs36024092 | TGCCACACCCAGCTAATTTGTGTGTG[-/G]TATGTGTGTGTATGTATGTGTGTGT |
| GBA | 2629 | rs35682967 | GTTCCTCCAGTAATTTTTTTTTTTTT[-/T]GGTTTTGAGACAGAGTCTTGCCCTG |
| GBA | 2629 | rs35033592 | ATCATGCCCAGATAATTTTTTTTTTT[-/T]GTATTTTAGTAGACACAGGGTTTCA |
| GBA | 2629 | rs34732744 | CGAGCGAGAGAGAGAGAGAGAGAGAG[-/AG]GAGCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs34620635 | CCTGTGAGGGGCACATTCCTTAGTAG[-/C]TAAGGAGTTGGGGGTGTGAAGATCC |
| GBA | 2629 | rs34302637 | ACAGGCTACTGGCTGGGCCCAGGCAA[-/A]GGGGGCCTTGGCAGGAAAAGTTCCT |
| GBA | 2629 | rs33949225 | GCGAGAGAGAGAGAGAGAGAGAGAGG[-/AG]AGCCGGCGCGAGAACTACGCATGCG |
| GBA | 2629 | rs28678003 | AAGAAGAAAAATAAAAAGAAAGTGGG[C/T]CAGACCGAGAGAACAGGAA |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | GCCTGA |
| GBA | 2629 | rs28559737 | AAGGACAAAGGCAAAGAGACAAAGGC[G/T]CAACACTGGGGGTCCCCAG AGAGTG |
| GBA | 2629 | rs28373017 | TACCTAGTCACTTCCTGCCTCCATGG[C/T]GCAAAAGGGGATGGGTGTGCC TCTT |
| GBA | 2629 | rs12752133 | CTCTTCCGAGGTTCCACCCTGAACAC[C/T]TTCCTGCTCCCTCGTGGTGTAG AGT |
| GBA | 2629 | rs12747811 | TTCTGACTGGCAACCAGCCCCACTCT[C/T]TGGGAGCCCTCAGGAATGAAC TTGC |
| GBA | 2629 | rs12743554 | gctcagcctcccaggctggagtgcag[A/T]ggcgcgatctcggctcaccgcaacc |
| GBA | 2629 | rs12041778 | CATGAACCACATCAAATGAGATTTAG[C/T]GGGAGTGGCACACACAGTCA TGACC |
| GBA | 2629 | rs12034326 | AAGCAGCCCTGGGGAGTCGGGGCGGG[A/G]CCTGGATTGGAAAAGAGAC GGTCAC |
| GBA | 2629 | rs11558184 | CTCCAAGTTCTGGGAGCAGAGTGTGC[A/G]GCTAGGCTCCTGGGATCGAG GGATG |
| GBA | 2629 | rs11430678 | GTTCCTCCAGTAAttttttttttttt[-/G/T]gttttgagacagagtcttgccctgt |
| GBA | 2629 | rs11264345 | CTAGTACCTTACTTCCCTCAAGTTCA[A/T]TCATCTCACAGATATTTCCTGA GCA |
| GBA | 2629 | rs10908459 | aattagccgtgcgtggtggcgggtgc[C/T]tgtaatcccacgtacttgggaggct |
| GBA | 2629 | rs10796940 | CCATGGCCAGCCGGGGAGGGGACGGG[A/C]ACACACAGACCCACACAGA GACTCA |
| GBA | 2629 | rs10668496 | agcgagagagagagagagagagagag[-/AG]gagCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs7416991 | CGTAGCAGTTAGCAGATGATAGGCGG[C/G/T]GAAATCTTATTTCACAGGG CATTAA |
| GBA | 2629 | rs4024049 | CTGGCCCTGGTGACAGTGGGGCTGTG[C/T]GTGGGGCCAGAGCCTTCTCAG AGGT |
| GBA | 2629 | rs4024048 | CAGATACTGGCCCTGGTGACAGTGGG[A/G]CTGTGCGTGGGGCCAGAGCC TTCTC |
| GBA | 2629 | rs4024047 | GACAGATACTGGCCCTGGTGACAGTG[G/T]GGCTGTGCGTGGGGCCAGAG CCTTC |
| GBA | 2629 | rs3841430 | GGCTCctctctctctctctctctctc[-/TC]gctcgctctctcgctctctcgctct |
| GBA | 2629 | rs3754485 | GTTTCAGACCAGCCTGGCCAACATAG[C/T]GAAACCCCATCTCTACTAAAA ATAA |
| GBA | 2629 | rs3205619 | AGTGGGCGATTGGATGGAGCTGAGTA[C/T]GGGGCCCATCCAGGCTAATC ACACC |
| GBA | 2629 | rs2990227 | CCGGGCTCCGTGAATGTTTGTCACAT[C/G]TCTGAAGAACGTATGAATTAC ATAA |
| GBA | 2629 | rs2990226 | GAATCCCAACCCCGACGCTCGTCGCC[C/G]GGCTCCGTGAATGTTTGTCAC ATGT |
| GBA | 2629 | rs2990225 | GCGAATCCCAACCCCGACGCTCGTCG[C/T]CGGGCTCCGTGAATGTTTGTC ACAT |
| GBA | 2629 | rs2990224 | TGGGCAGAAGTCAGGGTCCAAAGAAA[G/T]GGCAAAGAAAAGTGTcagtggct ca |
| ASPA | 443 | rs63751297 | TAAGAAAGACGTTTTTGATTTTTTTC[A/G]GACTTCTCTGGCTCCACTACCC TGC |
| ASPA | 443 | rs62071301 | CTGATTCCTGGCCAGGAGCGGTGGCT[C/T]ACGCCTGTAATCCCAGCGCTT TGGG |
| ASPA | 443 | rs62071300 | TAAAAATGCTGATTCCTGGCCAGGAG[C/T]GGTGGCTCACGCCTGTAATCC CAGC |
| ASPA | 443 | rs62071299 | TTTAAAAATGCTGATTCCTGGCCAGG[A/C]GCGGTGGCTCACGCCTGTAAT CCCA |
| ASPA | 443 | rs62071297 | CAAGACCTGTCAAAGATCTGAGAAAT[A/T]TTACCCGACTTACAAGCTAAC |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | CATT |
| ASPA | 443 | rs61697033 | ACTGTAATAAGTGCTGTAAAAGAAAT[A/G]CACAAAATAATATAGCAGAG GGTAT |
| ASPA | 443 | rs60743592 | CTTGAGGTCAGGAGTTCAAGACCAGT[C/T]TGGGCAACATGGGGAAAACC TTGTC |
| ASPA | 443 | rs60666840 | AGGTTGCAGTGAGCCGAGATCATGCC[A/G]TTGCACTCCAGCCGGGGCAA CAAAA |
| ASPA | 443 | rs60147514 | ACAAGTGTCTTGAAATTATCTGTGAT[C/T]TGCTATAGAGCAATACTTTTGT AAA |
| ASPA | 443 | rs59930743 | GTGGGTATATGCAGCTCTATGCACTA[C/T]CTGCTCATTTATTTGGTAAATC TAA |
| ASPA | 443 | rs59690349 | TGTGTGTGTGTGCGTGTGTGTGTGTG[-/TGTGTGTG]ATCATAAGAGTGGCTGCAGCAAACT |
| ASPA | 443 | rs59676360 | AGTCTGGAGTGCAATGGTGCAATCTC[A/G]GCTCACTGCAGCCTCCACCTC CGGG |
| ASPA | 443 | rs59335404 | CTCCTAATGGATATTTCCTAAATTTT[G/T]CTGAACAGAATTTAACTTGAGC TGG |
| ASPA | 443 | rs58879097 | ATTTAAAAATGGATTTCTAGAAAAAC[A/G]ATCACATACTTGAATATTTTA GCAA |
| ASPA | 443 | rs58686774 | CTATAAATGGGTAGCATGAGGGATTC[A/G]AGGAGGTGGCTGAAAGAAGC ACGTA |
| ASPA | 443 | rs57511162 | AAGAAACCAAGCATAGTAGAGTGTTA[A/G]AAAACCAAAGCAACTAAACA ACTGT |
| ASPA | 443 | rs55859596 | CGGGGCTCAGAACTTGTAACAGAAAA[A/T]TAAAATATACTCCACTCAAG GGAAT |
| ASPA | 443 | rs55742972 | TACTACACTTCACGGATACTGTACTT[-/GTACTT]TTTTTCCAAATTGAAGGTTTTTGGC |
| ASPA | 443 | rs55640436 | TTGTTTTTGTTTTTGTTTTTGTTTTT[-/GTTTTTGTTTTT]TGAGATGGAGTCTCGCTCTGTCGCC |
| ASPA | 443 | rs36225687 | TTTGCCTTACTACACTTCACGGATAC[-/TGTACT]TGTACTTTTTTTCCAAATTGAAGGT |
| ASPA | 443 | rs36051310 | GAGGTGGCTGAAAGAAGCACGTATCC[-/C]TGATGGCATGGTTGCGGGTTATATG |
| ASPA | 443 | rs36034906 | GAGAAAAGCAGTTCCTGGAACACCCC[-/C]ACCCCTTAACCCCTTATCTCTGCTT |
| ASPA | 443 | rs36033666 | TTACATATGTATACATGTGCCATGTT[-/T]GGTGTGCCGCACCCATTAACTCGTC |
| ASPA | 443 | rs35730123 | CTTTTTCCAGATTTTTTTTTTTTTTT[-/T]GAGACAGAGTTTCACTCTTGTTGCC |
| ASPA | 443 | rs35629100 | TTTGGAAATCTTAAGCTTTTATTTGG[-/G]TGTCACAGAGAAACAGGATCTGTAT |
| ASPA | 443 | rs35614631 | TACTTTAAGTTTTAGGGTACATGTGC[-/A]CCATGTGCAGGTTTGTTACATATGT |
| ASPA | 443 | rs35225782 | ATTCATGACCAGCCACATAAATGCAC[-/A]GTATTACTTCGCAAGCATGCCAATG |
| ASPA | 443 | rs35178659 | GTGCACTAGAATTAGCTAAAGTGGGG[-/G]AAAAAAAGATGCATTTGATGGTCTA |
| ASPA | 443 | rs35095578 | AACCTCCACCTCCCAGGTTCAAGAGA[-/A]TTCTCCTGCCTCAGCCTCCCAAGTA |
| ASPA | 443 | rs35002210 | CCTCCCTGTGATCCGAAGTAGCAGAC[A/G]TACTTAACTTCCATGGTGGAT TGTT |
| ASPA | 443 | rs34744839 | AAAACATTATTATATCTAGAAAAAAA[-/A]TGTATCTTAACCATTGTGGGAAGTG |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| ASPA | 443 | rs34680506 | TTGAAGGTAAAATCATAGGGAGTTGG[-/G]AGCTGTCCTCTTGCGCTGAATCAGT |
| ASPA | 443 | rs34365618 | ACTTGTGGCCTTTTTGGAGAGGTTAG[-/CA]ACTCTGAAAACTCTGTCCCTGGACC |
| ASPA | 443 | rs34275920 | GAAGGAGAAAAAGAGAGGAAATAAGT[-/T]AAAATAATAAACACAATTAATAAAG |
| ASPA | 443 | rs34109510 | TGTATACATGTGCCATGTTGGTGTGC[C/T]GCACCCATTAACTCGTCATTTAGCA |
| ASPA | 443 | rs34054576 | TCACCTGTCACCTCCTATAGAACTTT[-/C]CCCTGACCCTCCTCTATAGCATTAA |
| ASPA | 443 | rs34015272 | ATAAATGATCATCATTCACAGTAGGG[-/G]TTTTGTTTTGTTTTTTTTCTGGAAA |
| ASPA | 443 | rs34002091 | ACAGACATATCTACAAACACACTTTT[-/T]CACATATTTGTGTAAGTCATTTATG |
| ASPA | 443 | rs28940574 | AAAGACAACTAAACTAACGCTCAATG[A/C]AAAAAGTATTCGCTGCTGTTTACAT |
| ASPA | 443 | rs28940279 | TACCGTGTACCCCGTGTTTGTGAATG[A/C]GGCCGCATATTACGAAAAGAAAGAA |
| ASPA | 443 | rs17850703 | CAGGGCTGGAGGTAAAACCATTTATT[A/G]CTAACCCCAGAGCAGTGAAGAAGTG |
| ASPA | 443 | rs17222495 | TTCTTCATTGCCTATTGAAGAGAGAG[C/T]GGAATGCTTTGGTTGCCAGATATGG |
| ASPA | 443 | rs17175228 | CACAAGATCTCATTACTCAGGAGCTG[C/T]CCAAGTGTCTAATGTACTTAGTTAA |
| ASPA | 443 | rs16953074 | TTCTGTGTAACATTTCATTTAAGCAA[A/G]GGATTCGGCAAATCAAAAATTGTCA |
| ASPA | 443 | rs16953070 | TAAAACGTATTGAAGGTATTATTGAC[G/T]CTGTTGAAGCAAAGAGAACAAAACA |
| HEXA | 3073 | rs62022858 | ATCTGCTCTTCCAGTTGGATGACAAG[C/T]CTTGCTGTCTAACACCTGCTGCAGA |
| HEXA | 3073 | rs62022857 | CCATTTTTTGTTGTATTTTTTTTTTC[C/T]TGAATACTTTTTATCGCAGTTGGTT |
| HEXA | 3073 | rs62017872 | CCCTGTCTCTAAAAGAAAAAAAAAAA[A/G]AAAAAAAAAAGAAAACAAAACCCAA |
| HEXA | 3073 | rs62017871 | AGTGGCTCCAAAAAGGTCATGGAACC[C/T]CTTGAGGATGATGCAAATTGACTCT |
| HEXA | 3073 | rs61662730 | TAAAGTTACTTTTCTTTTATTGACTT[C/T]CCCTTATTTTTTAACCTTATGCTTT |
| HEXA | 3073 | rs61329913 | CAGAGTTAAAAAAAAAAAAAAAAAA[-/A]GGAAGTAGCAGCAACAGCTTGGAAA |
| HEXA | 3073 | rs60920713 | GTTGCCCAGGGTTGAGTGCAGAGGCA[C/T]ATCTTGGCTCACAGCAACCTCTGCC |
| HEXA | 3073 | rs60783213 | AAGGCTTTTTTTTTTTTTTTTTTT[-/TTTT]GAGACAGAGTCTTGCTGTGTCACCC |
| HEXA | 3073 | rs60644867 | GCCTACATTCTGCAAAGAGGAGGGAA[C/G]ATTCACAGCTCCATACTTGAACCCT |
| HEXA | 3073 | rs60288568 | CCAAAGGAGAATAGCTCTAGGGGAGG[C/G]AGGTGGATGAGTATGCATGGGGGAG |
| HEXA | 3073 | rs59888548 | GACTCCATCTCAAAAAAAAAAAAAA[-/A]TGCAGTCTAATGGCAGAATTAGACT |
| HEXA | 3073 | rs59733856 | TTATTTATTTATTTATTTATTTTTGA[A/G]ACAGGGTCTCTGTTGTCCAGGCTGG |
| HEXA | 3073 | rs59427837 | TTTTGAGGCAGGGTCTCACTCTGTTG[C/T]CCAGGGTTGAGTGCAGAGGCACATC |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| HEXA | 3073 | rs59171976 | CGCCTTGCGAAGGCCCCACAGCTTGC[C/T]TGTGACAAACGTTCATAGGCAAATG |
| HEXA | 3073 | rs58706602 | GGAGGTCTGTACAAAGCACCACCTAC[C/T]TCATGGGTCAGTTTCCACAGCAGAA |
| HEXA | 3073 | rs58696963 | GAATCTTATAATTCACTGTGTACCTC[-/CCTC]TGTTTCATATTTTCGCAATTGAACT |
| HEXA | 3073 | rs58610850 | AACATAGTATCTAATATAGCTTTACA[C/T]CCAAAGCCAAAATATGAATACACTG |
| HEXA | 3073 | rs58016062 | TTGTTTTGTTTTGTTTGGGGGGGGGG[-/G]TTGTTTTTCTGAGAGGGAGTCTTGC |
| HEXA | 3073 | rs57733983 | CATACCAAAGGGCAGCTGGAGGGATA[C/T]AGACGGAAGTCATGTGGAGAGTGAA |
| HEXA | 3073 | rs57476645 | CAGGTGTGAGCCACCACGACCACCAA[A/T]TTAGCTCTTTTTACTCCTTCCCTTC |
| HEXA | 3073 | rs56870003 | AGTGGTAGCTGATTTTGCTTCTGGAT[A/C]CTTTGCCACCTTCCCACTCTTTAAT |
| HEXA | 3073 | rs56338339 | AAAGACCTGTTTCTTAAAAAAAAAAA[-/AGAAAAAAAAAAAA]GAAAGAAAAGAAAAGAAAAAAACAG |
| HEXA | 3073 | rs55995352 | TAAAAAATCTTTCAATGAGGAGATGT[C/T]CCCAGAGCAAGACAGCTGTAGGATG |
| HEXA | 3073 | rs55860138 | AAAAGAAAAAAAAAAAAAAAAAAAA[-/A]GAAAACAAAACCCAAACCCATAAAG |
| HEXA | 3073 | rs55743646 | CCTGTCTCTAAAAGAAAAAAAAAAAA[A/G]AAAAAAAAAGAAAACAAAACCCAAA |
| HEXA | 3073 | rs55665666 | GTTATCATAGAAAAATATCACACTCT[-/GT]CTGTATCCCCACTTCCAGAAACTGT |
| HEXA | 3073 | rs36106892 | CAGGAGCTCATAGAATTACATACAAT[-/C]TTTTTTTTTTTTTTTTTGAGACAGCG |
| HEXA | 3073 | rs36091525 | TTGAGAATCTTATAATTCACTGTGTA[-/CCTC]CCTCTGTTTCATATTTTCGCAATTG |
| HEXA | 3073 | rs35949555 | CCACTACCACAGTGCCTAGAGAACAA[C/T]ATGTGTTTAATAATATTTAAATAAT |
| HEXA | 3073 | rs35827424 | CCCTGTCTCTAAAAGAAAAAAAAAAA[-/A]AAAAAAAAAAGAAAACAAAACCCAA |
| HEXA | 3073 | rs35729578 | CCATTATATCATTCATTTCCCACTCA[-/T]TTTCTTCATTCCAACCAAGATATAT |
| HEXA | 3073 | rs35649102 | TCCGTCTCAAAAAAAAAAAAAAAAAG[-/A]GAAAGGAATTATTCTCATGTATACA |
| HEXA | 3073 | rs35118677 | CTGGGGCAGTTAAAAAGAAAAACAAA[-/C]CCCTGGTCCCTGCCCTTGAGGAGAT |
| HEXA | 3073 | rs35005352 | CTCCAGGGTCCCATTCCAGGACCACA[-/C]GCCTGCTACCTCTGCAGCTCACTCA |
| HEXA | 3073 | rs34736306 | GGATTGACATATACCAGTTAGACGGA[-/T]TTTTTTTTTCCATAAACCAGGCTCA |
| HEXA | 3073 | rs34607939 | ACAAATAATTACTACATATCTACAAC[A/G]TTCCAGATACAGAAGAAATGGCCAA |
| HEXA | 3073 | rs34496117 | TAAACACACTTGAAACATCATATAAA[-/ATG]ATATTACTACAAGACTTAACCGTAA |
| HEXA | 3073 | rs34300017 | ACACAGGTAATCCATGTTTATTATAG[-/A]AAAATGCCACATTACTCTTTATTGA |
| HEXA | 3073 | rs34206496 | AGTTATCATAGAAAAATATCACACTC[-/TG]TCTGTATCCCCACTTCCAGAAACTG |
| HEXA | 3073 | rs34110830 | AATGAACTTACAGGAAGGTAATATAT[- |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | /G]GGAAATAAACATCTTATTGAATTTA |
| HEXA | 3073 | rs34093438 | GGACCCCTGAAAGGCACAAGACACCC[-/T]TTCAGGTTCACACTTCCTGAAAGCT |
| HEXA | 3073 | rs34085965 | CCACCAATCACCAGAGCCTTCTGCTC[A/G]GGGGTACCTGAGGGAAAACAAGCAA |
| HEXA | 3073 | rs34004907 | AAAGACTGAAAAAACATTCATAACTA[-/T]TTTTCTTGTTATCCTCGGAAATGTC |
| HEXA | 3073 | rs28942072 | TATCTTCATCTTGGAGGAGATGAGGT[C/T]GATTTCACCTGCTGGAAGTCCAACC |
| HEXA | 3073 | rs28942071 | TTGCCTATGAACGTTTGTCACACTTC[C/T]GCTGTGAGTTGCTGAGGCGAGGTGT |
| HEXA | 3073 | rs28941771 | GCTTGCTGTTGGATACATCTCGCCAT[C/T]ACCTGCCACTCTCTAGCATCCTGGA |
| HEXA | 3073 | rs28941770 | CCGGGGCTTGCTGTTGGATACATCTC[G/T]CCATTACCTGCCACTCTCTAGCATC |

## Examples

The following examples illustrate non-limiting embodiments of the invention.

***Design a set of capture probes for a human target exon***

All targets are captured as a set of partially-overlapping subtargets. For example, in the tiling approach, a 200 bp target exon might be captured as a set of 12 subtargets, each 60bp in length (Figure 1). Each subtarget is chosen such that it partially overlaps two or three other targets.

All probes are composed of three regions: 1) a 20 bp 'targeting arm' comprised of sequence which hybridizes immediately upstream from the sub-target, 2) a 30 bp 'constant region' comprised of sequence used as a pair of amplification priming sites, and 3) a second 20 bp 'targeting arm' comprised of sequence which hybridizes immediately downstream from the sub-target. Targeting arm sequences will be different for each capture probe in a set, while constant region sequence will be the same for all probes in the set, allowing all captured targets to be amplified with a single set of primers. Targeting arm sequences should be designed such that any given pair of 20 bp sequences is unique in the target genome (to prevent spurious capture of undesired sites). Additionally, melting temperatures should be matched for all probes in the set such that hybridization efficiency is uniform for all probes at a constant temperature (e.g. 60C). Targeting arm sequences should be computationally screened to ensure they do not form



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strong secondary structure that would impair their ability to basepair with the genomic target.

***Hybridize capture probes to human genomic sample***

Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dH2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

***Convert hybridized probes into covalently-closed circular products containing subtargets***

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

***Purify circularized probe / subtarget products from un-reacted probes and genomic DNA***

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubte at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.



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***Amplify circular material by PCR using primers specific to the 'constant region' of the probes***

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3')
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3')
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

1. 95C for 5 min
2. 95C for 30 sec
3. 58C for 60 sec
4. 72C for 60 sec
5. goto 2, N more times

***Prepare a shotgun next-generation sequencing library for analysis***

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.
- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.



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It will be clear that the methods may be practiced other than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present disclosure are possible in light of the above teachings and, therefore, are within the scope of the claims. Preferred features of each aspect of the disclosure are as for each of the other aspects mutatis mutandis. The documents including patents, patent applications, journal articles, or other disclosures mentioned herein are hereby incorporated by reference in their entirety. In the event of conflict, the disclosure of present application controls, other than in the event of clear error.

What is claimed is:



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## Claims

1. A method of analyzing a plurality of genetic loci, the method comprising:

contacting each of a plurality of target nucleic acids with a probe set,

wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion,

isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

2. A method of analyzing a plurality of genetic loci, the method comprising:

contacting each of a plurality of target nucleic acids with a probe set,

wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe,

isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

3. The method of claim 1 or 2, wherein the target nucleic acids are selected from Table 1.

4. The method of claim 1 or 2, wherein the analyzing comprises genotyping a polymorphism of the target nucleic acid.



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5. The method of claim 4, wherein the polymorphism is selected from Table 2.



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**Abstract of the Disclosure**

The invention relates to methods that reduce bias and increase reproducibility in multiplex detection of genetic loci.



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| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 5256599 |
| **Application Number:** | 61174470 |
| **International Application Number:** | |
| **Confirmation Number:** | 9948 |
| **Title of Invention:** | METHOD FOR REDUCING REPRESENTATIONAL BIAS IN MULTIPLEX AMPLIFICATION OF NUCLEIC ACIDS |
| **First Named Inventor/Applicant Name:** | Greg Porreca |
| **Customer Number:** | 23628 |
| **Filer:** | Patrick R.H. Waller/Trish McDonald |
| **Filer Authorized By:** | Patrick R.H. Waller |
| **Attorney Docket Number:** | G0814.70000US00 |
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| The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges) | |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Provisional Cover Sheet (SB16) | G081470000US00-TRN-PRW.pdf | 45766<br>/99f/dda40/5aae/4b61345afeb24939ed454dcf | no | 2 |
| **Warnings:** | | | | | |
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| 2 | Application Data Sheet | G081470000US00-ADS-PRW.pdf | 21051<br>3ff643d5b782464c0efc876893fbd9073a52b873 | no | 3 |
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| 3 | Fee Worksheet (PTO-875) | G081470000US00-FEE-PRW.pdf | 44552<br>44488229c858c4aebb0639279770da5a6399f070 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | | G081470000US00-PROAPL-PRW.pdf | 152979<br>eeb127549597b76eda1921d075f086c9e19b6440 | yes | 28 |

**Multipart Description/PDF files in .zip description**

| Document Description | Start | End |
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| Specification | 1 | 25 |
| Claims | 26 | 27 |
| Abstract | 28 | 28 |

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| **Information:** | | | | | |
| 5 | Drawings-only black and white line drawings | G081470000US00-DRW-PRW.pdf | 602374<br>1c1bc60f2f4d40735e0ea98e3b041cb2841cf82a | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Fee Worksheet (PTO-875) | fee-info.pdf | 29731<br>5fe0719ef53f9849eaee0bec67eee060cce91b40 | no | 2 |
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**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

**Figure 1**

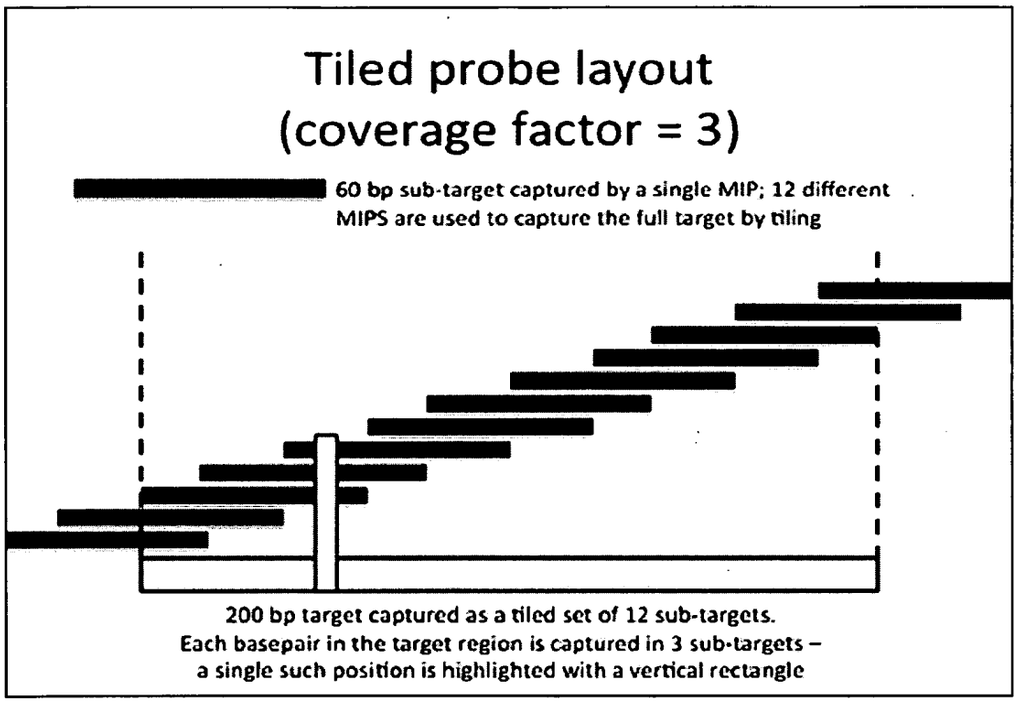



**Figure 1. Tiled probe layout, showing ten captured sub-targets tiled across a single target. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes.**

**Figure 2**

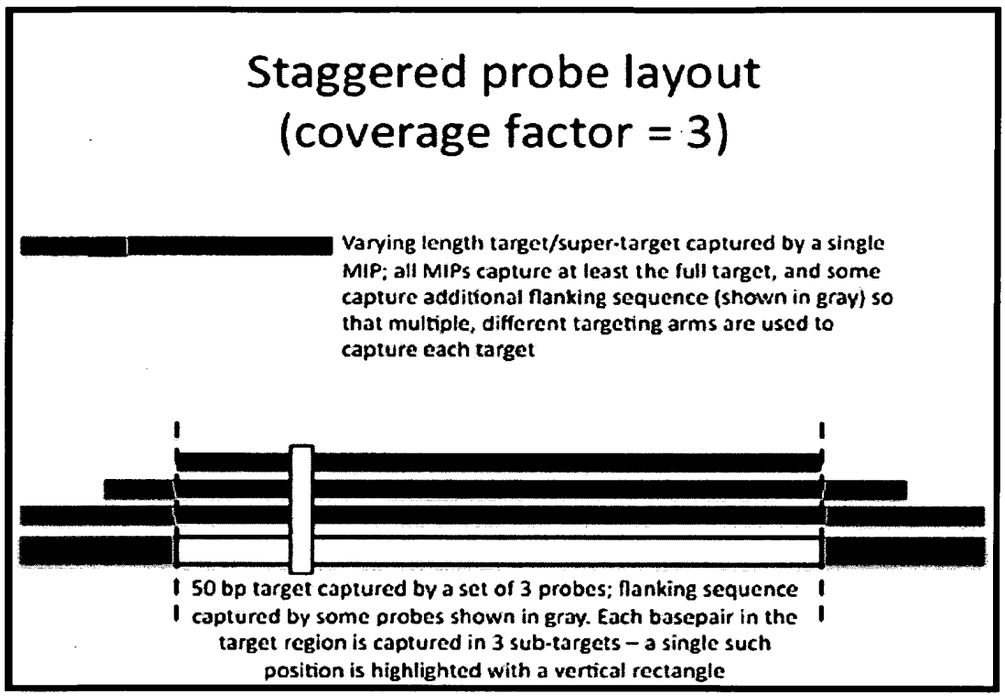



Figure 2. Staggered probe layout, showing the targets captured by a set of three MIPs. Each MIP captures the full target, shown in black, plus (in some cases) additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes.

**Figure 3**

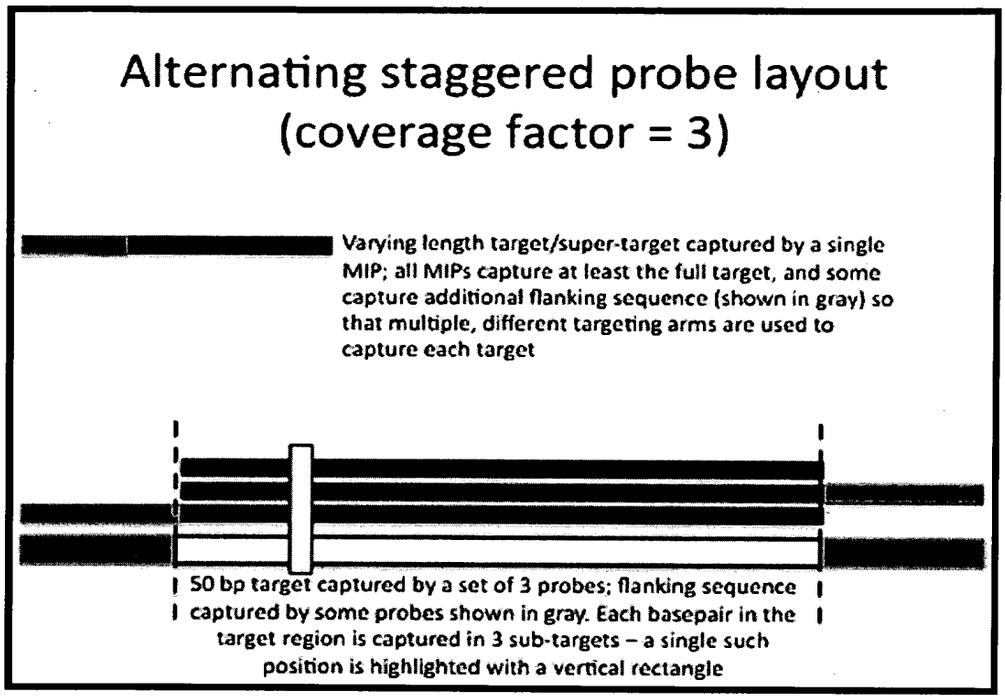



**Figure 3. Alternating staggered probe layout, showing the targets captured by a set of three MIPs. Each MIP captures the full target, shown in black, plus (in some cases) additional extra-target sequence, shown in gray, such that the targeting arms of each MIP fall on different sequence. Each position in the target is covered by three sub-targets such that MIP performance per basepair is averaged across three probes.**

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**APPLICATION NUMBER:** ***61/182,089***
**FILING DATE:** ***May 28, 2009***
**RELATED PCT APPLICATION NUMBER:** ***PCT/US10/01293***

**THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS** ***US61/182,089***

Certified by

David W. Kappos

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for Intellectual Property
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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

**Express Mail Label No.** VIA EFS

## INVENTOR(S)

| Given Name (first and middle [if any] ) | Family Name or Surname | Residence (City and either State or Foreign Country) |
|---|---|---|
| Jin Billy | Li | Medford, Massachusetts 02155 |
| Gregory | Porreca | Ocean City, New Jersey 08226 |
| Uri | Laserson | Boston, Massachusetts 02215 |
| | | |
| | | |

*Additional inventors are being named on the* _____ *separately numbered sheets attached hereto.*

**TITLE OF THE INVENTION (500 characters max):**

EVALUATION OF TARGET LENGTH IN NUCLEIC ACID CAPTURE-BASED ASSAYS

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

[X] The address corresponding to Customer Number: 23628

**OR**

[ ] Firm or Individual Name

Address

| City | State | Zip |
|---|---|---|
| Country | Telephone | Email |

**ENCLOSED APPLICATION PARTS (*check all that apply*)**

[X] Application Data Sheet. See 37 CFR 1.76 [ ] CD(s), Number of CDs _____

[X] Drawing(s) *Number of Sheets* 2 [ ] Other (specify) _____

[X] Specification (e.g. description of the invention) *Number of Pages* 25

Fees Due: Filing Fee of $220 ($110 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

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***PROVISIONAL APPLICATION COVER SHEET***
***Page 2 of 2***

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SIGNATURE Pat R H Waller Date May 28, 2009

TYPED or PRINTED NAME Patrick R.H. Waller REGISTRATION NO. *(if appropriate)* 41,418

TELEPHONE 617.646.8000 Docket Number: G0814.70001US00

**Application Data Sheet**

**Application Information**

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| Sequence submission?:: | None |
| Computer Readable Form (CRF)?:: | No |
| Title:: | EVALUATION OF TARGET LENGTH IN NUCLEIC ACID CAPTURE-BASED ASSAYS |
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| Secrecy Order in Parent Appl.?:: | No |

**Applicant Information**

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | CHINA |
| Status:: | Full Capacity |
| Given Name:: | Jin |
| Middle Name:: | Billy |
| Family Name:: | Li |
| City of Residence:: | Medford |
| State or Province of Residence:: | MA |
| Country of Residence:: | US |
| Street of mailing address:: | 100 Station Landing, Unit 204 |
| City of mailing address:: | Medford |

| | |
|---|---|
| State or Province of mailing address:: | MA |
| Postal or Zip Code of mailing address:: | 02155 |

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Status:: | Full Capacity |
| Given Name:: | Gregory |
| Family Name:: | Porreca |
| City of Residence:: | Ocean City |
| State or Province of Residence:: | NJ |
| Country of Residence:: | US |
| Street of mailing address:: | 911 Gardens Parkway |
| City of mailing address:: | Ocean City |
| State or Province of mailing address:: | NJ |
| Postal or Zip Code of mailing address:: | 08226 |

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | US |
| Status:: | Full Capacity |
| Given Name:: | Uri |
| Family Name:: | Laserson |
| City of Residence:: | Boston |
| State or Province of Residence:: | MA |
| Country of Residence:: | US |
| Street of mailing address:: | 645 Beacon Street, #215 |
| City of mailing address:: | Boston |
| State or Province of mailing address:: | MA |
| Postal or Zip Code of mailing address:: | 02215 |





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**Correspondence Information**

Correspondence Customer Number:: 23628

**Representative Information**

Representative Customer Number:: 23628

**Domestic Priority Information**

**Foreign Priority Information**

**Assignee Information**

| | |
|---|---|
| Assignee Name:: | Good Start Genetics, Inc. |
| Street of Mailing Address:: | 312 Marlborough St., #4 |
| City of Mailing Address:: | Boston |
| State or Province of Mailing Address:: | Massachusetts |
| Country of Mailing Address:: | United States |
| Postal or Zip Code of Mailing Address:: | 02116 |

# EVALUATION OF TARGET LENGTH IN NUCLEIC ACID CAPTURE-BASED ASSAYS

## Field of Invention

The invention relates to methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats.

## Background of the Invention

Genomic regions that contain nucleic acid sequence repeats are often the site of genetic instability due to the amplification or contraction of the number of sequence repeats (e.g., the insertion or deletion of one or more units of the repeated sequence). of Instability in the length of genomic regions that contain high numbers of repeat sequences has been associated with a number of hereditary and non hereditary diseases and conditions.

For example, "Fragile X syndrome, or Martin-Bell syndrome, is a genetic syndrome which results in a spectrum of characteristic physical, intellectual, emotional and behavioral features which range from severe to mild in manifestation. The syndrome is associated with the expansion of a single trinucleotide gene sequence (CGG) on the X chromosome, and results in a failure to express the FMR-1 protein which is required for normal neural development. There are four generally accepted forms of Fragile X syndrome which relate to the length of the repeated CGG sequence; Normal (29-31 CGG repeats), Premutation (55-200 CGG repeats), Full Mutation (more than 200 CGG repeats), and Intermediate or Gray Zone Alleles (40 - 60 repeats)."

Other examples include cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26).

The are currently many sequencing-based assays for determining the number of nucleic acid sequence repeats at a particular locus and identifying the presence of nucleic acid insertions or deletions.

## Summary of the Invention

Aspects of the invention provide methods and compositions for identifying nucleic acid insertions or deletions in genomic regions of interest without determining



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the nucleotide sequences of these regions. Aspects of the invention are particularly useful for detecting nucleic acid insertions or deletions in genomic regions containing nucleic acid sequence repeats (e.g., di- or tri-nucleotide repeats). However, the invention is not limited to analyzing nucleic acid repeats and may be used to detect insertions or deletions in any target nucleic acid of interest. Aspects of the invention are particularly useful for analyzing multiple loci in a multiplex assay.

In some embodiments, aspects of the invention relate to determining whether an amount of target nucleic acid that is captured in a genomic capture assay is higher or lower than expected. In some embodiments, a statistically significant deviation from an expected amount (e.g., higher or lower) is indicative of the presence of a nucleic acid insertion or deletion in the genomic region of interest. In some embodiments, the amount is a number of nucleic acid molecules that are captured. In some embodiments, the amount is a number of independently captured nucleic acid molecules in a sample. It should be appreciated that the captured nucleic acids may be literally captured from a sample, or their sequences may be captured without actually capturing the original nucleic acids in the sample. For example, nucleic acid sequences may be captured in an assay that involves a template-based extension of nucleic acids having the region of interest, in the sample.

Aspects of the invention are based on the recognition that the efficiency of certain capture techniques is affected by the length of the nucleic acid being captured. Accordingly, an increase or decrease in the length of a target nucleic acid (e.g., due to an insertion or deletion of a repeated sequence) can alter the capture efficiency of that nucleic acid. In some embodiments, a difference in the capture efficiency (e.g., a statistically significant difference in the capture efficiency) of a target nucleic acid is indicative of an insertion or deletion in the target nucleic acid. It should be appreciated that the capture efficiency for a target nucleic acid may be evaluated based on an amount of captured nucleic acid (e.g., number of captured nucleic acid molecules) relative to a control amount (e.g., based on an amount of control nucleic acid that is captured). However, the invention is not limited in this respect and other techniques for evaluating capture efficiency also may be used.

According to aspects of the invention, evaluating the capture efficiency as opposed to determining the sequence of the entire repeat region reduces errors associated

with sequencing through repeat regions. Repeat sequences often give rise to stutters or skips in sequencing reactions that make it very difficult to accurately determine the number of repeats in a target region without running multiple sequencing reactions under different conditions and carefully analyzing the results. Such procedures are cumbersome and not readily scalable in a manner that is consistent with high throughput analyses of target nucleic acids. Accordingly, aspects of the invention are useful to increase the sensitivity of detecting insertions or deletions in target regions, particularly target regions containing repeated sequences.

In some embodiments, aspects of the invention relate to capturing genomic nucleic acid sequences using a molecular inversion probe (e.g., MIP or Padlock probe) technique, and determining whether the amount (e.g., number) of captured sequences is higher or lower than expected. In some embodiments, the amount (e.g., number) of captured sequences is compared to an amount (e.g., number) of sequences captured in a control assay. The control assay may involve analyzing a control sample that contains a nucleic acid from the same genetic locus having a known sequence length (e.g., a known number of nucleic acid repeats). However, a control may involve analyzing a second (e.g., different) genetic locus that is not expected to contain any insertions or deletions. The second genetic locus may be analyzed in the same sample as the locus being interrogated. The second genetic locus may be a locus that is not characterized by the presence of nucleic acid repeats (and thus not expected to contain insertions or deletions of the repeat sequence).

In some embodiments, a target nucleic acid region that is being evaluated may be determined by the identify of the targeting arms of a probe that is designed to capture the target region (or sequence thereof). For example, the targeting arms of a MIP probe may be designed to be complementary (e.g., sufficiently complementary for selective hybridization) to genomic regions flanking a target region suspected of containing an insertion or deletion. It should be appreciated that two targeting arms may be designed to be complementary (e.g., sufficiently complementary for selective hybridization) to the two flanking regions that are immediately adjacent (e.g., immediately 5' and 3', respectively) to a region of a sequence repeat on one strand of a genomic nucleic acid. However, one or both targeting arms may be designed to hybridize several bases (e.g., 1-5, 5-10, 10-25, 25-50, or more) upstream or downstream from the repeat region in such a

way that the captured sequence includes a region of unique genomic sequence that on one or both sides of the repeat region.

In some embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) different loci may be interrogated in parallel in a single assay (e.g., in a multiplex assay). In some embodiments, the ratio of captured nucleic acids for each locus may be used to determine whether a nucleic acid insertion or deletion is present in one locus relative to the other. For example, the ratio may be compared to a control ratio that is representative of the two loci when neither one has an insertion or deletion relative to control sequences (e.g., sequences that are normal or known to be associated with healthy phenotypes for those loci). However, the amount of captured nucleic acids may be compared to any suitable control as discussed herein.

The locus of a captured sequence may be identified by determining a portion of unique sequence 5' and/or 3' to the repeat region in the target nucleic acid suspected of containing a deletion or insertion. This does not require sequencing the captured repeat region itself. However, some or all of the repeat region also could be sequenced as aspects of the invention are not limited in this respect.

Aspects of the invention may be combined with one or more sequence-based assays (e.g., SNP detection assays), for example in a multiplex format, to determine the genotype of one or more regions of a subject.

According to some aspects of the invention, methods of detecting a polymorphism in a nucleic acid in a biological sample are provided. In some embodiments, the methods comprise evaluating the efficiency of capture at one or more loci and determining whether one or both alleles at that locus contain an insertion or deletion relative to a control locus (e.g., a locus indicative of a length of repeat sequence that is associated with a healthy phenotype).

Accordingly, aspects of the invention relate to methods for determining whether a target nucleic acid has an abnormal length by evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length. It should be appreciated that the term "abnormal" is a relative term based on a comparison to a "normal" length. In some embodiments, a normal length is a length that is associated

with a normal (e.g., healthy or non-carrier phenotype). Accordingly, an abnormal length is a length that is either shorter or longer than the normal length. In some embodiments, the presence of an abnormal length is indicative of an increased risk that the locus is associated with a disease or a disease carrier phenotype. In some embodiments, the abnormal length is indicative that the subject is either has a disease or condition or is a carrier of a disease or condition (e.g., associated with the locus). However, it should be appreciated that the description of embodiments relating to detecting the presence of an abnormal length also support detecting the presence of a length that is different from an expected or control length.

In some embodiments, aspects of the invention relate to estimating the length of a target nucleic acid (e.g., of a sub-target region within a target nucleic acid). In some embodiments, aspects of the invention relate to methods for estimating the length of a target nucleic acid by contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid, wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid, wherein the first and second regions are on a common strand of the target nucleic acid, and wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid; and capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes; and measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the sub-target nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. It should be appreciated that methods for estimating a nucleic acid length may involve comparing a capture efficiency for a target nucleic acid region to two or more reference efficiencies for known nucleic acid lengths in order to determine whether the target nucleic acid region is smaller, intermediate, or larger in size than the known control lengths. In some embodiments, a series of nucleic acids of known different lengths may be used to provide a calibration curve for evaluating the length of a target nucleic acid region of interest.

In some embodiments, the capture efficiency of a target region suspected of having a deletion or insertion is determined and comparing the capture efficiency to a reference indicative of a normal capture efficiency. In some embodiments, the capture efficiency is lower than the reference capture efficiency. In some embodiments, the subject is identified as having an insertion in the target region. In some embodiments, the capture efficiency is higher than the reference capture efficiency. In some embodiments, the subject is identified as having a deletion in the target region. In some embodiments, the subject is identified as being heterozygous for the insertion. In some embodiments, the subject is identified as being heterozygous for the deletion.

In some embodiments, aspects of the invention relate to capturing a sub-target nucleic acid (or a sequence of a sub-target nucleic acid). In some embodiments, a molecular inversion probe technique is used. In some embodiments, a molecular inversion probe is a single linear strand of nucleic acid that comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid. In some embodiments, the first and second targeting arms are between about 10 and about 100 nucleotides long. In some embodiments, the first and second targeting arms are about 10-20, 20-30, 30-40, or 40-50 nucleotides long. In some embodiments, the first and second targeting arms are about 20 nucleotides long. In some embodiments, the first and second targeting arms have the same length. In some embodiments, the hybridization Tms of the first and second targeting arms are similar. In some embodiments, the hybridization Tms of the first and second targeting arms are within 2-5 C of each other. In some embodiments, the hybridization Tms of the first and second targeting arms are identical. In some embodiments, the sub-target nucleic acid contains a nucleic acid repeat. In some embodiments, the nucleic acid repeat is a dinucleotide or trinucleotide repeat. In some embodiments, the sub-target nucleic acid contains 10-100 copies of the nucleic acid repeat in the absence of an abnormal increase or decrease in nucleic acid repeats. In some embodiments, the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat. In some embodiments, one or both targeting arms hybridize to a region on the target nucleic acid

that is immediately adjacent to a region of nucleic acid repeats. In some embodiments, one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats. In some embodiments, the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

In some embodiments, aspects of the invention relate to evaluating the length of a plurality of different target nucleic acids in a biological sample. In some embodiments, the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes. In some embodiments, each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends. In some embodiments, each different molecular inversion probe comprises the same primer-binding sequence.

In some embodiments, aspects of the invention relate to analyzing nucleic acid from a biological sample obtained from a subject. In some embodiments, the biological sample is a blood sample. In some embodiments, the biological sample is a tissue sample. In some embodiments, nucleic acids are analyzed in biological samples obtained from a plurality of different subjects. In some embodiments, nucleic acids from a biological sample are analyzed in multiplex reactions. It should be appreciated that a biological sample contains a plurality of copies of a genome derived from a plurality of cells in the sample. Accordingly, a sample contains a plurality of independent copies of a target nucleic acid region of interest, the capture efficiency of which can be used to evaluate its size as described herein.

In some embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1 or 2) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample. A patient or subject may be a human. However, aspects of the invention are not limited to humans and may be applied to other species (e.g., mammals, birds, reptiles, other vertebrates or invertebrates) as aspects of the invention are not limited in this respect. A subject or patient may be male or female. In some embodiments, in connection with

reproductive genetic counseling, samples from a male and female member of a couple may be analyzed. In some embodiments, for example, in connection with an animal breeding program, samples from a plurality of male and female subjects may be analyzed to determine compatible or optimal breeding partners or strategies for particular traits or to avoid one or more diseases or conditions.

In some embodiments, aspects of the invention relate to evaluating a nucleic acid capture efficiency by determining an amount of target nucleic acid that is captured (e.g., an amount of sub-target nucleic acid sequences that are captured). In some embodiments, the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules (e.g., the amount of independently captured molecules that have the sequence of the sub-target region). In some embodiments, the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid. In some embodiments, the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid. In some embodiments, the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion. In some embodiments, the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction. As described herein, a number of independently captured nucleic acid sequences can be determined by contacting a nucleic acid sample with a preparation of a probe (e.g., a MIP probe as described herein). It should be appreciated that the preparation comprises a plurality of copies of the same probe and accordingly a plurality of independent copies of the target region may be captured by different probe molecules. The number of probe molecules that actually capture a sequence can be evaluated by determining an amount or number of captured molecules using any suitable technique. This number is a reflection of both the number of target molecules in the sample and the efficiency of capture of those target molecules, which in turn is related to the size of the target molecules as describedf herein. Accordingly, the capture efficiency can be evaluated by controlling for the abundance of the target nucleic acid, for example by comparing the number or amount of captured target molecules to an appropriate control (e.g., a known size and amount of control nucleic acid, or a different locus that should be present in the same amount in the biological sample and is not expected to contain any insertions or

deletions). It should be appreciated that other factors may affect the capture efficiency of a particular target nucleic acid region (e.g., the sequence of the region, the GC content, the presence of secondary structures, etc.). However, these factors also can be accounted for by using appropriate controls (e.g., known sequences having similar properties, the same sequences, other genomic sequences expected to be present in the biological sample at the same frequency, etc., or any combination thereof).

In some embodiments, aspects of the invention relate to identifying a subject as having an insertion or deletion in one or more alleles of a genetic locus if the capture efficiency for that genetic locus is statistically significantly different than a reference capture efficiency.

It should be appreciated that hybridization conditions used for any of the capture techniques described herein (e.g., MIP capture techniques) can be based on known hybridization buffers and conditions.

In some embodiments, a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject). In some embodiments, an assessment is provided to a couple, based on their respective genotypes at one or more genetic loci, of the risk of their having one or more children having a genotype associated with a disease or condition (e.g., a homozygous or heterozygous genotype associated with a disease or condition). In some embodiments, a subject or a couple may seek genetic or reproductive counseling in connection with a genotype determined according to embodiments of the invention. The methods disclosed herein are useful for any application where the detection of deletions or insertions is important.

## Brief Description of the Drawings

Figure 1 is reproduced from Deng et al., Nature Biotech. 27:353-60, (see Supplemental Fig. 1G) and illustrates: An "analysis of the effects of probe characteristics on capturing efficiency. The statistical package R and its *effects* module were used for this analysis. A linear model was used, and each individual factor was assumed to be independent. The dashed lines represent a 95% confidence interval.

Shorter target sequences were captured with higher efficiency than long target sequences ($p < 2x10^{-16}$)."

Figure 2 illustrates a non-limiting scheme of padlock capture of a region that includes both repetitive regions (thick wavy line) and the adjacent unique sequence (thick strait line). The regions of the probe are indicated with the targeting arms shown as regions "1" and "3." An intervening region that may be, or include, a sequencing primer binding site is shown as "2." After the padlock is circularized and amplified, it can be end-sequenced to obtain the sequence of the unique sequence, which represents the repetitive region of interest.

Figure 3 illustrates a non-limiting hypothetical relationship between target gap size and the relative number of reads of the repetitive region, which is measured by the ratio of the repeat "representative" reads vs. the "control" region reads. The unit of y-axis is arbitrary.

## Description of the Invention

Aspects of the invention relate to detecting the presence of an insertion or deletion at a genomic locus without requiring the locus to be sequenced. Aspects of the invention are particularly useful for detecting an insertion or deletion in a nucleic acid region that contains high levels of sequence repeats. The presence of sequence repeats at a genetic locus is often associated with relatively high levels of polymorphism in a population due to insertions or deletions of one or more of the sequence repeats at the locus. The polymorphisms can be associated with diseases or predisposition to diseases (e.g., certain polymorphic alleles are recessive alleles associated with a disease or condition). However, the presence of sequence repeats often complicates the analysis of a genetic locus and increases the risk of errors when using sequencing techniques to determine the precise sequence and number of repeats at that locus.

Aspects of the invention relate to determining the size of a genetic locus by evaluating the capture frequency of a portion of that locus suspected of containing an insertion or deletion (e.g., due to the presence of sequence repeats) using a nucleic acid capture technique (e.g., a nucleic acid sequence capture technique based on molecular inversion probe technology). According to aspects of the invention, a statistically significant difference in capture efficiency for a genetic locus of interest in different

biological samples (e.g., from different subjects) is indicative of different relative lengths in those samples. It should be appreciated that the length differences may be at one or both alleles of the genetic locus. Accordingly, aspects of the invention may be used to identify polymorphisms regardless of whether biological samples being interrogated at heterozygous or homozygous for the polymorphisms. According to aspects of the invention, subjects that contain one or more loci with an insertion or deletion can be identified by analyzing capture efficiencies for nucleic acids obtained from one or more biological samples using appropriate controls (e.g., capture efficiencies for known nucleic acid sizes, capture efficiencies for other regions that are not suspected of containing an insertion or deletion in the biological sample(s), or predetermined reference capture efficiencies, or any combination thereof. However, it should be appreciated that aspects of the invention are not limited by the nature or presence of the control. In some embodiments, if a statistically significant variation in capture efficiency is detected, a subject may be identified as being at risk for a disease or condition associated with insertions or deletions at that genetic locus. In some embodiments, the subject may be analyzed in greater detail in order to determine the precise nature of the insertion or deletion and whether the subject is heterozygous or homozygous for one or more insertions or deletions. For example, gel electrophoresis of an amplification (e.g., PCR) product of the locus, or Southern blotting, or any combination thereof can be used as an orthogonal approach to verify the length of the locus. In some embodiments, a more exhaustive and detailed sequence analysis of the locus can be performed to identify the number and types of insertions and deletions. However, other techniques may be used to further analyze a locus identified as having an abnormal length according to aspects of the invention.

Accordingly, aspects of the invention relate to detecting abnormal nucleic acid lengths in genomic regions of interest. In some embodiments, the invention aims to estimate the size of genomic regions that are hard to be accessed, such as repetitive elements. However, it should be appreciated that methods of the invention do not require that the precise length be estimated. In some embodiments, it is sufficient to determine that one or more alleles with abnormal lengths are present at a locus of interest (e.g., based on the detection of abnormal capture efficiencies).

In a non-limiting example, fragile X can be used to illustrate aspects of the invention where the size of trinucleotide repeats (genotype) is linked to a symptom (phenotype). However, it should be appreciated that fragile X is a non-limiting example and similar analyses may be performed for other genetic loci (e.g., independently or simultaneously in multiplex analyses).

According to aspects of the invention, repetitive regions are surrounded by non-repetitive unique sequences, which can be used to amplify the repeat-containing regions using, for example, PCR or padlock (MIP)-based method.

In addition to the repetitive regions, a probe (e.g., a MIP or padlock probe) can be designed to include at least a sequence that is sufficient to be uniquely identified in the genome (or target pool). After the probe is circularized and amplified, the amplicon can be end-sequenced so that the unique sequence can be identified and served as the "representative" of the repetitive region (Figure 2). Although capturing efficiency is overall negatively correlated with target length, different probe sequences may have unique features. Therefore, multiple probes could be designed and tested so that an optimal one is chosen to be sensitive enough to differentiate repetitive sizes of roughly 0-150bp, 150-600bp, and beyond, which represent normal, premutation and full mutation of fragile X syndrome, respectively. However, it should be appreciated that other probe sizes and sequences can be designed, and optionally optimized, to distinguish a range of repeat region size differences (e.g., length differences of about 3-30 bases, about 30-60 bases, about 60-90 bases, about 90-120 bases, about 120-150 bases, about 150-300 bases, about 300-600 bases, about 600-900 bases, or any intermediate or longer length difference). It should be appreciated that a length difference may be an increase in size or a decrease in size.

In some embodiments, an initial determination of an unexpected capture frequency is indicative of the presence of size difference. In some embodiments, an increase in capture frequency is indicative of a deletion. In some embodiments, a decrease in capture frequency is indicative of an insertion. However, it should be appreciated that depending on specific sequence parameters and the relative sizes of the capture probes, the target region, and the deletions or insertions, a change in capture frequency can be associated with either an increase or decrease in target region length.

In some embodiments, the precise nature of the change can be determined using one or more additional techniques as described herein.

Use of molecular inversion probes has been demonstrated for detection of single nucleotide polymorphisms (Hardenbol et al. 2005 Genome Res 15:269-75) and for preparative amplification of large sets of exons (Porreca et al. 2007 Nat Methods 4:931-6, Krishnakumar et al. 2008 Proc Natl Acad Sci USA 105:9296-301). In both cases, oligonucleotide probes are designed which have ends ('targeting arms') that hybridize up-stream and down-stream of the locus that is to be amplified. Following hybridization, polymerase fill-in and ligation reactions are performed to convert the hybridized probe to a covalently-closed, circular molecule containing the desired target. PCR or rolling circle amplification plus exonuclease digestion of non-circularized material is performed to isolate and amplify the circular targets from the starting nucleic acid pool. Since one of the main benefits of the method is the potential for a high degree of multiplexing, generally thousands of targets are captured in a single reaction containing thousands of probes.

Accordingly, in some aspects a MIP probe includes a linear nucleic acid strand that contains two hybridization sequences or targeting arms, one at each end of the linear probe, wherein each of the hybridization sequences is complementary to a separate sequence on a the same strand of a target nucleic acid, and wherein these sequences on the target nucleic acid flank the two ends of the target nucleic acid sequence of interest. It should be appreciated that upon hybridization, the two ends of the probe are inverted with respect to each other in the sense that both 5' and 3' ends of the probe hybridize to the same strand to separate regions flanking the target region (as illustrated in Figure 2 for example).

In some embodiments, the hybridization sequences are between about 10-100 nucleotides long, for example between about 10-30, about 30-60, about 60-90, or about 20, about 30, about 40, or about 50 nucleotides long. However, other lengths may be used depending on the application. In some embodiments, the hybridization Tms of both targeting arms of a probe are designed or selected to be similar. In some embodiments, the hybridization Tms of the targeting arms of a plurality of probes designed to capture different target regions are selected or designed to be similar so that they can be used together in a multiplex reaction. Accordingly, a typical size of a MIP probe prior to fill-

in is about 60-80 nucleotides long. However, other sizes can be used depending on the sizes of the targeting arms and any other sequences (e.g., primer binding or tag sequences) that are present in the MIP probe. In some embodiments, MIP probes are designed to avoid sequence-dependent secondary structures. In some embodiments, MIP probes are designed such that the targeting arms do not overlap with known polymorphic regions. In some embodiments, targeting arms that can be used for capturing the repeat region of the Fragile X locus can have the following sequences or complementary to these sequences depending on the strand that is captured.

left: CTCCGTTTCGGTTTCACTTC

right: ATCTTCTCTTCAGCCCTGCT

The typical captured size using these targeting arms is about 100 nucleotides in length (e.g., about 30 repeats of a tri-nucleotide repeat).

In some embodiments, the number of reads obtained for the "representative" of the repetitive region is not informative to estimate the target length because it is dependent on the total number of reads obtained. To overcome this, it is useful to include one or more probes that target other "control" regions where no or minimal polymorphism exists among populations. Because of the systematic consistency of capturing efficiency (Figure 2), the ratio of reads obtained for the repetitive "representative" to reads obtained for the control region(s) will be tuned using DNA with defined numbers of repeats. Ultimately, the ratio can serve as a measure of the repeat length (Figure 3).

In some embodiments, to better tell targets with similar size range apart, the whole repetitive region can be sequenced by making a shotgun library (e.g., by making a shotgun library from a captured sequence, for example a sequence captured using a MIP probe). The longer the repeat is, the more short reads of repeats will be obtained. Therefore, the target length will contribute twice to the relative number of "repetitive" reads, which will gainer better resolution of differentiating targets. In some embodiments, the expectation is that the number of reads from any given repeat will be a direct function of the number of repeats present. However, in some embodiments, a Poisson sampling-induced spread may need to be considered and in some embodiments may be sufficiently large to limit the resolution.

Other non-limiting examples of loci at which insertions or deletions or repeat sequences may be associated with a disease or condition are provided in Tables 1 and 2. It should be appreciated that the presence of an abnormal length at any one or more of these loci may be evaluated according to aspects of the invention. In some embodiments, two or more of these loci or other loci may be evaluated in a single multiplex reaction using different probes designed to hybridize under the same reaction conditions to different target nucleic acid in a biological sample.

Table 1.

**Polyglutamine (PolyQ) Diseases**

| Type | Gene | Normal/wildtype | Pathogenic |
|---|---|---|---|
| DRPLA (Dentatorubropallidoluysian atrophy) | ATN1 or DRPLA | 6 - 35 | 49 - 88 |
| HD (Huntington's disease) | HTT (Huntingtin) | 10 - 35 | 35+ |
| SBMA (Spinobulbar muscular atrophy or Kennedy disease) | Androgen receptor on the X chromosome. | 9 - 36 | 38 - 62 |
| SCA1 (Spinocerebellar ataxia Type 1) | ATXN1 | 6 - 35 | 49 - 88 |
| SCA2 (Spinocerebellar ataxia Type 2) | ATXN2 | 14 - 32 | 33 - 77 |
| SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease) | ATXN3 | 12 - 40 | 55 - 86 |
| SCA6 (Spinocerebellar ataxia Type 6) | CACNA1A | 4 - 18 | 21 - 30 |
| SCA7 (Spinocerebellar ataxia Type 7) | ATXN7 | 7 - 17 | 38 - 120 |
| SCA17 (Spinocerebellar ataxia Type 17) | TBP | 25 - 42 | 47 - 63 |

Table 2.

**Non-Polyglutamine Diseases**

| Type | Gene | Codon | Normal/wildtype | Pathogenic |
|---|---|---|---|---|
| FRAXA (Fragile X syndrome) | *FMR1*, on the X-chromosome | **CGG** | 6 - 53 | 230+ |
| FXTAS (Fragile X-associated tremor/ ataxia syndrome) | *FMR1*, on the X-chromosome | **CGG** | 6 - 53 | 55-200 |
| FRAXE (Fragile XE mental retardation) | AFF2 or FMR2, on the X-chromosome | **GCC** | 6 - 35 | 200+ |
| FRDA (Friedreich's ataxia) | FXN or X25, (frataxin) | **GAA** | 7 - 34 | 100+ |
| DM (Myotonic dystrophy) | DMPK | **CTG** | 5 - 37 | 50+ |
| SCA8 (Spinocerebellar ataxia Type 8) | OSCA or SCA8 | **CTG** | 16 - 37 | 110 – 250 |
| SCA12 (Spinocerebellar ataxia Type 12) | PPP2R2B or SCA12 | **CAG** On 5' end | 7 - 28 | 66 - 78 |

The following examples illustrate aspects and embodiments of the invention and are not intended to be limiting or restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

## Examples

**MIP capture of 'target' locus and 'control' loci**

In some embodiments, to accurately quantify the efficiency of target locus capture, at least three sets of control loci are captured in parallel that have *a priori* been shown to serve as proxies for various lengths of target locus. For example, if the target

locus is expected to have a length between 50 and 1000 bp, then sets of control loci having lengths of 50, 250, and 1000 bp could be captured (e.g. 20 loci per set should provide adequate protection from outliers), and their abundance digitally measured by sequencing. These loci should be chosen such that minimal variation in efficiency between samples and on multiple runs of the same sample is observed (and are therefore 'efficiency invariant'). These will serve as 'reference' points that define the shape of the curve of abundance-vs-length. Determining the length of the target is then simply a matter of 'reading' the length from the appropriate point on the calibration curve.

In some embodiments, the statistical confidence one has in the estimate of target length from this method is driven largely by three factors: 1) reproducibility / variation of the abundance data used to generate the calibration curve; 2) goodness of fit of the regression to the 'control' datapoints; 3) reproducibility of abundance data for the target locus being measured. Statistical bounds on 1) and 2) will be known in advance, having been measured during development of the assay. Additionally, statistical bounds on 3) will be known in general in advance, since assay development should include adequate population sampling and measure of technical reproducibility. Standard statistical methods should be used to combine these three measures into a single *P* value for any given experimental measure of target abundance.

In some embodiments, given the set of calibration observations, and a linear regression fit to that data, the regression can be used to predict the length value for n observations of the target locus whose length is unknown. First, choose an acceptable range for the confidence interval of the length estimate. For example, in the case of distinguishing "normal" (87-93bp) from "premutation" (165-600bp) potential cases of Fragile X, the goal is to measure length to sufficient precision to distinguish 93 bp from 165 bp. The predicted response value, computed when n observations is substituted into the equation for the regressed line, will have arbitrary precision. However, if for example a 95% confidence level is desired, that 95% confidence interval must be sufficiently short that it does not overlap both the "normal" and "premutation" length ranges. Continuing the example, if one calculates a length of 190 from n=400 MIP observations, and based on the regression from calibration data, the 95% confidence interval is 190 +/- 20 bp, one can conclude the sample represents a "premutation" length with 95% certainty. Conversely, if the calibration data were less robust, error estimates

of the regression would be higher, leading to larger confidence intervals on the predicted response value. In some embodiments, if the 95% CI were calculated as 190 +/- 100 bp from n=400, one could not determine whether the predicted response value corresponds to a "normal" or "premutation" length.

In some embodiments, the confidence interval for a predicted response is calculated as:

*The estimate for the response* $\hat{y}$ *is identical to the estimate for the mean of the response:* $\hat{y} = b_0 + b_1 x^*$. *The confidence interval for the predicted value is given by* $\hat{y} \pm t^* s_{\hat{y}}$, *where* $\hat{y}$ *is the fitted value corresponding to* $x^*$. *The value* $t^*$ *is the upper (1 - C)/2 critical value for the t(n - 2) distribution.*

In some embodiments, a technique for analyzing a locus of interest can involve the following steps.

***Convert hybridized probes into covalently-closed circular products containing subtargets***

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

***Purify circularized probe / subtarget products from un-reacted probes and genomic DNA***

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)
- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubte at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

***Amplify circular material by PCR using primers specific to the 'constant region' of the probes***

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA-Ilmn (platform-specific amplification sequence plus 'circle constant region'-specific sequence)
- 1.5 ul 10 uM CP-2-RA-Ilmn (platform-specific amplification sequence plus 'circle constant region'-specific sequence)
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

1. 95C for 5 min
2. 95C for 30 sec
3. 58C for 60 sec
4. 72C for 60 sec
5. goto 2, N more times

***Perform sequencing (e.g., next-generation sequencing) on sample for digital quantitation according to manufacturer's instructions (e.g., Illumina, ABI)***

It should be appreciated that the preceding examples are non-limiting and aspects of the invention may be implemented as described herein using alternative techniques and/or protocols that are available to one or ordinary skill in the art.

What is claimed is:

**Claims**

1. A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

2. The method of claim 1, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

3. The method of claim 1 or 2, wherein the capture efficiency is lower than the reference capture efficiency.

4. The method of claim 3, wherein the subject is identified as having an insertion in the target region.

5. The method of claim 1 or 2, wherein the capture efficiency is higher than the reference capture efficiency.

6. The method of claim 5, wherein the subject is identified as having a deletion in the target region.

7. The method of claim 4, wherein the subject is identified as being heterozygous for the insertion.

8. The method of claim 6, wherein the subject is identified as being heterozygous for the deletion.

9. The method of any prior claim, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

10. The method of claim 9, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

11. The method of claim 10, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

12. The method of claim 11, wherein the first and second targeting arms are about 10-20, 20-30, 30-40, or 40-50 nucleotides long.

13. The method of claim 12, wherein the first and second targeting arms are about 20 nucleotides long.

14. The method of any one of claims 10-13, wherein the first and second targeting arms have the same length.

15. The method of any one of claims 10-14, wherein the hybridization Tms of the first and second targeting arms are similar.

16. The method of claim 15, wherein the hybridization Tms of the first and second targeting arms are within 2-5 C of each other.

17. The method of claim 15, wherein the hybridization Tms of the first and second targeting arms are identical.

18. The method of any one of claims 9-17, wherein the sub-target nucleic acid contains a nucleic acid repeat.

19. The method of claim 18, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

20. The method of claim 18, wherein the sub-target nucleic acid contains 10-100 copies of the nucleic acid repeat in the absence of an abnormal increase or decrease in nucleic acid repeats.

21. The method of claim 18, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

22. The method of any one of claims 10-17, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.

23. The method of any one of claims 10-17, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.

24. The method of any one of claims 10-17, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

25. The method of any of the prior claims, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

26. The method of claim 25, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

27. The method of claim 26, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

28. The method of claim 27, wherein each different molecular inversion probe comprises the same primer-binding sequence.

29. The method of any prior claim, wherein the biological sample is a blood sample.

30. The method of any prior claim, wherein the biological sample is a tissue sample.

31. The method of any prior claim, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

32. The method of claim 31, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.

33. The method of claim 31 or 32, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.

34. The method of claim 33, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

35. The method of claim 34, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.

36. The method of claim 34, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

37. The method of any prior claim, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

38. A method for estimating the length of a target nucleic acid, the method comprising:

(i) contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid,

wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid,

wherein the first and second regions are on a common strand of the target nucleic acid, and

wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid; and

(ii) capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes; and

(iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the sub-target nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid.

**Abstract**

Aspects of the invention relate to methods for detecting abnormal nucleic acid lengths in a biological sample by using assays capable of detecting abnormal sequence capture efficiencies.

Figure 1

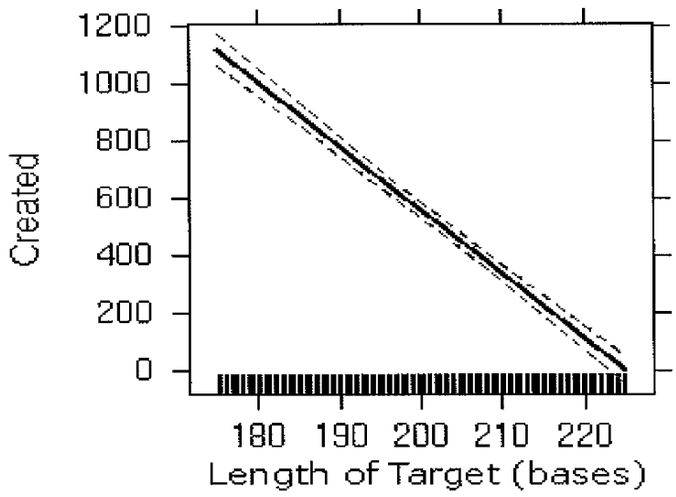

1200
1000
800
600
400
200
0
Created
180 190 200 210 220
Length of Target (bases)

Figure 2

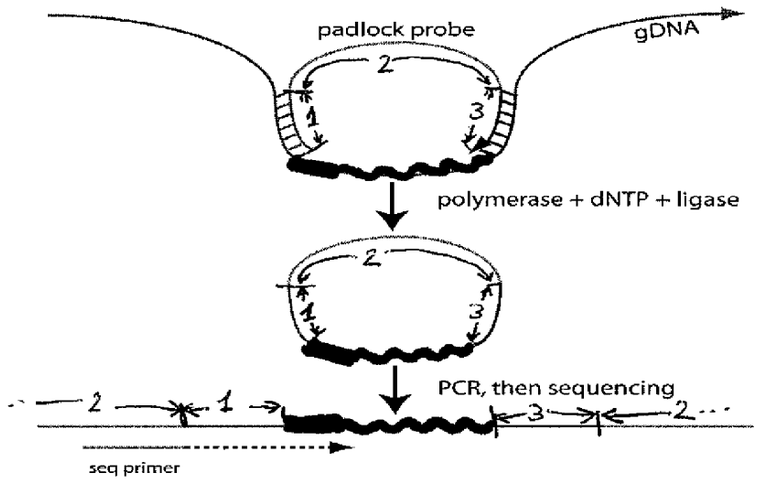
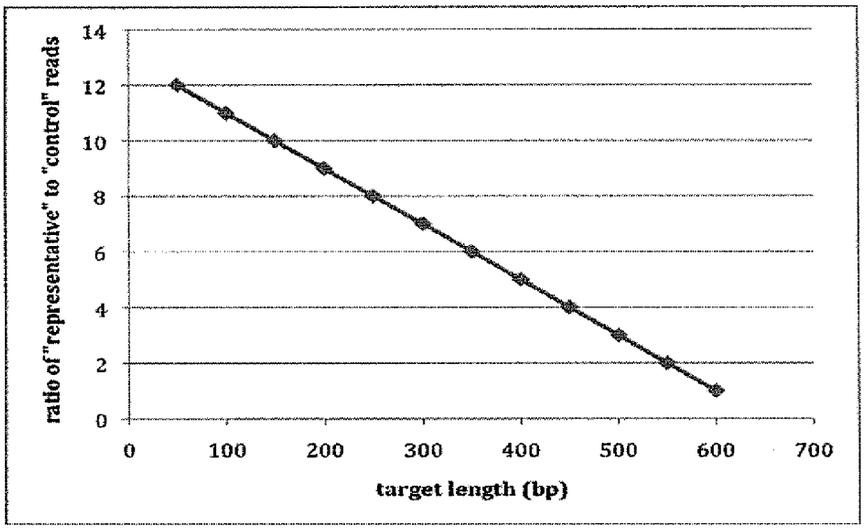

padlock probe
gDNA
2
1
3
polymerase + dNTP + ligase
2
1
3
PCR, then sequencing
2
1
3
2
seq primer

Figure 3


ratio of "representative" to "control" reads
14
12
10
8
6
4
2
0
0
100
200
300
400
500
600
700
target length (bp)

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 5417300 |
| **Application Number:** | 61182089 |
| **International Application Number:** | |
| **Confirmation Number:** | 1183 |
| **Title of Invention:** | EVALUATION OF TARGET LENGTH IN NUCLEIC ACID CAPTURE-BASED ASSAYS |
| **First Named Inventor/Applicant Name:** | Jin Billy Li |
| **Customer Number:** | 23628 |
| **Filer:** | Patrick R.H. Waller/Eileen MacKenzie |
| **Filer Authorized By:** | Patrick R.H. Waller |
| **Attorney Docket Number:** | G0814.70001US00 |
| **Receipt Date:** | 28-MAY-2009 |
| **Filing Date:** | |
| **Time Stamp:** | 22:44:06 |
| **Application Type:** | Provisional |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | Credit Card |
| Payment was successfully received in RAM | $110 |
| RAM confirmation Number | 5402 |
| Deposit Account | 232825 |
| Authorized User | WALLER,PATRICK R.H. |
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## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Provisional Cover Sheet (SB16) | G081470001US00-PROCVR-PRW.pdf | 54167<br>3b8fdf4365e3970a0a2d4e6fc52dde2e37753a61 | no | 2 |
| **Warnings:** | | | | | |
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| 2 | Fee Worksheet (PTO-875) | G081470001US00-FEETRN-PRW.pdf | 36372<br>8eb7db65a89a29551ead62634c6d7cc925261f94 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Application Data Sheet | G081470001US00-ADS-PRW.pdf | 17838<br>4b774fc30e425babefa5c772b3b9162cb093535c | no | 3 |
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| **Information:** | | | | | |
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| 4 | | G081470001US00-PROAPL-PRW.pdf | 158678<br>6753dc9343cf44df049eac0fa16e81ac8c6bc5bb | yes | 25 |

**Multipart Description/PDF files in .zip description**

| Document Description | Start | End |
|---|---|---|
| Specification | 1 | 19 |
| Claims | 20 | 24 |
| Abstract | 25 | 25 |

| | | | | | |
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| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Drawings-only black and white line drawings | G081470001US00-DRW-PRW.pdf | 27039<br>91f087c1e4d975a3310cea927c64b8a37b58f57e | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Fee Worksheet (PTO-875) | fee-info.pdf | 29854<br>94a6a97672eca06a4e246acb53e058c26804e4b9 | no | 2 |
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# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US2010/001293

International filing date: 30 April 2010 (30.04.2010)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 61/179,358
Filing date: 18 May 2009 (18.05.2009)

Date of receipt at the International Bureau: 20 May 2010 (20.05.2010)

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**APPLICATION NUMBER:** ***61/179,358***
**FILING DATE:** ***May 18, 2009***
**RELATED PCT APPLICATION NUMBER:** ***PCT/US10/01293***

**THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS** ***US61/179,358***

Certified by

David W. Kappos

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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

**Express Mail Label No.** _____

| INVENTOR(S) | | |
|---|---|---|
| Given Name (first and middle [if any] ) | Family Name or Surname | Residence (City and either State or Foreign Country) |
| Uri | Laserson | Boston, Massachusetts 02215 |
| Greg | Porreca | Ocean City, New Jersey 08226 |
| | | |
| | | |
| | | |

*Additional inventors are being named on the* _____ *separately numbered sheets attached hereto.*

**TITLE OF THE INVENTION (500 characters max):**

METHODS AND COMPOSITIONS FOR REDUCING DIAGNOSTIC ERRORS ASSOCIATED WITH BIAS IN PREPARATIONS OF NUCLEIC ACIDS

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

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| Country | Telephone | Email |

**ENCLOSED APPLICATION PARTS (*check all that apply*)**

[x] Application Data Sheet. See 37 CFR 1.76
[ ] CD(s), Number of CDs _____
[x] Drawing(s) *Number of Sheets* 4
[ ] Other (specify) _____
[x] Specification (e.g. description of the invention) *Number of Pages* 36

Fees Due: Filing Fee of $220 ($110 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

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***PROVISIONAL APPLICATION COVER SHEET***
***Page 2 of 2***

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| | | | |
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| SIGNATURE | /Patrick R.H. Waller/ | Date | May 18, 2009 |
| TYPED or PRINTED NAME | Patrick R.H. Waller | REGISTRATION NO. *(if appropriate)* | 41,418 |
| TELEPHONE | 617.646.8000 | Docket Number: | G0814.70002US00 |

**Abstract**

The invention relates to preparative steps in DNA sequencing-related technologies that reduce the impact of bias in a genetic analysis and increase the reliability and accuracy of downstream quantitative evaluations or applications.



Petitioner Illumina, Inc., EX1002, p. 499

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 5356732 |
| **Application Number:** | 61179358 |
| **International Application Number:** | |
| **Confirmation Number:** | 9208 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR REDUCING DIAGNOSTIC ERRORS ASSOCIATED WITH BIAS IN PREPARATIONS OF NUCLEIC ACIDS |
| **First Named Inventor/Applicant Name:** | Uri Laserson |
| **Customer Number:** | 23628 |
| **Filer:** | Patrick R.H. Waller/Danielle Calder |
| **Filer Authorized By:** | Patrick R.H. Waller |
| **Attorney Docket Number:** | G081470002US00 |
| **Receipt Date:** | 18-MAY-2009 |
| **Filing Date:** | |
| **Time Stamp:** | 23:08:38 |
| **Application Type:** | Provisional |

## Payment information:

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|---|---|
| Submitted with Payment | yes |
| Payment Type | Credit Card |
| Payment was successfully received in RAM | $110 |
| RAM confirmation Number | 5791 |
| Deposit Account | 232825 |
| Authorized User | WALLER,PATRICK R.H. |
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| **File Listing:** | | | | | |
| **Document Number** | **Document Description** | **File Name** | **File Size(Bytes)/ Message Digest** | **Multi Part /.zip** | **Pages (if appl.)** |
| 1 | Provisional Cover Sheet (SB16) | G081470002US00-TRN-PRW.pdf | 45169<br>8026c9499a1ae593613f664b1130c4f5606f4e18 | no | 2 |
| **Warnings:** | | | | | |
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| 2 | Fee Worksheet (PTO-875) | G081470002US00-FEE-PRW.pdf | 44797<br>e6461f8ad46d85f890a7e15adf09c327cba85ea0 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Application Data Sheet | G081470002US00-ADS-PRW.pdf | 17233<br>d8f4796e15a3286187e8de38aa13fd32becb0db3 | no | 3 |
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| 4 | | G081470002US00-PROAPL-PRW.pdf | 208502<br>8f9482a22c3bff72a7f5fb1fbeb082a6879d08b2 | yes | 36 |
| | **Multipart Description/PDF files in .zip description** | | | | |
| | **Document Description** | | **Start** | **End** | |
| | Specification | | 1 | 33 | |
| | Claims | | 34 | 35 | |
| | Abstract | | 36 | 36 | |
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| 5 | Drawings-only black and white line drawings | G081470002US00-DRW-PRW.pdf | 180154<br>1176ebc4ecbf15a567351771a70950f18a8cd6c3 | no | 4 |
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| 6 | Fee Worksheet (PTO-875) | fee-info.pdf | 29603<br>6690662c6ae0d98d3f105ae5c90bce3ae13dd6e6 | no | 2 |
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**National Stage of an International Application under 35 U.S.C. 371**
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Claims

1. A method of genotyping a subject, the method comprising:

determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence,

wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject.

2. The method of claim 1, wherein the isolated nucleic acids are molecular inversion probe capture products.

3. A method of genotyping a subject, the method comprising:

determining the sequence of molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, and wherein the target nucleic acid is a captured genomic locus of a subject, and

genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products.

4. A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject,

amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.



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5. The method of claim 4, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

6. The method of claim 4 or 5, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

7. The method of any one of claims 4-6, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

8. The method of any one of claims 4-6, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

9. A method of analyzing a plurality of genetic loci, the method comprising:

obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence,

amplifying the plurality of molecular inversion probe capture products,

determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and

if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination.

10. The method of claim 9, further comprising genotyping target sequences in the plurality, wherein the genotyping comprises correcting for bias, if detected.

11. The method of any of claims 1-10, wherein the target nucleic acid sequence is a sequence of a gene selected from Table 1.



Petitioner Illumina, Inc., EX1002, p. 504

**Application Data Sheet**

**Application Information**

| | |
|---|---|
| Application Type:: | Provisional |
| Subject Matter:: | Utility |
| Suggested Group Art Unit:: | N/A |
| CD-ROM or CD-R?:: | None |
| Sequence submission?:: | None |
| Computer Readable Form (CRF)?:: | No |
| Title:: | METHODS AND COMPOSITIONS FOR REDUCING DIAGNOSTIC ERRORS ASSOCIATED WITH BIAS IN PREPARATIONS OF NUCLEIC ACIDS |
| Attorney Docket Number:: | G0814.70002US00 |
| Request for Early Publication?:: | No |
| Request for Non-Publication?:: | No |
| Small Entity?:: | Yes |
| Petition included?:: | No |
| Secrecy Order in Parent Appl.?:: | No |

**Applicant Information**

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | US |
| Status:: | Full Capacity |
| Given Name:: | Uri |
| Family Name:: | Laserson |
| City of Residence:: | Boston |
| State or Province of Residence:: | MA |
| Country of Residence:: | US |
| Street of mailing address:: | 645 Beacon Street, #215 |

| | |
|---|---|
| City of mailing address:: | Boston |
| State or Province of mailing address:: | MA |
| Postal or Zip Code of mailing address:: | 02215 |

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | US |
| Status:: | Full Capacity |
| Given Name:: | Greg |
| Family Name:: | Porreca |
| City of Residence:: | Ocean City |
| State or Province of Residence:: | NJ |
| Country of Residence:: | US |
| Street of mailing address:: | 911 Gardens Parkway |
| City of mailing address:: | Ocean City |
| State or Province of mailing address:: | NJ |
| Postal or Zip Code of mailing address:: | 08226 |

**Correspondence Information**

| | |
|---|---|
| Correspondence Customer Number:: | 23628 |

**Representative Information**

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|---|---|
| Representative Customer Number:: | 23628 |

**Domestic Priority Information**

**Foreign Priority Information**

**Assignee Information**

| | |
|---|---|
| Assignee Name:: | Good Start Genetics, Inc. |
| Street of Mailing Address:: | 8 St. Mary's Street, Suite 621 |
| City of Mailing Address:: | Boston |
| State or Province of Mailing Address:: | Massachusetts |
| Country of Mailing Address:: | United States |
| Postal or Zip Code of Mailing Address:: | 02115 |





Petitioner Illumina, Inc., EX1002, p. 507

# METHODS AND COMPOSITIONS FOR REDUCING DIAGNOSTIC ERRORS ASSOCIATED WITH BIAS IN PREPARATIONS OF NUCLEIC ACIDS

## Field of the Invention

The invention relates to preparative steps in DNA sequencing-related technologies that reduce bias and increase the reliability and accuracy of downstream quantitative applications.

## Background of the Invention

There are currently many genomics assays that utilize next-generation (polony-based) sequencing to generate data, including genome resequencing, RNA-seq for gene expression, bisulphite sequencing for methylation, and Immune-seq, among others. In order to make quantitative measurements (including genotype calling), these methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids. The majority of these techniques require a preparative step to construct a high-complexity library of DNA molecules that is representative of a sample of interest. This may include chemical or biochemical treatment of the DNA (e.g., bisulphite treatment), capture of a specific subset of the genome (e.g., padlock probe capture, solution hybridization), and a variety of amplification techniques (e.g., polymerase chain reaction, whole genome amplification, rolling circle amplification).

Systematic and random errors are common problems associated with genome amplification and sequencing library construction techniques. For example, genomic sequencing library may contain an over- or under-representation of particular sequences from a source genome as a result of errors (bias) in the library construction process. Such bias can be particularly problematic when it results in target sequences from a genome being absent or undetectable in the sequencing libraries. For example, an under-representation of particular allelic sequences (e.g., heterozygotic alleles) from a genome in a sequencing library can result in an apparent homozygous representation in a sequencing library. As most downstream sequencing library quantification techniques depend on stochastic counting processes, these problems have typically been addressed by sampling enough (over-sampling) to obtain a minimum number of observations necessary to make statistically significant decisions. However, the strategy of oversampling is generally limited to



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elimination of low-count Poisson noise, and the approach wastes resources and increases the expense required to perform such experiments. Moreover, oversampling can result in a reduced statistical confidence in certain conclusions (e.g., diagnostic calls) based on the data. Accordingly, new approaches are needed for overcoming bias in sequencing library preparatory methods.

## Brief Summary of the Invention

Aspects of the disclosure are based, in part, on the discovery of methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, namely high variability in the capture and amplification of nucleic acids and disproportionate representation of heterozygous alleles in sequencing libraries. Accordingly, in some embodiments, the disclosure provides methods that reduce errors associated with the variability in the capture and amplification of nucleic acids. In other embodiments, the methods improve allelic representation in sequencing libraries and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of differentiator tag sequences to uniquely tag individual nucleic acid molecules. In some embodiments, the differentiator tag sequence permit the detection of bias based on the occurrence of combinations of differentiator tag and target sequences observed in a sequencing reaction. In other embodiments, the methods reduce errors caused by bias, or the risk of bias, associated with the capture, amplification and sequencing of genetic loci, e.g., for diagnostic purposes.

Aspects of the invention relate to providing sequence tags (referred to as differentiator tags) that are useful to determine whether target nucleic acid sequences identified in an assay are from independently isolated target nucleic acids or from multiple copies of the same target nucleic acid molecule (e.g., due to bias in a preparative step, for example, amplification). This information can be used to help analyze a threshold number of independently isolated target nucleic acids from a biological sample in order to obtain sequence information that is reliable and can be used to make a genotype conclusion (e.g., call) with a desired degree of confidence. This information also can be used to detect bias in one or more nucleic acid preparative steps.

In some embodiments, a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject). In some



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embodiments, an assessment is provided to a couple, based on their respective genotypes at one or more genetic loci, of the risk of their having one or more children having a genotype associated with a disease or condition (e.g., a homozygous or heterozygous genotype associated with a disease or condition). In some embodiments, a subject or a couple may seek genetic or reproductive counseling in connection with a genotype determined according to embodiments of the invention. The methods disclosed herein are useful for any application where reduction of bias, e.g., associated with genomic isolation, amplification, sequencing, is important. For example, detection of cancer mutations in a heterogeneous tissue sample, detection of mutations in maternally-circulating fetal DNA, and detection of mutations in cells isolated during a preimplantation genetic diagnostic procedure.

Accordingly, in some aspects, methods of genotyping a subject are provided. In some embodiments, the methods comprise determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject.

In some embodiments, the isolated nucleic acids are products of a circularization selection-based preparative method, e.g., molecular inversion probe capture products. In other embodiments, the isolated nucleic acids are products of an amplification-based preparative methods. In other embodiments, the isolated nucleic acids are products of hybridization-based preparative methods.

Circularization selection-based preparative methods selectively convert regions of interest (target nucleic acids) into a covalently-closed circular molecule which is then isolated typically by removal (usually enzymatic, e.g. with exonuclease) of any non-circularized linear nucleic acid. Oligonucleotide probes (e.g., molecular inversion probes) are designed which have ends that flank the region of interest (target nucleic acid) and, optionally, primer sites, e.g., sequencing primer sites. The probes are allowed to hybridize to the genomic target, and enzymes are used to first (optionally) fill in any gap between probe ends and second ligate the probe closed. Following circularization, any remaining (non-target) linear nucleic acid is typically removed, resulting in isolation (capture) of target nucleic acid. Circularization selection-based preparative methods include molecular inversion probe



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capture reactions and 'selector' capture reactions. In some embodiments, molecular inversion probe capture of a target nucleic acid is indicative of the presence of a polymorphism in the target nucleic acid.

In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using a pair of oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or both of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and differentiator tag sequences. Amplification-based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel).

Hybridization-based preparative methods involve selectively immobilizing target nucleic acids for further manipulation. It is to be understood that one or more oligonucleotides (immobilization oligonucleotides), which comprise differentiator tag sequences, and which may be from 15 to 170 nucleotides in length, are used which hybridize along the length of a target region of a genetic locus to immobilize it. In some embodiments, immobilization oligonucleotides, are either immobilized before hybridization is performed (e.g., Roche/Nimblegen 'sequence capture'), or are prepared such that they include a moiety (e.g. biotin) which can be used to selectively immobilize the target nucleic acid after hybridization by binding to e.g., streptavidin-coated microbeads (e.g. Agilent 'SureSelect').

In some embodiments, the methods disclosed herein comprise determining the sequence of molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, and wherein the target nucleic acid is a captured genomic locus of a subject, and genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products.

In some embodiments, the methods disclosed herein comprise obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target



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nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products. In certain embodiments, obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence. In specific embodiments, capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

In one embodiment, the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype. In some embodiments, the methods further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

According to some aspects, methods of analyzing a plurality of genetic loci are provided. In some embodiments, the methods comprise obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, amplifying the plurality of molecular inversion probe capture products, determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. In some embodiments, the methods further comprise genotyping target sequences in the plurality, wherein the genotyping comprises correcting for bias, if detected.

In some embodiments, the target nucleic acid is a gene (or portion thereof) selected from Table 1. In some embodiments, the genotyping comprises determining the sequence of a target nucleic acid (e.g., a polymorphic sequence) at one or more (both) alleles of a genome (a diploid genome) of a subject. In certain embodiments, the genotyping comprises



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determining the sequence of a target nucleic acid at both alleles of a diploid genome of a subject, wherein in the target nucleic acid comprises, or consists of, a sequence if Table 2.

## Brief Description of the Drawings

Figure 1a, b, and c depict various methods for combining differentiator tag sequence and target sequences (NNNN depicts a differentiator tag sequence).

Figure 2 depicts a method for genotyping based on target and differentiator tag sequences. Sequencing reads of target and differentiator tags sequences are collapsed to make diploid genotype calls.

Figure 3 depicts results of a simulation of a MIP capture reaction in which MIP probes, each having a differentiator tag sequence of 15 nucleotides, are combined with 10000 target sequence copies (e.g., genome equivalents). In this simulated reaction, the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is 0.05. The Y axis reflects the number of observations. The X axis reflects the number of independent occurrences of target:differentiator tag combinations.

Figure 4 depicts a graph of sequencing coverage, which is needed to ensure that alleles are sampled to sufficient depth (either 10x or 20x minimum sampling per allele, assuming 1000 targets). X axis is total per-target coverage required, and Y axis is the probability that a given total coverage will result in at least 10x or 20x coverage for each allele.

## Detailed Description of the Invention

Aspects of the disclosure are based, in part, on the discovery of methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, namely high variability in the capture and amplification of nucleic acids and disproportionate representation of heterozygous alleles in sequencing libraries. Accordingly, in some embodiments, the disclosure provides methods that reduce variability in the capture and amplification of nucleic acids. In other embodiments, the methods improve allelic representation in sequencing libraries and, thus, improve variant detection outcomes. In certain embodiments, the disclosure provides preparative methods for capturing target nucleic acids (e.g., genetic loci) that involve the use of differentiator tag sequences to uniquely tag individual nucleic acid molecules. In some



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embodiments, the differentiator tag sequence permits the detection of bias based on the frequency with which pairs of differentiator tag and target sequences are observed in a sequencing reaction. In other embodiments, the methods reduce errors caused by bias, or the risk of bias, associated with the capture, amplification and sequencing of genetic loci, e.g., for diagnostic purposes.

Aspects of the invention relate to associating unique sequence tags (referred to as differentiator tag sequences) with individual target molecules that are independently captured and/or analyzed (e.g., prior to amplification or other process that may introduce bias). These tags are useful to distinguish independent target molecules from each other thereby allowing an analysis to be based on a known number of individual target molecules. For example, if each of a plurality of target molecule sequences obtained in an assay is associated with a different differentiator tag, then the target sequences can be considered to be independent of each other and a genotype likelihood can be determined based on this information. In contrast, if each of the plurality of target molecule sequences obtained in the assay is associated with the same differentiator tag, then they probably all originated from the same target molecule due to over-representation (e.g., due to biased amplification) of this target molecule in the assay. This provides less information than the situation where each nucleic acid was associated with a different differentiator tag. In some embodiments, a threshold number of independently isolated molecules (e.g., unique combinations of differentiator tag and target sequences) is analyzed to determine the genotype of a subject.

According to some aspects, the disclosure relates to multiplex diagnostic methods. In some embodiments, the multiplex diagnostic methods comprise capturing a plurality of target nucleic acids (genetic loci) in parallel (e.g., a genetic locus of Table 1) using capture probes having unique differentiator tag sequences. In some embodiments, the genetic loci possess one or more polymorphisms (e.g., a polymorphism of Table 2) the genotypes of which correspond to disease causing alleles. Accordingly, in some embodiments, the disclosure provides methods for assessing multiple heritable disorders in parallel. In some embodiments, methods are provided for diagnosing multiple heritable disorders in parallel at a pre-implantation, prenatal, perinatal, or postnatal stage. In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample. A patient or subject may be a human. However, aspects of the invention are not limited to humans



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and may be applied to other species (e.g., mammals, birds, reptiles, other vertebrates or invertebrates) as aspects of the invention are not limited in this respect. A subject or patient may be male or female. In some embodiments, in connection with reproductive genetic counseling, samples from a male and female member of a couple may be analyzed. In some embodiments, for example, in connection with an animal breeding program, samples from a plurality of male and female subjects may be analyzed to determine compatible or optimal breeding partners or strategies for particular traits or to avoid one or more diseases or conditions.

In some embodiments, the invention relates to compositions comprising pools (libraries) of preparative nucleic acids that each comprise "differentiator tag sequences" for detecting and reducing the effects of bias, and for genotyping target nucleic acid sequences. As used herein, a "differentiator tag sequence" is a sequence of a nucleic acid (a preparative nucleic acid), which in the context of a plurality different isolated nucleic acids, identifies a unique, independently isolated nucleic acid. Typically, differentiator tag sequences are used to identify the origin of a target nucleic acid at one or more stages of a nucleic acid preparative method. For example, in the context of a multiplex nucleic acid capture reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, target nucleic acid capture events. Also, in the context of a multiplex nucleic acid amplification reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, primary amplicons of a target nucleic acid, for example. Thus, combinations of target nucleic acid and differentiator tag sequence (target:diffentiator tag sequences) of an isolated nucleic acid of a preparative method provide a basis for identifying unique, independently isolated target nucleic acids. Differentiator tags may be synthesized by random nucleotide addition, for example. Differentiator tag sequences are typically of a predefined length, which is selected to control the likelihood of producing unique target:diffentiator tag sequences in a preparative reaction (e.g., amplification-based reaction, a circularization selection-based reaction, e.g., a MIP reaction). Differentiator tag sequences may be, up to 5, up to 6, up to 7 up to 8, up to 9, up to 10, up to 11, up to 12, up to 13, up to 14, up to 15, up to 16, up to 17, up to 18, up to 19, up to 20, up to 21, up to 22, up to 23, up to 24, up to 25, or more nucleotides in length. For purposes of genotyping, isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and observance of threshold numbers of unique



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combinations of target nucleic acid and differentiator tag sequences provide a certain statistical confidence in the genotype.

During a library preparation process, each nucleic acid molecule may be tagged with a unique differentiator tag sequence in a configuration that permits the differentiator tag sequence to be sequenced along with the target nucleic acid sequence of interest (the nucleic acid sequence for which the library is being prepared, e.g., a polymorphic sequence). The incorporation of the nucleic acid comprising a differentiator tag sequence at a particular step allows the detection and correction of biases in subsequent steps of the protocol.

A large library of unique differentiator tag sequences may be created by using degenerate, random-sequence polynucleotides of defined length. The differentiator tag sequences of the polynucleotides may be read at the final stage of the sequencing. The observations of the differentiator tag sequences may be used to detect and correct biases in the final sequencing read-out of the library. For example, the total possible number of differentiator tag sequences, which may be produced, e.g., randomly, is $4^N$, where N is the length of the differentiator tag sequence. Thus, it is to be understood that the length of the differentiator tag sequence may be adjusted such that the size of the population of MIPs having unique differentiator tag sequences is sufficient to produce a library of MIP capture products in which identical independent combinations of target nucleic acid and differentiator tag sequence are rare. As used herein combinations of target nucleic acid and differentiator tag sequences, may also be referred to as "target:differentiator tag sequences".

In the final readout of a sequencing process, each read may have an additional unique differentiator tag sequence. In some embodiments, when differentiator tag sequences are distributed randomly in a library, all the unique differentiator tag sequences will be observed about an equal number of times. Accordingly, the number of occurrences of a differentiator tag sequence may follow a Poisson distribution.

In some embodiments, overrepresentation of target:differentiator tag sequences in a pool of preparative nucleic acids (e.g., amplified MIP capture products) is indicative of bias in the preparative process (e.g., bias in the amplification process). For example, target:differentiator tag sequence combinations that are statistically overrepresented are indicative of bias in the protocol at one or more steps between the incorporation of the differentiator tag sequences into MIPs and the actual sequencing of the MIP capture products.

The number of reads of a given target:differentiator tag sequence may be indicative (may serve as a proxy) of the amount of that target sequence present in the originating sample. In some embodiments, the numbers of occurrence of sequences in the originating



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sample is the quantity of interest. For example, using the methods disclosed herein, the occurrence of differentiator tag sequences in a pool of MIPs may be predetermined (e.g., may be the same for all differentiator tag sequences). Accordingly, changes in the occurrence of differentiator tag sequences after amplification and sequencing may be indicative of bias in the protocol. Bias may be corrected to provide an accurate representation of the composition of the original MIP pool, e.g., for diagnostic purposes.

According to some aspects, a library of preparative nucleic acid molecules (e.g., MIPs, each nucleic acid in the library having a unique differentiator tag sequence, may be constructed such that the number of nucleic acid molecules in the library is significantly larger than the number prospective target nucleic acid molecules to be captured using the library. This ensures that products of the preparative methods include only unique target:differentiator tag sequence; e.g., in a MIP reaction the capture step would undersample the total population of unique differentiator tag sequences in the MIP library. For example, an experiment utilizing 1 μg of genomic DNA will contain about ~150,000 copies of a diploid genome. For a MIP library, each MIP in the library comprising a randomly produced 12-mer differentiator tag sequence (~1.6 million possible unique differentiator tag sequences), there would be more than 100 unique differentiator tag sequences per genomic copy. For a MIP library, each MIP in the library comprising a randomly produced 15-mer differentiator tag sequence (~1 billion possible unique differentiator tag sequences), there would be more than 7000 unique differentiator tag sequences per genomic copy. Therefore, the probability of the same differentiator tag sequence being incorporated multiple times is incredibly small. Thus, it is to be appreciated that the length of the differentiator tag sequence is to be selected based on the amount of target sequence in a MIP capture reaction and the desired probability for having multiple, independent occurrences of target:differentiator tag sequence combinations.

The skilled artisan will appreciate that as part of a MIP library preparation process, adapters may be ligated onto the ends of the molecules of interest. Adapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites. In addition, barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007/0020640 A1 (McCloskey et al.)

The actual incorporation of the random differentiator tag sequences can be performed through various methods known in the art. For example, nucleic acids comprising



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differentiator tag sequences may be incorporated by ligation. This is a flexible method, because molecules having differentiator tag sequence can be ligated to any blunt-ended nucleic acids. The sequencing primers must be incorporated subsequently such that they sequence both the differentiator tag sequence and the target sequence. Alternatively, the sequencing adaptors can be synthesized with the random differentiator tag sequences at their 3' end (as degenerate bases), so that only one ligation must be performed. Another method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags. Another method to incorporate the differentiator tag sequences is to synthesize them into a padlock probe prior to performing a gene capture reaction. The differentiator tag sequence is incorporated 3' to the targeting arm but 5' to the amplification primer that will be used downstream in the protocol. Another method to incorporate the differentiator tag sequences is as a tag on a gene-specific or poly-dT reverse-transcription primer. This allows the differentiator tag sequence to be incorporated directly at the cDNA level.

In some embodiments, at the incorporation step, the distribution of differentiator tag sequences can be assumed to be uniform. In this case, bias in any part of the protocol would change the uniformity of this distribution, which can be observed after sequencing. This allows the differentiator tag sequence to be used in any preparative process where the ultimate output is sequencing of many molecules in parallel.

Differentiator tag sequences may be incorporated into probes (e.g., MIPs) of a plurality when they are synthesized on-chip in parallel, such that degeneracy of the incorporated nucleotides is sufficient to ensure near-uniform distribution in the plurality of probes. It is to be appreciated that amplification of a pool of unique differentiator tag sequences may itself introduce bias in the initial pool. However, in most practical cases, the scale of synthesis (e.g., by column synthesis, chip based synthesis, etc.) is large enough that amplification of an initial pool of differentiator tag sequences is not necessary. By avoiding amplification or selection steps on the pool of unique differentiator tag sequences, potential bias may be minimized.

One example of the use of the differentiator tag sequences is in genome re-sequencing. Considering that the raw accuracy of most next-generation sequencing instruments is relatively low, it is crucial to oversample the genomic loci of interest.



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Furthermore, since there are two alleles at every locus, it is important to sample enough to ensure that both alleles have been observed a sufficient number of times to determine with a sufficient degree of statistical confidence whether the sample is homozygous or heterozygous. Indeed, the sequencing is performed to sample the composition of molecules in the originating sample. However, after multiple reads have been collected for a given locus, it is possible that due to bias (e.g., caused by PCR amplification steps), a large fraction of the reads are derived from a single originating molecule. This would skew the population of target sequences observed, and would affect the outcome of the genotype call. For example, it is possible that a locus that is heterozygous is called as homozygous, because there are only a few observations of the second allele out of many observations of that locus. However, if information is available on differentiator tag sequences, this situation could be averted, because the over-represented allele would be seen to also have an over-represented differentiator tag sequence (i.e., the sequences with the overrepresented differentiator tag sequence all originated from the same single molecule). Therefore, the sequences and corresponding distribution of differentiator tag sequences can be used as an additional input to the genotype-calling algorithm to significantly improve the accuracy and confidence of the genotype calls.

In some aspects, the disclosure provides methods for analyzing a plurality of target sequences which are genetic loci or portions of genetic loci (e.g., a genetic locus of Table 1). The genetic loci may be analyzed by sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs). Exemplary polymorphisms are disclosed in Table 2. The skilled artisan will appreciate that other polymorphisms are known in the art and may be identified, for example, by querying the Entrez Single Nucleotide Polymorphism database, for example, by searching with a GeneID from Table 1.

The mutations listed in Table 2 are documented polymorphisms in several disease-associated genes (CFTR is mutated in cystic fibrosis, GBA is mutated in Gaucher disease, ASPA is mutated in Canavan disease, HEXA is mutated in Tay Sachs disease). The polymorphisms are of several types: insertion/deletion polymorphisms which will cause frameshifts (and thus generally interrupt protein function) unless the insertion/deletion length is a multiple of 3bp, and substitutions which can alter the amino acid sequence of the protein and in some cases cause complete inactivation by introduction of a stop codon.

The methods disclosed here are also useful for diagnosis of one or more heritable disorders. In some embodiments, a heritable disorder that may be diagnosed with the methods disclosed herein is a genetic disorder that is prevalent in the Ashkenazi Jewish



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population. In some embodiments, the heritable disorders are selected from: 21-Hydroxylase-Defiocient Congenital Adrenal Hyperplasia; ABCC8-Related Hyperinsulinism; Alpha-Thalassemia, includes Constant Spring, & MR associated; Arylsulfatase A Deficiency-Metyachromatic Leukodystrophy; Biotinidase Deficiency-Holocarboxylase Synthetase Deficiency; Bloom's Syndrome; Canavan Disease; CFTR-Related Disorders-cystic fibrosis; Citrullinemia Type I; Combined MMA & Homocystinuria-dblC; Dystrophinopathies (DMD & BMD); Familial Dysautonomia; Fanconi Anemia-FANCC; Galactosemia-Classical: Galactokinase Defiency & Galactose Epimerase Deficiency; Gaucher Disease; GJB2-Related DFNB 1 Nonsyndromic Hearing Loss and Deafness; Glutaric acidemia Type 1; Hemoglobinopathies beta-chain disorders; Glycogen Storage Disease Type 1A; Maple Syrup Urine Disease; Types 1A, 1B, 2, 3; Medium Chain Acyl-Coenzyme A; Dehydrogenase Deficiency-MCADD; Methylmalonic Acidemia; Mucolipidosis IV; Nemaline Myopathy; Nieman-Pick Type A-Acid Sphingomyelinase Deficiency; Non-Ketotic Hyperglycinemia-Glycine Encephalopathy; Ornithine Transcarbamylase Deficiency; PKU Phenylalanine Hydroxylase Deficiency; Propionic Acidemia; Short Chain Acyl-CoA Dehydrogenase Deficiency-SCADD; Smith-Lemli-Opitz Syndrome; Spinal Muscular Atrophy (SMN1)-SMA; Tay Sachs-HexA Deficiency; Usher Synbdrome-Type I (Type IB, Type IC, Type ID, Type IF, Type IG) ; X-Linked Mental Retardation ARX-Related Disorders; X-Linked Mental Retardation with Cerebellar Cypoplasia and sistinctive Facial Appearance; X-Linked Mental Retardation; includes 9, 21, 30, 46, 58, 63, 88, 89; X-linked mental retardation: FM1-Related Disorders-FRXA, Fragile X MR; X-linked SMR: Renpenning Syndrome 1; Zellweger Spectrum disorders - Peroxisomal Bifunctional Enzyme Deficiencies including Zellweger, NALD, infantile Refsums.

**Table 1: Target Nucleic Acids**

| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| CYP21A2 | 1589 | cytochrome P450, family 21, subfamily A, polypeptide 2 | CAH1; CPS1; CA21H; CYP21; CYP21B; P450c21B; MGC150536; MGC150537; CYP21A2 | 201910 | NG_008337.1 | 6p21.3 |
| ABCC8 | 6833 | ATP-binding cassette, sub-family C (CFTR/MRP), member | HI; SUR; HHF1; MRP8; PHHI; SUR1; ABC36; | 600509 | NG_008867.1 | 11p15.1 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| | | 8 | HRINS; TNDM2; ABCC8 | | | |
| ATRX | 546 | alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) | SHS; XH2; XNP; ATR2; SFM1; RAD54; MRXHF1; RAD54L; ZNF-HX; MGC2094; ATRX | 300032 | NG_008838.1 | Xq13.1-q21.1 |
| ARSA | 410 | arylsulfatase A | MLD; ARSA | 607574 | NG_009260.1 | 22q13.31-qter; 22q13.33 |
| PSAP | 5660 | prosaposin | GLBA; SAP1; FLJ00245; MGC110993; PSAP | 176801 | NG_008835.1 | 10q21-q22 |
| BTD | 686 | biotinidase | BTD | 609019 | NG_008019.1 | 3p25 |
| HLCS | 3141 | holocarboxylase synthetase (biotin-(proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)) ligase) | HCS; HLCS | 609018 | NC_000021.7 | 21q22.1; 21q22.13 |
| BLM | 641 | Bloom syndrome, RecQ helicase-like | BS; RECQ2; RECQL2; RECQL3; MGC126616; MGC131618; MGC131620; BLM | 604610 | NG_007272.1 | 15q26.1 |
| ASPA | 443 | aspartoacylase (Canavan disease) | ASP; ACY2; ASPA | 608034 | NG_008399.1 | 17pter-p13 |
| CFTR | 1080 | cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) | CF; MRP7; ABC35; ABCC7; CFTR/MRP; TNR-CFTR; dJ760C5.1; CFTR | 602421 | NC_000007.12 | 7q31.2 |
| ASS1 | 445 | argininosuccinate synthetase 1 | ASS; CTLN1; ASS1 | 603470 | NG_011542.1 | 9q34.1 |
| MMACHC | 25974 | methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria | cblC; FLJ25671; DKFZp564I122; RP11-291L19.3; MMACHC | 609831 | NC_000001.9 | 1p34.1 |
| IKBKAP | 8518 | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein | FD; DYS; ELP1; IKAP; IKI3; TOT1; FLJ12497; DKFZp781H1425; IKBKAP | 603722 | NG_008788.1 | 9q31 |
| FANCC | 2176 | Fanconi anemia, complementation group | FA3; FAC; FACC; | 227645 | NG_011707.1 | 9q22.3 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| | | C | FLJ14675; FANCC | | | |
| GALK1 | 2584 | galactokinase 1 | GK1; GALK; GALK1 | 604313 | NG_008079.1 | 17q24 |
| GALT | 2592 | galactose-1-phosphate uridylyltransferase | GALT | 606999 | NC_000009.10 | 9p13 |
| GALE | 2582 | UDP-galactose-4-epimerase | SDR1E1; FLJ95174; FLJ97302; GALE | 606953 | NG_007068.1 | 1p36-p35 |
| GBA | 2629 | glucosidase, beta; acid (includes glucosylceramidase) | GCB; GBA1; GLUC; GBA | 606463 | NG_009783.1 | 1q21 |
| GJB2 | 2706 | gap junction protein, beta 2, 26kDa | HID; KID; PPK; CX26; DFNA3; DFNB1; NSRD1; DFNA3A; DFNB1A; GJB2 | 121011 | NG_008358.1 | 13q11-q12 |
| GCDH | 2639 | glutaryl-Coenzyme A dehydrogenase | GCD; ACAD5; GCDH | 608801 | NG_009292.1 | 19p13.2 |
| G6PC | 2538 | glucose-6-phosphatase, catalytic subunit | G6PT; GSD1; GSD1a; MGC163350; G6PC | 232200 | NG_011808.1 | 17q21 |
| HBB | 3043 | hemoglobin, beta | CD113t-C; beta-globin; HBB | 141900 | NG_000007.3 | 11p15.5 |
| BCKDHA | 593 | branched chain keto acid dehydrogenase E1, alpha polypeptide | MSU; MSUD1; OVD1A; BCKDE1A; FLJ45695; BCKDHA | 608348 | NC_000019.8 | 19q13.1-q13.2 |
| BCKDHB | 594 | branched chain keto acid dehydrogenase E1, beta polypeptide | E1B; FLJ17880; dJ279A18.1; BCKDHB | 248611 | NG_009775.1 | 6q13-q15 |
| DBT | 1629 | dihydrolipoamide branched chain transacylase E2 | E2; E2B; BCATE2; MGC9061; DBT | 248610 | NG_011852.1 | 1p31 |
| DLD | 1738 | dihydrolipoamide dehydrogenase | E3; LAD; DLDH; GCSL; PHE3; DLD | 238331 | NG_008045.1 | 7q31-q32 |
| ACADM | 34 | acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain | MCAD; ACAD1; MCADH; FLJ18227; FLJ93013; FLJ99884; ACADM | 607008 | NG_007045.1 | 1p31 |
| MMAA | 166785 | methylmalonic aciduria (cobalamin deficiency) cblA type | cblA; MGC120010; MGC120011; MGC120012; MGC120013; MMAA | 607481 | NG_007536.1 | 4q31.22 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| MMAB | 326625 | methylmalonic aciduria (cobalamin deficiency) cblB type | ATR; cblB; MGC20496; MMAB | 607568 | NG_007096.1 | 12q24 |
| MUT | 4594 | methylmalonyl Coenzyme A mutase | MCM; MUT | 609058 | NG_007100.1 | 6p12.3 |
| MCOLN1 | 57192 | mucolipin 1 | ML4; MLIV; MST080; TRPML1; MSTP080; TRP-ML1; TRPM-L1; MCOLN1 | 605248 | NC_000019.8 | 19p13.3-p13.2 |
| ACTA1 | 58 | actin, alpha 1, skeletal muscle | ACTA; ASMA; CFTD; MPFD; NEM1; NEM2; NEM3; CFTD1; CFTDM; ACTA1 | 102610 | NG_006672.1 | 1q42.13 |
| TPM3 | 7170 | tropomyosin 3 | TM3; TRK; NEM1; TM-5; TM30; TM30nm; TPMsk3; hscp30; MGC3261; FLJ41118; MGC14582; MGC72094; OK/SW-cl.5; TPM3 | 191030 | NG_008621.1 | 1q21.2 |
| TNNT1 | 7138 | troponin T type 1 (skeletal, slow) | ANM; TNT; STNT; TNTS; FLJ98147; MGC104241; TNNT1 | 191041 | NG_011829.1 | 19q13.4 |
| NEB | 4703 | nebulin | NEM2; NEB177D; FLJ11505; FLJ36536; FLJ39568; FLJ39584; DKFZp686C1456; NEB | 161650 | NG_009382.1 | 2q22 |
| SMPD1 | 6609 | sphingomyelin phosphodiesterase 1, acid lysosomal | ASM; NPD; SMPD1 | 607608 | NG_011780.1 | 11p15.4-p15.1 |
| GLDC | 2731 | glycine dehydrogenase (decarboxylating) | GCE; NKH; GCSP; HYGN1; MGC138198; MGC138200; GLDC | 238300 | NC_000009.10 | 9p22 |
| GCSH | 2653 | glycine cleavage system protein H (aminomethyl carrier) | GCE; NKH; GCSH | 238330 | NC_000016.8 | 16q23.2 |
| AMT | 275 | aminomethyltransferase | GCE; NKH; GCST; AMT | 238310 | NC_000003.10 | 3p21.2-p21.1 |
| OTC | 5009 | ornithine carbamoyltransferase | OCTD; MGC129967; MGC129968; MGC138856; OTC | 300461 | NG_008471.1 | Xp21.1 |
| PAH | 5053 | phenylalanine hydroxylase | PH; PKU; PKU1; PAH | 612349 | NG_008690.1 | 12q22-q24.2 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| DHPR | 5860 | quinoid dihydropteridine reductase | DHPR; PKU2; SDR33C1; FLJ42391; QDPR | 612676 | NG_008763.1 | 4p15.31 |
| PTS | 5805 | 6-pyruvoyltetrahydropterin synthase | PTPS; FLJ97081; PTS | 261640 | NG_008743.1 | 11q22.3-q23.3 |
| PCCA | 5095 | propionyl Coenzyme A carboxylase, alpha polypeptide | PCCA | 232000 | NG_008768.1 | 13q32 |
| PCCB | 5096 | propionyl Coenzyme A carboxylase, beta polypeptide | DKFZp451E113; PCCB | 232050 | NG_008939.1 | 3q21-q22 |
| ACADS | 35 | acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain | SCAD; ACAD3; ACADS | 606885 | NG_007991.1 | 12q22-qter |
| DHCR7 | 1717 | 7-dehydrocholesterol reductase | SLOS; DHCR7 | 602858 | NC_000011.8 | 11q13.2-q13.5 |
| SMN1 | 6606 | survival of motor neuron 1, telomeric | SMA; SMN; SMA1; SMA2; SMA3; SMA4; SMA@; SMNT; BCD541; T-BCD541; SMN1 | 600354 | NG_008691.1 | 5q13 |
| HEXA | 3073 | hexosaminidase A (alpha polypeptide) | TSD; MGC99608; HEXA | 606869 | NG_009017.1 | 15q23-q24 |
| MYO7A | 4647 | myosin VIIA | DFNB2; MYU7A; NSRD2; USH1B; DFNA11; MYOVIIA; MYO7A | 276903 | NG_009086.1 | 11q13.5 |
| USH1C | 10083 | Usher syndrome 1C (autosomal recessive, severe) | PDZ73; AIE-75; DFNB18; PDZ-45; PDZ-73; NY-CO-37; NY-CO-38; ush1cpst; PDZ-73/NY-CO-38; USH1C | 605242 | NC_000011.8 | 11p15.1-p14 |
| CDH23 | 64072 | cadherin-like 23 | USH1D; DFNB12; FLJ00233; FLJ36499; KIAA1774; KIAA1812; MGC102761; DKFZp434P2350; CDH23 | 605516 | NG_008835.1 | 10q21-q22 |
| PCDH15 | 65217 | protocadherin 15 | USH1F; DFNB23; DKFZp667A1711; PCDH15 | 605514 | NG_009191.1 | 10q21.1 |
| SANS | 124590 | Usher syndrome 1G (autosomal recessive) | SANS; ANKS4A; FLJ33924; USH1G | 607696 | NG_007882.1 | 17q25.1 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| ARX | 170302 | aristaless related homeobox | ISSX; PRTS; MRX29; MRX32; MRX33; MRX36; MRX38; MRX43; MRX54; MRX76; MRX87; MRXS1; ARX | 300382 | NG_008281.1 | Xp21 |
| OPHN1 | 4983 | oligophrenin 1 | OPN1; MRX60; OPHN1 | 300127 | NG_008960.1 | Xq12 |
| JARID1C | 8242 | lysine (K)-specific demethylase 5C | MRXJ; SMCX; MRXSJ; XE169; JARID1C; DXS1272E; KDM5C | 314690 | NG_008085.1 | Xp11.22-p11.21 |
| FTSJ1 | 24140 | FtsJ homolog 1 (E. coli) | JM23; MRX9; SPB1; TRM7; CDLIV; MRX44; FTSJ1 | 300499 | NG_008879.1 | Xp11.23 |
| SLC6A8 | 6535 | solute carrier family 6 (neurotransmitter transporter, creatine), member 8 | CRT; CT1; CRTR; MGC87396; SLC6A8 | 300036 | NC_000023.9 | Xq28 |
| DLG3 | 1741 | discs, large homolog 3 (Drosophila) | MRX; MRX90; NEDLG; NE-Dlg; SAP102; SAP-102; KIAA1232; DLG3 | 300189 | NC_000023.9 | Xq13.1 |
| TM4SF2 | 7102 | tetraspanin 7 | A15; MXS1; CD231; MRX58; CCG-B7; TM4SF2; TALLA-1; TM4SF2b; DXS1692E; TSPAN7 | 300096 | NG_009160.1 | Xp11.4 |
| ZNF41 | 7592 | zinc finger protein 41 | MRX89; MGC8941; ZNF41 | 314995 | NG_008238.1 | Xp11.23 |
| FACL4 | 2182 | acyl-CoA synthetase long-chain family member 4 | ACS4; FACL4; LACS4; MRX63; MRX68; ACSL4 | 300157 | NG_008053.1 | Xq22.3-q23 |
| PQBP1 | 10084 | polyglutamine binding protein 1 | SHS; MRX55; MRXS3; MRXS8; NPW38; RENS1; PQBP1 | 300463 | NC_000023.9 | Xp11.23 |
| PEX1 | 5189 | peroxisomal biogenesis factor 1 | ZWS1; PEX1 | 602136 | NG_008341.1 | 7q21.2 |
| PXMP3 | 5828 | peroxisomal membrane protein 3, 35kDa | PAF1; PEX2; PMP3; PAF-1; PMP35; RNF72; PXMP3 | 170993 | NG_008371.1 | 8q21.1 |
| PEX6 | 5190 | peroxisomal biogenesis factor 6 | PAF2; PAF-2; PXAAA1; PEX6 | 601498 | NG_008370.1 | 6p21.1 |
| PEX10 | 5192 | peroxisomal biogenesis | NALD; RNF69; | 602859 | NG_008342.1 | 1p36.32 |



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| Gene name | Gene ID | Description | Gene aliases | OMIM | RefSeqGene | Chromosome map position |
|---|---|---|---|---|---|---|
| | | factor 10 | MGC1998; PEX10 | | | |
| PEX12 | 5193 | peroxisomal biogenesis factor 12 | PAF-3; PEX12 | 601758 | NG_008447.1 | 17q12 |
| PEX5 | 5830 | peroxisomal biogenesis factor 5 | PXR1; PTS1R; PTS1-BP; FLJ50634; FLJ50721; FLJ51948; PEX5 | 600414 | NG_008448.1 | 12p13.31 |
| PEX26 | 55670 | peroxisomal biogenesis factor 26 | FLJ20695; PEX26M1T; Pex26pM1T; PEX26 | 608666 | NG_008339.1 | 22q11.21 |

**Table 2: Polymorphisms**

| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs63500661 | TCACATCACCAAGTTAAAAAAAAAAA[A/G]GGGGCGGGGGGGCAGAATGAAAATT |
| CFTR | 1080 | rs63107760 | AAACAAGGATGAATTAAGTTTTTTTT[-/T]AAAAAAGAAACATTTGGTAAGGGGA |
| CFTR | 1080 | rs62469443 | ATCACCAAGTTAAAAAAAAAAAAGGG[A/G]CGGGGGGGCAGAATGAAAATTGCAT |
| CFTR | 1080 | rs62469442 | CTATTGAACCAGAACCAAACAGGAAT[A/G]CCATAGCATTTTGTAAACTAAACTG |
| CFTR | 1080 | rs62469441 | CAGGAGTTCAAGACCAGCCTACTAAA[A/C]CACACACACACACACACACACAC |
| CFTR | 1080 | rs62469439 | GATTAAATAATAGTGTTTATGTACCC[C/G]GCTTATAGGAGAAGAGGGTGTGTGT |
| CFTR | 1080 | rs62469438 | ATTGTTATCTTTTCATATAAGGTAAC[A/T]GAGGCCCAGAGAGATTAAATAACAT |
| CFTR | 1080 | rs62469437 | TAATTTTAATTAAGTAAATTTAATTG[A/G]TAGATAAATAAGTAGATAAAAAATA |
| CFTR | 1080 | rs62469436 | GTATAAAAAAAAAAAAAAAAAAAGTT[A/T]GAATGTTTTCTTGCATTCAGAGCCT |
| CFTR | 1080 | rs62469435 | ATACTAAAAATTTAAAGTTCTCTTGC[A/G]ATATATTTTCTTAATATCTTACATC |
| CFTR | 1080 | rs62469434 | TGCTGGGATTACAGGCGTGAGCCACC[A/G]CGCCTGGCCTGATGGGACATATTTT |
| CFTR | 1080 | rs62469433 | CTACAATATAAGTATAGTATTGCAAA[A/C]CCATCAGGAAGGGTGTTAACTATTT |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs61763210 | GTTGTCTCCAAACTTTTTTTCAGGTG[-/AGA]AGGTGGCCAACCGAGCTTCGGAAAG |
| CFTR | 1080 | rs61720488 | TTTTTTCATAAAAGATTATATAAAGG[A/C]TATTGCTTTTGAATCACAAACACTA |
| CFTR | 1080 | rs61481156 | ATCTAGTGAGCAGTCAGGAAAGAGAA[C/T]TTCCAGATCCTGGAAATCAGGGTTA |
| CFTR | 1080 | rs61443875 | TAGAGTATAAAAAAAAAAAAAAAAAA[-/A]GTTTGAATGTTTTCTTGCATTCAGA |
| CFTR | 1080 | rs61312222 | TGCAAATGCCAACTATCAAAGATATT[C/G]GAGTATACTGTCAATAAACTTCATA |
| CFTR | 1080 | rs61159372 | TCCTCAACAGTTAGAAACAATATTTT[C/G]AGTGATTTCCCATGCCAACTTTACT |
| CFTR | 1080 | rs61094145 | TTTTTGGTATTGTTGTTAAATAAGTG[A/G]GAATTCAATACAGTATAATGTCTGT |
| CFTR | 1080 | rs61086387 | CTTGAAATCGGATATATATATATATA[-/TGTATATATATATATATATATATATATACATATATATATATA]GTATTATCCCTGTTTTCACAGTTTT |
| CFTR | 1080 | rs60996744 | AGAGGGGCTGTGAAGGACACCAAGGA[A/G]GAGACTAAGAGCCAGGAGGGAAAAC |
| CFTR | 1080 | rs60960860 | TAGAGTTTATTAGCTTTTACTACTCT[A/G]CTTAGTTACTTTGTGTTACAGAATA |
| CFTR | 1080 | rs60923902 | ACTAGTGATGATGAGCTTCTTTTCAT[-/AT]GTTTGTTGGCTGCATAAATGTCTTC |
| CFTR | 1080 | rs60912824 | GCAGAGAAAAGAGGGGCTGTGAAGGA[C/G]ACCAAGGAGGAGACTAAGAGCCAGG |
| CFTR | 1080 | rs60887846 | TTCAGAGGTCTACCACTGGTGCATAC[G/T]CTAATCACAGTGTCGAAAATTTTAC |
| CFTR | 1080 | rs60793174 | AAGAAAGAGCAAAAGAGGGCAAACTT[C/T]TCATACATTTTTGATGTCGAAACCA |
| CFTR | 1080 | rs60788575 | CCTAAAGTTTAAAAAGAAAAAAAAAA[-/A]GGAAGAAGGAATTAAAAATCCAAAG |
| CFTR | 1080 | rs60760741 | GTGTGTGTGTGTATATATATATATATAT[A/T]TATATATTTTTTTTTTCCTGAGCCA |
| CFTR | 1080 | rs60456599 | AAACTGTTGATGTTTTCATTTATTTA[C/G]ATCATTGGAAAACTTTAGATTCTAG |
| CFTR | 1080 | rs60363249 | TTTATCCATTCTTAACCAGAACAGAC[A/G]TTTTTTCAGAGCTGGTCCAGGAAAA |
| CFTR | 1080 | rs60355115 | TTGAAATCGGATATATATATATATAT[A/G]TATATATATATATATATATATATAT |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| CFTR | 1080 | rs60308689 | TAGTTTTTTATTTCCTCATATTATTT[-/T]CAGTGGCTTTTTCTTCCACATCTTT |
| CFTR | 1080 | rs60271242 | ACATAGTTCTCAGTGGTACAACTACA[A/G]GTGATTTCTCTTTTCTTATTTCTGG |
| CFTR | 1080 | rs60010318 | AGAGCAATGGCATCCCTTGTCTTGTG[C/T]TATACAGGATGCAGCAATTTATAGG |
| CFTR | 1080 | rs59961323 | TTCTGTCTACATAAGATGTCATACTA[A/G]ATTATCTTTTCCAGCATGCATTCAG |
| CFTR | 1080 | rs59961270 | CAGGGTGGCATGTTAGGCAGTGCTTA[A/G]AATAAATGAGTTGGTTATACAAGTA |
| CFTR | 1080 | rs59837506 | AGGACACACACACACACACACACACA[-/CA]TGCACACACATTTAAATAGATGCAT |
| CFTR | 1080 | rs59572090 | TAAAAAATTGGTATAATGAAATTGCA[C/T]TTGTAGTCTTTGGACATTTAAATCC |
| CFTR | 1080 | rs59548252 | TTTCAATACTTAAGAGGTACGCAGAG[A/G]AAAGAGGGGCTGTGAAGGACACCAA |
| CFTR | 1080 | rs59519859 | CAGCAATGAATATTTTGAGGCTGAGG[C/T]GCTGAGGGGTAAAATTGCAGCCTGG |
| CFTR | 1080 | rs59509837 | TTATGGTTTATATTTTTGTGTCTTCT[-/CTTT]AACACATCTTTTCTAGCAGAATTCA |
| CFTR | 1080 | rs59417037 | GTATTTTAGTTTTTTTTTTTTGTTTG[-/T]TTTGTTTTGTTTTGTTTTGTTTTTG |
| CFTR | 1080 | rs59159458 | TGGGTGACTCCATTTTTACTTTTAGT[C/T]TGGTCTGTTGAGGCCTCGTGAGAGA |
| CFTR | 1080 | rs59048119 | TATTTTCATGTATTTTAGTTTTTTTT[-/TTTT]GTTTGTTTTGTTTTGTTTTGTTTTG |
| CFTR | 1080 | rs58970500 | GTGTGTGTGTATATATATATATATAT[A/T]TATATTTTTTTTTTCCTGAGCCAAA |
| CFTR | 1080 | rs58942292 | AACCTATTAGCATGTCTGGCAGAAAA[-/A]TAGATACTTAATAAATTTCTTAAAT |
| CFTR | 1080 | rs58917054 | GAGGCTTAGACAGTTTAAGTAACTCA[A/G]GCATGGTTACACAACTAGCTAGGGC |
| CFTR | 1080 | rs58837484 | GTGTGAGTATTATGAGACCATATGTT[A/G]GGAGATTTTATTTGGTATTGAGGAT |
| CFTR | 1080 | rs58829491 | GAAACCCCACCCCTTCTATAGTTTTC[C/T]CTTTAATATTTACAATGGAACCATT |
| CFTR | 1080 | rs58805195 | CATATATATATAGTGTGTGTGTGTGT[A/G]TATATATATATATATATATATTTTT |
| GBA | 2629 | rs60866785 | CGAGCGAGAGAGAGAGAGAGAGAGAG[- |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | /AG]GAGCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs60239603 | GGCAGGTAATATCTAGTACCTTACTT[A/T]TATTTCCTGAGCACATTCTAC ATTT |
| GBA | 2629 | rs56310840 | GGCCAGGAATGGGAGTGCTTAGGTGC[A/G]GAGGTGGCACTGTTCCCGC AGCTGC |
| GBA | 2629 | rs41264927 | TGAAAACTCCATCCCCTCAGGGTCAT[C/T]AGATGAAGAGAAGACCACA GGGGTT |
| GBA | 2629 | rs41264925 | TGTAGGTAAGGGTCACATGTGGGAGA[C/G]GCAGCTGTGGGTAGGTCAG CCCTGT |
| GBA | 2629 | rs36024691 | CCAAGAAGGCGCCATTACACTCCAGC[- /C]TGGGCGACAGGGCGAGACTCCCTCA |
| GBA | 2629 | rs36024092 | TGCCACACCCAGCTAATTTGTGTGTG[- /G]TATGTGTGTGTATGTATGTGTGTGT |
| GBA | 2629 | rs35682967 | GTTCCTCCAGTAATTTTTTTTTTTTT[- /T]GGTTTTGAGACAGAGTCTTGCCCTG |
| GBA | 2629 | rs35033592 | ATCATGCCCAGATAATTTTTTTTTTT[- /T]GTATTTTAGTAGACACAGGGTTTCA |
| GBA | 2629 | rs34732744 | CGAGCGAGAGAGAGAGAGAGAGAGAG[- /AG]GAGCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs34620635 | CCTGTGAGGGGCACATTCCTTAGTAG[- /C]TAAGGAGTTGGGGGTGTGAAGATCC |
| GBA | 2629 | rs34302637 | ACAGGCTACTGGCTGGGCCCAGGCAA[- /A]GGGGGCCTTGGCAGGAAAAGTTCCT |
| GBA | 2629 | rs33949225 | GCGAGAGAGAGAGAGAGAGAGAGAGG[- /AG]AGCCGGCGCGAGAACTACGCATGCG |
| GBA | 2629 | rs28678003 | AAGAAGAAAAATAAAAAGAAAGTGGG[C/T]CAGACCGAGAGAACAGGA AGCCTGA |
| GBA | 2629 | rs28559737 | AAGGACAAAGGCAAAGAGACAAAGGC[G/T]CAACACTGGGGGTCCCCA GAGAGTG |
| GBA | 2629 | rs28373017 | TACCTAGTCACTTCCTGCCTCCATGG[C/T]GCAAAAGGGGATGGGTGTGC CTCTT |
| GBA | 2629 | rs12752133 | CTCTTCCGAGGTTCCACCCTGAACAC[C/T]TTCCTGCTCCCTCGTGGTGTA GAGT |
| GBA | 2629 | rs12747811 | TTCTGACTGGCAACCAGCCCCACTCT[C/T]TGGGAGCCCTCAGGAATGAA CTTGC |
| GBA | 2629 | rs12743554 | gctcagcctcccaggctggagtgcag[A/T]ggcgcgatctcggctcaccgcaacc |
| GBA | 2629 | rs12041778 | CATGAACCACATCAAATGAGATTTAG[C/T]GGGAGTGGCACACACAGTC ATGACC |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| GBA | 2629 | rs12034326 | AAGCAGCCCTGGGGAGTCGGGGCGGG[A/G]CCTGGATTGGAAAAGAGACGGTCAC |
| GBA | 2629 | rs11558184 | CTCCAAGTTCTGGGAGCAGAGTGTGC[A/G]GCTAGGCTCCTGGGATCGAGGGATG |
| GBA | 2629 | rs11430678 | GTTCCTCCAGTAAttttttttttttt[-/G/T]gttttgagacagagtcttgccctgt |
| GBA | 2629 | rs11264345 | CTAGTACCTTACTTCCCTCAAGTTCA[A/T]TCATCTCACAGATATTTCCTGAGCA |
| GBA | 2629 | rs10908459 | aattagccgtgcgtggtggcgggtgc[C/T]tgtaatcccacgtacttgggaggct |
| GBA | 2629 | rs10796940 | CCATGGCCAGCCGGGGAGGGGACGGG[A/C]ACACACAGACCCACACAGAGACTCA |
| GBA | 2629 | rs10668496 | agcgagagagagagagagagagagag[-/AG]gagCCGGCGCGAGAACTACGCATGC |
| GBA | 2629 | rs7416991 | CGTAGCAGTTAGCAGATGATAGGCGG[C/G/T]GAAATCTTATTTCACAGGGCATTAA |
| GBA | 2629 | rs4024049 | CTGGCCCTGGTGACAGTGGGGCTGTG[C/T]GTGGGGCCAGAGCCTTCTCAGAGGT |
| GBA | 2629 | rs4024048 | CAGATACTGGCCCTGGTGACAGTGGG[A/G]CTGTGCGTGGGGCCAGAGCCTTCTC |
| GBA | 2629 | rs4024047 | GACAGATACTGGCCCTGGTGACAGTG[G/T]GGCTGTGCGTGGGGCCAGAGCCTTC |
| GBA | 2629 | rs3841430 | GGCTCctctctctctctctctctc[-/TC]gctcgctctctcgctctctcgctct |
| GBA | 2629 | rs3754485 | GTTTCAGACCAGCCTGGCCAACATAG[C/T]GAAACCCCATCTCTACTAAAAATAA |
| GBA | 2629 | rs3205619 | AGTGGGCGATTGGATGGAGCTGAGTA[C/T]GGGGCCCATCCAGGCTAATCACACC |
| GBA | 2629 | rs2990227 | CCGGGCTCCGTGAATGTTTGTCACAT[C/G]TCTGAAGAACGTATGAATTACATAA |
| GBA | 2629 | rs2990226 | GAATCCCAACCCCGACGCTCGTCGCC[C/G]GGCTCCGTGAATGTTTGTCACATGT |
| GBA | 2629 | rs2990225 | GCGAATCCCAACCCCGACGCTCGTCG[C/T]CGGGCTCCGTGAATGTTTGTCACAT |
| GBA | 2629 | rs2990224 | TGGGCAGAAGTCAGGGTCCAAAGAAA[G/T]GGCAAAGAAAAGTGTcagtggctca |
| ASPA | 443 | rs63751297 | TAAGAAAGACGTTTTTGATTTTTTTC[A/G]GACTTCTCTGGCTCCACTACCCTGC |
| ASPA | 443 | rs62071301 | CTGATTCCTGGCCAGGAGCGGTGGCT[C/T]ACGCCTGTAATCCCAGCGCTTTGGG |
| ASPA | 443 | rs62071300 | TAAAAATGCTGATTCCTGGCCAGGAG[C/T]GGTGGCTCACGCCTGTAATCCCAGC |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| ASPA | 443 | rs62071299 | TTTAAAAATGCTGATTCCTGGCCAGG[A/C]GCGGTGGCTCACGCCTGTAATCCCA |
| ASPA | 443 | rs62071297 | CAAGACCTGTCAAAGATCTGAGAAAT[A/T]TTACCCGACTTACAAGCTAACCATT |
| ASPA | 443 | rs61697033 | ACTGTAATAAGTGCTGTAAAAGAAAT[A/G]CACAAAATAATATAGCAGAGGGTAT |
| ASPA | 443 | rs60743592 | CTTGAGGTCAGGAGTTCAAGACCAGT[C/T]TGGGCAACATGGGGAAAACCTTGTC |
| ASPA | 443 | rs60666840 | AGGTTGCAGTGAGCCGAGATCATGCC[A/G]TTGCACTCCAGCCGGGGCAACAAAA |
| ASPA | 443 | rs60147514 | ACAAGTGTCTTGAAATTATCTGTGAT[C/T]TGCTATAGAGCAATACTTTTGTAAA |
| ASPA | 443 | rs59930743 | GTGGGTATATGCAGCTCTATGCACTA[C/T]CTGCTCATTTATTTGGTAAATCTAA |
| ASPA | 443 | rs59690349 | TGTGTGTGTGTGCGTGTGTGTGTGTG[-/TGTGTGTG]ATCATAAGAGTGGCTGCAGCAAACT |
| ASPA | 443 | rs59676360 | AGTCTGGAGTGCAATGGTGCAATCTC[A/G]GCTCACTGCAGCCTCCACCTCCGGG |
| ASPA | 443 | rs59335404 | CTCCTAATGGATATTTCCTAAATTTT[G/T]CTGAACAGAATTTAACTTGAGCTGG |
| ASPA | 443 | rs58879097 | ATTTAAAAATGGATTTCTAGAAAAAC[A/G]ATCACATACTTGAATATTTTAGCAA |
| ASPA | 443 | rs58686774 | CTATAAATGGGTAGCATGAGGGATTC[A/G]AGGAGGTGGCTGAAAGAAGCACGTA |
| ASPA | 443 | rs57511162 | AAGAAACCAAGCATAGTAGAGTGTTA[A/G]AAAACCAAAGCAACTAAACAACTGT |
| ASPA | 443 | rs55859596 | CGGGGCTCAGAACTTGTAACAGAAAA[A/T]TAAAATATACTCCACTCAAGGGAAT |
| ASPA | 443 | rs55742972 | TACTACACTTCACGGATACTGTACTT[-/GTACTT]TTTTTCCAAATTGAAGGTTTTTGGC |
| ASPA | 443 | rs55640436 | TTGTTTTTGTTTTTGTTTTTGTTTTT[-/GTTTTTGTTTTT]TGAGATGGAGTCTCGCTCTGTCGCC |
| ASPA | 443 | rs36225687 | TTTGCCTTACTACACTTCACGGATAC[-/TGTACT]TGTACTTTTTTTCCAAATTGAAGGT |
| ASPA | 443 | rs36051310 | GAGGTGGCTGAAAGAAGCACGTATCC[-/C]TGATGGCATGGTTGCGGGTTATATG |
| ASPA | 443 | rs36034906 | GAGAAAAGCAGTTCCTGGAACACCCC[-/C]ACCCCTTAACCCCTTATCTCTGCTT |
| ASPA | 443 | rs36033666 | TTACATATGTATACATGTGCCATGTT[- |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | /T]GGTGTGCCGCACCCATTAACTCGTC |
| ASPA | 443 | rs35730123 | CTTTTTCCAGATTTTTTTTTTTTTTT[-<br>/T]GAGACAGAGTTTCACTCTTGTTGCC |
| ASPA | 443 | rs35629100 | TTTGGAAATCTTAAGCTTTTATTTGG[-<br>/G]TGTCACAGAGAAACAGGATCTGTAT |
| ASPA | 443 | rs35614631 | TACTTTAAGTTTTAGGGTACATGTGC[-<br>/A]CCATGTGCAGGTTTGTTACATATGT |
| ASPA | 443 | rs35225782 | ATTCATGACCAGCCACATAAATGCAC[-<br>/A]GTATTACTTCGCAAGCATGCCAATG |
| ASPA | 443 | rs35178659 | GTGCACTAGAATTAGCTAAAGTGGGG[-<br>/G]AAAAAAAGATGCATTTGATGGTCTA |
| ASPA | 443 | rs35095578 | AACCTCCACCTCCCAGGTTCAAGAGA[-<br>/A]TTCTCCTGCCTCAGCCTCCCAAGTA |
| ASPA | 443 | rs35002210 | CCTCCCTGTGATCCGAAGTAGCAGAC[A/G]TACTTAACTTCCATGGTGGA<br>TTGTT |
| ASPA | 443 | rs34744839 | AAAACATTATTATATCTAGAAAAAAA[-<br>/A]TGTATCTTAACCATTGTGGGAAGTG |
| ASPA | 443 | rs34680506 | TTGAAGGTAAAATCATAGGGAGTTGG[-<br>/G]AGCTGTCCTCTTGCGCTGAATCAGT |
| ASPA | 443 | rs34365618 | ACTTGTGGCCTTTTTGGAGAGGTTAG[-<br>/CA]ACTCTGAAAACTCTGTCCCTGGACC |
| ASPA | 443 | rs34275920 | GAAGGAGAAAAAGAGAGGAAATAAGT[-<br>/T]AAAATAATAAACACAATTAATAAAG |
| ASPA | 443 | rs34109510 | TGTATACATGTGCCATGTTGGTGTGC[C/T]GCACCCATTAACTCGTCATTT<br>AGCA |
| ASPA | 443 | rs34054576 | TCACCTGTCACCTCCTATAGAACTTT[-<br>/C]CCCTGACCCTCCTCTATAGCATTAA |
| ASPA | 443 | rs34015272 | ATAAATGATCATCATTCACAGTAGGG[-<br>/G]TTTTGTTTTGTTTTTTTTCTGGAAA |
| ASPA | 443 | rs34002091 | ACAGACATATCTACAAACACACTTTT[-<br>/T]CACATATTTGTGTAAGTCATTTATG |
| ASPA | 443 | rs28940574 | AAAGACAACTAAACTAACGCTCAATG[A/C]AAAAAGTATTCGCTGCTGT<br>TTACAT |
| ASPA | 443 | rs28940279 | TACCGTGTACCCCGTGTTTGTGAATG[A/C]GGCCGCATATTACGAAAAGA<br>AAGAA |
| ASPA | 443 | rs17850703 | CAGGGCTGGAGGTAAAACCATTTATT[A/G]CTAACCCCAGAGCAGTGAA<br>GAAGTG |
| ASPA | 443 | rs17222495 | TTCTTCATTGCCTATTGAAGAGAGAG[C/T]GGAATGCTTTGGTTGCCAGA<br>TATGG |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| ASPA | 443 | rs17175228 | CACAAGATCTCATTACTCAGGAGCTG[C/T]CCAAGTGTCTAATGTACTTAGTTAA |
| ASPA | 443 | rs16953074 | TTCTGTGTAACATTTCATTTAAGCAA[A/G]GGATTCGGCAAATCAAAAATTGTCA |
| ASPA | 443 | rs16953070 | TAAAACGTATTGAAGGTATTATTGAC[G/T]CTGTTGAAGCAAAGAGAACAAAACA |
| HEXA | 3073 | rs62022858 | ATCTGCTCTTCCAGTTGGATGACAAG[C/T]CTTGCTGTCTAACACCTGCTGCAGA |
| HEXA | 3073 | rs62022857 | CCATTTTTTGTTGTATTTTTTTTTTC[C/T]TGAATACTTTTTATCGCAGTTGGTT |
| HEXA | 3073 | rs62017872 | CCCTGTCTCTAAAAGAAAAAAAAAAA[A/G]AAAAAAAAAAGAAAACAAAACCCAA |
| HEXA | 3073 | rs62017871 | AGTGGCTCCAAAAAGGTCATGGAACC[C/T]CTTGAGGATGATGCAAATTGACTCT |
| HEXA | 3073 | rs61662730 | TAAAGTTACTTTTCTTTTATTGACTT[C/T]CCCTTATTTTTTAACCTTATGCTTT |
| HEXA | 3073 | rs61329913 | CAGAGTTAAAAAAAAAAAAAAAAAAA[-/A]GGAAGTAGCAGCAACAGCTTGGAAA |
| HEXA | 3073 | rs60920713 | GTTGCCCAGGGTTGAGTGCAGAGGCA[C/T]ATCTTGGCTCACAGCAACCTCTGCC |
| HEXA | 3073 | rs60783213 | AAGGCTTTTTTTTTTTTTTTTTTTTT[-/TTTT]GAGACAGAGTCTTGCTGTGTCACCC |
| HEXA | 3073 | rs60644867 | GCCTACATTCTGCAAAGAGGAGGGAA[C/G]ATTCACAGCTCCATACTTGAACCCT |
| HEXA | 3073 | rs60288568 | CCAAAGGAGAATAGCTCTAGGGGAGG[C/G]AGGTGGATGAGTATGCATGGGGGAG |
| HEXA | 3073 | rs59888548 | GACTCCATCTCAAAAAAAAAAAAAAA[-/A]TGCAGTCTAATGGCAGAATTAGACT |
| HEXA | 3073 | rs59733856 | TTATTTATTTATTTATTTATTTTTGA[A/G]ACAGGGTCTCTGTTGTCCAGGCTGG |
| HEXA | 3073 | rs59427837 | TTTTGAGGCAGGGTCTCACTCTGTTG[C/T]CCAGGGTTGAGTGCAGAGGCACATC |
| HEXA | 3073 | rs59171976 | CGCCTTGCGAAGGCCCCACAGCTTGC[C/T]TGTGACAAACGTTCATAGGCAAATG |
| HEXA | 3073 | rs58706602 | GGAGGTCTGTACAAAGCACCACCTAC[C/T]TCATGGGTCAGTTTCCACAGCAGAA |
| HEXA | 3073 | rs58696963 | GAATCTTATAATTCACTGTGTACCTC[-/CCTC]TGTTTCATATTTTCGCAATTGAACT |
| HEXA | 3073 | rs58610850 | AACATAGTATCTAATATAGCTTTACA[C/T]CCAAAGCCAAAATATGAATA |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| | | | CACTG |
| HEXA | 3073 | rs58016062 | TTGTTTTGTTTTGTTTGGGGGGGGGG[-/G]TTGTTTTTCTGAGAGGGAGTCTTGC |
| HEXA | 3073 | rs57733983 | CATACCAAAGGGCAGCTGGAGGGATA[C/T]AGACGGAAGTCATGTGGAGAGTGAA |
| HEXA | 3073 | rs57476645 | CAGGTGTGAGCCACCACGACCACCAA[A/T]TTAGCTCTTTTTACTCCTTCCCTTC |
| HEXA | 3073 | rs56870003 | AGTGGTAGCTGATTTTGCTTCTGGAT[A/C]CTTTGCCACCTTCCCACTCTTTAAT |
| HEXA | 3073 | rs56338339 | AAAGACCTGTTTCTTAAAAAAAAAAA[-/AGAAAAAAAAAAA]GAAAGAAAAGAAAAGAAAAAAACAG |
| HEXA | 3073 | rs55995352 | TAAAAAATCTTTCAATGAGGAGATGT[C/T]CCCAGAGCAAGACAGCTGTAGGATG |
| HEXA | 3073 | rs55860138 | AAAAGAAAAAAAAAAAAAAAAAAAA[-/A]GAAAACAAAACCCAAACCCATAAAG |
| HEXA | 3073 | rs55743646 | CCTGTCTCTAAAAGAAAAAAAAAAAA[A/G]AAAAAAAAAGAAAACAAAACCCAAA |
| HEXA | 3073 | rs55665666 | GTTATCATAGAAAAATATCACACTCT[-/GT]CTGTATCCCCACTTCCAGAAACTGT |
| HEXA | 3073 | rs36106892 | CAGGAGCTCATAGAATTACATACAAT[-/C]TTTTTTTTTTTTTTTTGAGACAGCG |
| HEXA | 3073 | rs36091525 | TTGAGAATCTTATAATTCACTGTGTA[-/CCTC]CCTCTGTTTCATATTTTCGCAATTG |
| HEXA | 3073 | rs35949555 | CCACTACCACAGTGCCTAGAGAACAA[C/T]ATGTGTTTAATAATATTTAAATAAT |
| HEXA | 3073 | rs35827424 | CCCTGTCTCTAAAAGAAAAAAAAAAA[-/A]AAAAAAAAAAGAAAACAAAACCCAA |
| HEXA | 3073 | rs35729578 | CCATTATATCATTCATTTCCCACTCA[-/T]TTTCTTCATTCCAACCAAGATATAT |
| HEXA | 3073 | rs35649102 | TCCGTCTCAAAAAAAAAAAAAAAAAG[-/A]GAAAGGAATTATTCTCATGTATACA |
| HEXA | 3073 | rs35118677 | CTGGGGCAGTTAAAAAGAAAAACAAA[-/C]CCCTGGTCCCTGCCCTTGAGGAGAT |
| HEXA | 3073 | rs35005352 | CTCCAGGGTCCCATTCCAGGACCACA[-/C]GCCTGCTACCTCTGCAGCTCACTCA |
| HEXA | 3073 | rs34736306 | GGATTGACATATACCAGTTAGACGGA[-/T]TTTTTTTTTCCATAAACCAGGCTCA |
| HEXA | 3073 | rs34607939 | ACAAATAATTACTACATATCTACAAC[A/G]TTCCAGATACAGAAGAAATGGCCAA |



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| Gene name | Gene ID | SNP ID | Mutation |
|---|---|---|---|
| HEXA | 3073 | rs34496117 | TAAACACACTTGAAACATCATATAAA[-/ATG]ATATTACTACAAGACTTAACCGTAA |
| HEXA | 3073 | rs34300017 | ACACAGGTAATCCATGTTTATTATAG[-/A]AAAATGCCACATTACTCTTTATTGA |
| HEXA | 3073 | rs34206496 | AGTTATCATAGAAAAATATCACACTC[-/TG]TCTGTATCCCCACTTCCAGAAACTG |
| HEXA | 3073 | rs34110830 | AATGAACTTACAGGAAGGTAATATAT[-/G]GGAAATAAACATCTTATTGAATTTA |
| HEXA | 3073 | rs34093438 | GGACCCCTGAAAGGCACAAGACACCC[-/T]TTCAGGTTCACACTTCCTGAAAGCT |
| HEXA | 3073 | rs34085965 | CCACCAATCACCAGAGCCTTCTGCTC[A/G]GGGGTACCTGAGGGAAAACAAGCAA |
| HEXA | 3073 | rs34004907 | AAAGACTGAAAAAACATTCATAACTA[-/T]TTTTCTTGTTATCCTCGGAAATGTC |
| HEXA | 3073 | rs28942072 | TATCTTCATCTTGGAGGAGATGAGGT[C/T]GATTTCACCTGCTGGAAGTCCAACC |
| HEXA | 3073 | rs28942071 | TTGCCTATGAACGTTTGTCACACTTC[C/T]GCTGTGAGTTGCTGAGGCGAGGTGT |
| HEXA | 3073 | rs28941771 | GCTTGCTGTTGGATACATCTCGCCAT[C/T]ACCTGCCACTCTCTAGCATCCTGGA |
| HEXA | 3073 | rs28941770 | CCGGGGCTTGCTGTTGGATACATCTC[G/T]CCATTACCTGCCACTCTCTAGCATC |

Examples

**Example 1: Use of differentiator tag sequences to detect and correct bias in a MIP-capture reaction of a set of exon targets**

The first step in performing the detection/correction is to determine how many differentiator tag sequences are necessary for the given sample. In this example, 1000 genomic targets corresponding to 1000 exons were captured. Since the differentiator tag sequence is part of the probe, it will measure/report biases that occur from the earliest protocol steps. Also, being located in the backbone, the differentiator tag sequence can easily be sequenced from a separate priming site, and therefore not impact the total achievable read-length for the target sequence. MIP probes are synthesized using standard column-based oligonucleotide synthesis by any number of vendors (e.g. IDT), and differentiator tag sequences are introduced as 'degenerate' positions in the backbone. Each degenerate

position increases the total number of differentiator tag sequences synthesized by a factor of 4, so a 10 nt degenerate region implies a differentiator tag sequence complexity of ~1e6 species.

*Hybridize capture probes to human genomic sample*

Assemble hybridization reaction:

- 1.0 ul capture probe mix (~2.5 pmol)
- 2.0 ul 10X Ampligase buffer (Epicentre)
- 6.0 ul 500 ng/ul human genomic DNA (~16.7 fmol)
- 11 ul dII2O

In a thermal cycler, heat reaction to 95C for 5 min to denature genomic DNA, then cool to 60C. Allow to incubate at 60C for 40 hours.

*Convert hybridized probes into covalently-closed circular products containing subtargets*

Prepare fill-in / ligation reaction mixture:

- 0.25 ul 2 mM dNTP mix (Invitrogen)
- 2.5 ul 10X Ampligase buffer (Epicentre)
- 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems)
- 12.5 ul 5 U/ul Ampligase (Epicentre)
- 4.75 ul dH2O

Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60C for 10 hours.

*Purify circularized probe / subtarget products from un-reacted probes and genomic DNA*

Prepare exonuclease reaction mixture:

- 21 ul fill-in / ligation reaction product
- 2.0 ul 10X exonuclease I buffer (New England Biolabs)
- 2.0 ul 20 U/ul exonuclease I (New England Biolabs)



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- 2.0 ul 100 U/ul exonuclease III (New England Biolabs)

Incubate at 37C for 60 min, then heat-inactivate by incubating at 80C for 15 min. Immediately cool to 4C for storage.

*Amplify circular material by PCR using primers specific to the 'constant region' of the probes*

Prepare PCR mixture:

- 5.0 ul 10X Accuprime reaction buffer (Invitrogen)
- 1.5 ul 10 uM CP-2-FA (5'-GCACGATCCGACGGTAGTGT-3')
- 1.5 ul 10 uM CP-2-RA (5'-CCGTAATCGGGAAGCTGAAG-3')
- 0.4 ul 25 mM dNTP mix (Invitrogen)
- 2.0 ul heat-inactivated exonuclease reaction mix
- 1.5 ul 10X SybrGreen (Invitrogen)
- 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)
- 37.7 ul dH2O

Thermal cycle in real-time thermal cycler according to the following protocol, but stop cycling before amplification yield plateaus (generally 8-12 cycles):

1. 95C for 5 min
2. 95C for 30 sec
3. 58C for 60 sec
4. 72C for 60 sec
5. goto 2, N more times

*Prepare a shotgun next-generation sequencing library for analysis*

- Purify desired amplicon population from non-specific amplification products by gel extraction.
- Concatemerize amplicons into high-molecular weight products suitable for shearing
- Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in lenth.



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- Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

*Perform sequencing of library according to manufacturer's directions (e.g. Illumina,ABI, etc), reading both the target sequence and the differentiator tag sequence.*

*Analyze data by correcting for any biases detected by quantitation of differentiator tag sequence abundance*

Construct a table of target:differentiator tag abundances from the read data, e.g.:

| Target ID | Differentiator tag sequence ID | Count |
|---|---|---|
| 1 | 3547 | 1 |
| 2 | 4762 | 1 |
| 1 | 9637 | 1 |
| 1 | 1078 | 5 |
| 3 | 4762 | 1 |
| 1 | 2984 | 1 |

All 'count' entries should be '1', since any particular target:differentiator tag mapping will not occur more than once by chance, and therefore will only be observed if bias was present somewhere in the sample preparation process. For any target:differentiator tag combination observed more than once, all such reads are 'collapsed' into a single read before consensus basecalls are determined. This will cancel the effect of bias on consensus basecall accuracy. Figure 2 depicts a method for making diploid genotype calls in which repeat target:differentiator tag combination are collapsed.

**Example 2: Differentiator tag sequence design for MIP capture reactions**

For a set of targets, the number of differentiator tag sequences necessary to be confident (within some statistical bounds) that a certain differentiator tag sequence will not be observed more than once by chance in combination with a certain target sequence was



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determined. The total number of unique differentiator tag sequences for a certain differentiator tag sequence length is determined as $4^{(\text{Length in nucleotides of the differentiator tag sequence})}$. For a molecular inversion probe capture reaction that uses MIP probes having differentiator tag sequences, the probability of performing the capture reaction and capturing one or more copies of a target sequence having the same differentiator tag sequence is calculated as: p = 1 - [N! / (N-M)!] / [N^M], wherein N is the total number of possible unique differentiator tag sequences and M is the number of target sequence copies in the capture reaction. Thus, by varying the differentiator tag sequence length it is possible to perform a MIP capture reaction in which the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is set at a predetermined probability value.

For example, for a differentiator tag sequence of 15 nucleotides in length, there are 1,073,741,824 possible differentiator tag sequences. A MIP capture reaction in which MIP probes, each having a differentiator tag sequence of 15 nucleotides, are combined with 10000 target sequence copies (e.g., genome equivalents), the probability of capturing one or more copies of a target sequence having the same differentiator tag sequence is 0.05. In this example, the MIP reaction will produce very few (usually 0, but occasionally 1 or more) targets where multiple copies are tagged with the same differentiator tag sequence. Figure 3 depicts results of a simulation for 100000 capture reactions having 15 nucleotide differentiator tag sequences and 10000 target sequences.

**Example 3: Assessment of the probability for obtaining enough sequencing reads to make accurate base-calls at multiple independent loci, as a function of sequencing coverage.**

Monte Carlo simulations were performed to determine sequencing coverage requirements. The simulations assume 10000 genomic copies of a given locus (target) half mom alleles and half dad alleles. The simulations further assume 1% efficiency of capture for the MIP reaction. The simulation samples from a capture mix 100 times without replacement to create a set of 100 capture products. The simulation then samples from the set of 100 capture products with replacement (assuming unbiased amplification) to generate 'reads' from either mom or dad. The number of reads sampled depends on the coverage. The number of independent reads from both mom and dad necessary to make a high-quality base-call (assumed to be 10 or 20 reads) were then determined. The process was repeated 1000 times for each coverage level, and the fraction of times that enough reads from both parents were



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successfully obtained was determined. This fraction was raised to the power 1000, assuming we have 1000 independent loci that must obtain successful base-calls, plotted (See Figure 4). Result show that roughly 50x coverage is required to capture each allele >= 10x with > 0.95 probability.

It will be clear that the methods may be practiced other than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present disclosure are possible in light of the above teachings and, therefore, are within the scope of the claims. Preferred features of each aspect of the disclosure are as for each of the other aspects mutatis mutandis. The documents including patents, patent applications, journal articles, or other disclosures mentioned herein are hereby incorporated by reference in their entirety. In the event of conflict, the disclosure of present application controls, other than in the event of clear error.

What is claimed is:



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PTO/SB/17 (10-08)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number

*Effective on 12/08/2004.*
*Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).*

# FEE TRANSMITTAL
## For FY 2009

[x] Applicant claims small entity status. See 37 CFR 1.27

**TOTAL AMOUNT OF PAYMENT** ($) 110.00

| *Complete if Known* | |
|---|---|
| Application Number | Not Yet Assigned |
| Filing Date | Concurrently Herewith |
| First Named Inventor | Uri Laserson |
| Examiner Name | Not Yet Assigned |
| Art Unit | N/A |
| Attorney Docket No. | G0814.70002US00 |

**METHOD OF PAYMENT** (check all that apply)

[ ] Check [x] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[x] Deposit Account Deposit Account Number: 23/2825 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

[ ] Charge fee(s) indicated below
[ ] Charge fee(s) indicated below, **except for the filing fee**
[x] Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17
[x] Credit any overpayments

**FEE CALCULATION**

**1. BASIC FILING, SEARCH, AND EXAMINATION FEES**

| | FILING FEES | | SEARCH FEES | | EXAMINATION FEES | | |
|---|---|---|---|---|---|---|---|
| **Application Type** | **Fee ($)** | **Small Entity Fee ($)** | **Fee ($)** | **Small Entity Fee ($)** | **Fee ($)** | **Small Entity Fee ($)** | **Fees Paid ($)** |
| Utility | 330 | 165 | 540 | 270 | 220 | 110 | |
| Design | 220 | 110 | 100 | 50 | 140 | 70 | |
| Plant | 220 | 110 | 330 | 165 | 170 | 85 | |
| Reissue | 330 | 165 | 540 | 270 | 650 | 325 | |
| Provisional | 220 | 110 | 0 | 0 | 0 | 0 | 110.00 |

**2. EXCESS CLAIM FEES**

| **Fee Description** | **Fee ($)** | **Small Entity Fee ($)** |
|---|---|---|
| Each claim over 20 (including Reissues) | 52 | 26 |
| Each independent claim over 3 (including Reissues) | 220 | 110 |
| Multiple dependent claims | 390 | 195 |

| **Total Claims** | **Extra Claims** | **Fee ($)** | **Fee Paid ($)** |
|---|---|---|---|
| _____ - 20 or HP | _____ x | _____ = | _____ |

HP = highest number of total claims paid for, if greater than 20.

| **Indep. Claims** | **Extra Claims** | **Fee ($)** | **Fee Paid ($)** |
|---|---|---|---|
| _____ - 3 or HP = | _____ x | _____ = | _____ |

HP = highest number of independent claims paid for, if greater than 3.

**Multiple Dependent Claims**

| **Fee ($)** | **Fee Paid ($)** |
|---|---|
| | |

**3. APPLICATION SIZE FEE**

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

| **Total Sheets** | **Extra Sheets** | **Number of each additional 50 or fraction thereof** | **Fee ($)** | **Fee Paid ($)** |
|---|---|---|---|---|
| _____ - 100 = | _____ /50 = | _____ (round **up** to a whole number) x | _____ = | _____ |

**4. OTHER FEE(S)** **Fees Paid ($)**

Non-English Specification, $130 fee (no small entity discount)

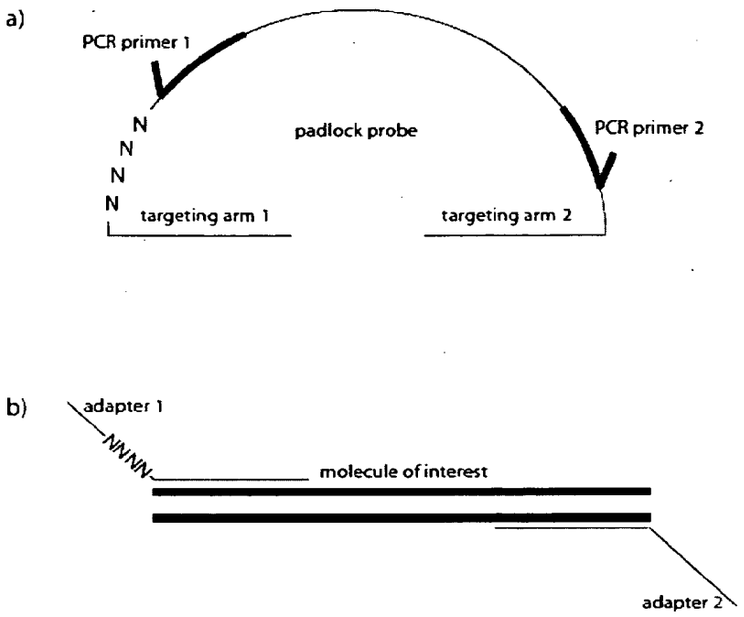
Other (e.g., late filing surcharge): _____

**SUBMITTED BY**

| | | | | | |
|---|---|---|---|---|---|
| Signature | /Patrick R.H. Waller/ | Registration No. (Attorney/Agent) | 41,418 | Telephone | 617.646.8000 |
| Name (Print/Type) | Patrick R.H. Waller | | | Date | May 18, 2009 |

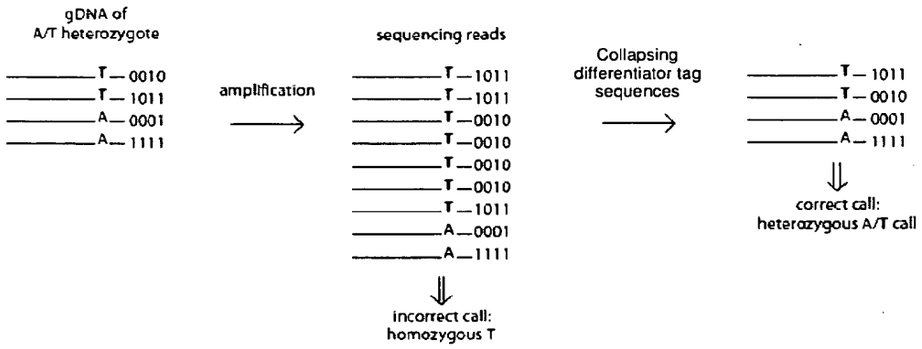
1667853.1

FIGURE 1



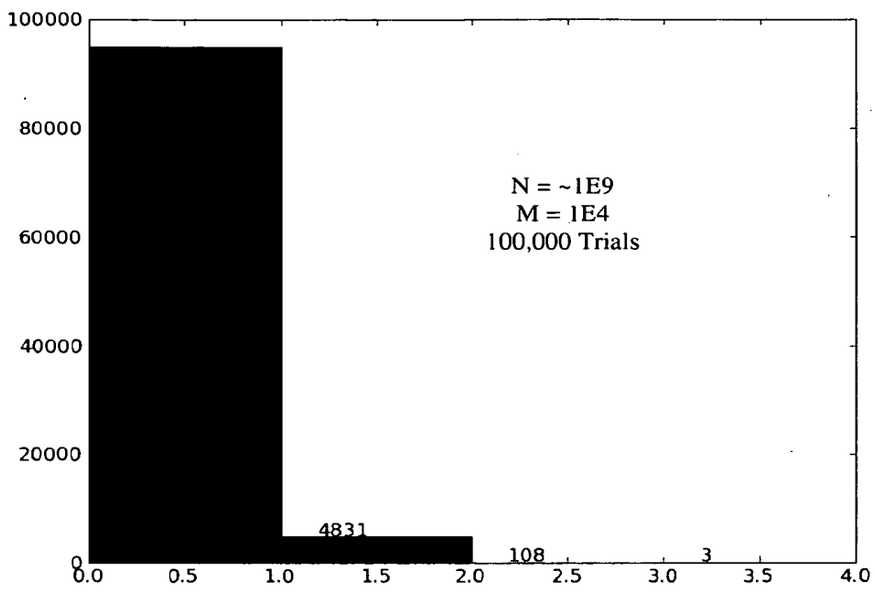

1661347.1

FIGURE 2




1661347.1

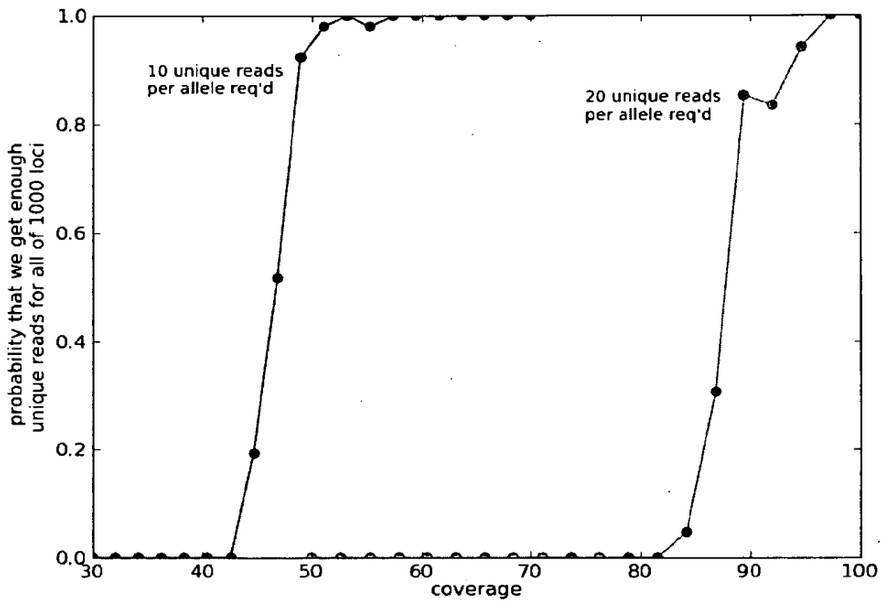
FIGURE 3




100,000 Trials

1661347.1

FIGURE 4




1661347.1

UNITED STATES PATENT AND TRADEMARK OFFICE

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| U.S. APPLICATION NUMBER NO. | FIRST NAMED APPLICANT | ATTY. DOCKET NO. |
|---|---|---|
| 13/266,862 | Gregory Porreca | GSGE-002/01US 29258/20 |

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| INTERNATIONAL APPLICATION NO. | |
|---|---|
| PCT/US10/01293 | |
| I.A. FILING DATE | PRIORITY DATE |
| 04/30/2010 | 04/30/2009 |

**CONFIRMATION NO. 6082**
**371 FORMALITIES LETTER**

*OC000000050953374*

Date Mailed: 11/15/2011

## NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as a Designated Office (37 CFR 1.494):

- Indication of Small Entity Status
- Priority Document
- Copy of the International Application filed on 10/28/2011
- Copy of the International Search Report filed on 10/28/2011
- Preliminary Amendments filed on 10/28/2011
- U.S. Basic National Fees filed on 10/28/2011
- Assignee Statement for PGPUB filed on 10/28/2011
- Priority Documents filed on 10/28/2011
- Specification filed on 10/28/2011
- Claims filed on 10/28/2011
- Abstracts filed on 10/28/2011
- Drawings filed on 10/28/2011

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.

SUMMARY OF FEES DUE:

Total additional fees required for this application is $**-155** for a Small Entity:

(A previous payment of $**155** will be applied to the additional fees indicated above.)

**ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE OR BY 32 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.**

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)



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DIAN S GORDON

Telephone: (571) 272-3915



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| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 12295130 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Franziska Salcedo |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 13-MAR-2012 |
| **Filing Date:** | |
| **Time Stamp:** | 17:34:15 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $345 |
| RAM confirmation Number | 5010 |
| Deposit Account | 500369 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

| | |
|---|---|
| Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees) | |
| Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees) | |
| Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges) | |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Applicant Response to Pre-Exam Formalities Notice | GSGE_002_01US_Response_to_NTFMP.pdf | 20456<br>d95e57448e9354c968380ccb7ea5e402b4261eec | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Oath or Declaration filed | GSGE_002_01US_Laserson_Declaration.pdf | 182351<br>9247806d0c4783fc8619173651789720 75fd4fc2 | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Oath or Declaration filed | GSGE_002_01US_Li_Declaration.pdf | 191107<br>98e78755ca26ff2c0d2515c3ddf35e5c7bebd50d | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Oath or Declaration filed | GSGE_002_01US_Porreca_Declaration.pdf | 190895<br>5f312a6519d53891004756a30b5682d377002fdf | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Oath or Declaration filed | GSGE_002_01US_Wassman_Declaration.pdf | 190056<br>4d541c03ef7d09a8d673613e18317de8c662e88b | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Fee Worksheet (SB06) | fee-info.pdf | 32201<br>da21195122a35be4d3317afb01cbc3ab235827ae | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 807066 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Attorney Docket No.: GSGE-002/01US 29258/20
Serial No.: 13/266,862

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANTS: | Porreca et al. | ART UNIT: | NOT YET ASSIGNED |
| SERIAL NUMBER: | 13/266,862 | CONFIRMATION NO.: | 6082 |
| FILING DATE: | October 28, 2011 | EXAMINER: | NOT YET ASSIGNED |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

**FILED ELECTRONICALLY**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Dear Sirs:

In response to the Notice to File Missing Parts of Non-provisional Application dated November 15, 2011, Applicants submit herewith executed Declarations that identify the above application by international application number and international filing date.

Applicants respectfully petition for and request a two-month extension of time to bring the response deadline up to and including Thursday, March 15, 2012. Applicants hereby authorize the extension fee and any other fees associated with this response to be charged to Deposit Account No. 500369.

Dated: March 13, 2012

Respectfully submitted,
**BROWN RUDNICK LLP**

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/s/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

60271811

**Attorney Docket No: GSGE-002/01US 29258/20** **PATENT**

## DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS**

the specification of which:

(check one)

[] is attached hereto;

[X] was filed as PCT International Application No. **PCT/US2010/001293** on **April 30, 2010**, and is now United States Application Serial No. **13/266,862**, filed on **October 28, 2011**;

[] was filed as PCT International Application No. ___ on ___ and was amended under PCT Article 19 or Article 34 on ___ (if applicable);

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information which is known to me to be material to the patentability of said invention in accordance with 37 C.F.R. §1.56;

I hereby claim foreign priority benefits under 35 U.S.C. §119 and/or §365 of any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America listed below; I have also identified below any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Prior Foreign Application(s)

| COUNTRY/INTERNATIONAL | APPLICATION NUMBER | DATE OF FILING (day, month, year) | PRIORITY CLAIMED |
|---|---|---|---|
| | | | [] YES [] NO |
| | | | [] YES [] NO |

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

| | |
|---|---|
| 61/174,470 (Application Number) | 30 April 2009 (Filing Date) (day, month, year) |
| 61/178,923 (Application Number) | 15 May 2009 (Filing Date) (day, month, year) |
| 61/179,358 (Application Number) | 18 May 2009 (Filing Date) (day, month, year) |
| 61/182,089 (Application Number) | 28 May 2009 (Filing Date) (day, month, year) |

I hereby claim the benefit under 35 U.S.C. §120 and/or §365 of any United States application(s) or of any international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date(s) of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Application(s) or PCT International Applications Designating the U.S. for benefit under 35 U.S.C. §120

| U.S. APPLICATIONS | | | STATUS (*check one*) | | |
|---|---|---|---|---|---|
| U.S. APPLICATION NO. | U.S. FILING DATE (day, month, year) | | Pending | Patented | Expired |
| | | | [] | [] | [] |
| | | | [] | [] | [] |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | | | |
| PCT APPLICATION NO. | PCT FILING DATE (day, month, year) | U.S. APPLICATION NOS. (if any) | | | |
| PCT/US2010/001293 | 30 April 2010 | | [] | [] | [x] |
| | | | [] | [] | [] |



Petitioner Illumina, Inc., EX1002, p. 553

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**Full name of inventor:** Gregory Porreca

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 57 Reservoir Street, Cambridge, MA 02138

**Full name of inventor:** Uri Laserson

Inventor's signature _____ Date 2/28/12
Residence: US
Citizen of: US
Post Office Address: 645 Beacon Street #215, Boston, MA 02215

**Full name of inventor:** Jin Billy Lee

Inventor's signature _____ Date _____
Residence: US
Citizen of: CN
Post Office Address: 100 Station Landing, Unit 204, Medford, MA 02155

**Full name of inventor:** E. Robert Wassman

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 90 West Shore Drive, Marblehead, MA 01945

1864782



2/2 02-28-2012 12:38:28 6174326513

Petitioner Illumina, Inc., EX1002, p. 554

**Attorney Docket No: GSGE-002/01US 29258/20** **PATENT**

## DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS**

the specification of which:

(check one)

[] is attached hereto;

[X] was filed as PCT International Application No. **PCT/US2010/001293** on **April 30, 2010**, and is now United States Application Serial No. **13/266,862**, filed on **October 28, 2011**;

[] was filed as PCT International Application No. ___ on ___ and was amended under PCT Article 19 or Article 34 on ___ (if applicable);

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information which is known to me to be material to the patentability of said invention in accordance with 37 C.F.R. §1.56;

I hereby claim foreign priority benefits under 35 U.S.C. §119 and/or §365 of any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America listed below; I have also identified below any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Prior Foreign Application(s)

| COUNTRY/INTERNATIONAL | APPLICATION NUMBER | DATE OF FILING (day, month, year) | PRIORITY CLAIMED |
|---|---|---|---|
| | | | [] YES [] NO |
| | | | [] YES [] NO |

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

| | |
|---|---|
| 61/174,470 (Application Number) | 30 April 2009 (Filing Date) (day, month, year) |
| 61/178,923 (Application Number) | 15 May 2009 (Filing Date) (day, month, year) |
| 61/179,358 (Application Number) | 18 May 2009 (Filing Date) (day, month, year) |
| 61/182,089 (Application Number) | 28 May 2009 (Filing Date) (day, month, year) |

I hereby claim the benefit under 35 U.S.C. §120 and/or §365 of any United States application(s) or of any international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date(s) of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Application(s) or PCT International Applications Designating the U.S. for benefit under 35 U.S.C. §120

| U.S. APPLICATIONS | | | STATUS (*check one*) | | |
|---|---|---|---|---|---|
| U.S. APPLICATION NO. | U.S. FILING DATE (day, month, year) | | Pending | Patented | Expired |
| | | | [] | [] | [] |
| | | | [] | [] | [] |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | | | |
| PCT APPLICATION NO. | PCT FILING DATE (day, month, year) | U.S. APPLICATION NOS. (if any) | | | |
| PCT/US2010/001293 | 30 April 2010 | | [] | [] | [x] |
| | | | [] | [] | [] |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**Full name of inventor:** Gregory Porreca

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 57 Reservoir Street, Cambridge, MA 02138

**Full name of inventor:** Uri Laserson

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 645 Beacon Street #215, Boston, MA 02215

**Full name of inventor:** Jin Billy Li

Inventor's signature _____ Date 3/5/2012
Residence: US
Citizen of: CN
Post Office Address: 29 Peter Coutts Circle, Stanford, CA 94305

**Full name of inventor:** E. Robert Wassman

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 90 West Shore Drive, Marblehead, MA 01945

1884782



Petitioner Illumina, Inc., EX1002, p. 557

**Attorney Docket No: GSGE-002/01US 29258/20** **PATENT**

## DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS**

the specification of which:

(check one)

[] is attached hereto;

[X] was filed as PCT International Application No. **PCT/US2010/001293** on **April 30, 2010**, and is now United States Application Serial No. **13/266,862**, filed on **October 28, 2011**;

[] was filed as PCT International Application No. ___ on ___ and was amended under PCT Article 19 or Article 34 on ___ (if applicable);

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information which is known to me to be material to the patentability of said invention in accordance with 37 C.F.R. §1.56;

I hereby claim foreign priority benefits under 35 U.S.C. §119 and/or §365 of any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America listed below; I have also identified below any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Prior Foreign Application(s)

| COUNTRY/INTERNATIONAL | APPLICATION NUMBER | DATE OF FILING (day, month, year) | PRIORITY CLAIMED |
|---|---|---|---|
| | | | [] YES [] NO |
| | | | [] YES [] NO |

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

| | |
|---|---|
| 61/174,470 (Application Number) | 30 April 2009 (Filing Date) (day, month, year) |
| 61/178,923 (Application Number) | 15 May 2009 (Filing Date) (day, month, year) |
| 61/179,358 (Application Number) | 18 May 2009 (Filing Date) (day, month, year) |
| 61/182,089 (Application Number) | 28 May 2009 (Filing Date) (day, month, year) |

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Prior U.S. Application(s) or PCT International Applications Designating the U.S. for benefit under 35 U.S.C. §120

| U.S. APPLICATIONS | | | STATUS (*check one*) | | |
|---|---|---|---|---|---|
| U.S. APPLICATION NO. | U.S. FILING DATE (day, month, year) | | Pending | Patented | Expired |
| | | | [] | [] | [] |
| | | | [] | [] | [] |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | | | |
| PCT APPLICATION NO. | PCT FILING DATE (day, month, year) | U.S. APPLICATION NOS. (if any) | | | |
| PCT/US2010/001293 | 30 April 2010 | | [] | [] | [x] |
| | | | [] | [] | [] |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**Full name of inventor:** Gregory Porreca

Inventor's signature _____ Date 3/1/2012
Residence: US
Citizen of: US
Post Office Address: 57 Reservoir Street, Cambridge, MA 02138

**Full name of inventor:** Uri Laserson

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 645 Beacon Street #215, Boston, MA 02215

**Full name of inventor:** Jin Billy Lee

Inventor's signature _____ Date _____
Residence: US
Citizen of: CN
Post Office Address: 100 Station Landing, Unit 204, Medford, MA 02155

**Full name of inventor:** E. Robert Wassman

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 90 West Shore Drive, Marblehead, MA 01945

1664782



Petitioner Illumina, Inc., EX1002, p. 560

**Attorney Docket No: GSGE-002/01US 29258/20** **PATENT**

## DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS**

the specification of which:

(check one)

[] is attached hereto;

[X] was filed as PCT International Application No. **PCT/US2010/001293** on **April 30, 2010**, and is now United States Application Serial No. **13/266,862**, filed on **October 28, 2011**;

[] was filed as PCT International Application No. ___ on ___ and was amended under PCT Article 19 or Article 34 on ___ (if applicable);

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information which is known to me to be material to the patentability of said invention in accordance with 37 C.F.R. §1.56;

I hereby claim foreign priority benefits under 35 U.S.C. §119 and/or §365 of any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America listed below; I have also identified below any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Prior Foreign Application(s)

| COUNTRY/INTERNATIONAL | APPLICATION NUMBER | DATE OF FILING (day, month, year) | PRIORITY CLAIMED |
|---|---|---|---|
| | | | [] YES [] NO |
| | | | [] YES [] NO |

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

| | |
|---|---|
| 61/174,470 (Application Number) | 30 April 2009 (Filing Date) (day, month, year) |
| 61/178,923 (Application Number) | 15 May 2009 (Filing Date) (day, month, year) |
| 61/179,358 (Application Number) | 18 May 2009 (Filing Date) (day, month, year) |
| 61/182,089 (Application Number) | 28 May 2009 (Filing Date) (day, month, year) |

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Prior U.S. Application(s) or PCT International Applications Designating the U.S. for benefit under 35 U.S.C. §120

| U.S. APPLICATIONS | | | STATUS (*check one*) | | |
|---|---|---|---|---|---|
| U.S. APPLICATION NO. | U.S. FILING DATE (day, month, year) | | Pending | Patented | Expired |
| | | | [] | [] | [] |
| | | | [] | [] | [] |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | | | |
| PCT APPLICATION NO. | PCT FILING DATE (day, month, year) | U.S. APPLICATION NOS. (if any) | | | |
| PCT/US2010/001293 | 30 April 2010 | | [] | [] | [x] |
| | | | [] | [] | [] |



Petitioner Illumina, Inc., EX1002, p. 562

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**Full name of inventor:** Gregory Porreca

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 57 Reservoir Street, Cambridge, MA 02138

**Full name of inventor:** Uri Laserson

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 645 Beacon Street #215, Boston, MA 02215

**Full name of inventor:** Jin Billy Lee

Inventor's signature _____ Date _____
Residence: US
Citizen of: CN
Post Office Address: 100 Station Landing, Unit 204, Medford, MA 02155

**Full name of inventor:** E. Robert Wassman

Inventor's signature _____ Date 3/6/12
Residence: US
Citizen of: US
Post Office Address: 90 West Shore Drive, Marblehead, MA 01945

1884782



Petitioner Illumina, Inc., EX1002, p. 563

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 13266862 |
| **Filing Date:** | |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Filer:** | Thomas C. Meyers/Franziska Salcedo |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |

Filed as Small Entity

## U.S. National Stage under 35 USC 371 Filing Fees

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| Oath/decl > 30 mo. from priority date | 2617 | 1 | 65 | 65 |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| Extension - 2 months with $0 paid | 2252 | 1 | 280 | 280 |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **345** |

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| U.S. APPLICATION NUMBER NO. | FIRST NAMED APPLICANT | ATTY. DOCKET NO. |
|---|---|---|
| 13/266,862 | Gregory Porreca | GSGE-002/01US 29258/20 |

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| INTERNATIONAL APPLICATION NO. | |
|---|---|
| PCT/US10/01293 | |
| I.A. FILING DATE | PRIORITY DATE |
| 04/30/2010 | 04/30/2009 |

**CONFIRMATION NO. 6082**
**371 ACCEPTANCE LETTER**

*OC000000053210207*

Date Mailed: 03/20/2012

## NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

| 03/13/2012 | 03/13/2012 |
|---|---|
| DATE OF RECEIPT OF 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) REQUIREMENTS | DATE OF COMPLETION OF ALL 35 U.S.C. 371 REQUIREMENTS |

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Indication of Small Entity Status
- Copy of the International Application filed on 10/28/2011
- Copy of the International Search Report filed on 10/28/2011
- Preliminary Amendments filed on 10/28/2011
- Oath or Declaration filed on 03/13/2012
- U.S. Basic National Fees filed on 10/28/2011
- Assignee Statement for PGPUB filed on 10/28/2011
- Priority Documents filed on 10/28/2011
- Specification filed on 10/28/2011
- Claims filed on 10/28/2011
- Abstracts filed on 10/28/2011
- Drawings filed on 10/28/2011



FORM PCT/DO/EO/903 (371 Acceptance Notice)

Petitioner Illumina, Inc., EX1002, p. 566

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

BRIAN A HARDEN

Telephone: (703) 756-1815



FORM PCT/DO/EO/903 (371 Acceptance Notice)

Petitioner Illumina, Inc., EX1002, p. 567

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | | 1230 | GSGE-002/01US 29258/20 | 39 | 3 |

**CONFIRMATION NO. 6082**

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

**FILING RECEIPT**

*OC000000053210206*

Date Mailed: 03/20/2012

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Applicant(s)**

Gregory Porreca, Cambridge, MA;
Uri Laserson, Boston, MA;
Jin Billy Lee, Medford, MA;
E. Robert Wassman, Marblehead, MA;

**Assignment For Published Patent Application**

GOOD START GENETICS, INC., Cambridge, MA

**Power of Attorney:**

Thomas Meyers--36989

**Domestic Priority data as claimed by applicant**

This application is a 371 of PCT/US10/01293 04/30/2010
which claims benefit of 61/174,470 04/30/2009
and claims benefit of 61/174,923 05/01/2009
and claims benefit of 61/179,358 05/18/2009
and claims benefit of 61/182,089 05/28/2009

**Foreign Applications** (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

**If Required, Foreign Filing License Granted:** 03/19/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/266,862**

**Projected Publication Date:** 06/28/2012

**Non-Publication Request:** No



Petitioner Illumina, Inc., EX1002, p. 568

**Early Publication Request:** No
**** SMALL ENTITY ****
**Title**

METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

**Preliminary Class**

## PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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## Title 37, Code of Federal Regulations, 5.11 & 5.15

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The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

**NOT GRANTED**

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| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 12513774 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Robin Wilkes |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 11-APR-2012 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 14:34:09 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| Submitted with Payment | no |
|---|---|

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Request for Corrected Filing Receipt | GSGE-002-01US-Request-for-Corrected-Filing-Receipt-041112.pdf | 17290<br>42a4220db910e758c15af50103bc4f1192d5410b | no | 1 |

| **Warnings:** |
|---|
| **Information:** |

| | | | | | |
|---|---|---|---|---|---|
| 2 | Request for Corrected Filing Receipt | GSGE-002-Amendments-to-Filing-Receipt-041012.PDF | 167516<br>2c40e66f5032fcbd1a5c1e6586a314a663fddb41 | no | 3 |

| | |
|---|---|
| **Warnings:** | |
| **Information:** | |
| **Total Files Size (in bytes):** | 184806 |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Attorney Docket No.: GSGE-002/01US 29258/20

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANTS: | Porreca, Gregory, et al. | ART UNIT: | - |
| SERIAL NUMBER: | 13/266,862 | CONFIRMATION NO.: | 6082 |
| FILING DATE: | March 13, 2012 | EXAMINER: | - |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

**FILED ELECTRONICALLY**

**REQUEST FOR CORRECTION OF FILING RECEIPT**

Sir:

An error is noted on the Filing Receipt that was mailed from the Office on March 20, 2012, for the above-referenced patent application.

The name of the third inventor should read **Jin Billy Li** as shown under Applicant 3 on the Application Data Sheet submitted with the application to the USPTO on October 29, 2011, and not "Jin Billy Lee", as was shown on the March 20, 2012, Filing Receipt. A marked-up copy of the Filing Receipt showing the requested change accompanies this submission.

Applicants kindly request that the Office correct the inventor's name and issue a corrected filing receipt. Applicants believe that no fees are required for this action.

Dated: April 11, 2012

Respectfully submitted,
BROWN RUDNICK LLP

/Thomas C. Meyers/
Thomas C. Meyers Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: 617-856-8124
Fax: 617-856-8201
60453595

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | | 1230 | GSGE-002/01US 29258/20 | 39 | 3 |

**CONFIRMATION NO. 6082**

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

**FILING RECEIPT**

*OC000000053210206*

Date Mailed: 03/20/2012

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Applicant(s)**

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Uri Laserson, Boston, MA;
Jin Billy Li ~~Jin Billy Lee~~, Medford, MA;
E. Robert Wassman, Marblehead, MA;

**Assignment For Published Patent Application**

GOOD START GENETICS, INC., Cambridge, MA

**Power of Attorney:**

Thomas Meyers--36989

**Domestic Priority data as claimed by applicant**

This application is a 371 of PCT/US10/01293 04/30/2010
which claims benefit of 61/174,470 04/30/2009
and claims benefit of 61/174,923 05/01/2009
and claims benefit of 61/179,358 05/18/2009
and claims benefit of 61/182,089 05/28/2009

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**If Required, Foreign Filing License Granted:** 03/19/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/266,862**

**Projected Publication Date:** 06/28/2012

**Non-Publication Request:** No



Petitioner Illumina, Inc., EX1002, p. 574

**Early Publication Request:** No
**** SMALL ENTITY ****
**Title**

METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

**Preliminary Class**

## PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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## Title 37, Code of Federal Regulations, 5.11 & 5.15

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**NOT GRANTED**

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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www.uspto.gov

| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | 1636 | 1230 | GSGE-002/01US 29258/20 | 39 | 3 |

**CONFIRMATION NO. 6082**

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

**CORRECTED FILING RECEIPT**

*OC000000053695045*

Date Mailed: 04/12/2012

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

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E. Robert Wassman, Marblehead, MA;

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**Power of Attorney:**

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**Projected Publication Date:** 06/28/2012

**Non-Publication Request:** No



Petitioner Illumina, Inc., EX1002, p. 577

**Early Publication Request:** No
**** SMALL ENTITY ****
**Title**

METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

**Preliminary Class**

506

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Petitioner Illumina, Inc., EX1002, p. 578

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| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|---|---|---|---|
| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 |

**CONFIRMATION NO. 6082**

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

**PUBLICATION NOTICE**

*OC000000055103360*

**Title:**METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

**Publication No.**US-2012-0165202-A1
**Publication Date:**06/28/2012

## NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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Petitioner Illumina, Inc., EX1002, p. 580

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 | 6082 |

21710 7590 11/08/2012

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| EXAMINER | |
|---|---|
| POHNERT, STEVEN C | |
| ART UNIT | PAPER NUMBER |
| 1634 | |

| MAIL DATE | DELIVERY MODE |
|---|---|
| 11/08/2012 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 13/266,862 | PORRECA ET AL. | |
| | Examiner | Art Unit | |
| | STEVEN POHNERT | 1634 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *1* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *10/28/2011*.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

5)☒ Claim(s) *1-4,12-16,20-30,33,36,37,41-47 and 50-56* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *1-4,12-16,20-30,33,36,37,41-47 and 50-56* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.



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***Election/Restrictions***

1. Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group 1, claim(s) 1-4, drawn to methods of analyzing a plurality of genetic loci.

Group 2, claim(s) 12-16, drawn to methods of genotyping a subject.

Group 3, claim(s) 20(in part), 21 (in part) 22, 23, 26, and 56 (in part) drawn to methods of determining if a target nucleic acid has an insertion.

Group 4, claim(s) 20(in part), 21 (in part), 24, 25, 27, 56 (in part) drawn to methods of determining if a target nucleic acid has an deletion.

Group 5, claim(s) 20(in part), 28(in part), 29 -30, 33,36, 41-43 drawn to methods of determining if a target nucleic acid has abnormal length using molecular inversion probes.

Group 6, claim(s) 20(in part), 28(in part), 37-38 and 40, drawn to methods of determining if a target nucleic acid has nucleotide repeat.

Group 7, claim(s) 20(in part), 44-47, drawn to methods of determining if a target nucleic acid has an abnormal length in a biological sample.

Group 8, claim(s) ) 20(in part), 50-55, drawn to methods of determining if a target nucleic acid has an abnormal length by determining amount of target nucleic acid captured.

2. The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract;

para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface).

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention or species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention or species.

Should applicant traverse on the ground that the inventions have unity of invention (37 CFR 1.475(a)), applicant must provide reasons in support thereof. Applicant may submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. Where such evidence or admission is provided by applicant, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

3. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-5:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634

| Notice of References Cited | Application/Control No. 13/266,862 | Applicant(s)/Patent Under Reexamination PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | Classification |
|---|---|---|---|---|---|
| * | A | US-2001/0046673 | 11-2001 | French et al. | 435/6 |
| | B | US- | | | |
| | C | US- | | | |
| | D | US- | | | |
| | E | US- | | | |
| | F | US- | | | |
| | G | US- | | | |
| | H | US- | | | |
| | I | US- | | | |
| | J | US- | | | |
| | K | US- | | | |
| | L | US- | | | |
| | M | US- | | | |

**FOREIGN PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Country | Name | Classification |
|---|---|---|---|---|---|---|
| | N | | | | | |
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**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
|---|---|---|
| | U | |
| | V | |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.



Petitioner Illumina, Inc., EX1002, p. 587

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 14362658 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Thomas Hellenthal |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 03-DEC-2012 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 09:39:10 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

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|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | | GSGE_002_01US_Response_to_restriction_requirement.pdf | 39090<br>772393c404986e02ed230be3e422837869a32ffd | yes | 10 |

| | Multipart Description/PDF files in .zip description | | |
|---|---|---|---|
| | **Document Description** | **Start** | **End** |
| | Response to Election / Restriction Filed | 1 | 1 |
| | Specification | 2 | 2 |
| | Claims | 3 | 9 |
| | Applicant Arguments/Remarks Made in an Amendment | 10 | 10 |
| **Warnings:** | | | |
| **Information:** | | | |
| | **Total Files Size (in bytes):** | 39090 | |

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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Attorney Docket No.: GSGE-002/01US 29258/20
Serial No.: 13/266,862

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANT: | Porreca et al. | ART UNIT: | 1634 |
| SERIAL NUMBER: | 13/266,862 | CONFIRMATION NO.: | 6082 |
| FILING DATE: | March 13, 2012 | EXAMINER: | Pohner, Steven C. |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

**FILED ELECTRONICALLY**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

## RESPONSE TO RESTRICTION REQUIREMENT

This paper is in response to a Restriction Requirement mailed November 8, 2012 from the United States Patent and Trademark Office.

Applicant believes that no fees are due with this Response. However, Applicant hereby authorizes any and all required fees due with this response to be charged to Deposit Account No. 500369.

**Amendments to the specification** begin on page 2.

**Amendments to the claims** begin on page 3.

**Remarks** begin on page 10.

Amendments to the specification

No amendments to the specification are made herein.



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Amendment to the claims

Please withdraw claims 16-16, 20-30, 33, 36-38, 40-47, and 50-56 as follows. This list of claims replaces all prior versions and listings of claims in this application.

1. (Original) A method of analyzing a plurality of genetic loci, the method comprising:

contacting each of a plurality of target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion,

isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

2. (Original) The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion.

3. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.

4. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5-11. (Canceled)



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12. (Withdrawn) A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. (Withdrawn) The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

14. (Withdrawn-Previously presented) The method of claim 12, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

15. (Withdrawn-Previously presented) The method of claim 12, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. (Withdrawn-Previously presented) The method of claim 12, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

17-19. (Canceled)

20. (Withdrawn) A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. (Withdrawn) The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is lower than the reference capture efficiency.

23. (Withdrawn) The method of claim 22, wherein the subject is identified as having an insertion in the target region.

24. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is higher than the reference capture efficiency.

25. (Withdrawn) The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. (Withdrawn) The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.



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27. (Withdrawn) The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. (Withdrawn-Previously presented) The method of claim 20, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. (Withdrawn) The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

30. (Withdrawn) The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31-32. (Canceled)

33. (Withdrawn-Previously presented) The method of claim 29, wherein the first and second targeting arms have the same length.

34-35. (Canceled)

36. (Withdrawn-Previously presented) The method of claim 29, wherein the hybridization Tms of the first and second targeting arms are identical.

37. (Withdrawn-Previously presented) The method of claim 28, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. (Withdrawn) The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. (Canceled)

40. (Withdrawn) The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.

42. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.

43. (Withdrawn-Previously presented) The method of claim 29, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. (Withdrawn-Previously presented) The method of claim 20, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. (Withdrawn) The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. (Withdrawn) The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. (Withdrawn) The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48-49. (Canceled)

50. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. (Withdrawn) The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.

52. (Withdrawn-Previously presented) The method of claim 50, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.

53. (Withdrawn) The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.



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55. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. (Withdrawn-Previously presented) The method of claim 20, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57-67 (Canceled)



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Remarks

In response to the Restriction Requirement mailed November 8, 2012, Applicants elect Group I (claims 1-4) drawn to methods of analyzing a plurality of genetic loci. Accordingly, claims 16-16, 20-30, 33, 36-38, 40-47, and 50-56 are withdrawn.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: December 3, 2012

Respectfully submitted,
**BROWN RUDNICK LLP**

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/s/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

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Document code: WFEE

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 | 6082 |

21710 7590 01/10/2013

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| EXAMINER | |
|---|---|
| POHNERT, STEVEN C | |
| **ART UNIT** | **PAPER NUMBER** |
| 1634 | |

| MAIL DATE | DELIVERY MODE |
|---|---|
| 01/10/2013 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| *Office Action Summary* | **Application No.** 13/266,862 | **Applicant(s)** PORRECA ET AL. | |
|---|---|---|---|
| | **Examiner** STEVEN POHNERT | **Art Unit** 1634 | |

***-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --***

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE ____ MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

5)☐ Claim(s) _____ is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.



Petitioner Illumina, Inc., EX1002, p. 602

## DETAILED ACTION

### *Election/Restrictions*

1. Applicant's election of group 1, claims 1-4, in the reply filed on 12/3/2012 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 12-16, 20-30, 33, 36-38, 40-47 and 50-56 withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention , there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 12/3/2012.

### *Priority*

The instant application was filed 3/13/2012 and is a national stage entry of PCT?US10/01293 filed 4/30/2010 and claims priority to provisional application 61/147,470 filed 4/30/2009; provisional application 61/147923 filed 5/1/2009; provisional application 61179358 filed 5/18/2009; and 61/182,089 filed 5/28/2009.

### *Drawings*

2. The drawings are objected to because the figures are not labeled consistent with 37 CFR 1.84 (u) (1) which requires, "The different views must be numbered in consecutive Arabic numerals, starting with 1,independent of the numbering of the sheets and, if possible, in the order in which they appear on the drawing sheet(s). Partial views intended to form one complete view, on one or several sheets, must be identified by the same number followed by a capital letter. View numbers must be

preceded by the abbreviation "FIG. " Where only a single view is used in an application to illustrate the claimed invention, it must not be numbered and the abbreviation "FIG. " must not appear.. Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

***Claim Rejections - 35 USC § 112***

3. Claims 1-4 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The Board has held, “If a claim is amenable to two or more plausible constructions, applicant is required to amend the claim to more precisely define the metes and bounds of the claimed invention or the claim is indefinite under §112, ¶2.” Ex parte Miyazaki, 89 USPQ2d 1207 (BPAI 2008) (expanded panel).

Claim 1 recites the limitation "the same strand of one of the plurality of subregions of the target nucleic acid" in 4th line. There is insufficient antecedent basis for this limitation in the claim as the claim does not previously recite,” same strand of one of the plurality of subregions of the target nucleic acid.” The claim previously recited, “target nucleic acids” thus it is unclear if "the same strand" is intended to be limited to a single target nucleic acid or all the target nucleic acids. Further 3’ region and 5’ region appear to be relative terms. It is unclear if the 5’ region ( or 3’ region) is intended to require the 5' terminal (or 3’ terminal) nucleotide of the probe or merely any nucleotide that is 5’( or 3’) of the central region.

Claims 2-4 are rejected as they depend from claim 1.

Claim 3 recites, “wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.” The recitation appears an attempt to exclude the probes from overlapping, however claim 1 defines the probes with respect to the target nucleic acid and requires, " wherein each subregion overlaps with at least one other subregion.” Thus claim 1 appears to require the probes are to overlapping subregions of the target nucleic acid, which appears to require the probes overlap. Thus it is unclear how claim 1 can require the probes to be overlapping and claim 3 requires the the probes are non-

overlapping. Further it is unclear if non-overlapping is intended to exclude a single nucleotide that is identical, 2 identical nucleotides, 10 identical nucleotides, etc.

***Claim Rejections - 35 USC § 102***

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 1-2 and 4 are rejected under 35 U.S.C. 102(b) as being anticipated by Chee et al (WO 95/11995).

This rejection is presented in view of the 112-2nd paragraph issues.

With regards to claim 1, Chee teaches, “The invention provides a number of strategies for comparing a polynucleotide of known sequence (a reference sequence) with variants of that sequence (target sequences). The comparison can be performed at the level of entire genomes, chromosomes, genes, exons or introns, or can focus on individual mutant sites and immediately adjacent bases” (page 18). Chee teaches the use of an array of capture probes (see figure 16,and page 79 lines 23-39) and block tiling arrays to determine nucleic acid sequences (see Figure7 and page 37 line 10-page 38 line 34). The tiling array of Chee has probes that are complementary to a 3' and 5' region relative to an interrogation site of target nucleic acid and the subregions overlap. Chee teaches hybridization of the nucleic acids to an array and analyzing images.

With regards to claim 2, Chee teaches the subregions completely overlap.

With regards to claim 4, Chee teaches each probe of the figure 13 is different in the 3' region and 5' region from all other probes.

***Claim Rejections - 35 USC § 103***

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 1-4 rejected under 35 U.S.C. 103(a) as being unpatentable over Hardenbohl et al (Nature Biotechnology(2003) volume 21, pages 673-678) in view of Mockler et al (Genomics (2005) volume 85, pages 1-15).

This rejection is presented in view of the 112-2$^{nd}$ paragraph issues.

With regards to claim 1, Hardenbol discloses a method of analyzing a plurality of genetic loci by contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1).

Hardenbohl does not specifically teach wherein each subregion overlaps with at least one other subregion. Hardenbohl does not specifically teach, "wherein at least one subregion entirely overlaps at least one subregion."

Mockler, teaches each subregion overlaps with at least one other subregion (Fig. 2a).

With regards to claim 2, Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other Subregion)).

With regards to claim 3, Mockler teaches the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

With regards to claim 4, Mockler teaches sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the tiling probes of Mockler in the method of Hardenbol. The artisan would be motivated as Mockler teaches the probe tiling approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1). The artisan would

have a reasonable expectation of success as the artisan is merely combining known probes and methods for analysis of genetic information.

8. Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (WO 95/11995) in view of Mockler et al (Genomics (2005) volume 85, pages 1-15).

This rejection is presented in view of the 112-2nd paragraph issues.

With regards to claim 1, Chee teaches, “The invention provides a number of strategies for comparing a polynucleotide of known sequence (a reference sequence) with variants of that sequence (target sequences). The comparison can be performed at the level of entire genomes, chromosomes, genes, exons or introns, or can focus on individual mutant sites and immediately adjacent bases” (page 18). Chee teaches the use of an array of capture probes (see figure 16,and page 79 lines 23-39) and block tiling arrays to determine nucleic acid sequences (see Figure7 and page 37 line 10-page 38 line 34). The tiling array of Chee has probes that are complementary to a 3' and 5' region relative to an interrogation site of target nucleic acid and the subregions overlap. Chee teaches hybridization of the nucleic acids to an array and analyzing images.

With regards to claim 2, Chee teaches the subregions completely overlap.

With regards to claim 4, Chee teaches each probe of the figure 13 is different in the 3’ region and 5’ region from all other probes.

Chee does not specifically teach “wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.”

However, Mockler teaches the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use to the true whole genome tiling array of Mockler in the methods of Chee. The artisan would be merely substituting one art accepted tiling strategy for another art accepted tiling strategy. The artisan would have a reasonable expectation of success as the artisan is merely substituting one art accepted tiling strategy for another art accepted tiling strategy.

**Summary**

No claims are allowed.

***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-5:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner’s supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634

| Notice of References Cited | Application/Control No. 13/266,862 | Applicant(s)/Patent Under Reexamination PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | Classification |
|---|---|---|---|---|---|
| | A | US- | | | |
| | B | US- | | | |
| | C | US- | | | |
| | D | US- | | | |
| | E | US- | | | |
| | F | US- | | | |
| | G | US- | | | |
| | H | US- | | | |
| | I | US- | | | |
| | J | US- | | | |
| | K | US- | | | |
| | L | US- | | | |
| | M | US- | | | |

**FOREIGN PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Country | Name | Classification |
|---|---|---|---|---|---|---|
| | N | WO 9511995 A1 | 05-1995 | World Intellect | CHEE et al. | |
| | O | | | | | |
| | P | | | | | |
| | Q | | | | | |
| | R | | | | | |
| | S | | | | | |
| | T | | | | | |

**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
|---|---|---|
| | U | Hardenbohl et al (Nature Biotechnology(2003) volume 21, pages 673-678) |
| | V | Mockler et al (Genomics (2005) volume 85, pages 1-15) |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.



Petitioner Illumina, Inc., EX1002, p. 612

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 | 6082 |

21710 7590 03/26/2013

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| EXAMINER | |
|---|---|
| POHNERT, STEVEN C | |
| ART UNIT | PAPER NUMBER |
| 1634 | |

| MAIL DATE | DELIVERY MODE |
|---|---|
| 03/26/2013 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| ***Examiner-Initiated Interview Summary*** | **Application No.** 13/266,862 | **Applicant(s)** PORRECA ET AL. | |
|---|---|---|---|
| | **Examiner** STEVEN POHNERT | **Art Unit** 1634 | |

All participants (applicant, applicant's representative, PTO personnel):

(1) *STEVEN POHNERT*. (3)_____.

(2) *Adam Schoen*. (4)_____.

Date of Interview: *18 March 2013*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☐ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *none*.

Identification of prior art discussed: *none*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

*Applicant's representative contacted the examiner about the objection to the drawings. The examiner indicated the statute requires,"FIG." not "figure" as the filed figures present. The representative concurred based on the objection. No discussion of patentability occurred.* .

**Applicant recordation instructions**: It is not necessary for applicant to provide a separate record of the substance of interview.

**Examiner recordation instructions**: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

| /Steven C Pohnert/<br>Primary Examiner, Art Unit 1634 | |
|---|---|

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 15377707 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Kelley Froehlich |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 28-MAR-2013 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 14:29:01 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $90 |
| RAM confirmation Number | 845 |
| Deposit Account | 500369 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

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|---|---|
| Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees) | |
| Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees) | |
| Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges) | |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Non Patent Literature | P12159EP00_D5_1.PDF | 1645412<br>cd0486c592e0a9645577e0c255d59b4f8c027285 | no | 6 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Non Patent Literature | P12159EP00_D6_2.PDF | 2371834<br>4efaa7ee467cc86ee4981a6ee0c62bf6b49db3de | no | 10 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Non Patent Literature | P12159EP00_D8_3.PDF | 1252685<br>444c0eb5e4f1a036710f93fcb99889a6997c8085 | no | 6 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Non Patent Literature | 61137186_1-P12159EP00_D1_4.PDF | 2269150<br>81774057f3b9b703a7d478c1c29b069625133c2e | no | 8 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Non Patent Literature | P12159EP00_D1_cont_5.PDF | 1030383<br>8c195ad4c986d59c551dcf8ef47729d9e322a069 | no | 12 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Non Patent Literature | P12159EP00_SR_6.PDF | 2012319<br>216e05240e5f1bb2aea6d53e49f09b7a46e52e10 | no | 17 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 7 | Non Patent Literature | Hardenbol_article_7.PDF | 824768<br>969e0bce054409479ade9ed5bb14fc93913550b6 | no | 6 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 8 | Non Patent Literature | IPRP_for_WO2010126614_8.PDF | 583011<br>3c8b4a93a140b80154de21fb3ea8bc5c9f5f33d0 | no | 9 |

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| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 9 | Non Patent Literature | ISR_for_WO2010126614_9.PDF | 203925<br>d981d7d3cf6a87125b622f786ccfa3471541dd66 | no | 4 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 10 | Non Patent Literature | P12159EP00_D3_10.PDF | 2629955<br>de9e6b2d0ed1b28eb8ba2d045db058f9ab20e9a9 | no | 12 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 11 | Non Patent Literature | Mockler_article_11.PDF | 1972694<br>b7fcda97be8d632474d7b35fc384bed601a7ee96 | no | 15 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 12 | Non Patent Literature | P12159EP00_D4_12.PDF | 1473160<br>d13ebe55123d17017dd2e01d70af9a4208a6a94a | no | 6 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 13 | Non Patent Literature | P12159EP00_D2_13.PDF | 525412<br>e1f5252df6098a78e9eaf5f5f1cb4fbca21043b9 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 14 | Non Patent Literature | P12159EP00_D2_cont_14.PDF | 1348360<br>44b17ae3abd6b0fb61a453d6cb0574543d3c26b6 | no | 12 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 15 | Foreign Reference | 61136799_1-WO9511995A1_16.PDF | 9618445<br>6805a6e7beb0db8d6207dbd6247410c0598c8b43 | no | 223 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 16 | Foreign Reference | WO2007107717A1_17.PDF | 1920747<br>aa2830f2208caf4c3598f672fd0a6df9bd07a8e3 | no | 43 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 17 | Information Disclosure Statement (IDS) Form (SB08) | GSGE-002-01US_29258-20_SB08.pdf | 613192<br>45eaf040f390910976ce020e497b75c02ee32ef1 | no | 5 |

| | | | | | |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 18 | Fee Worksheet (SB06) | fee-info.pdf | 30344<br>689fda8dd0a10d50121bbfdb9b0d49c32386d1e4 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | | **Total Files Size (in bytes):** | 32325796 | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

**OR**

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2013-03-28 |
|---|---|---|---|
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

## Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** ( **Not for submission under 37 CFR 1.99)** | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| | 1 | 20030203370 | | 2003-10-30 | Yakhini et al. | |
| | 2 | 20080280955 | | 2008-11-13 | McCamish | |
| | 3 | 20090099041 | | 2009-04-16 | CHURCH et al. | |
| | 4 | 20050059048 | | 2005-03-17 | Gunderson et al. | |
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| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
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| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 1995/011995 | WO | A1 | 1995-05-04 | Affymax Tech Nv | | ☐ |
| | 2 | 2007/107717 | WO | A1 | 2007-09-27 | Ucl Business Plc | | ☐ |

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| Examiner Initials* | Cite No | Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published. | T5 |
|---|---|---|---|
| | 1 | Akhras (PLOS ONE, 2(9):e915-e915, 2007) | ☐ |
| | 2 | Ball (Nature Biotechnology, 27(4):361-368, 2009) | ☐ |
| | 3 | BAU (Analytical and Bioanalytical chemistry, 393(1):171-175, 2008) | ☐ |
| | 4 | Deng et al. (Nature Biotechnology, 27(4):353-360, 2009) | ☐ |
| | 5 | Deng et al. (Nature Biotechnology) SUPPLEMENT | ☐ |
| | 6 | European Search Report for EP app. no. 10770071.8 | ☐ |
| | 7 | Hardenbol (Nature Biotechnology, 21:673-678, 2003) | ☐ |

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

| | | | |
|---|---|---|---|
| | 8 | International Preliminary Report on Patentability for WO 2010/126614 | ☐ |
| | 9 | International Search Report and Written Opinion for WO 2010/126614 | ☐ |
| | 10 | Krishnakumar (Proceedings of the National Acaemy of Sciences, 105(27):9296-9301, 2008) | ☐ |
| | 11 | Mockler (Genomics, 85:1-15, 2005) | ☐ |
| | 12 | Porreca (Nature Methods, 4(11): 931-936, 2007) | ☐ |
| | 13 | Turner (Nature Methods, 6(5):315-316, 2009) | ☐ |
| | 14 | Turner (Nature Methods, SUPPLEMENT | ☐ |

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**EXAMINER SIGNATURE**

| Examiner Signature | | Date Considered | |
|---|---|---|---|

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

**PATENT COOPERATION TREATY**

# PCT

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

| | | |
|---|---|---|
| Applicant's or agent's file reference<br>G0814.70000 | **FOR FURTHER ACTION** | See item 4 below |
| International application No.<br>PCT/US2010/001293 | International filing date *(day/month/year)*<br>30 April 2010 (30.04.2010) | Priority date *(day/month/year)*<br>30 April 2009 (30.04.2009) |
| International Patent Classification (8th edition unless older edition indicated)<br>See relevant information in Form PCT/ISA/237 | | |
| Applicant<br>GOOD START GENETICS, INC. | | |

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the report |
| ☐ | Box No. II | Priority |
| ☒ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☒ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☐ | Box No. VIII | Certain observations on the international application |

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

| | |
|---|---|
| | Date of issuance of this report<br>01 November 2011 (01.11.2011) |
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No. +41 22 338 82 70 | Authorized officer<br>Philippe Bécamel<br>e-mail: pt01.pct@wipo.int |

Form PCT/IB/373 (January 2004)

**PATENT COOPERATION TREATY**

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK R.H. WALLER
WOLF, GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 28 OCT 2010 |
| Applicant's or agent's file reference<br>G0814.70000 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 10/01293 | 30 April 2010 (30.04.2010) | 30 April 2009 (30.04.2009) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/94

Applicant GOOD START GENETICS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 07 Oct. 2010 (07.10.2010) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/01293

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No. PCT/US 10/01293

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 15, 16, 19, 22-56, 64, 65, 67

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 15, 16, 19, 22-56, 64, 65, 67 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims are not formed according to the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 15, 16, 19, 22-56, 64, 65, 67

☐ a meaningful opinion could not be formed without the sequence listing: the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. IV Lack of unity of invention**

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☒ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-8, and 57, directed to a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different.

Group II: claims 9-14, 17, 18, and 66, directed to a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject.

Group III: claims 20-21, directed to a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject.

Group IV: claims 58-63, directed to a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The inventions listed as Groups I - IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different. The special technical feature of the Group II claims is a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject. The special technical feature of the Group III claims is a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject. The special technical feature of the Group IV claims is a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract; para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface). Therefore, the inventions of Groups I - IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-14, 17, 18, 57 and 66

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-14, 17, 18, 57 and 66 | YES |
| | Claims | None | NO |
| Inventive step (IS) | Claims | 17, 18, 57 | YES |
| | Claims | 1-14 and 66 | NO |
| Industrial applicability (IA) | Claims | 1-14, 17, 18, 57 and 66 | YES |
| | Claims | None | NO |

2. Citations and explanations:

Claim 66 lacks an inventive step under PCT Article 33(3) as being obvious over the article entitled 'Multiplexed genotyping with sequence-tagged molecular inversion probes' by Hardenbol et al. (hereinafter 'Hardenbol').

As per claim 66, Hardenbol discloses a method of genotyping a nucleic acid in a biological sample, the method comprising: obtaining a nucleic acid preparation using a preparative method on a biological sample (pg 674, col 2, para 2--25 different individuals were genotyped, pg 677, col 1, para 3), sequencing a target nucleic acid of the nucleic acid preparation (pg 674, col 2, para 3), and performing a molecular inversion probe capture reaction on the biological sample (pg 677, col 2, para 2), genotyping the target nucleic acid based on the results of the capture reaction (pg 674, col 2, para 2). Hardenbol further teaches that the MIPs are engineered such that when annealed to a target nucleic acid, a gap between the flanking regions comprises only one nucleotide for detection of a single nucleotide polymorphism (pg 674, col 1, para 2) and it would have been obvious to one skilled in the art during the polymerization reaction to add only the nucleotide that would have indicated an SNP at this locus if the molecular inversion probe captured the target nucleic acid in the biological sample to save reagents and time. It further would have been obvious to one skilled in the art to utilize the sequence information in addition to the capture reaction information to ensure accurate genotyping.

Claim 9 lacks an inventive step under PCT Article 33(3) as being obvious over US 2003/0203370 A1 to Yakhini et al. (hereinafter "Yakhini").

As per claim 9, although Yakhini does not explicitly teach a method of genotyping a subject, the method comprising: determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject, Yakhini discloses a method of genotyping using mass tags (abstract), wherein "As discussed above, there are generally a much smaller number of mass tags than primer sequences, and many different primers may be therefore identically labeled. Thus, fingerprinting SNPs using the CMT/PEA technique involves extracting a small fingerprint signal from complex mass spectra in which the presence of a particular mass tag may indicate extension of many different oligonucleotide primers. Moreover, even considering only SNP-characteristic primers, there may be a number of different genotypes that map to a particular mass spectrum. For example, the mass spectrum shown in FIG. 10 corresponds to mass tags removed from primers, extended as shown in FIG. 8, characteristic for the genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6. However, the same mass spectrum is produced by the above-illustrated CMT/PEA technique when applied to the example genotype S1/S1', S2/S2', S3/S3', S4'/S4', S5/S5', S6/S6, as can be see noting that, in FIG. 6, the characteristic primers for polymorphism S3 610-613 carry the same mass tags as the primer sequences characteristic for polymorphism S4' 614-617 and the characteristic primers for polymorphism S3' carry the same mass tags as the primer sequences characteristic for polymorphism S4. Therefore, the genotyping assay illustrated in FIGS. 4-10 needs to be carried out in multiple assay steps in order to distinguish genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6 from genotype S1/S1', S2/S2', S3'/S3', S4'/S4', S5/S5', S6/S6. Instead of hybridizing the set of oligonucleotide primers to all chromosome fragments, including fragments produced from all six polymorphisms, the chromosome fragments need to be partitioned into sets that generate unambiguous mask-tag fingerprints under the CMT/PEA assay technique". (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini.

----Continued in Supplemental Box------

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V(2): Citations and Explanations

Claims 1- 5, and 7 lack an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of the article entitled 'Applications of DNA tiling arrays for whole-genome analysis' by Mockler et al. (hereinafter 'Mockler').

As per claim 1, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein each subregion overlaps with at least one other subregion. Mockler, however, discloses wherein each subregion overlaps with at least one other subregion (Fig. 2a). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 2, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other subregion)).

As per claim 3, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

As per claim 4, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

As per claim 5, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising: contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3--1,517 loci were genotyped in a 1,517 probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe. Mockler, however, discloses a tiling array wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe (Fig. 2a, d). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 7, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol further teaches wherein the analyzing comprises genotyping a polymorphism of the target nucleic acid (abstract).

Claims 10-14 lack an inventive step under PCT Article 33(3) as being obvious over Yakhihi in view of Hardenbol.

As to claim 10, although Yakhini does not explicitly teach wherein the isolated nucleic acids are molecular inversion probe capture products, Hardenbol teaches the use of said products for the genotyping of SNP's (abstract). It would have been obvious to a person skilled in the art that the mollecular inversion probe capture products taught by Hardenbol could have substituted for the mass spectrometric tags taught by Yakhini, with a similar type of degeneracy requiring the determination of sets to generate unambiguous sequence-tag fingerprints (para [0026]) in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

-------Continued in Subsequent Supplemental Box-------

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No. PCT/US 10/01293

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2: Citations and Explanations

As to claim 11, Hardenbol discloses a method of genotyping a subject, the method comprising:
determining the sequence of molecular inversion probe capture products (abstract, analyzed using universal sequence tag DNA microarrays), each comprising a molecular inversion probe and a target nucleic acid (Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a--P1,P2,Tag), and wherein the target nucleic acid is a captured genomic locus of a subject (pg 674, col 2, para 2), and genotyping the subject at the captured genomic locus based on the sequence of the captured target nucleic acid (abstract, pg 674, col 2, para 2), but fails to specifically teach genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 12, Hardenbol discloses a method of genotyping a subject, the method comprising: obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid , wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), wherein the target nucleic acid is a captured genomic locus of the subject (abstract, pg 674, col 2, para 2), amplifying the molecular inversion probe capture products (pg 674, col 1, para 2), and genotyping the subject by determining, for each target nucleic acid, the sequence of the target nucleic acid (abstract--analzed using unversal sequence tag DNA microarrays), but fails to specifically disclose genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 13, Hardenbol discloses wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence (Each amplified probe contains a unique tag sequence that is complementary to a sequence on the universal tag array; pg. 270, col 1, para 2).

As to claim 14, although neither Hardenbol nor Yakhini explcitly teaches wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05, Hardenbol teaches wherein repeatability and trio accuracy were greater than 99%, which corresponds to an error rate of less than 0.01. A person skilled in the art would have known that a likelihood of ambiguity (obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences) of the results for a genotyping assay, particularly assays associated with either prognostication of disease or of the potential utility of pharmaceuticals to treat a disease, would have been required to be at least less than 5%.

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of Mockler, further in view of US 2008/0280955 A1 to McCamish et al. (hereinafter 'McCamish').

As per claim 6, the combination of Hardenbol and Mockler discloses the method of claim 1, but fails to teach that which McCamish teaches, that is wherein the target nucleic acids are selected from Table 1 (Table 9- NC_000011.8 (DHCR7), NC_000016.8 (GCSH), NC_000019.8 (BCKDH), NC_000021.7 (HLCS)). As McCamish teaches that the SNPs in Table 9 are related to drug response to blood glucose regulation (para [0258], claims 1, 15), it would have been obvious to one skilled in the art to analyze the loci taught by McCamish using the combined method of Hardenbol and Mockler in order to ascertain whether a particular subject would be susceptible to drug therapy for blood glucose regulation.

Claim 8 lacks inventive step under PCT Article 33(3) as being obvious over Hardenbol, in view of Mockler, in further view of WO 2007/107717 A1 to Chamberlain et al. (hereinafter 'Chamberlain').

As per claim 8, the combination of Hardenbol and Mockler teaches the method of claim 7, but fails to specifically teach that which Chamberlain teaches, that is wherein the polymorphism is selected from Table 2 (Table 3, 4). As Chamberlain teaches that the listed SNP's are related to responsiveness to treatment with bisphosphonate (pg 4, ln 16-33), it would have been obvious to one skilled in the art to analyze the loci and polymorphisms as taught by Chamberlain using the combined method of Hardenbol and Mockler in order to determine who would be responsive to treatment with bisphosphonate.

-------Continued in Subsequent Supplemental Box------

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No. PCT/US 10/01293

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2: Citations and Explanations

Claims 17-18 meet the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Hardenbol teaches a a method of analyzing a plurality of genetic loci, the method comprising:
obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid (pg 674, col 2, para 2; Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), amplifying the plurality of molecular inversion probe capture products (pg 674, col 1, para 2), but fails to teach: determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Therefore, claim 17 and claim 18, which depends therefrom, meet the aforementioned requirements for the above reasons.

Claim 57 meets the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Hardenbol discloses a method comprising:
(i) contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid (pg 674, col 1, para 2), wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid, wherein the first and second regions are on a common strand of the target nucleic acid, and wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid (Fig. 2a, b); and
(ii) capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes (pg 674, col 1, para 3 to pg 674, col 2, para 1), but fails to specifically teach (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Therefore claim 57 meets the aforementioned requirements for the above reasons.

Claims 1-14, 17, 18, 57, 66, have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference G0814.70000 | **FOR FURTHER ACTION** | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US 10/01293 | International filing date *(day/month/year)* 30 April 2010 (30.04.2010) | (Earliest) Priority Date *(day/month/year)* 30 April 2009 (30.04.2009) |
| Applicant GOOD START GENETICS | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 10/01293

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 15, 16, 19, 22-56, 64, 65, 67
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-8, and 57, directed to a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-14, 17, 18, 57 and 66

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/01293

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/94
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 436/94

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, Google Scholar
Search Terms:molecular inversion probe, multiplex, overlap, tile, stack, probe, DNA, bias, reduce, circular, padlock, genotyping, probe set

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>-----<br>Y<br>---<br>A | Hardenbol et al., 'Multiplexed genotyping with sequence-tagged molecular inversion probes' Nature Biotechnology, 2003, Vol. 21, No. 6, pages 673-678; abstract, Fig. 2; pg 673, col 2, para 2pg 674, col 1, para 2-3; pg 674, col 2, para 1-3; pg 677, col 1, para 3 | 66<br>----<br>1-8, 10-14<br>----------<br>17-18, 57 |
| X<br>---<br>Y | US 2003/0203370 A1 (YAKHINI et al.) 30 October 2003 (30.10.2003) abstract, para [0026] | 9<br>-------<br>10-14 |
| Y | Mockler et al., 'Applications of DNa tiling arrays for while-genome analysis', Genomics, 2005, Vol. 85, pages 1-15; Fig. 2; pg 2, col 1, para 4 to pg 2, col 2, para 1 | 1-8 |
| Y | US 2008/0280955 A1 (MCCAMISH et al), 13 Nov. 2008 (13.11.2008), Table 9, para [0258], claims 1, 15 | 6 |
| Y | WO 2007/107717 A1 (CHAMBERLAIN et al) 27 Sep. 2007 (27.09.2007), Tables 3, 4; pg 4, ln 16-33 | 8 |
| & | US 2010/0129896 A1 (KNAPP) 27 May 2010 (27.05.2010) | 57 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 15 Oct. 2010 (15.10.2010) | 28 OCT 2010 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/01293

Continuation of Box III: Unity of Invention

Group II: claims 9-14, 17, 18, and 66, directed to a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject.

Group III: claims 20-21, directed to a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject.

Group IV: claims 58-63, directed to a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The inventions listed as Groups I - IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different. The special technical feature of the Group II claims is a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject. The special technical feature of the Group III claims is a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject. The special technical feature of the Group IV claims is a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract; para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface). Therefore, the inventions of Groups I - IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

| Electronic Patent Application Fee Transmittal | |
|---|---|
| **Application Number:** | 13266862 |
| **Filing Date:** | 13-Mar-2012 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Filer:** | Thomas C. Meyers/Kelley Froehlich |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| Filed as Small Entity | |

**U.S. National Stage under 35 USC 371 Filing Fees**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Miscellaneous:** | | | | |
| Submission- Information Disclosure Stmt | 2806 | 1 | 90 | 90 |
| | | **Total in USD ($)** | | **90** |

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 15455056 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Thomas Hellenthal |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 08-APR-2013 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 13:04:16 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| Submitted with Payment | no |
|---|---|

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | | GSGE_002_01US_Amendment_and_response_to_OA_mailed_01_10_2013.pdf | 56870<br>c47e38767ee496ea7cf56622f6f39cfe7896f49f | yes | 14 |

| | Multipart Description/PDF files in .zip description | | |
|---|---|---|---|
| | **Document Description** | **Start** | **End** |
| | Amendment/Req. Reconsideration-After Non-Final Reject | 1 | 1 |
| | Specification | 2 | 2 |
| | Claims | 3 | 9 |
| | Applicant Arguments/Remarks Made in an Amendment | 10 | 14 |

| | | | | | |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Drawings-only black and white line drawings | GSGE_002_01US_Replacement_Figures.pdf | 397931<br>296a038cc1f13292d4f3347730073e576b0d1806 | no | 16 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 454801 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANT: | Porreca et al. | ART UNIT: | 1634 |
| SERIAL NUMBER: | 13/266,862 | CONFIRMATION NO.: | 6082 |
| FILING DATE: | March 13, 2012 | EXAMINER: | Pohner, Steven C. |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

**FILED ELECTRONICALLY**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

**AMENDMENT AND RESPONSE**

This paper is in response to a non-final Office Action mailed January 10, 2013 from the United States Patent and Trademark Office. Applicants authorize the Commissioner to charge any required fees due with this response to Deposit Account No. 500369.

**Amendments to the specification** begin on page 2.

**Amendments to the claims** begin on page 3.

**Remarks** begin on page 10.

Amendments to the specification

No amendments to the specification are made herein.



Petitioner Illumina, Inc., EX1002, p. 642

Amendment to the claims

Please amend claim 1 as follows. This list of claims replaces all prior versions and listings of claims in this application.

1. (Currently amended) A method of analyzing a plurality of genetic loci, the method comprising:

contacting each of a plurality of target nucleic acids with a ~~probe set, wherein each probe set comprises a~~ plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking ~~the same strand of~~ one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, ~~and~~ wherein each subregion overlaps with at least one other subregion, and wherein when hybridized to the target nucleic acid in a circular configuration, the 5' region and the 3' region of each probe are separated by a plurality of nucleic acids on the target nucleic acid,

converting hybridized probes into closed circularized probes,

isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

2. (Original) The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion.

3. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.

4. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5-11. (Canceled)

12. (Withdrawn) A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. (Withdrawn) The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

14. (Withdrawn-Previously presented) The method of claim 12, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.



Petitioner Illumina, Inc., EX1002, p. 644

15. (Withdrawn-Previously presented) The method of claim 12, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. (Withdrawn-Previously presented) The method of claim 12, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

17-19. (Canceled)

20. (Withdrawn) A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. (Withdrawn) The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is lower than the reference capture efficiency.

23. (Withdrawn) The method of claim 22, wherein the subject is identified as having an insertion in the target region.



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24. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is higher than the reference capture efficiency.

25. (Withdrawn) The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. (Withdrawn) The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.

27. (Withdrawn) The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. (Withdrawn-Previously presented) The method of claim 20, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. (Withdrawn) The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

30. (Withdrawn) The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31-32. (Canceled)

33. (Withdrawn-Previously presented) The method of claim 29, wherein the first and second targeting arms have the same length.

34-35. (Canceled)

36. (Withdrawn-Previously presented) The method of claim 29, wherein the hybridization Tms of the first and second targeting arms are identical.

37. (Withdrawn-Previously presented) The method of claim 28, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. (Withdrawn) The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. (Canceled)

40. (Withdrawn) The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.

42. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.



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43. (Withdrawn-Previously presented) The method of claim 29, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. (Withdrawn-Previously presented) The method of claim 20, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. (Withdrawn) The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. (Withdrawn) The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. (Withdrawn) The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48-49. (Canceled)

50. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. (Withdrawn) The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.

52. (Withdrawn-Previously presented) The method of claim 50, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.



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53. (Withdrawn) The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.

55. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. (Withdrawn-Previously presented) The method of claim 20, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57-67 (Canceled)



Petitioner Illumina, Inc., EX1002, p. 649

Remarks

Claim 1 is amended to recite molecular inversion probes and the step of circularizing hybridized probes. Support for those amendments is found at p. 5 line 11 to p. 8 line 31 and p. 32 line 10 to p. 38 line 2 of the as-filed application. Claim 1 is also amended to recite that when hybridized to the target nucleic acid, the 5' region and the 3' region of each probe are separated by a plurality of nucleic acids on the target. Support for that amendment is found at p. 12 lines 10-24, p. 60 line 28 to p. 62 line 22, p. 74 line 30 to p. 75 line 17, and FIG. 9 of the as-filed application. Claim 1 is also amended to correct a clerical error in that claim. No new matter is added.

Examiner Interview

A telephonic interview was held on March 18, 2013 between Examiner Pohnert and Applicants' representative, Adam Schoen, regarding the objection to the drawings in the non-final Office Action mailed January 10, 2013. Applicants' representative thanks the Examiner for his time and his helpful comments.

Claims are Novel

The Office rejects claims 1-2 and 4 under 35 U.S.C. §102(b) in view of Chee et al. (WO 95/11995). Applicants respectfully disagree.

The law requires that a reference show each and every element of a claim to be used as a reference against the novelty of that claim. See M.P.E.P. §2131. Amended claim 1 requires that the method be performed with molecular inversion probes and also requires the element of converting hybridized probes into closed circularized probes. Chee does not disclose molecular inversion probes and does not disclose converting hybridized probes into closed circularized probes. Rather, Chee is limited to reporting general probe tiling strategies using immobilized linear probes. Since Chee does not disclose the claim elements of molecular inversion probes

and converting hybridized probes into closed circularized probes, it is not possible for Chee to be a novelty reference against the amended claims.

Applicants respectfully requests withdrawal of the rejection of claims 1-2 and 4 under 35 U.S.C. §102(b) in view of Chee.

Claims are not Obvious

Chee and Mockler

The Office rejects claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Chee et al. and Mockler (Genomics, 85:1-15, 2005). Applicants respectfully disagree.

In assessing a claim for obviousness, all the claim limitations must be considered. M.P.E.P. § 2143.03. Moreover, to establish prima facie obviousness of a claimed invention, all the claim features must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974). The amended claims are not obvious in view of the combination of Chee and Mockler because that combination does not teach or suggest the required elements of amended claim 1 of molecular inversion probes and circularizing hybridized probes.

As discussed above, Chee is limited to reporting general probe tiling strategies using immobilized linear probes. Chee does not teach or suggest molecular inversion probes, and does not teach or suggest converting hybridized probes into closed circularized probes.

Similar to Chee, Mockler is limited to reporting general tiling strategies using immobilized linear probes. Mockler also does not teach or suggest the elements of amended claim 1 of molecular inversion probes and converting hybridized probes into closed circularized probes.

Therefore, the combination of Chee and Mockler does not teach or suggest all of the subject matter of amended claim 1, and therefore that amended claim is not obvious in view of the combination of Chee and Mockler. Claims 2-4 depend from amended claim 1, and therefore also are not obvious in view of the combination of Chee and Mockler.

Applicants respectfully requests withdrawal of the rejection of claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Chee and Mockler.

Hardenbol and Mockler

The Office rejects claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Hardenbohl (Nature Biotechnology, 21:673-678, 2003) and Mockler (Genomics, 85:1-15, 2005). Applicants respectfully disagree.

Amended claim 1 requires that when hybridized to a target nucleic acid in circular configuration, a 5' region and a 3' region of each molecular inversion probe are separated by a plurality of nucleic acids on the target nucleic acid. The amended claims are not obvious in view of the combination of Hardenbohl and Mockler because that combination does not teach or suggest that required element of amended claim 1.

Hardenbohl is limited to reporting the use of padlock probes that hybridize to a target in a manner such that a 5' region and a 3' region are separated from each other by only a single nucleotide (Hardenbohl, p. 674, section entitled "Molecular inversion probe (MIP) genotyping"; p. 675, Fig. 2; p. 677, Methods section entitled "Molecular inversion probe assay"). Hardenbohl states:

> A mixture of Genomic DNA, 1,000 or more probes, thermostable ligase and polymerase is heat-denatured and brought to annealing temperature. Two sequences targeting each terminus of the probe hybridize to complementary sites in the genome, creating a circular conformation with a single-nucleotide gap between the termini of the probe. Ibid., p. 675, right column, Description of Figure 2.

According, Hardenbohl does not teach or suggest the element of amended claim 1 of a molecular inversion probe having a 5' region and a 3' region that hybridizes to a target nucleic such that in a circular configuration, the 5' region and the 3' region are separated by a plurality of nucleic acids on the target nucleic acid. Rather, Hardenbohl is limited to methods in which padlock

probes hybridize to a target in a manner that a 5' region and a 3' region are separated from each other by only a single nucleotide.

Mockler does not cure the deficiencies of Hardenbohl. As discussed above, Mockler is limited to reporting general tiling strategies using immobilized linear probes. Mockler does not teach or suggest molecular inversion probes. Since Mockler does not teach or suggest molecular inversion probes, it is not possible for Mockler to teach or suggest the subject matter of amended claim 1 of a molecular inversion probe having a 5' region and a 3' region that hybridizes to a target nucleic such that in a circular configuration, the 5' region and the 3' region are separated by a plurality of nucleic acids on the target nucleic acid.

Therefore, the combination of Hardenbohl and Mockler does not teach or suggest all of the subject matter of amended claim 1, and therefore that amended claim is not obvious in view of the combination of Hardenbohl and Mockler. Claims 2-4 depend from amended claim 1, and therefore also are not obvious in view of the combination of Hardenbohl and Mockler.

Applicants respectfully requests withdrawal of the rejection of claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Hardenbohl and Mockler.

Clarity of Claims

The Office Action Rejects claims 1-4 under 35 U.S.C. §112 ¶2 stating that the phrase "the same strand of one of a plurality of subregions of the target nucleic acid" lacks antecedent basis. Claim 1 is amended to correct that error. Applicants respectfully request withdrawal of that rejection.

The Office Action rejects claim 3 under 35 U.S.C. §112 ¶2 stating that the claim is unclear because it is contradictory to the subject matter in claim 1. Applicant respectfully disagrees.

Claim 1 requires that the probes hybridize to a plurality of different subregions in which the subregions are different and each of the subregions overlaps with another subregion. Accordingly, the subject matter of claim 1 refers to subregions that partially overlap with each

other. Since each subregion includes a portion that overlaps with a portion of another subregion and a portion that is different for the other subregions, it is consistent to have probes where the 5' and 3' regions of each probe are all different and non-overlapping of each other, as recited in claim 3. Stated another way, claim 3 recites that the 5' and 3' regions of each probe hybridize to the portions of the subregions that do not overlap with each other. Therefore, claim 3 does not contradict the subject matter of claim 1. Applicants respectfully request withdrawal of that rejection.

Objection to the Drawings

The Office Action objects to the drawings because each figure is not preceded by the term "FIG.". Replacement drawings are provided in which each figure is preceded by the term "FIG.". Applicants respectfully request withdrawal of that objection.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

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Respectfully submitted,
**BROWN RUDNICK LLP**

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

60919995

**FIG. 1**

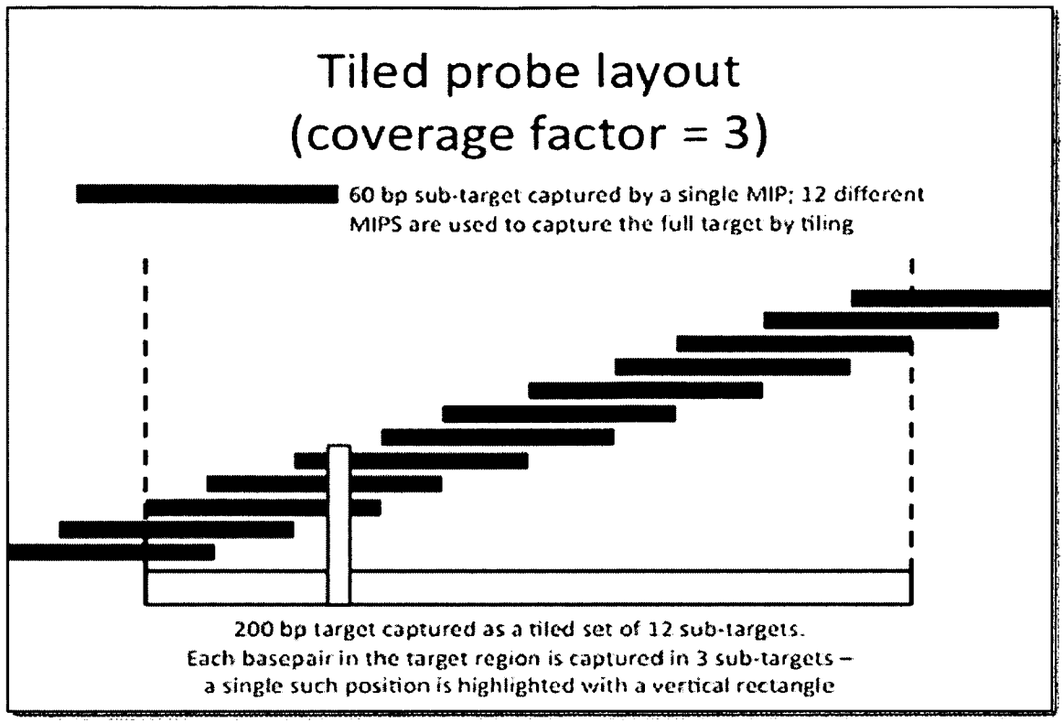

Tiled probe layout
(coverage factor = 3)
60 bp sub-target captured by a single MIP; 12 different MIPS are used to capture the full target by tiling
200 bp target captured as a tiled set of 12 sub-targets.
Each basepair in the target region is captured in 3 sub-targets –
a single such position is highlighted with a vertical rectangle

**FIG. 2**

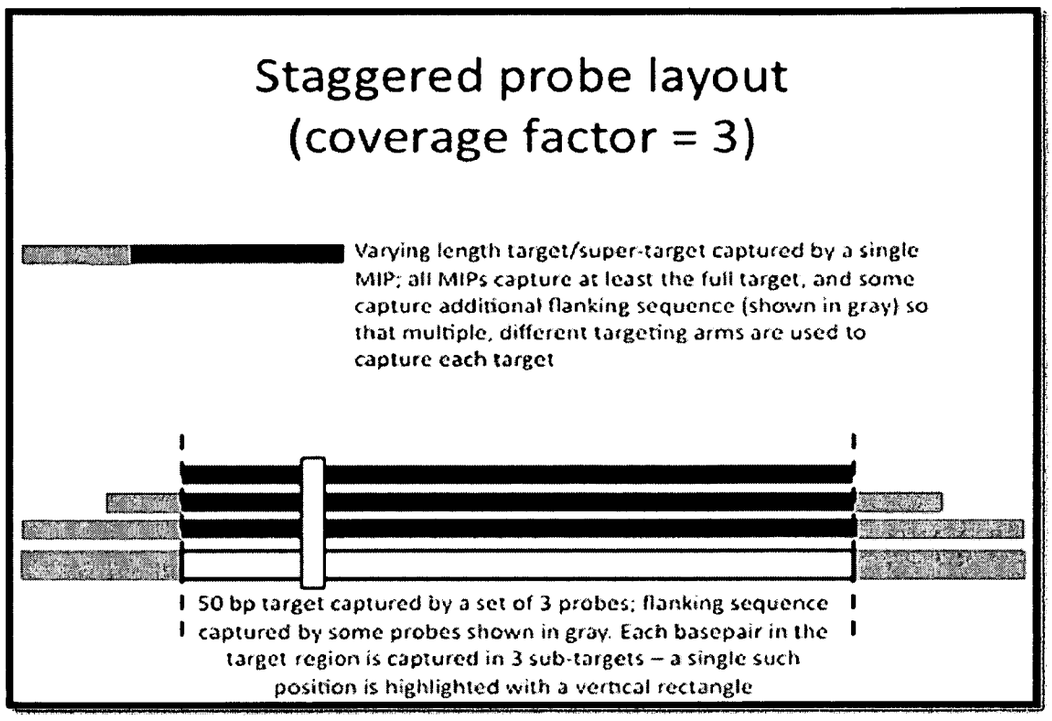

Staggered probe layout
(coverage factor = 3)
Varying length target/super-target captured by a single MIP; all MIPs capture at least the full target, and some capture additional flanking sequence (shown in gray) so that multiple, different targeting arms are used to capture each target
50 bp target captured by a set of 3 probes; flanking sequence captured by some probes shown in gray. Each basepair in the target region is captured in 3 sub-targets – a single such position is highlighted with a vertical rectangle

**FIG. 3**

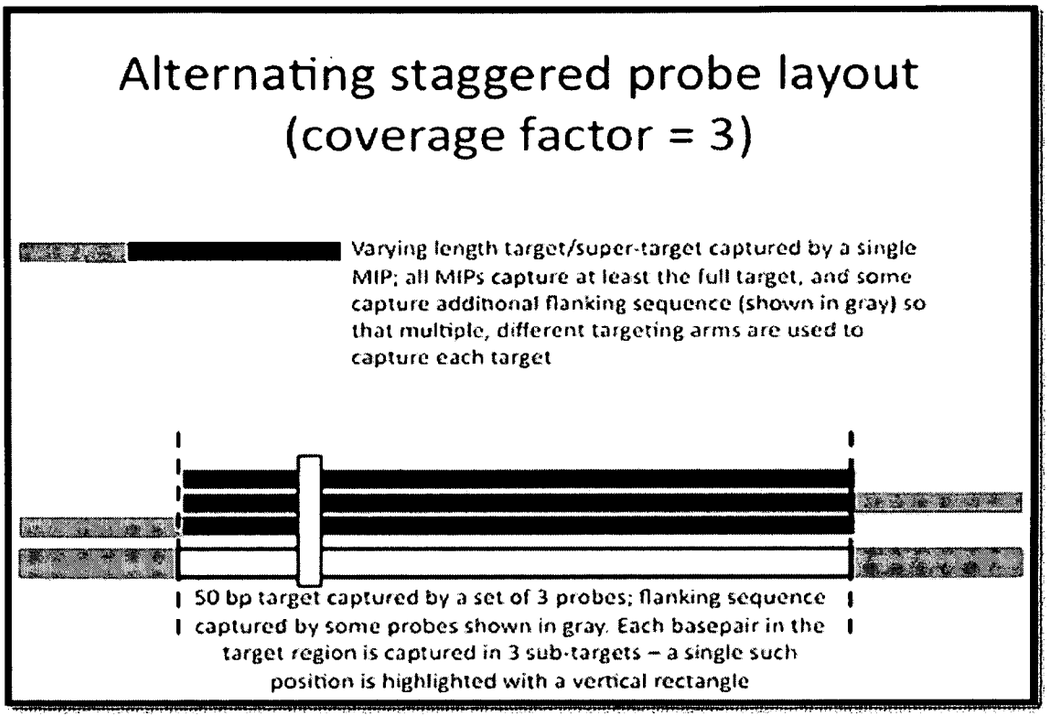

Alternating staggered probe layout
(coverage factor = 3)
Varying length target/super-target captured by a single MIP; all MIPs capture at least the full target, and some capture additional flanking sequence (shown in gray) so that multiple, different targeting arms are used to capture each target
50 bp target captured by a set of 3 probes; flanking sequence captured by some probes shown in gray. Each basepair in the target region is captured in 3 sub-targets – a single such position is highlighted with a vertical rectangle

**FIG. 4**

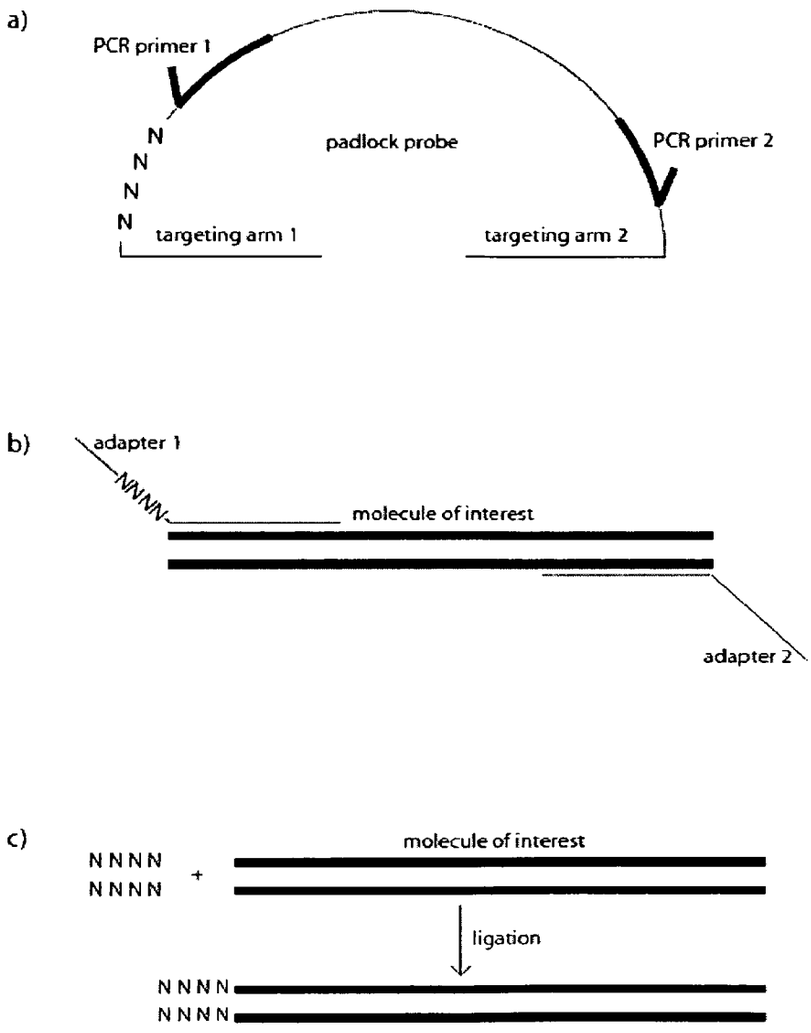

a)
PCR primer 1
N
N
N
N
padlock probe
PCR primer 2
targeting arm 1
targeting arm 2
b)
adapter 1
NNNN
molecule of interest
adapter 2
c)
NNNN
NNNN
+
molecule of interest
ligation
NNNN
NNNN

**FIG. 5**

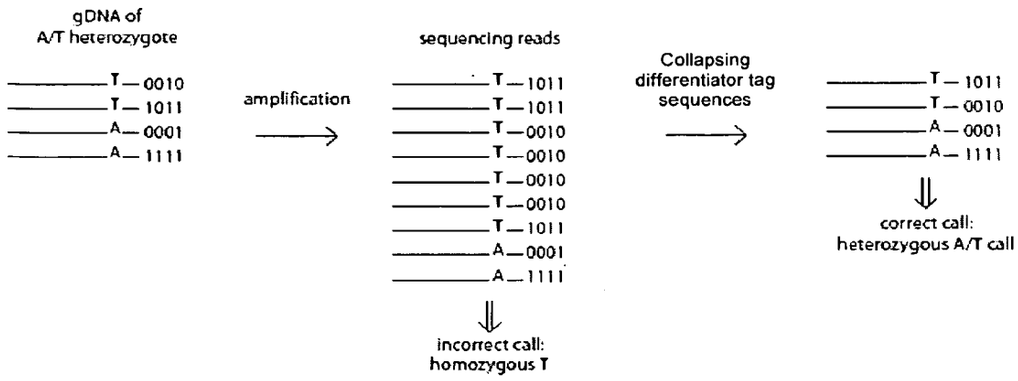

gDNA of
A/T heterozygote
T — 0010
T — 1011
A — 0001
A — 1111
amplification
sequencing reads
T — 1011
T — 1011
T — 0010
T — 0010
T — 0010
T — 0010
T — 1011
A — 0001
A — 1111
incorrect call:
homozygous T
Collapsing
differentiator tag
sequences
T — 1011
T — 0010
A — 0001
A — 1111
correct call:
heterozygous A/T call

**FIG. 6**

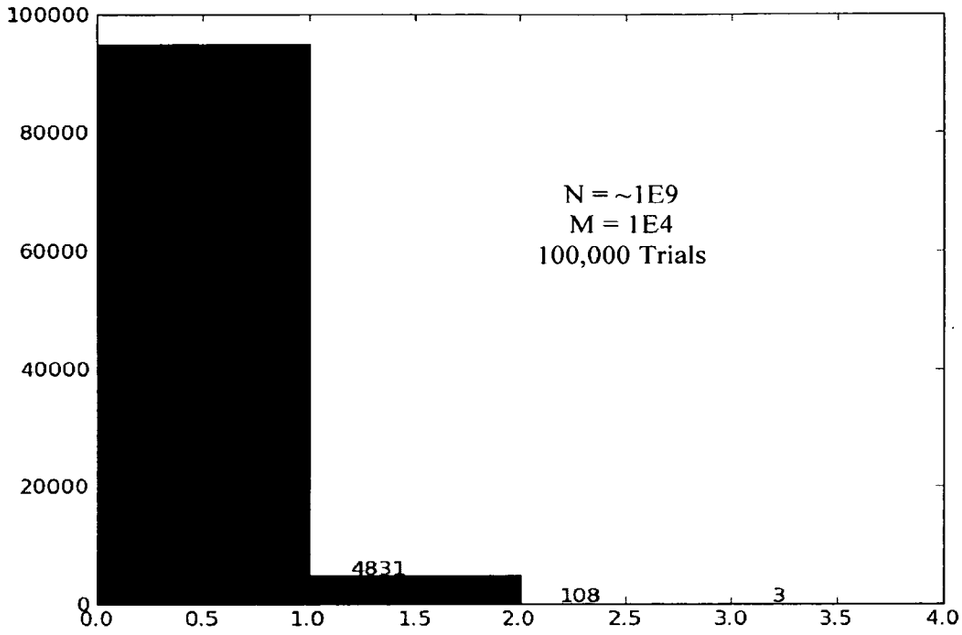

100000
80000
60000
40000
20000
0
N = ~1E9
M = 1E4
100,000 Trials
4831
108
3
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Independent Occurrences of
Target:Differentiator Tag Sequence
100,000 Trials

**FIG. 7**

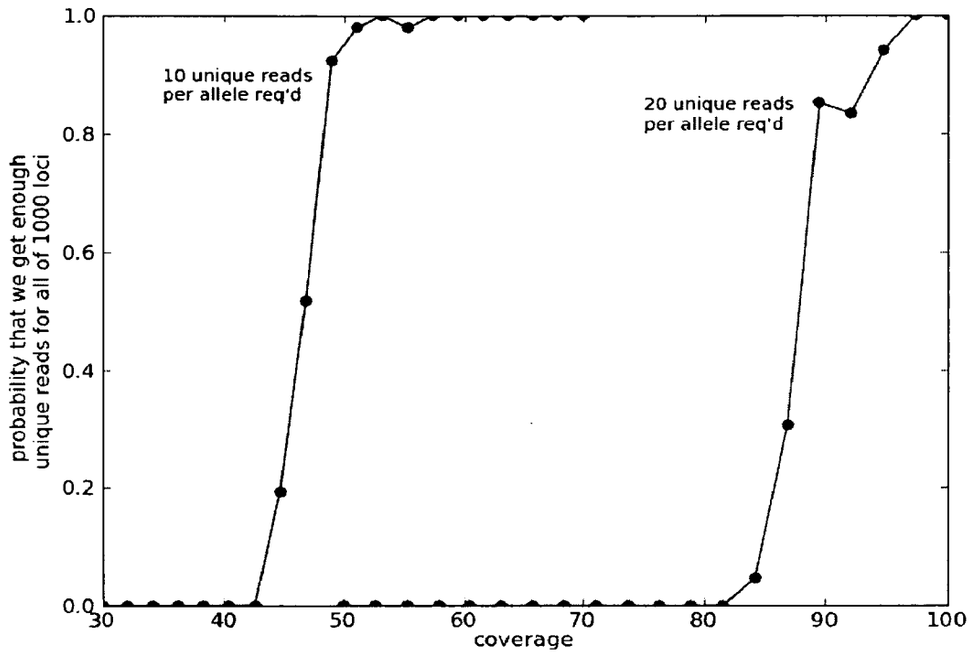

1.0
0.8
0.6
0.4
0.2
0.0
probability that we get enough unique reads for all of 1000 loci
10 unique reads per allele req'd
20 unique reads per allele req'd
30
40
50
60
70
80
90
100
coverage

**FIG. 8**

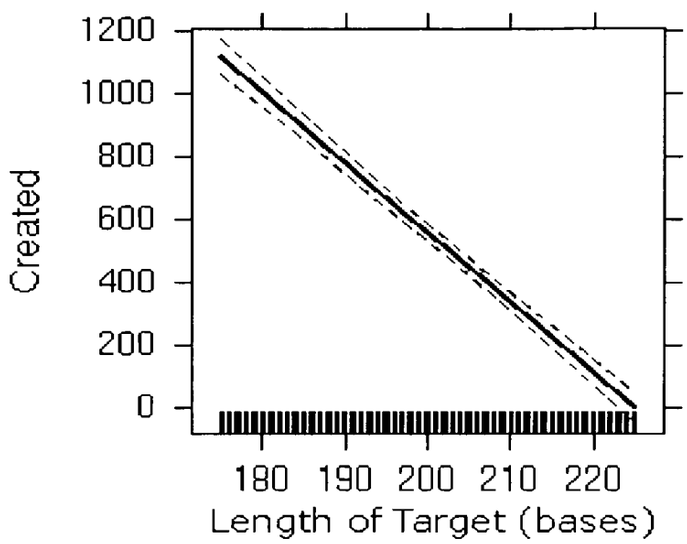

1200
1000
800
600
400
200
0
Created
180
190
200
210
220
Length of Target (bases)

**FIG. 9**

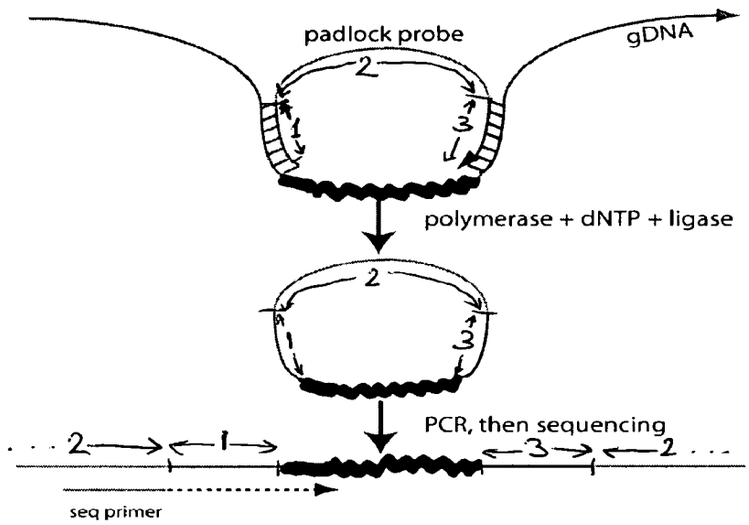

padlock probe
gDNA
2
1
3
polymerase + dNTP + ligase
2
1
3
PCR, then sequencing
2
1
3
2
seq primer

**FIG. 10**

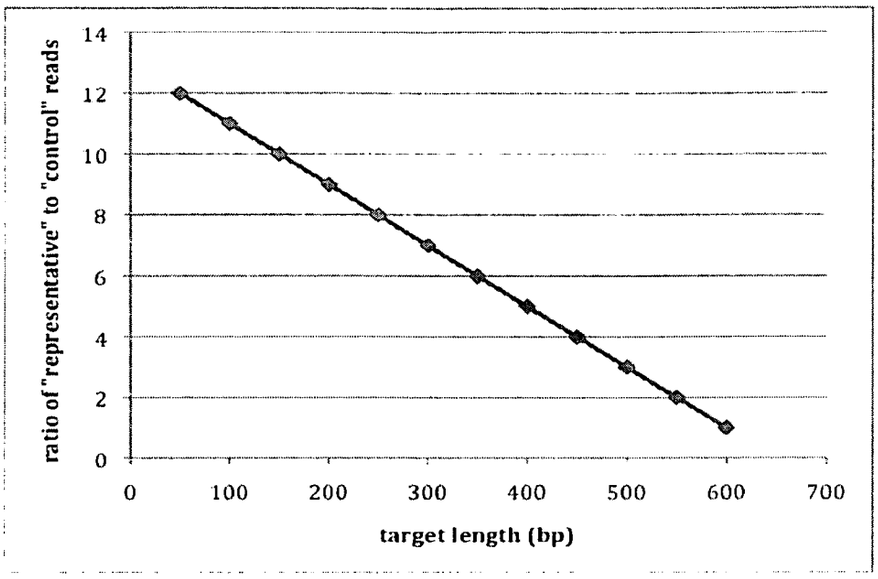

ratio of "representative" to "control" reads
14
12
10
8
6
4
2
0
0
100
200
300
400
500
600
700
target length (bp)

**FIG. 11**

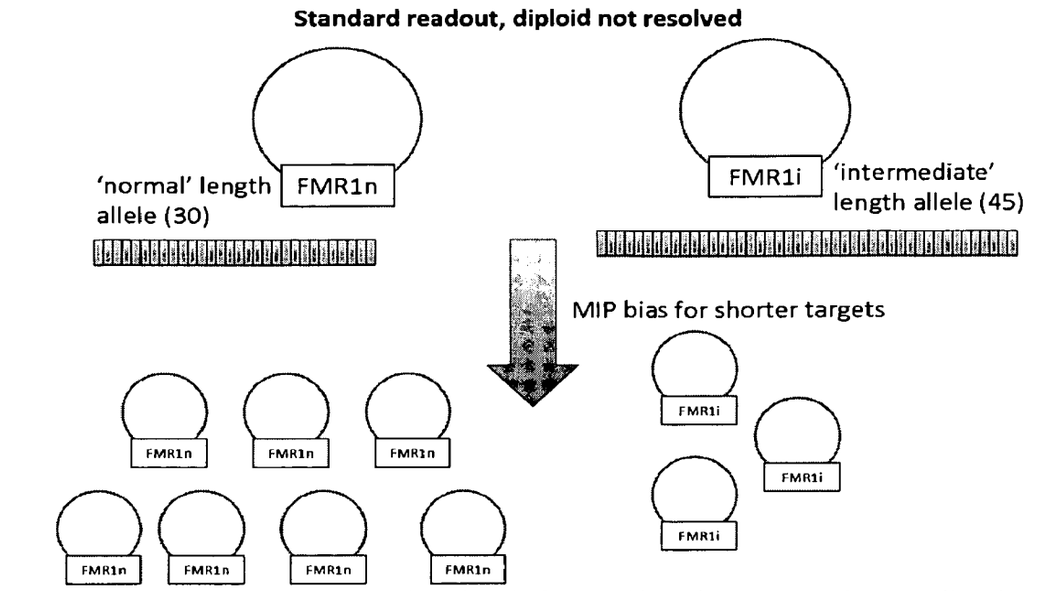

Standard readout, diploid not resolved
'normal' length allele (30)
FMR1n
FMR1i
'intermediate' length allele (45)
MIP bias for shorter targets
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1n
FMR1i
FMR1i
FMR1i
Readout: sum of alleles appears as 'long' end of normal length range, not intermediate het

**FIG. 11B**

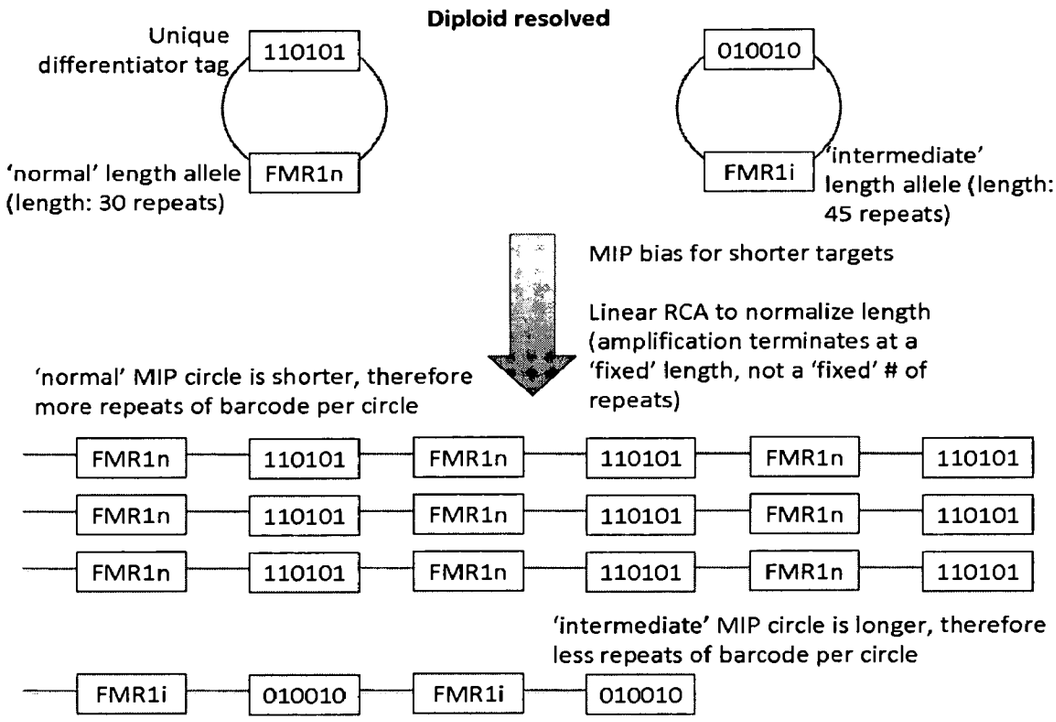

Diploid resolved
Unique differentiator tag
110101
'normal' length allele (length: 30 repeats)
FMR1n
010010
FMR1i
'intermediate' length allele (length: 45 repeats)
MIP bias for shorter targets
Linear RCA to normalize length (amplification terminates at a 'fixed' length, not a 'fixed' # of repeats)
'normal' MIP circle is shorter, therefore more repeats of barcode per circle
FMR1n 110101 FMR1n 110101 FMR1n 110101
FMR1n 110101 FMR1n 110101 FMR1n 110101
FMR1n 110101 FMR1n 110101 FMR1n 110101
'intermediate' MIP circle is longer, therefore less repeats of barcode per circle
FMR1i 010010 FMR1i 010010

**FIG. 11C**

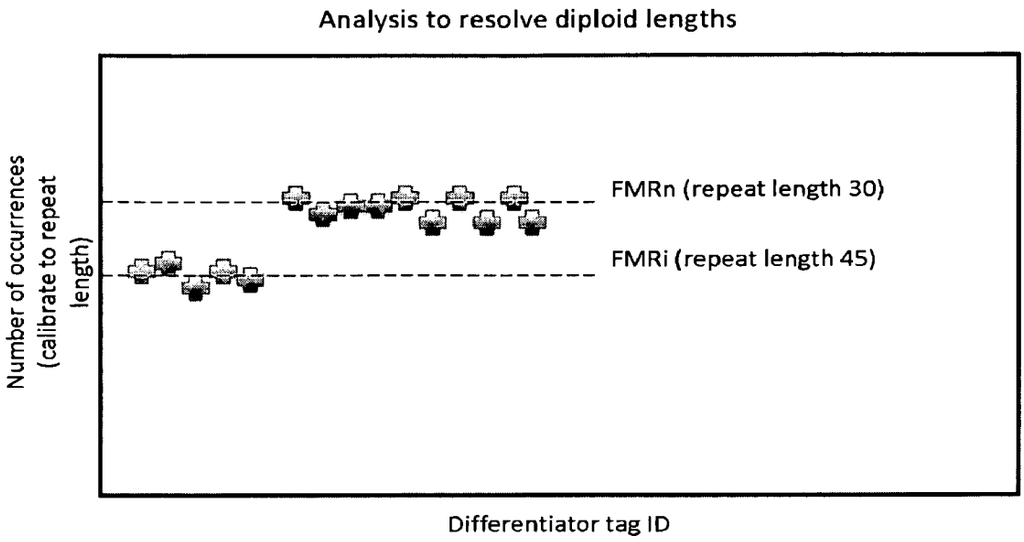

Analysis to resolve diploid lengths
Number of occurrences (calibrate to repeat length)
FMRn (repeat length 30)
FMRi (repeat length 45)
Differentiator tag ID

**FIG. 12**

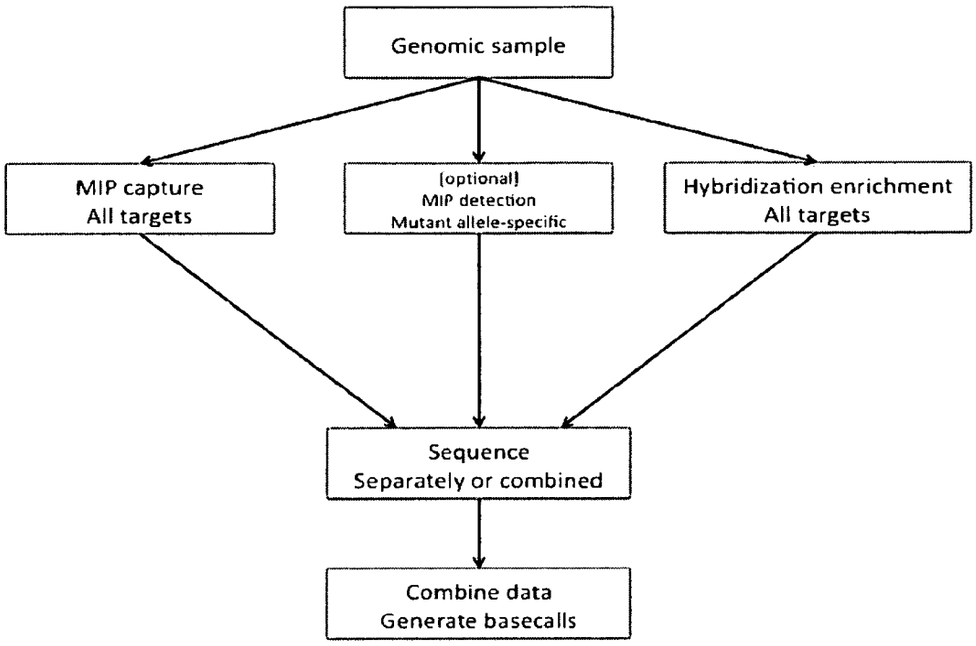

Genomic sample
MIP capture
All targets
(optional)
MIP detection
Mutant allele-specific
Hybridization enrichment
All targets
Sequence
Separately or combined
Combine data
Generate basecalls

**FIG. 13**

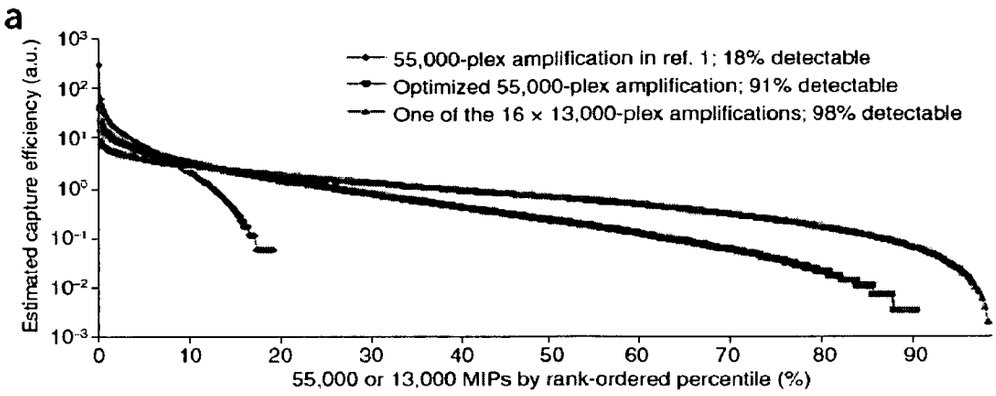
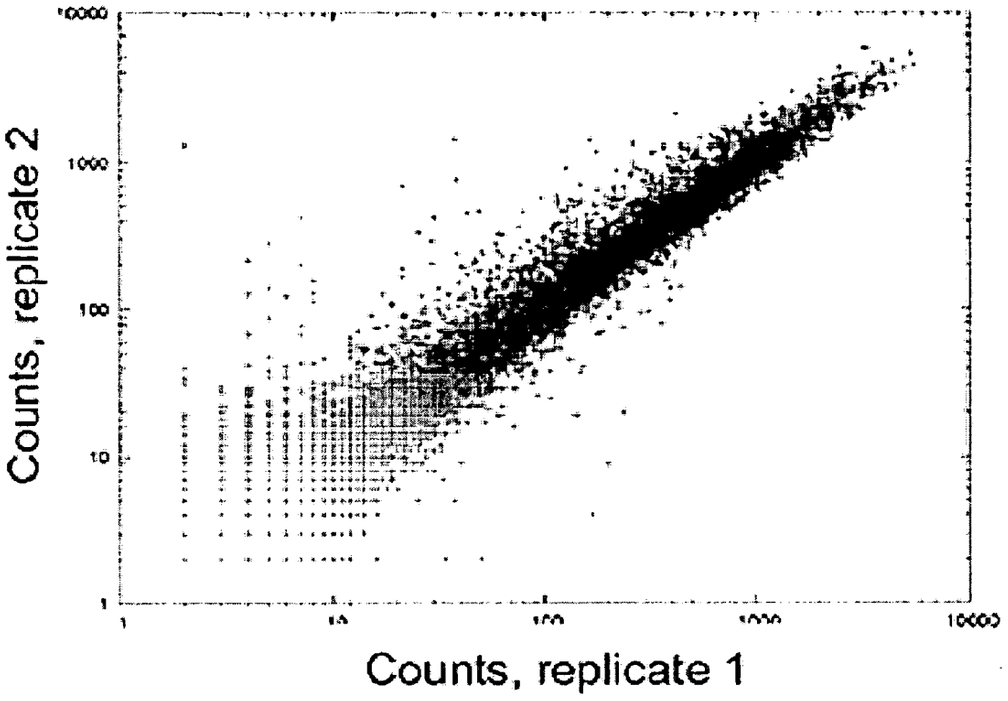

a
Estimated capture efficiency (a.u.)
$10^3$
$10^2$
$10^1$
$10^0$
$10^{-1}$
$10^{-2}$
$10^{-3}$
0
10
20
30
40
50
60
70
80
90
55,000 or 13,000 MIPs by rank-ordered percentile (%)
55,000-plex amplification in ref. 1; 18% detectable
Optimized 55,000-plex amplification; 91% detectable
One of the 16 × 13,000-plex amplifications; 98% detectable

**FIG. 14**



UNITED STATES PATENT AND TRADEMARK OFFICE

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 | 6082 |

21710 7590 06/07/2013

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| EXAMINER | |
|---|---|
| POHNERT, STEVEN C | |
| ART UNIT | PAPER NUMBER |
| 1634 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 06/07/2013 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

| Office Action Summary | Application No. 13/266,862 | Applicant(s) PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | AIA (First Inventor to File) Status No |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *8 April 2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

5)☒ Claim(s) *1-4,12-16,20-30,33,36-38,40-47 and 50-56* is/are pending in the application.

5a) Of the above claim(s) *12-16,20-30,33,36-38,40-47 and 50-56* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-4* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *08 April 2013* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a)☐ All b)☐ Some * c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Interim copies:**

a)☐ All b)☐ Some c)☐ None of the: Interim copies of the priority documents have been received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *3/28/2013*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

## DETAILED ACTION

### *Claim Status and Formal matters*

This action is in response to papers filed 4/8/2013.

Claim 1 has been amended.

Claims 1-4, 12-16, 20-30, 33, 36-38, 40-47 and 50-56 are pending.

Claims 5-11, 17-19,31-32, 34-35, 39-38, 48-49 and 57-67 have been canceled.

Claims 12-16, 20-30, 33, 36-38, 40-47 and 50-56 withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention , there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 12/3/2012.

This action is FINAL.

Claims 1-4 are being examined.

The objection to the drawings has been withdrawn in view of the replacement drawings.

The rejection based on Chee has been withdrawn in view of the amendment to require, "wherein when hybridized to a target nucleic acid in a circular configuration."

### *Information Disclosure Statement*

Non patent literature items 5-6, 8-9 and 14 have been marked through as the IDS filed 3/28/2013 does not provide a date for the publication of the items and thus the non-patent literature items cannot be published on the face of a US patent.

### *Priority*

The instant application was filed 3/13/2012 and is a national stage entry of PCT/US10/01293 filed 4/30/2010 and claims priority to provisional application 61/147,470 filed 4/30/2009; provisional application 61/147923 filed 5/1/2009; provisional application 61179358 filed 5/18/2009; and 61/182,089 filed 5/28/2009.

***Claim Rejections - 35 USC § 112***

1. Claims 1-4 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The Board has held, "If a claim is amenable to two or more plausible constructions, applicant is required to amend the claim to more precisely define the metes and bounds of the claimed invention or the claim is indefinite under §112, ¶2." Ex parte Miyazaki, 89 USPQ2d 1207 (BPAI 2008) (expanded panel).

The instant rejection has been modified in view of the amendments.

Claim 1 recites "3' region" and "5' region" appear to be relative terms. It is unclear if the 5' region (or 3' region) is intended to require the 5' terminal (or 3' terminal) nucleotide of the probe or merely any nucleotide that is 5' (or 3') of the central region.

Claim 1 further recites, "The 5' region and the 3' region of each probe are separated by a plurality of nucleic acids on the target molecule." The metes and bounds of the claim are unclear as to what is required of "a plurality of target nucleic acid on a target molecule." "Nucleic acids" are generally construed in the art to require multiple nucleic acid molecules comprising nucleotides. Thus the specification and

Hardenbohl appear to suggest the molecular inversion probes hybridize to a target nucleic with a separation of a nucleotide or plurality of nucleotides between the 5' terminus and 3' terminus of the probe. Thus it is unclear if the intent of the claim is to require a separation of nucleotides or nucleic acids. Thus it is further unclear if the intent of the claim is to have the target nucleic acid being a single nucleic acid or the target nucleic acid comprises multiple nucleic acids. Further as it is unclear if the 5' region and 3' region require the 5' terminal and 3' terminal nucleotide, respectively, it is unclear if the claim requires a gap equivalent to a plurality of nucleotides between the 5' terminal nucleotide and 3' terminal nucleotide as the instant claims are define the gap relative to the target nucleic acid. Further it is unclear if the 5' region and 3' region require the 5' terminal and 3' terminal nucleotides, it is thus unclear if the hybridization of the 3' and 5' region to a target nucleic acid in which the 3' nucleotide and 5' nucleotide are adjacent to each other or have a single nucleotide gap meet this limitation.

Claims 2-4 are rejected as they depend from claim 1.

Claim 3 recites, "wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe." The recitation appears an attempt to exclude the probes from overlapping, however claim 1 defines the probes with respect to the target nucleic acid and requires, "wherein each subregion overlaps with at least one other subregion." Thus claim 1 appears to require the probes are to overlapping subregions of the target nucleic acid, which appears to require the probes overlap. Thus it is unclear how claim 1

can require the probes to be overlapping and claim 3 requires the probes are non-overlapping. Further it is unclear if non-overlapping is intended to exclude a single nucleotide that is identical, 2 identical nucleotides, 10 identical nucleotides, etc.

**Response to Arguments.**

The response provides no arguments or indication to the 112-2nd paragraph rejection of claim as to what is required or encompassed of the "3' region" or "5' region." Thus the rejection is maintained and leads to the issues presented in the rejection with respect to claim 3.

The response traverses the rejection asserting, "Accordingly, the subject matter of claim 1 refers to subregions that partially overlap with each other. Since each subregion includes a portion that overlaps with a portion of another subregion and a portion that is different for the other subregions, it is consistent to have probes where the 5' and 3' regions of each probe are all different and non-overlapping of each other, as recited in claim 3. Stated another way, claim 3 recites that the 5' and 3' regions of each probe hybridize to the portions of the subregions that do not overlap with each other. Therefore, claim 3 does not contradict the subject matter of claim 1." These arguments have been thoroughly reviewed but are not considered persuasive as the claims do not specifically define what is encompassed or required of the 5' region and the 3' region, thus the 5' region and 3' region can encompass the subregions and thus the metes and bounds of the claims are unclear. Further the subregions of claim 1 are defined relative to the target nucleic acid, thus making the metes and bounds of the requirements of the 5' region, 3'regions and subregions unclear as indicated in the 112-

2nd paragraph rejection of claim 1 from the non-final rejection that was not specifically addressed by the response.

***Claim Rejections - 35 USC § 103***

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 1-4 rejected under 35 U.S.C. 103(a) as being unpatentable over Hardenbohl et al (Nature Biotechnology(2003) volume 21, pages 673-678) in view of Mockler et al (Genomics (2005) volume 85, pages 1-15).

This rejection is presented in view of the 112-2nd paragraph issues with respect to the "5' region and the 3' region of each probe are separated by a plurality of nucleic acids on the target molecule" and with regard to claim 3.

MPEP 2111 states:

The court held that the PTO is not required, in the course of prosecution, to interpret claims in applications in the same manner as a court would interpret claims in an infringement suit. Rather, the "PTO applies to verbiage of the proposed claims the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in applicant's specification."

MPEP 2111.01 states

Though understanding the claim language may be aided by explanations contained in the written description, it is important not to import into a claim limitations that are not part of the claim.

With regards to claim 1, Hardenbol discloses a method of analyzing a plurality of genetic loci by contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1).

Hardenbohl teaches in figure 2b the joining of the molecular inversion probes which results in a circular type configuration. Hardenbohl exemplifies in fig 2b a probe that has a 5’ region and a 5’ terminal nucleotide, a 3’ region and a 3’ terminal nucleotide, and a single base gap. Thus the 5’ terminal nucleotide, 3’ terminal nucleotide and single base gap are a plurality of nucleotides between the 3’ and 5' region of the probe.

Hardenbohl does not specifically teach wherein each subregion overlaps with at least one other subregion. Hardenbohl does not specifically teach, "wherein at least one subregion entirely overlaps at least one subregion.”

Mockler teaches each subregion overlaps with at least one other subregion (Fig. 2a).

With regards to claim 2, Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other Subregion)).

With regards to claim 3, Mockler teaches the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

With regards to claim 4, Mockler teaches sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the tiling probes of Mockler in the method of Hardenbol. The artisan would be motivated as Mockler teaches the probe tiling approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1). The artisan would have a reasonable expectation of success as the artisan is merely combining known probes and methods for analysis of genetic information.

**Response to Arguments**

The response asserts that the Hardenbohl does not teach or suggest every limitation of the claim as Hardenbohl teaches a single base gap between the termini of

the probe. This argument has been thoroughly reviewed but is not considered persuasive as the claims require "5' region and the 3' region of each probe are separated by a plurality of nucleic acids on the target molecule." As indicated in the 112-2nd paragraph rejection this recitation is indefinite for numerous reasons explained in more detail above. Further the claims do not require a gap of a plurality of nucleotides between the termini as asserted, but between the 3' region and 5' region which is indefinite as well. Thus these arguments are beyond the scope of the claimed invention.

The response asserts the teachings of Mockler do not overcome the alleged deficiencies of Hardenbohl. These arguments have been thoroughly reviewed but are not considered persuasive as deficiencies of Hardenbohl asserted by the response are limitations not required of the claim.

The rejection is thus maintained.

4. Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhras et al (PLOS one (2007) volume 2 (9) e915) (IDS 3/28/2013), Nilsson et al (Trends in Biotechnology (2006 ) volume 24, pages 83-88), Hardenbohl et al (Nature Biotechnology(2003) volume 21, pages 673-678) in view of Mockler et al (Genomics (2005) volume 85, pages 1-15).

MPEP 2111 states:

The court held that the PTO is not required, in the course of prosecution, to interpret claims in applications in the same manner as a court would interpret claims in an infringement suit. Rather, the "PTO applies to verbiage of the proposed claims the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever

enlightenment by way of definitions or otherwise that may be afforded by the written description contained in applicant's specification."

MPEP 2111.01 states

Though understanding the claim language may be aided by explanations contained in the written description, it is important not to import into a claim limitations that are not part of the claim.

It is noted that the specification lacks a limiting definition of a molecular inversion probe. Thus "molecular inversion probe" is being given the broadest reasonable interpretation in view of the claims and art as a probe that allows for formation of a circular probe upon hybridization to a target nucleic acid.

It is further noted the 112-2nd issues of claim 3 are considered in rendering this rejection.

Akhras teaches a connector inversion probe method (CIPer) that is a combination of Gap-LCR and padlock probes. Akhras teaches, "The Connector Inversion Probe (CIPer) reaction (Figure 1) shares some basic design features with a recently described assay, the PathogenMip assay [17], which includes: i) probe hybridization, single "G" base gap fill and probe circularization, ii) exonuclease based circular DNA selection, iii) probe inversion by re-linearization, and iv) universal PCR amplification. The distinguishing and key feature of the CIPer method is a modified first reaction step involving a complete filling of an extensive gap formed by the probe and target." Akhaus further teaches, " Connector Inversion Probe (CIPer) technology is a powerful method for multiplex amplification, and has been validated here in genetic pathogen diagnostics, typing, and antibiotic resistance detection arenas as a proof-of-concept. This technology can be designed and optimized for use in numerous other

scientific areas and applications. Other areas suitable for use of the CIPer method include cancer genomics [32], human SNP detection [15], and selective exon amplification [7], among numerous other promising applications. Modified CIPers may also in the future offer an alternative strategy for gateway cloning [33]. In this paper, we focused on “lowplex” systems amplifying shorter stretches of DNA of up to 100 base pair lengths. To achieve higher degrees of multiplexing and longer clone reads, one must take into account i) clone GC content, ii) clone length variability, and iii) steric effects for longer clones. Analog amplification and downstream validation are delimiting factors for multiplex detection methods. We believe that the future of CIPer technology will rely on digital amplification [34] and high throughput sequencing validation (Figure S5). The CIPers universal primer sites can be formatted to fit any amplification method, e.g. RCA [12], [13], emulsion PCR [34], SPIA [35] LAMP [36] and SMAP 2 [37]; universal adaptor ligation, a tedious and sample-consuming procedure [38], is therefore optional. Proposed digital amplification methods include emulsion PCR, RCA, or a combination of the two [39]. Suitable upcoming high throughput validation methods include next-generation sequence systems such as the 454 Life Sciences platform [38] (http://www.454.com), the Solexa platform [40] (http://www.illumina.com), polony sequencing [41] (http://www.agentcourt.com), Helicos Biosciences [42] (http://www.helicosbio.com), or use of a resequencing array (GeneChip CustomSeq, www.affymetrix.com). Recent digital quantification methods [43] open the possibility for quantitative CIPer technology. With the addition of a molecular barcode to the padlocks [14], MIPs were used for ultra high-throughput SNP detection based on microarray

barcode hybridization [15]. Because CIPers can also include a barcode, they could be used for microarray validation, or amplicon tracing with sequencing methods [17], [44] allowing for longer read lengths [38]. With the introduction of a third universal sequence flanking the barcode and the universal primer sites, one can begin to envision a two-dimension informational barcode, a concept we call “multiplexing multiplex padlocks” (MMP) (Figure S6). Probe construction based on ligation of three smaller constructs [45] would allow for variability in fragment assembly. By combining the one-genotype detection pair of AS/ES constructs with multiple unique barcodes, we can create patient and genotype specific probes with one barcode per probe. This will allow for analysis of multiple parallel samples, essentially lowering the cost of microarray chips and sequencing reactions.”

Nilsson teaches the use of padlock probes and “selector” probes that form circles with gaps of several nucleotides between the 5’ terminal nucleotide and the 3’ terminal nucleotide.

Akhras and Nilsson demonstrate probes were known that result in a circular probe upon hybridization to a target sequence with a gap between the 3' terminal nucleotide and 5' terminal nucleotide were known. Akhras suggests the use of CIPer methods similar to the use of MIPs, including the use of “barcodes to allow detection by microarrays.”

Akhras and Nilsson do not specifically teach, “contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to

nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion."

However, Hardenbol discloses a method of analyzing a plurality of genetic loci by contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1). Hardenbohl teaches these probes are MIP or padlock probes.

Hardenbohl teaches in figure 2b the joining of the molecular inversion probes which results in a circular type configuration. Hardenbohl exemplifies in fig 2b a probe that has a 5' region and a 5' terminal nucleotide, a 3' region and a 3' terminal nucleotide, and a single base gap. Thus the 5' terminal nucleotide, 3' terminal nucleotide and single base gap are a plurality of nucleotides between the 3' and 5' region of the probe.

Hardenbohl does not specifically teach wherein each subregion overlaps with at least one other subregion. Hardenbohl does not specifically teach, "wherein at least one subregion entirely overlaps at least one subregion."

Mockler, teaches each subregion overlaps with at least one other subregion (Fig. 2a).

With regards to claim 2, Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other Subregion)).

With regards to claim 3, Mockler teaches the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

With regards to claim 4, Mockler teaches sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the CIPer or selector probes taught by Akhras and Nilsson with a gap of a plurality of nucleotides between the 5' terminal nucleotide and 3' terminal of the padlock probes with tiling probes of Mockler in the method of Hardenbol. The artisan would be motivated as Akhras suggests the use of CIPer methods in MIP methods. The teachings of Hardenbohl set forth one

embodiment of the use of MIP or padlock probes. The artisan further would be motivated as Mockler teaches the probe tiling approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1). The artisan would have a reasonable expectation of success as the artisan is merely increasing the number of nucleotides between the 3' terminal nucleotide and 5' terminal nucleotide of the probes of Mockler, which Akhras demonstrates were known and worked. Thus the combination of references suggests all the limitations of claims and suggests the use of CIPer in place of the similar MIP methods.

**Response to Arguments**

This is a new grounds of rejection presented in view of the amendment of claim 1 as interpreted in view of the responses interpretation of the claims. Thus there are no arguments to the instant rejection.

**Summary**

No claims are allowed.

***Conclusion***

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not

mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-5:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Steven C Pohnert/
Primary Examiner, Art Unit 1634

| Notice of References Cited | Application/Control No. 13/266,862 | Applicant(s)/Patent Under Reexamination PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | Classification |
|---|---|---|---|---|---|
| | A | US- | | | |
| | B | US- | | | |
| | C | US- | | | |
| | D | US- | | | |
| | E | US- | | | |
| | F | US- | | | |
| | G | US- | | | |
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| | J | US- | | | |
| | K | US- | | | |
| | L | US- | | | |
| | M | US- | | | |

**FOREIGN PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Country | Name | Classification |
|---|---|---|---|---|---|---|
| | N | | | | | |
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| | P | | | | | |
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| | R | | | | | |
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| | T | | | | | |

**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
|---|---|---|
| | U | Nilsson et al (Trends in Biotechnology (2006 ) volume 24, pages 83-88) |
| | V | |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

**OR**

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2013-03-28 |
|---|---|---|---|
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
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|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| /S.P./ | 2 | 20080280955 | | 2008-11-13 | McCamish | |
| /S.P./ | 3 | 20090099041 | | 2009-04-16 | CHURCH et al. | |
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|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| /S.P./ | 1 | 1995/011995 | WO | A1 | 1995-05-04 | Affymax Tech Nv | | ☐ |
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| /S.P./ | 1 | Akhras (PLOS ONE, 2(9):e915-e915, 2007) | ☐ |
| /S.P./ | 2 | Ball (Nature Biotechnology, 27(4):361-368, 2009) | ☐ |
| /S.P./ | 3 | BAU (Analytical and Bioanalytical chemistry, 393(1):171-175, 2008) | ☐ |
| /S.P./ | 4 | Deng et al. (Nature Biotechnology, 27(4):353-360, 2009) | ☐ |
| | 5 | Deng et al. (Nature Biotechnology) SUPPLEMENT | ☐ |
| | 6 | European Search Report for EP app. no. 10770071.8 | ☐ |
| /S.P./ | 7 | Hardenbol (Nature Biotechnology, 21:673-678, 2003) | ☐ |



/Steven Pohnert/ (05/30/2013)

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
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| | Application Number | 13266862 |
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| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

| | | | |
|---|---|---|---|
| | 8 | International Preliminary Report on Patentability for WO 2010/126614 | ☐ |
| | 9 | International Search Report and Written Opinion for WO 2010/126614 | ☐ |
| /S.P./ | 10 | Krishnakumar (Proceedings of the National Acaemy of Sciences, 105(27):9296-9301, 2008) | ☐ |
| /S.P./ | 11 | Mockler (Genomics, 85:1-15, 2005) | ☐ |
| /S.P./ | 12 | Porreca (Nature Methods, 4(11): 931-936, 2007) | ☐ |
| /S.P./ | 13 | Turner (Nature Methods, 6(5):315-316, 2009) | ☐ |
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|---|---|
| **EFS ID:** | 16793998 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Thomas Hellenthal |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 09-SEP-2013 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 11:55:36 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

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## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Request for Continued Examination (RCE) | GSGE_002_01US_RCE.pdf | 697519<br>618df3964e4d092ae8da80dcbe26332a13411761 | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | | GSGE_002_01US_Amendment_and_response_to_OA_mailed_6_7_2013.pdf | 57804<br>cdc70e50e3fc1e400d14e23ec07b6a0b42c4f895 | yes | 14 |

**Multipart Description/PDF files in .zip description**

| Document Description | Start | End |
|---|---|---|
| Amendment Submitted/Entered with Filing of CPA/RCE | 1 | 1 |
| Specification | 2 | 2 |
| Claims | 3 | 9 |
| Applicant Arguments/Remarks Made in an Amendment | 10 | 14 |

| | | | | | |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Fee Worksheet (SB06) | fee-info.pdf | 30409<br>21b81113d07935c9115137fe769a7652c62d7933 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
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Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (07-09)
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# REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
## (Submitted Only via EFS-Web)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Application Number | 13266862 | Filing Date | 2012-03-13 | Docket Number (if applicable) | GSGE-002/01US 29258/20 | Art Unit | 1634 |
| First Named Inventor | Gregory Porreca | | | Examiner Name | S. C. Pohnert | | |

**This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.**
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

### SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

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- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☒ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

### MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

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### SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

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|---|---|---|---|
| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2013-09-09 |
| Name | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

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4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANT: | Porreca et al. | ART UNIT: | 1634 |
| SERIAL NUMBER: | 13/266,862 | CONFIRMATION NO.: | 6082 |
| FILING DATE: | March 13, 2012 | EXAMINER: | Pohner, Steven C. |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

**FILED ELECTRONICALLY**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

**AMENDMENT AND RESPONSE**

This paper is in response to a final Office Action mailed June 7, 2013 from the United States Patent and Trademark Office. This response is submitted with a Request for Continued Examination (RCE). Applicants authorize the Commissioner to charge any required fees due with this response to Deposit Account No. 500369.

**Amendments to the specification** begin on page 2.

**Amendments to the claims** begin on page 3.

**Remarks** begin on page 10.

Amendments to the specification

No amendments to the specification are made herein.



Petitioner Illumina, Inc., EX1002, p. 702

Amendment to the claims

Please amend claim 1 as follows. This list of claims replaces all prior versions and listings of claims in this application.

1. (Currently amended) A method of analyzing a plurality of genetic loci, the method comprising:

contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region that comprises a 5' terminal nucleotide and a 3' region that comprises a 3' terminal nucleotide, the 5' region and 3' region being ~~are~~ complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion, ~~and~~ wherein when hybridized to the target nucleic acid in a circular configuration, the 5' ~~region~~ terminal nucleotide and the 3' ~~region~~ terminal nucleotide of each probe are separated by a plurality of ~~nucleic acids~~ nucleotides on the target nucleic acid, and wherein at least two of the plurality of molecular inversion probes are present at different concentrations,

converting hybridized probes into closed circularized probes,

isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

2. (Original) The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion.

3. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.

4. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5-11. (Canceled)

12. (Withdrawn) A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. (Withdrawn) The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

14. (Withdrawn-Previously presented) The method of claim 12, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

15. (Withdrawn-Previously presented) The method of claim 12, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. (Withdrawn-Previously presented) The method of claim 12, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

17-19. (Canceled)

20. (Withdrawn) A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. (Withdrawn) The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is lower than the reference capture efficiency.

23. (Withdrawn) The method of claim 22, wherein the subject is identified as having an insertion in the target region.



Petitioner Illumina, Inc., EX1002, p. 705

24. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is higher than the reference capture efficiency.

25. (Withdrawn) The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. (Withdrawn) The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.

27. (Withdrawn) The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. (Withdrawn-Previously presented) The method of claim 20, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. (Withdrawn) The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

30. (Withdrawn) The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31-32. (Canceled)

33. (Withdrawn-Previously presented) The method of claim 29, wherein the first and second targeting arms have the same length.

34-35. (Canceled)

36. (Withdrawn-Previously presented) The method of claim 29, wherein the hybridization Tms of the first and second targeting arms are identical.

37. (Withdrawn-Previously presented) The method of claim 28, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. (Withdrawn) The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. (Canceled)

40. (Withdrawn) The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.

42. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.



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43. (Withdrawn-Previously presented) The method of claim 29, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. (Withdrawn-Previously presented) The method of claim 20, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. (Withdrawn) The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. (Withdrawn) The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. (Withdrawn) The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48-49. (Canceled)

50. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. (Withdrawn) The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.

52. (Withdrawn-Previously presented) The method of claim 50, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.

53. (Withdrawn) The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.

55. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. (Withdrawn-Previously presented) The method of claim 20, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57-67 (Canceled)

Remarks

Claim 1 is amended to recite that the 5' region includes the 5' terminal nucleotide, that the 3' region includes the 3' terminal nucleotide, and that the 5' terminal nucleotide and the 3' terminal nucleotide are separated by a plurality of nucleotides. Support for those amendments is found at p. 12 lines 10-24, p. 33 line 16 to p. 34 line 8, p. 60 line 28 to p. 62 line 22, p. 74 line 30 to p. 75 line 17, and FIG. 9 of the as-filed application. Claim 1 is also amended to recite that at least two of the plurality of molecular inversion probes are present at different concentrations. Support for that amendment is found at p. 69 line 28 to p. 70 line 23. No new matter is added.

Clarity of Claims

The Office Action rejects claim 1 under 35 U.S.C. §112 ¶2 stating that it is unclear if the phrases "5' region" and "3' region" include the 5' and the 3' terminal nucleotides. Claim 1 is amended to recite that the 5' region includes the 5' terminal nucleotide and that the 3' region includes the 3' terminal nucleotide. Based on that amendment, Applicants respectfully request withdrawal of that rejection.

The Office Action rejects claim 1 under 35 U.S.C. §112 ¶2 stating that the phrase "separated by a plurality of nucleic acids on the target nucleic acid" is unclear. That phrase is amended to state "separated by a plurality of nucleotides on the target nucleic acid". Based on that amendment, Applicants respectfully request withdrawal of that rejection.

The Office Action rejects claim 3 under 35 U.S.C. §112 ¶2 stating that the claim is unclear because it is contradictory to the subject matter in claim 1. The rejection appears to be based on the Office Action interpreting claim 3 to require non-overlapping probes. Applicants respectfully disagree with that interpretation of claim 3.

Claim 3 only requires that the 5' region and the 3' region of each probe are non-overlapping. Using molecular inversion probes, it is not contradictory to having probes that overlap in which the 5' region and the 3' region of each probe are non-overlapping. An example of such a configuration is shown in Figure 1 of the as-filed application. Figure 1 shows a tiled

probe lay-out in which MIPs are used to capture 60 bp subregions. Figure 1 shows that between any two subregions that is a common portion of overlap and two distinct non-overlapping portions, one distinct to each subregion.

Since each subregion includes a portion that overlaps with a portion of another subregion and a portion that is different for the other subregions, it is consistent to have probes where the 5' and 3' regions of each probe are all different and non-overlapping of each other, as recited in claim 3. Further, as shown in Figure 1, such a probe configuration does not exclude overlapping probes. Therefore, one of skill in the art, reading the application at the time it was filed, would have understood that claims 1 and 3 are not contradictory of each other.

Applicants respectfully request withdrawal of the 35 U.S.C. §112 ¶2 rejection.

Claims are not Obvious

Hardenbohl and Mockler

The Office rejects claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Hardenbohl (Nature Biotechnology, 21:673-678, 2003) and Mockler (Genomics, 85:1-15, 2005). Applicants respectfully disagree.

In assessing a claim for obviousness, all the claim limitations must be considered. M.P.E.P. § 2143.03. Moreover, to establish prima facie obviousness of a claimed invention, all the claim features must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974). The amended claims require that when hybridized to a target nucleic acid in circular configuration, a 5' terminal nucleotide and a 3' terminal nucleotide of each molecular inversion probe are separated by a plurality of nucleotides on the target nucleic acid. The as-filed application at p. 33 lines 16-29 explains that a problem in the art is uniform capture of a target. The as-filed application at pp. 33-36 explains that tiling MIPs across a target at a specific coverage factor solves that problem. Tiling MIPs across a target at a specific coverage can only be accomplished if a 5' terminal nucleotide and a 3' terminal nucleotide of each molecular inversion probe are separated by a plurality of nucleotides on the target nucleic

acid. The amended claims are not obvious in view of the combination of Hardenbohl and Mockler because that combination does not teach or suggest that required element of amended claim 1.

Hardenbohl is limited to reporting the use of padlock probes that hybridize to a target in a manner such that a 5' terminal nucleotide and a 3' terminal nucleotide are separated from each other by only a single nucleotide (Hardenbohl, p. 674, section entitled "Molecular inversion probe (MIP) genotyping"; p. 675, Fig. 2; p. 677, Methods section entitled "Molecular inversion probe assay"). Hardenbohl states:

> A mixture of Genomic DNA, 1,000 or more probes, thermostable ligase and polymerase is heat-denatured and brought to annealing temperature. Two sequences targeting each terminus of the probe hybridize to complementary sites in the genome, creating a circular conformation with a single-nucleotide gap between the termini of the probe. Ibid., p. 675, right column, Description of Figure 2.

According, Hardenbohl does not teach or suggest the element of amended claim 1 of a molecular inversion probe having a 5' terminal nucleotide and a 3' terminal nucleotide that hybridizes to a target nucleic such that in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide are separated by a plurality of nucleotides on the target nucleic acid. Rather, Hardenbohl is limited to methods in which padlock probes hybridize to a target in a manner that a 5' terminal nucleotide and a 3' terminal nucleotide are separated from each other by only a single nucleotide.

Mockler does not cure the deficiencies of Hardenbohl. Mockler is limited to reporting general tiling strategies using immobilized linear probes. Mockler does not teach or suggest molecular inversion probes. Since Mockler does not teach or suggest molecular inversion probes, it is not possible for Mockler to teach or suggest the subject matter of amended claim 1 of a molecular inversion probe having a 5' terminal nucleotide and a 3' nucleotide that hybridizes to a target nucleic such that in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide are separated by a plurality of nucleotides on the target nucleic acid.

Accordingly, the combination of Hardenbohl and Mockler does not teach or suggest all of the subject matter of amended claim 1, and therefore that amended claim is not obvious in view of the combination of Hardenbohl and Mockler. Claims 2-4 depend from amended claim 1, and therefore also are not obvious in view of the combination of Hardenbohl and Mockler.

Applicants respectfully requests withdrawal of the rejection of claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Hardenbohl and Mockler.

Akhras, Nilsson, Hardenbohl and Mockler

The Office rejects claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Akhras et al. (PLOS one, 2(9):e915, 2007), Nilsson et al. (Trends in Biotechnology, 24:83-88, 2006), Hardenbohl, and Mockler. Applicants respectfully disagree.

The as-filed application at p. 64 line 24 to p. 69 line 28 explains that bias can be present in a capture reaction, which subsequently biases analysis results. That section of the application explains that the bias can result from alleles being present in the sample at ratios other than a 50:50 representation. The as-filed application at p. 69 line 28 to p. 70 line 23 explains that such bias can be accounted for by having individual probes present at different relative concentrations, such that under-represented targets are increased, and over-represented targets are suppressed. Accordingly, claim 1 is amended to recite that at least two of the plurality of molecular inversion probes are present at different concentrations. Amended claim 1 is not obvious in view of the combination of the cited references, because that combination of references fails to disclose or suggest the subject matter of amended claim 1 of at least two of the plurality of molecular inversion probes are present at different concentrations.

Akhras, Nilsson, and Hardenbohl are limited to reporting padlock and molecular inversion probes. Mockler is limited to reporting probe tiling strategies. None of those references is concerned with bias in a capture reaction, and none of those references appreciate the problem of bias in a capture reaction. Accordingly, none of those references discloses or suggests the element of the amended claim of at least two of the plurality of molecular inversion

probes are present at different concentrations.

Therefore, the amended claims are not obvious in view of the combination of the cited prior art, because that combination fails to disclose or suggest the element of amended claim 1 of at least two of the plurality of molecular inversion probes are present at different concentrations.

Applicants respectfully requests withdrawal of the rejection of claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Akhras, Nilsson, Hardenbohl, and Mockler.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: September 9, 2013

Respectfully submitted,
**BROWN RUDNICK LLP**

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

61294650



Petitioner Illumina, Inc., EX1002, p. 714

| Electronic Patent Application Fee Transmittal | |
|---|---|
| **Application Number:** | 13266862 |
| **Filing Date:** | 13-Mar-2012 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Filer:** | Thomas C. Meyers/Thomas Hellenthal |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| Filed as Small Entity | |

**U.S. National Stage under 35 USC 371 Filing Fees**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Miscellaneous:** | | | | |
| Request for Continued Examination | 2801 | 1 | 600 | 600 |
| | | **Total in USD ($)** | | **600** |

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 19112061 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Diane Riordan |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 27-MAY-2014 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 16:30:45 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

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## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Foreign Reference | WO2000018957.PDF | 4448929<br>fe4d7f96ab629f57703290579247da823191da76 | no | 104 |

| **Warnings:** |
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| **Information:** |

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| 2 | Foreign Reference | WO2004018497.PDF | 4640674<br>8cc191778df049047076aaa5ee4c6ad9554134a5 | no | 134 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Foreign Reference | WO2007010251.PDF | 4137941<br>4270020ecb45e82b0f4e563ccb6242dde19c5272 | no | 99 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Foreign Reference | WO2007123744.PDF | 5501090<br>5862ece798d51d688915149760ee3ab4318a6908 | no | 135 |
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| 6 | Foreign Reference | WO2009036525.PDF | 1724273<br>5c5b06018ea2f5d3aa8c32c69256f6624ecf74fa | no | 32 |
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| **Information:** | | | | | |
| 7 | Foreign Reference | WO2010126614.PDF | 5196725<br>e85748b22222448b7226ac9d64fee035d303cc25 | no | 116 |
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| **Information:** | | | | | |
| 8 | Foreign Reference | WO2012051208.PDF | 3296920<br>69c62e39de5e4ab46550f0ace40e9a19300e8638 | no | 69 |
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| **Information:** | | | | | |
| 9 | Foreign Reference | WO2012087736.PDF | 1947707<br>d5cb06997318b172358798fe35f07f83107b7e22 | no | 39 |
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| **Information:** | | | | | |
| 10 | Foreign Reference | WO2012109500.PDF | 10622992<br>674286d6435580d3f1839361c2183a55595711ef | no | 157 |
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| **Information:** | | | | | |

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|---|---|---|---|---|---|
| 11 | Foreign Reference | WO2012134884.PDF | 1890215<br>4f7d306f449a79886e176ba7e957437e34e39d70 | no | 38 |
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| 12 | Foreign Reference | WO2012040387.PDF | 6731930<br>3aa20f1503de814cd677ea08df6770114e9e7d28 | no | 57 |
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| 13 | Foreign Reference | WO2013058907.PDF | 1304899<br>5d97e52de554a9d1525f4013cbfabd11356cb8b0 | no | 27 |
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| 14 | Foreign Reference | WO2002093453.PDF | 2305981<br>a9d6ae59637ec821793c9adc48c93bb957480b72 | no | 49 |
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| **Information:** | | | | | |
| 15 | Foreign Reference | WO2010024894.PDF | 1608570<br>4c09301bd7af977ac8782266c9188b7433074627 | no | 42 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 16 | Information Disclosure Statement (IDS) Form (SB08) | GSGE-002-01US-IDS-2.pdf | 615802<br>c01f547f438f8600b7fc43936381fa012f46eddf | no | 8 |
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| 18 | Information Disclosure Statement (IDS) Form (SB08) | GSGE-002-01US-IDS-4.pdf | 613417<br>aeffe6980f263d29babc4f51c9329610837b22a4 | no | 5 |
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| 19 | Information Disclosure Statement (IDS) Form (SB08) | GSGE-002-01US-IDS-1.pdf | 621550<br>3daabedd8333cb23e4c200f7c47387b6b8733a9e | no | 27 |
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**New Applications Under 35 U.S.C. 111**

**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**

**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**

**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| | | | |
|---|---|---|---|
| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2014-05-27 |
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** ( **Not for submission under 37 CFR 1.99)** | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

| | | | |
|---|---|---|---|
| | 1 | International Search Report and Written Opinion mailed August 12, 2013, for International Patent Application No. PCT/US13/36575, filed April 15, 2013 (9 pages) | ☐ |
| | 2 | International Search Report and Written Opinion mailed February 25, 2013 for International Patent Application No. PCT/US12/55362 | ☐ |
| | 3 | International Search Report and Written Opinion mailed June 10, 2013, for International Patent Application No. PCT/US13/33435, filed March 22, 2013 (6 pages) | ☐ |
| | 4 | International Search Report and Written Opinion mailed June 14, 2012, for International Patent Application No. PCT/US12/29790, filed March 20, 2012 (8 pages) | ☐ |
| | 5 | International Search Report and Written Opinion mailed November 1, 2013, for International Patent Application No. PCT/US2013/044039, filed June 4, 2013 (6 pages) | ☐ |
| | 6 | International Search Report and Written Opinion mailed on February 4, 2014, for Patent Application No. PCT/US13/62842, filed October 1, 2013 (5 pages) | ☐ |
| | 7 | International Search Report and Written Opinion mailed on June 28, 2013, for Patent Application No. PCT/US2013/032885, filed March 19, 2013 (9 pages) | ☐ |
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**EXAMINER SIGNATURE**

| Examiner Signature | | Date Considered | |
|---|---|---|---|

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference GSGE-009/01WO 29258/54 | FOR FURTHER ACTION | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US2013/036575 | International filing date *(day/month/year)* 15 April 2013 | (Earliest) Priority Date *(day/month/year)* 16 April 2012 |
| Applicant GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☒ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US2013/036575

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)
   - [x] on paper
   - [x] in electronic form

   b. (time)
   - [ ] in the international application as filed
   - [ ] together with the international application in electronic form
   - [x] subsequently to this Authority for the purposes of search

2. [x] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036575

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2013.01).
USPC - 435/6.11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12N 15/10, 15/11; C12P 19/34; C12Q 1/68; C40B 40/08 (2013.01)
USPC - 435/6.1, 6.11, 91.1; 506/2, 7, 16; 702/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC Class/Subclass(es): C12Q 1/6869, 1/6876, 1/6883, 1/701

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, Google, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2010/126614 A2 (PORRECA et al) 04 November 2010 (04.11.2010) entire document | 1-6, 9-15, 18 |
| --- | | --- |
| Y | | 7, 8, 16, 17, 19, 20 |
| Y | EP 1564306 A2 (MCGALL et al) 17 August 2005 (17.08.2005) entire document | 7, 8, 16, 17, 19, 20 |
| A | WO 2012/040387 A1 (MYLLYKANGAS et al) 29 March 2012 (29.03.2012) entire document | 1-20 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 July 2013 | 12 AUG 2013 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Blaine R. Copenheaver<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 12 AUG 2013 |
| Applicant's or agent's file reference<br>GSGE-009/01WO 29258/54 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US2013/036575 | 15 April 2013 | 16 April 2012 |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 23 July 2013 | Blaine R. Copenheaver<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2013/036575

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☒ on paper

      ☒ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☒ subsequently to this Authority for the purposes of search

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2013/036575

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 3, 4, 7, 8, 12, 13, 16, 17 | YES |
| | Claims | 1, 2, 5, 6, 9-11, 14, 15, 18 | NO |
| Inventive step (IS) | Claims | None | YES |
| | Claims | 1-20 | NO |
| Industrial applicability (IA) | Claims | 1-20 | YES |
| | Claims | None | NO |

2. Citations and explanations:

Claims 1, 2, 5, 6, 9-11, 14, 15 and 18 lack novelty under PCT Article 33(2) as being anticipated by Porreca et al. (hereafter Porreca).

Regarding Claim 1, Porreca discloses a method of improving performance of molecular inversion probe capture reactions (In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes, Pg. 32, Lns. 30-32; capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, Pg. 9, Lns. 15-16; methods include molecular inversion probe capture reactions, Pg. 22, Lns. 32-33), the method comprising: obtaining a nucleic acid (Invention provides a method of obtaining a nucleic acid preparation, Pg. 23, Lns. 18-19); fragmenting the nucleic acid into nucleic acid fragments (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12); and capturing a target on a nucleic acid fragment with a molecular inversion probe (Capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, Pg. 9, Lns. 15-16; DNA should be sheared into fragments, Pg. 77, Ln.12).

Regarding Claim 2, Porreca discloses the method of claim 1, further comprising the step of denaturing the nucleic acid or at least one nucleic acid fragment prior to the capturing step (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12; heat reaction to 95C for 5 min to denature genomic DNA, Pg. 75, Lns. 20-26 (nucleic acids are denatured prior to capture reaction); prior to performing a gene capture reaction, Pg. 44, Ln. 2).

Regarding Claim 5, Porreca discloses the method of claim 1, wherein the nucleic acid is selected from the group of genomic DNA, genomic RNA, whole or partial genome amplification product, high molecular weight DNA, and high molecular weight RNA (An experiment utilizing 1 ug of genomic DNA, Pg. 42, Ln. 20).

Regarding Claim 6, Porreca discloses the method of claim 1, wherein the step of fragmenting comprises mechanical fragmenting, chemical fragmenting, or enzymatic fragmenting (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12).

Regarding Claim 9, Porreca discloses the method of claim 2, wherein the step of denaturing comprises pH-based denaturing, heat-based denaturing, exonuclease degradation, or endonuclease nicking (Exonuclease digestion of non-circularized material is performed, Pg. 60, Ln. 21).

Regarding Claim 10, Porreca discloses a method of improving performance of capture reactions (In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes, Pg. 32, Lns. 30-32; capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, Pg. 9, Lns. 15-16; methods include molecular inversion probe capture reactions, Pg. 22, Lns. 32-33), the method comprising: obtaining a genomic nucleic acid (An experiment utilizing 1 ug of genomic DNA, Pg. 42, Ln. 20; invention provides a method of obtaining a nucleic acid preparation, Pg. 23, Lns. 18-19); fragmenting the genomic nucleic acid into nucleic acid fragments; contacting a probe to a nucleic acid fragment (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12; contacting a nucleic acid sample with a preparation of a probe e.g., a MIP [molecular inversion probe], Pg. 19, Ln.23), the probe comprising at least two target arms designed to hybridize upstream and downstream of a target sequence located on the nucleic acid fragment; and isolating the target sequence between the target arms; and analyzing the target sequence (Targeting arms of a probe that is designed to capture the target region or sequence thereof, Pg. 12, Lns. 11-12; both targeting arms may be designed to hybridize several bases or more upstream or downstream from the repeat region in such a way that the captured sequence includes a region of unique genomic sequence that on one or both sides of the repeat region; this unique region can then be used to identify the captured target e.g., based on sequence or hybridization information, Pg. 12, Lns. 20-24; associating unique sequence tags referred to as differentiator tag sequences with individual target molecules that are independently captured and/or analyzed, Pg. 39, Lns. 21-23).

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2013/036575

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 11, Porreca discloses the method of claim 10, further comprising the step of denaturing the genomic nucleic acid or at least one nucleic acid fragment prior to the contacting step (Heat reaction to 95C for 5 min to denature genomic DNA (nucleic acids are denatured prior to contacting), Pg. 75, Ln. 25; prior to performing a gene capture reaction, Pg. 44, Ln. 2; wherein the target nucleic acid is a captured genomic locus, Pg. Ln. 3; analyzing a plurality of genetic loci are provided; the methods comprise contacting each of a plurality of target nucleic acids, Pg. 5, Lns. 26-28).

Regarding Claim 14, Porreca discloses the method of claim 10, wherein the nucleic acid is selected from the group of genomic DNA, genomic RNA and whole or partial genome amplification product (An experiment utilizing 1 ug of genomic DNA, Pg. 42, Ln. 20).

Regarding Claim 15, Porreca discloses the method of claim 10, wherein the step of fragmenting comprises mechanical fragmenting, chemical fragmenting, or enzymatic fragmenting (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12).

Regarding Claim 18, Porreca discloses the method of claim 10, wherein the step of denaturing comprises pH-based denaturing, heat-based denaturing, exonuclease degradation, or endonuclease nicking (Exonuclease digestion of non-circularized material is performed, Pg. 60, Ln. 21).

Claims 3, 4, 12, and 13 lack an inventive step under PCT Article 33(3) as being obvious over Porreca et al. (hereafter Porreca).

Regarding Claim 3, Porreca discloses the method of claim 1, but Porreca fails to explicitly disclose wherein the nucleic acid fragments are from about 5kb to about 100kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 5kb to about 100kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Regarding Claim 4, Porreca discloses the method of claim 1, wherein the nucleic acid fragments are about 1kb in length or more (Probes can be designed to capture targets [nucleic acids] having lengths in the range of up to 10, 20, 30.....1000, or more bps, [wherein 1000 base pair is equivalent to 1kb], Pg. 34, Lns. 7-8; DNA should be sheared into fragments, Pg. 77, Ln.12), but fails to explicitly disclose wherein the nucleic acid fragments are from about 1kb to about 10kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 1kb to about 10kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Regarding Claim 12, Porreca discloses the method of claim 10, but Porreca fails to explicitly disclose wherein the nucleic acid fragments are from about 5kb to about 100 kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 5kb to about 100kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Regarding Claim 13, Porreca discloses the method of claim 10, wherein the nucleic acid fragments are about 1kb in length or more (Probes can be designed to capture targets [nucleic acid] having lengths in the range of up to 10, 20, 30.....1000, or more bps, [wherein 1000 base pairs is equivalent to 1kb], Pg. 34, Lns. 7-8; DNA should be sheared into fragments, Pg. 77, Ln.12), but fails to explicitly disclose wherein the nucleic acid fragments are from about 1kb to about 10kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 1kb to about 10kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Claims 7, 8, 16, 17, 19 and 20 lack an inventive step under PCT Article 33(3) as being obvious over Porreca et al. (hereafter Porreca) in view of McGall et al. (hereafter McGall).

Regarding Claim 7, Porreca discloses the method of claim 5, but Porreca fails to explicitly disclose wherein the chemical fragmenting comprises pH-based sheering.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches chemical fragmenting comprising pH-based shearing (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105°C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation of ds cDNA with Tris Buffer at high temperature, Para. [0085]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include chemical fragmenting comprising pH-based shearing as taught by McGall as preparation by fragmentation helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe (McGall, Para. [0020] and [0049]).



Petitioner Illumina, Inc., EX1002, p. 735

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 8, Porreca discloses the method of claim 6, but Porreca fails to explicitly disclose wherein the pH-based shearing also denatures the nucleic acid.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches pH-based shearing also denaturing the nucleic acid (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105° C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation mixtures containing 10 mg ds cDNA, 10 mM Tris-HCl, pH 7.2 at room temperature were incubated at 95°C for 75, 90, 105 and 120 minutes, Para. [0086], [wherein the instant application the use of pH or heat during the fragmenting step can result in denatured nucleic acid fragments, Pg. 11, Lns. 10-11]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include pH-based shearing also denaturing the nucleic acid as taught by McGall as DNA preparation by fragmentation and heat denaturation at 95°C helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe (McGall, Paras. [0020], [0049], [0086]).

Regarding Claim 16, Porreca discloses the method of claim 15, but Porreca fails to explicitly disclose wherein the chemical fragmentation comprises pH-based shearing.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches chemical fragmenting comprising pH-based shearing (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105°C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation of ds cDNA with Tris Buffer at high temperature, Para. [0085]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include chemical fragmenting comprising pH-based shearing as taught by McGall as preparation by fragmentation helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe, (McGall, Paras. [0020] and [0049]).

Regarding Claim 17, Porreca discloses the method of claim 14, but Porreca fails to explicitly disclose wherein the pH-based shearing also denatures the nucleic acid.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches pH-based shearing also denaturing the nucleic acid (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105° C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation mixtures containing 10 mg ds cDNA, 10 mM Tris-HCl, pH 7.2 at room temperature were incubated at 95°C, Para. [0086], [wherein the instant application the use of pH or heat during the fragmenting step can result in denatured nucleic acid fragments, Pg. 11, Lns. 10-11]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include pH-based shearing also denaturing the nucleic acid as taught by McGall as DNA preparation by fragmentation and heat denaturation at 95°C helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe, (McGall, Paras. [0020], [0049], [0086]).

Regarding Claim 19, Porreca discloses a method of preparing a library for sequencing (During a library preparation process, each nucleic acid molecule may be tagged with a unique differentiator tag sequence in a configuration that permits the differentiator tag sequence to be sequenced along with the target nucleic acid sequence of interest, Pg. 41, Lns. 3-5; methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, Pg. 39, Lns. 6-8), the method comprising; amplifying a captured target nucleic acid to generate an amplicon (The target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products [nucleic acids], Pg. 9, Lns. 10-12; in the context of a multiplex nucleic acid amplification reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, primary amplicons of a target nucleic acid, Pg. 40, Lns. 13-16), but Porreca fails to explicitly disclose performing a hydrolysis reaction on the amplicon in the presence of heat to fragment the amplicon.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches performing a hydrolysis reaction on the amplicon in the presence of heat to fragment the amplicon (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105°C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; amplifying the sample to generate amplified DNA [amplicon], Pg. 18, Ln. 6; fragmentation can be accomplished by altering temperature and pH, Pg. 7, Lns. 2-5; chemical methods for fragmentation of nucleic acids that are known in the art. These methods include: hydrolytic methods, Para. [0058]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include performing a hydrolysis reaction on the amplicon in the presence of heat to fragment the amplicon as taught by McGall as fragmentation methods including heat and hydrolytic methods can be used to select fragments of a desired size range and may be further desirable to optimize the size of nucleic acid molecules for subsequent analysis and minimize three dimensional structure, (McGall, Para. [0007], [0049] and [0058]).



Petitioner Illumina, Inc., EX1002, p. 736

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2013/036575

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 20, modified Porreca discloses the method of claim 19, but Porreca fails to explicitly disclose wherein the step of performing the hydrolysis reaction comprises incubating the amplicon in a Tris buffer having a pH range from about 7.5 to about 8.0 at a temperature of about 65°C for 16 hours.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches the step of performing the hydrolysis reaction comprising incubating the amplicon in a Tris buffer having a pH range from about 7.5 to about 8.0 at a temperature of about 65°C for 16 hours (Amplifying the sample to generate amplified DNA [amplicon], Pg. 18, Ln. 6; the DNA is in a solution that includes about 10 mM Tris-HCl, pH ~7.2-7.5, Para. [0060]; the DNA is mixed with the buffer at a temperature within the first temperature range and then incubated at a temperature in the second temperature range; the buffer is acidic in the second temperature range and the DNA is fragmented, Para. [0063]; in a preferred embodiment the buffer is Tris-HCl, Para. [0064]; tris or phosphate buffer pH 7-8.5 at 65°C is used to fragment single and double stranded DNA, Para. [0076]; the fragmentation reaction was incubated at 65°C, Para. [0097]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include a step of performing the hydrolysis reaction comprising incubating the amplicon in a Tris buffer having a pH range from about 7.5 to about 8.0 at a temperature of about 65°C for 16 hours as taught by McGall as fragmentation using Tris buffer, heat at 65°C and hydrolytic methods can be used to select fragments of a desired size range and may be further desirable to optimize the size of nucleic acid molecules for subsequent analysis and minimize three dimensional structure (McGall, Para. [0007], [0049], [0058], [0064] and [0076]).

Claims 1-20 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.



Petitioner Illumina, Inc., EX1002, p. 737

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference GSGE-008/01WO 29258/45 | **FOR FURTHER ACTION** | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US 12/55362 | International filing date *(day/month/year)* 14 September 2012 (14.09.2012) | (Earliest) Priority Date *(day/month/year)* 17 October 2011 (17.10.2011) |
| Applicant GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

Methods of identifying mutations associated with diseases

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 12/55362

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-20, drawn to a method for identifying a novel mutation associated with a disease, the method comprising: obtaining nucleic acid from a subject having a disease; identifying at least one mutation in the nucleic acid; and comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations, as indicated in claims 1 or 11; or a method for determining if a mutation is causative of a disease, as indicated in claim 20. The first named invention (claims 1-5, 7-8, 11-14, 16-17) is limited to wherein identifying comprising: sequencing the nucleic acid; wherein sequencing is sequencing-by-synthesis, and wherein the disease is cystic fibrosis. **********Continued in the extra sheet****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 7-8, 11-14, 16-17, limited to wherein identifying comprising: sequencing the nucleic acid; wherein sequencing is sequencing-by-synthesis, and wherein the disease is cystic fibrosis

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 12/55362

**Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)**

The disclosure relates to methods for analyzing nucleic acids to identify mutations associated with diseases. More specifically, the methods of the invention involve obtaining nucleic acid from a subject having a disease, identifying at least one mutation in the nucleic acid, and comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as newly identified mutations.

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 12/55362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11, 435/6.12, 435/6.17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11, 435/6.12, 435/6.17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C12Q 1/68 (2013.01) - see keyword below
USPC - 435/6.11, 435/6.12, 435/6.17 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: sequencing-by-synthesis, SBS, identifying, determine, screen, novel, mutation, new, disorder, disease, cancer, cystic fibrosis, mutation database, compare, sequencing-by-synthesis, single-molecule, obtain, sample, control, normal, Hispanic, nucleic acid, nucleotide, polynucleotide, DNA, RNA,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | CASTELLANI et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros. 2008, Vol. 7(3), p. 179-96. Abstract; pg 180, col 1, and col 2, lower para; pg 181, col 2, para 2; pg 182, col 2, para 1 and 3, and Table 2; pg 183, col 1, para 2, and col 2, para 1 and 2, and last para; pg 185, col 1, last para, and col 2, para 1; pg 186, col 1, para 3; pg 187, Table 5; pg 191, Table 7 and Table 8; pg 192, col 1, last para; and pg 193, col 1, Ref 4 | 1-2, 5, 7-8, 11,14, 16-17<br>---<br>3-4, 12-13 |
| Y | US 2011/0166029 A1 (MARGULIES et al.) 07 July 2011 (07.07.2011), para [0044], [0048], [0057], [0059], and [0064] | 3-4, 12-13 |
| A | SCHADT et al. A window into third-generation sequencing. Hum Mol Genet. 2010, Vol. 19(R2), p. R227-40. Epub 2010 Sep 21. pg R227, col 1, and col 2, middle para; pg R230, Table 1; and pg R231, col 1, last para | 1-5, 7-8, 11-14, 16-17 |
| A | NAN et al. A novel CFTR mutation found in a Chinese patient with cystic fibrosis. Comparing with normal database. Chinese Med J. 2006, Vol. 119(2), p. 103-109. Entire documentation, especially Abstract | 1-5, 7-8, 11-14, 16-17 |
| A | CHOE et al. Novel CFTR Mutations in a Korean Infant with Cystic Fibrosis and Pancreatic Insufficiency. J Korean Med Sci. 2010; Vol. 25, p. 163-5. Entire documentation, especially Abstract | 1-5, 7-8, 11-14, 16-17 |
| A | US 2011/0230365 A1 (ROHLFS et al.) 22 September 2011 (22.09.2011), Abstract, para [0005], [0071], [011], [0138], and [0150] | 1-5, 7-8, 11-14, 16-17 |

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 07 February 2013 (07.02.2013) | 25 FEB 2013 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

| International application No. |
| --- |
| PCT/US 12/55362 |

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| --- | --- | --- |
| A | US 2009/0192047 A1 (PARR et al.) 30 July 2009 (30.07.2009), para [0010], [0030], [0045], [0158], and [0200] | 1-5, 7-8, 11-14, 16-17 |

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 12/55362

Continuation of:
Box No III (unity of invention is lacking)

(Continuation of Group I+) Applicant is invited to elect an additional features, selected from: 1) wherein prior to identifying, the method further comprises attaching a barcode sequence to the nucleic acid (claims 6 and 15); or/and 2) the method further comprising determining of the novel mutation is causative of the disease, or a method for determining if a mutation is causative of a disease, comprising: identifying at least one novel mutation in the sequence, annotating the mutation with appropriate functional information, as indicated in claim 20 (claims 9-10, 18-19, 20), to be searched by paying additional fee for each election. Further provided are exemplary elections: 1) if the an additional feature wherein the method further comprises attaching a barcode sequence to the nucleic acid (claims 6 and 15) is elected, claims 6 and 15 will be searched by paying an additional fee; 2) if the additional feature of the method further comprising determining of the novel mutation is causative of the disease, or a method for determining if a mutation is causative of a disease comprising identifying at least one novel mutation in the sequence, annotating the mutation with appropriate functional information (claims 9-10, 18-19, 20) is elected, claims 9-10, 18-20 will be searched by paying an additional fee; and 3) both additional features 1) and 2) are elected, all additional claims 6, 9-10, 15, and 18-20 will be searched by paying 2X additional fees.

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Among Group I+, claims 1-8 and 11-17 do not include the inventive concept of determining of the novel mutation is causative of the disease as required by the claims, annotating the mutation with appropriate functional information, as required by claims 9-10 and 18-20; and claims 1-5, 7-14, and 16-20 do not include the inventive concept of attaching a barcode sequence to the nucleic acid, as required by claims 6 and 15.

The inventions of Groups I+ share the technical feature of determining if a mutation associated with a disease, comprising: obtaining a nucleic acid sequence from a subject having a disease, identifying at least one mutation in the nucleic acid; and claims 1-19 of Group I+ further share the technical feature of identifying a novel mutation associated with a disease, the method comprising: obtaining nucleic acid from a subject having a disease; identifying at least one mutation in the nucleic acid; and comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations. However, this shared technical feature does not represent a contribution over prior art as being obvious over an article entitled 'Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice' by CASTELLANI et al. (hereinafter 'Castellani'; J Cyst Fibros. 2008, Vol. 7(3), p. 179-96) as follows:Castellani discloses a method for identifying a novel mutation associated with a disease (pg 183, col 1, para 2 ? 'Conclusion:...methods to detect mutations in the CFTR gene ... to extensive scanning/sequencing protocols that enable the detection of novel mutations', wherein 'novel mutations' 'in the CFTR gene' is 'associated with a disease' - 'cystic fibrosis (CF)'; pg 180, col 1 - 'cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene ... have resulted in the identification of more than 1500 CFTR mutations'; Abstract),
--- the method comprising: obtaining nucleic acid from a subject having a disease (pg 182, col 2, para 1 - 'identification of CFTR mutations in patients diagnosed with CF'; pg 180, col 1 - 'cystic fibrosis (CF)'; pg 191, Table 7 - 'Name of patient ... sample number'; pg 191, Table 8 - 'Molecular genetic analysis for cystic fibrosis....Sample type: DNA')
---identifying at least one mutation in the nucleic acid (pg 183, col 2, para 2 - 'diagnosis of CF is based on a consistent phenotype plus evidence of CFTR channel dysfunction ... or identification of two CF-disease causing mutation in trans'; pg 183, col 2, last para - 'the term "mutation" is defined as a molecular alteration in the DNA sequence of a gene'; pg 182, col 2, para 1 - 'identification of CFTR mutations in patients diagnosed with CF').
Although Castellani does not expressively teach comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations, one of ordinary skill in the art at the time the invention was made would have known to do so, because it is the first step that one would have done to compare the mutation to a database of mutations in order to classify the mutation or to determine if the mutation identified is a novel mutation, especially Castellani further provides a database of mutations known to be associated with the disease, which is widely used by researchers in the field (pg 192, col 1, last para - 'The traditional mutation nomenclature is currently used in the vast majority of research papers, and in the CFGAC Mutation Database...we advise using the mutation names of the routinely and widely used CFTR mutation database [4]', wherein 'CFGAC Mutation Database' is 'a database of mutations known to be associated with the disease' - 'cystic fibrosis (CF)'; pg 181, col 2, para 2 - 'more than 1500 sequence alterations have been identified in the CFTR gene and their listing is continuously updated within the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) database [4]'; pg 193, col 1, References - '[4] Cystic Fibrosis Mutation Database. www.genet.sickkids.on.ca/cftr'); the classification of the mutations (pg 187, Table 5), as well as teaches criteria for characterization of novel mutations for determining the potential pathogenic potential of the mutation identified (pg 185, col 1, last para - 'Other criteria can be used to asses the pathogenic potential,..include: - the sequence variation causes a novel amino acid sequence ...the sequence variant changes a highly evolutionarily conserved amino acid residue...the sequence variation creates a novel/cryptic splice site...similar sequence variations are found in other ABC genes'). Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to compare the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference GSGE-007/01WO29258/52 | **FOR FURTHER ACTION** | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US 13/33435 | International filing date *(day/month/year)* 22 March 2013 (22.03.2013) | (Earliest) Priority Date *(day/month/year)* 04 April 2012 (04.04.2012) |
| Applicant GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 13/33435

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G06F 19/22 (2013.01)
USPC - 702/19, 20
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G06F 19/22 (2013.01)
USPC - 702/19, 20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (PGPB,USPT,USOC,EPAB,JPAB); PubMed (MEDLINE);
Search terms: contig, reads, alignment, insertion, deletion, mutation, reference, reference, mutation, polymorphism, SNP, identify, determine, assay, evaluate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>-----<br>Y | US 2011/0257889 A1 (KLAMMER et al.) 20 October 2011 (20.10.2011) para [0003], [0014], [0016], [0040], [0041], [0043], [0049], [0050], [0061], [0073], [0085], [0086], [0083] | 1-6, 9-12<br>----------<br>7-8 |
| Y | US 2009/0192047 A1 (PARR et al.) 30 July 2009 (30.07.2009) para [0236]; Table 4 | 7-8 |
| X,P | US 8,209,130 B1 (KENNEDY et al.) 26 June 2012 (26.06.2012) entire document | 1-12 |
| A | KLEIN et al., LOCAS--a low coverage assembly tool for resequencing projects. PLoS One; 2011 (published online 15 August 2011); Vol 6, No 8, Document e23455; entire document | 1-12 |
| A | US 2004/0152108 A1 (KEITH et al.) 5 August 2004 (05.08.2004) entire document | 1-12 |
| A | US 2004/0142325 A1 (MINTZ et al.) 22 July 2004 (22.07.2004) entire document | 1-12 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 03 May 2013 (03.05.2013) | 10 JUN 2013 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| Date of mailing *(day/month/year)* | 10 JUN 2013 |
|---|---|
| Applicant's or agent's file reference<br>GSGE-007/01WO29258/52 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 13/33435 | 22 March 2013 (22.03.2013) | 04 April 2012 (04.04.2012) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06F 19/22 (2013.01)
USPC - 702/19, 20

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 03 May 2013 (03.05.2013) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/33435

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account **the rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:



Petitioner Illumina, Inc., EX1002, p. 747

WRIT. OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

Inter nal application No.
PCT/US 13/33435

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 7-9 | YES |
| | Claims | 1-6, 10-12 | NO |
| Inventive step (IS) | Claims | none | YES |
| | Claims | 1-12 | NO |
| Industrial applicability (IA) | Claims | 1-12 | YES |
| | Claims | none | NO |

2. Citations and explanations:

Claims 1-6 and 10-12 lack novelty under PCT Article 33(2) as being anticipated by US 2011/0257889 A1 to Klammer et al. (hereinafter 'Klammer').

Regarding claim 1, Klammer discloses a method for identifying a mutation in a nucleic acid, the method comprising: sequencing nucleic acid to generate a plurality of sequence reads (para [0049]); creating a contig based on the reads (para [0016], [0040], [0050]); aligning the contig to a reference sequence (para [0017], [0043]); aligning the individual reads back to the contig (para [0050]); and identifying a mutation based on the alignments to the contig and the reference sequence (para [0043] - mutation that distinguishes the nucleotide sequence of the reference sequence).

Regarding claim 2, Klammer discloses wherein the method of claim 1 may further comprise splitting the reads into subsets corresponding to known locations (para [0083] - "overlap detection algorithms described above generally require a "query" and a "target," and so for de novo sequencing the sequence read dataset must provide both. In FIG. 5, the sequence dataset is provided to the algorithm as a plurality of subsets of the input sequence data set ("split data"), and these subsets can be used as the "query" or the "target" in a single pairwise comparison. In certain preferred embodiments, multiple of the subsets are combined to form a larger target for the comparison"; para [0086] - identifying locations of alignments between multiple occurrences of a known sequence within the sequencing read).

Regarding claim 3, Klammer discloses wherein the method of claim 1 may further comprise identifying a mutation in the contig relative to the reference (para [0043] - "loci that have basecalls that differ from the reference sequence can be further analyzed to determine if a given locus was erroneously called in the sequence read, or if it represents a true variation (e.g., a mutation, SNP variant, etc.)").

Regarding claim 4, Klammer discloses wherein the method of claim 1 may further comprise determining a position of the mutation in the nucleic acid based on a position of the mutation in the reads and the alignments to the contig and the reference sequence (para [0043] - "methods for aligning and assembling sequence reads based at least in part on a known reference sequence, which is sometimes termed "resequencing" or "mapping." In such embodiments, the sequence reads can be mapped to the reference sequence, and loci that have basecalls that differ from the reference sequence can be further analyzed to determine if a given locus was erroneously called in the sequence read, or if it represents a true variation (e.g., a mutation, SNP variant, etc.)"; para [0061]; the fourth position is mutated).

Regarding claim 5, Klammer discloses wherein the method of claim 1 may further comprise wherein the mutation may be selected from the group consisting of: a single nucleotide polymorphism (SNP), an insertion, a deletion, a substitution, and a translocation (para [0041], [0043]).

Regarding claim 6, further to the method of claim 1, Klammer discloses wherein a plurality of mutations may be identified (para [0014] - multiple deletion or insertions).

Regarding claim 10, further to the method of claim 1, Klammer discloses wherein the mutation may be associated with a disease (para [0003], [0004]).

Regarding claim 11, further to the method according to claim 1, Klammer discloses wherein sequencing may be sequencing-by-synthesis (para [0085]).

Regarding claim 12, further to the method according to claim 11, Klammer discloses wherein sequencing-by-synthesis may be a single molecule sequencing-by-synthesis (para [0038] - single-molecule real-time (SMRT.TM.) sequencing).

- Please see Supplemental Box for continuation -

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/33435

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2: Citations and Explanations

Claim 9 lacks an inventive step under PCT Article 33(3) as being obvious over Klammer, as above.

Regarding claim 9, although Klammer does not expressly recite wherein the mutation is a deletion at an end of a sequence read, Klammer discloses wherein the mutation may be a deletion (para [0073]; k-mer ATGC may contain missing base, thereby producing deletion ATG). It would have been obvious to a person of ordinary skill in the art to anticipate wherein a mutation or errant base call may have been made at the end of a sequence read, or in a gap between sequence reads, due to a known tendency of polymerases to add bases beyond the end of a template, based on both the nucleotide mix available, and the preferences inherent in the particular polymerase.

Claims 7-8 lack inventive step under PCT Article 33(3) as being obvious over Klammer, as above, in view of US 2009/0192047 A1 to Parr et al. (hereinafter 'Parr').

Regarding claims 7-8, further to the method of claim 6, Klammer does not specifically teach the further limitations taught by Parr, wherein a first mutation may be within about 100 nucleotides of a second mutation, and wherein the first mutation may be a substitution and the second mutation may be a deletion (para [0236], Table 4). As Klammer further teaches wherein nucleotide sequence mutations may encompass multiple adjacent positions in the reference and/or sequencing reads (para [0043]), it would have been obvious to one having ordinary skill in the art that the method of claim 6 might further comprise wherein a first mutation may be within about 100 nucleotides of a second mutation, and wherein the first mutation may be a substitution and the second mutation may be a deletion, as taught by Parr.

Claims 1-12 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference<br>GSGE-006/01WO 29258/31 | **FOR FURTHER ACTION** | see Form PCT/ISA/220<br>as well as, where applicable, item 5 below. |
|---|---|---|
| International application No.<br>PCT/US 12/29790 | International filing date *(day/month/year)*<br>20 March 2012 (20.03.2012) | (Earliest) Priority Date *(day/month/year)*<br>31 March 2011 (31.03.2011) |
| Applicant<br>GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☐ the text is approved as submitted by the applicant.

   ☒ the text has been established by this Authority to read as follows:

IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☒ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

**INTERNATIONAL SEARCH REPORT** PCT/US2012/029790 14.06.2012

International application No.
PCT/US 12/29790

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C07H 21/04 (2012.01)
USPC - 435/6.1, 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/68; C07H 21/04 (2012.01)
USPC: 435/6.1, 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.11, 436/94, 536/22.1, 536/24.3, 536/24.33

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, Google Scholar: Barcode, template, identifier, multiplex sequencing, identical, tag, validate, solution, nucleic acid, synthesis, substrate, contact, detect, polymerase, duplex, quantification, contamination, oligonucleotide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2010/0330619 A1 (WILLIS et al.) 30 December 2010 (30.12.2010); para [0012], [0014], [0094], [0131], [0155], [0199], [0228]-[0229], [0273], [0279], [0286], [0294], [0297], [0355], [0365] | 1-21 |
| Y | | 22-33 |
| Y | US 2008/0269068 A1 (CHURCH et al.) 30 October 2008 (30.10.2008); para [0009] | 22-33 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 May 2012 (25.05.2012) | 14 JUN 2012 |

| Name and mailing address of the ISA/US | Authorized officer: |
|---|---|
| Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| Date of mailing *(day/month/year)* | 14 JUN 2012 |
|---|---|

| Applicant's or agent's file reference | FOR FURTHER ACTION |
|---|---|
| GSGE-006/01WO 29258/31 | See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 12/29790 | 20 March 2012 (20.03.2012) | 31 March 2011 (31.03.2011) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68; C07H 21/04 (2012.01)
USPC - 435/6.1, 536/23.1

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [x] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 25 May 2012 (25.05.2012) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 12/29790

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 12/29790

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 22-33 | YES |
| | Claims | 1-21 | NO |
| Inventive step (IS) | Claims | None | YES |
| | Claims | 1-33 | NO |
| Industrial applicability (IA) | Claims | 1-33 | YES |
| | Claims | None | NO |

2. Citations and explanations:

Claims 1-21 lack novelty under PCT Article 33(2) as being anticipated by US 2010/0330619 A1 Willis et al. (hereinafter Willis).

Regarding claim 1, concerning a method for validating the integrity of a nucleic acid template Willis teaches the method comprising:
obtaining a solution comprising a template nucleic acid (para [0012], [0101], [0114]);
introducing an identifier nucleic acid to the solution (para [0012], attach identifiable marker);
incorporating an identical barcode oligonucleotide into the template and the identifier nucleic acids (para [0131]); and
sequencing the template and the identifier nucleic acids (para [0283], [0284]).

Regarding claim 2, Willis further teaches the method according to claim 1, wherein prior to sequencing, the template and the identifier are attached to a substrate (para [0286], [0294]).

Regarding claim 3, Willis further teaches the method according to claim 2, wherein the template and the identifier are directly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 4, Willis further teaches the method according to claim 2, wherein the template and the identifier are indirectly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 5, Willis further teaches the method according to claim 1, further comprising, amplifying the template and the identifier (para [0014], [0138])

Regarding claim 6, Willis further teaches the method according to claim 1, wherein sequencing is sequencing by synthesis (para [0218], [0165], [0202]).

Regarding claim 7, Wills further teaches the method according to claim 6, wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0365]).

Regarding claim 8, Willis further teaches the method according to claim 1, wherein sequencing comprises: hybridizing a primer to both the template and the identifier to form a template/primer duplex and an identifier/primer duplex (para [0012], [0138]);
contacting both duplexes with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primers of both duplexes in a template-dependent manner (para [0228]-[0229]; [0138]);
detecting a signal from the incorporated labeled nucleotide (para [0225]-[0226]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the template nucleic acid and the identifier nucleic acid (para [0225]-[0226]).

Regarding claim 9, Willis further teaches the method according to claim 8, wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0199]).

Regarding claim 10, Willis further teaches the method according to claim 9, wherein the optically labeled nucleotide is a fluorescently labeled nucleotide (para [0199]).

*****Continued in Supplemental Box*****

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/29790

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fu lly supported by the description, are made:
Claims 1-10 are objected to as being unclear as to the term "integrity". The claims fail to clearly set forth the technical features of the invention for which protection is sought, as according to PCT Rule 6. Claim 1 is generally narrative and indefinite with regard to the tem "integrity". Specifically, the claim fail to define the matter for which protection is sought with sufficient detail such that the claims are not clear and consise. Further, the specifications fails to enable one of ordinary skill in the art to practice the claims because the term is not defined nor fully supported.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/29790

**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V(2):

Regarding claim 11 concerning a method for quantifying a contamination rate in a sequencing reaction, Willis teaches the method comprising:
determining a number of barcode reads in a sequencing reaction that are correctly correlated with an identifier nucleic acid (para [0012], [0131]), wherein the identifier nucleic acid is associated with a template nucleic acid (para [0012]), and the barcode reads are generated by a sequencing apparatus (para [0094]);
determining a number of barcode reads in the sequencing reaction that are incorrectly correlated with the identifier nucleic acid (para [0297]-[0299]); and
obtaining a fraction of the two numbers, thereby quantifying a contamination rate in the sequencing reaction (para [0298]).

Regarding claim 12, Willis further teaches the method according to claim 11, wherein determining comprises:
introducing an identifier nucleic acid to a solution (para [0012], attach identifiable marker) comprising a template nucleic acid (para [0131]);
incorporating an identical barcode oligonucleotide into the template and the identifier nucleic acids (para [0131]); and
sequencing the template and the identifier nucleic acids (para [0283]).

Regarding claim 13, Willis further teaches the method according to claim 12, wherein prior to sequencing, the template and the identifier are attached to a substrate (para [0286], [0294]).

Regarding claim 14, Willis further teaches the method according to claim 13, wherein the template and the identifier are directly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 15, Willis further teaches the method according to claim 13, wherein the template and the identifier are indirectly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 16, Willis further teaches the method according to claim 12, further comprising, amplifying the template and the identifier (para [0014], [0138])

Regarding claim 17, Willis further teaches the method according to claim 12, wherein sequencing is sequencing by synthesis (para [0218], [0165], [0202]).

Regarding claim 18, Wills further teaches the method according to claim 17, wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0365]).

Regarding claim 19, Willis further teaches the method according to claim 12, wherein sequencing comprises: hybridizing a primer to both the template and the identifier to form a template/primer duplex and an identifier/primer duplex (para [0012], [0138]);
contacting both duplexes with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primers of both duplexes in a template-dependent manner (para [0228]-[0229]; [0138]);
detecting a signal from the incorporated labeled nucleotide (para [0225]-[0226]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the template nucleic acid and the identifier nucleic acid (para [0225]-[0226]).

Regarding claim 20, Willis further teaches the method according to claim 19, wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0199

Regarding claim 21, Willis further teaches the method according to claim 20, wherein the optically labeled nucleotide is a fluorescently labeled nucleotide (para [0199]).

Claims 22-33 lack inventive step under PCT Article 33(3) as being obvious over Willis in view of 2008/0269068 A1 to Church et al. (hereinafter .Church.).

Regarding claim 22, concerning a method for identifying a contamination in a batch of barcode oligonucleotides, Willis teaches the method comprising:
preparing a plurality of batches of barcoded identifier nucleic acids, wherein each batch comprises a unique identifier nucleic acid attached to a unique barcode oligonucleotide (para [0131]); however fails to teach
pooling the batches;
sequencing the pooled batches;
and identifying barcode oligonucleotides that are improperly paired with identifier nucleic acids.
Church teaches a method of identifying a contamination in a batch of barcode oligonucleotides (para [0009], detect that barcodes have been correctly attached and thus also being able to detect incorrect attachment)
pooling the batches (para [0009]);
sequencing the pooled batches (para [0009]);
and identifying barcode oligonucleotides that are improperly paired with identifier nucleic acids (para [0009], detect that barcodes have been correctly attached and thus also being able to detect incorrect attachment). In light of the fact that Church teaches a method of confirming correct barcode attachment to an oligonucleotide attached to an identifier (para [0009]), it would have been obvious to one of ordinary skill in the art to confirm correct attachment of barcodes to the sequence of interest in order to further insure the validity of the results found in the method taught by Willis.

*****Continued in Supplemental Box*****

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/29790

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
*****Continuation of Previous Supplemental Box*****

Regarding claim 23, the combination of Willis and Church makes obvious the method according to claim 22, and Willis further teaches wherein attaching comprises a PCR reaction (para [0146]).

Regarding claim 24, the combination of Willis and Church makes obvious the method according to claim 22, and Wills further teaches wherein attaching comprises a ligation reaction (para [0381]).

Regarding claim 25, the combination of Willis and Church makes obvious the method according to claim 22, and Willis further teaches wherein prior to sequencing, the barcoded identifier is attached to a substrate (para [0286], [0294]).

Regarding claim 26, the combination of Willis and Church makes obvious the method according to claim 25, and Willis further teaches wherein the barcoded identifier is directly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 27, the combination of Willis and Church makes obvious the method according to claim 25, and Willis further teaches wherein the barcoded identifier is indirectly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 28, the combination of Willis and Church makes obvious the method according to claim 22, and Church further teaches the method further comprising, amplifying the barcoded identifier (para [0007]).

Regarding claim 29, the combination of Willis and Church makes obvious the method according to claim 22, and Church further teaches wherein sequencing is sequencing by synthesis (para [0045]).

Regarding claim 30, the combination of Willis and Church makes obvious the method according to claim 29, and Church further teaches wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0016]).

Regarding claim 31, the combination of Willis and Church makes obvious the method according to claim 22, and Willis further teaches wherein sequencing comprises:
hybridizing a primer to the barcoded identifier to form an identifier/primer duplex (para [0012], [0138]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0228]-[0229]; [0138]);
detecting a signal from the incorporated labeled nucleotide (para [0225]-[0226]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the barcoded identifier nucleic acid (para [0225]-[0226]).

Regarding claim 32, the combination of Willis and Church makes obvious the method according to claim 31, and Willis further teaches wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0199])

Regarding claim 33, the combination of Willis and Church makes obvious the method according to claim 32, and Willis further teaches wherein the optically labeled nucleotide is a fluorescently labeled nucleotide (para [0199]).

Claims 1-33 have industrial applicability under PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference GSGE-011/01WO 29258/55 | **FOR FURTHER ACTION** | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US 13/44039 | International filing date *(day/month/year)* 04 June 2013 (04.06.2013) | (Earliest) Priority Date *(day/month/year)* 04 June 2012 (04.06.2012) |
| Applicant GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/44039

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G06F 19/22, C12Q 1/68 (2013.01)
USPC - 702/20, 435/6.11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): G06F 19/22, C12Q 1/68 (2013.01)
USPC: 702/20, 435/6.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 702/19, 435/6.12, 536/23.1
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms used: clinical significance, variant sequence, sequence read, reference sequence, genetic profile, sequencing-by-synthesis, single molecule sequencing, equivalent indel region, insertion or deletion, tandem repeat, splice site frameshift mutation, functional region, DNA RNA eDNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | US 2011/0166029 A1 (MARGULIES et al.) 7 July 2011 (07.07.2011) para [0010], [0011], [0046], [0048], [0049], [0067], [0078], [0081], [0088], [0095], [0096], [0101], [0104] | 1-6, 8, 9<br>----------<br>7, 10 |
| Y | US 2009/0129647 A1 (DIMITROVA et al.) 21 May 2009 (21.05.2009) Abstract, para [0040] | 7 |
| Y | WO 2012/051208 A2 (NAZARENKO etal.) 19 April 2012 (19.04.2012) para [0007], [0009], [0040], [0041] | 10 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 October 2013 (14.10.2013) | 01 NOV 2013 |

| Name and mailing address of the ISA/US | Authorized officer: |
|---|---|
| Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

**PATENT COOPERATION TREATY**

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 01 NOV 2013 |
| Applicant's or agent's file reference<br>GSGE-011/01WO 29258/55 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 13/44039 | 04 June 2013 (04.06.2013) | 04 June 2012 (04.06.2012) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06F 19/22, C12Q 1/68 (2013.01)
USPC - 702/20, 435/6.11

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 14 October 2013 (14.10.2013) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 13/44039

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:



Petitioner Illumina, Inc., EX1002, p. 761

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 13/44039

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 7, 10 | YES |
| | Claims | 1-6, 8, 9 | NO |
| Inventive step (IS) | Claims | None | YES |
| | Claims | 1-10 | NO |
| Industrial applicability (IA) | Claims | 1-10 | YES |
| | Claims | None | NO |

2. Citations and explanations:

Claims 1-6, 8 and 9 lack novelty under PCT Article 33(2) as being anticipated by US 2011/0166029 A1 to Margulies et al. (hereinafter 'Margulies').

Regarding claim 1, Margulies discloses a method for determining the clinical significance of a variant sequence (para [0010] "The method may comprise the steps of obtaining a nucleic acid ... from a subject and conducting an assay to identify whether there is a variant sequence in the subject's nucleic acid.", para [0011] "the invention may comprise methods for identifying mutations (i.e., variants) correlated with the presence or increased risk of developing an autism spectrum disorder.", para [0078] "The method may further include the steps of assessing the clinical significance of a variant or variants in the nucleic acid sequence ... for autism spectrum disorders."), the method comprising: sequencing a nucleic acid to generate at least one sequence read (para [0046] "In some embodiments, the method may comprise obtaining a nucleic acid ... from a subject and sequencing at least a portion of a nucleic acid in order to obtain a sample nucleic acid sequence for at least one gene.");
identifying a variant sequence within the sequence read based on mapping the sequence read to a reference sequence (para [0081] "The nucleic acid may be sequenced ... in order to detect variants (i.e., mutations) in the nucleic acid compared to a reference sequence derived from one or more individuals not known to suffer from an autism spectrum disorder", para [0088] "In next generation (massively parallel sequencing), all regions of interest are sequenced together, and the origin of each sequence read is determined by comparison (alignment) to a reference sequence.");
determining the equivalent indel [i. e. insertion or deletion] region of the variant sequence (para [0101] "types of variants include deletions of one or more nucleotides ... insertions of one or more nucleotides");
identifying a functional region comprising at least a portion of the equivalent insertion/deletion region (para [0101] "As shown in Table 2, sequence variants are named according to the change they cause in the DNA sequence and the resultant change (if any) to the peptide sequence.", para [0104] "Frameshift mutations cause a shift in reading frame leading to a complete change of the amino acid sequence downstream of the mutation (i.e., the frameshift site). A frameshift mutation is caused by a net deletion or insertion of a number of nucleotides not divisible by 3."),
associating the equivalent indel region with the identified functional region, thereby to determine clinical significance of the variant (para [0101] "types of variants include deletions of one or more nucleotides ... insertions of one or more nucleotides", para [0095] "As depicted in FIG. 2, the variants and/or variant combinations may be assessed for their clinical significance for autism spectrum disorders based on one or more of the following methods. If a variant or a variant combination is reported or known to occur more often in nucleic acid from subjects with, than in subjects without, autism spectrum disorders, it is considered to be at least potentially predisposing to autism spectrum disorders. ... Conversely, if a variant is found in both populations at a similar frequency, it is less likely to be associated with the development of an autism syndrome disorder (ASD)", para [0096] " If a variant or a variant combination is predicted to have an overall deleterious effect on a protein or gene expression (i.e., resulting in a nonsense mutation, a frameshift mutation, or a splice site mutation ...), based on the predicted effect on the sequence and/or the structure of a protein or a nucleic acid ... it is considered to be at least potentially predisposing to autism spectrum disorders").

Regarding claim 2, Margulies further teaches nucleic acid that is DNA and RNA (para [0067] "The techniques for sequencing nucleic acids (both DNA and RNA) are highly sensitive and therefore, can be used almost any biological sample (i.e., tissue or body fluid) taken from subject.").

Regarding claim 3, Margulies further teaches sequencing that is sequencing-by-synthesis (para [0048] "In various embodiments of the methods of the invention ... the assay comprises ... nucleic acid sequencing ... Additionally and/or alternatively, the sequencing comprises at least one of single-molecule sequencing-by-synthesis.").

----------SEE FIRST SUPPLEMENTAL BOX TO CONTINUE----------

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 13/44039

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V No. (2) Citations and Explanations:

Regarding claim 4, Margulies further teaches sequencing that is single molecule sequencing (para [0081] "The nucleic acid may be sequenced ... in order to detect variants (i.e., mutations) in the nucleic acid compared to a reference sequence ... sequence variants ... can be detected by any sequencing method known in the art e.g., ... single molecule sequencing").

Regarding claim 5, Margulies further teaches variant sequences that comprise a genetic mutation (para [0081] "The nucleic acid may be sequenced and/or its genomic arrangement and/or copy number is determined in order to detect variants (i.e., mutations) in the nucleic acid compared to a reference sequence derived from one or more individuals not known to suffer from an autism spectrum disorder").

Regarding claim 6, Margulies further teaches genetic mutations that are an insertion or a deletion (para [0101] "types of variants include deletions of one or more nucleotides ... insertions of one or more nucleotides", para [0104] "Frameshift mutations cause a shift in reading frame leading to a complete change of the amino acid sequence downstream of the mutation (i.e., the frameshift site). A frameshift mutation is caused by a net deletion or insertion of a number of nucleotides not divisible by 3.");

Regarding claim 8, Margulies further teaches determining a functional region that comprises a gene, an exon, an intron, a splice site, a regulatory element (para [0010] "The method may comprise the steps of obtaining a nucleic acid ... and conducting an assay to identify whether there is a variant sequence in the ... nucleic acid.", para [0049] "the nucleic acid in the conducting step comprises a gene ... an exon, an intron, a gene regulatory element ... including splice sites ... may be evaluated for mutations (i.e., variants).").

Regarding claim 9, Margulies further teaches variant sequences is a splice site mutation, a nonsense mutation, or a frameshift mutation (para [0096] "if a variant or a variant combination is predicted to have an overall deleterious effect on a protein or gene expression (i.e., resulting in a nonsense mutation, a frameshift mutation, or a splice site mutation, or even a missense mutation), based on the predicted effect on the sequence and/or the structure of a protein or a nucleic acid, and if this variant or variant combination affects a gene or genes known to be associated with autism spectrum disorders, it is considered to be at least potentially predisposing to autism spectrum disorders").

Claim 7 lacks an inventive step under PCT Article 33(3) as being obvious over Margulies in view of US 2009/0129647 A1 to Dimitrova (hereinafter 'Dimitrova').

Regarding claim 7, Margulies does not expressly teach identifying a genetic mutation that is located in a tandem repeat, however, Dimitrova teaches methods for said identifying (Abstract "Spectrogram extraction from DNA sequence has been known since 2001. ...Given a DNA sequence or whole genomes, with this method it is easy to generate a large number of spectrogram images. However, the difficult part is to elucidate where are the repetitive patterns and to associate a biological and clinical meaning to them. The present disclosure provides systems and methods that facilitate the location and/or identification of repetitive DNA patterns, such as ..tandem repeats", para [0040] "As described herein, exemplary systems and methods support and/or utilize one or more of the following DNA spectral analysis techniques: (i) comparative histogram methodologies; (ii) selection/classification using support vector machines and genetic algorithms; and (iii) spectrovideo methodologies based on spectrogram extractions from DNA sequence data. Many benefits may be realized according to the present disclosure, e.g., (i) enhanced visualization of genomic information, (ii) identification of repetitive DNA patterns, e.g. .. tandem repeats ...to permit rapid visualization of an entire genome at a desired resolution.). It would have been obvious to one of ordinary skill in the art to add Dimitrova's methods for identifying tandem repeat mutations among mutations in a nucleic acid, because said mutations are difficult to characterize, as taught by Dimitrova, but may add new clinically significant mutations to those identified by the method of Margulies.

Claim 10 lacks an inventive step under PCT Article 33(3) as being obvious over Margulies in view of WO 2012/051208 A2 to Nazarenko et al. (hereinafter 'Nazarenko').

Regarding claim 10, Margulies does not expressly teach determining whether the equivalent indel region extends beyond the functional region, however, Nazarenko teaches said determination (para [0007] "Embodiments of the present invention can provide identification of junctions (e.g., resulting from large-scale rearrangements) of a sequenced genome with respect to a human genome reference sequence.", para [0009] "a method is provided for determining whether a clinically significant junction exists between a sample genome and a reference genome", para [0040] "To determine a genome of an organism, fragments from a biological sample can have their two ends sequenced ... These mated pairs of sequence reads can then be mapped to one or more reference genomes to determine the sample genome. ... in some cases, pairs cannot be mapped as expected to a reference genome, and are called discordant pairs. ... Discordant mate pairs can occur when a rearrangement, or a large insertion or deletion, has occurred in the sample genome relative to the reference genome.", para [0041] "The accurate identification of rearrangement locations is important as such cases typically are the result of disease, such as cancer, ... Such rearrangement can include, for example ... a piece of the genome being deleted. Such rearrangements can cause the loss of a gene, a function of the gene, and a protein created from the gene."). It would have been obvious to one of ordinary skill in the art to combine Nazarenko's method of determining whether the equivalent indel region extends beyond the functional region with Margulies' method for determining the clinical significance of a variant sequence having smaller or uncharacterized indel mutations, because said determining could reveal other clinically significant aspects of extended indel regions, as taught by Nazarenko (para [0041] "since there are changes in which portions of the genome become near each other, a new expression or disregulation can occur. ... the discovery of larger insertions, deletions, duplications, and rearrangements can add value beyond the discovery of small variations.").

Claims 1-10 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

**PATENT COOPERATION TREATY**

# PCT

**INTERNATIONAL SEARCH REPORT**

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference GSGE-014/01 WO 29258/64 | **FOR FURTHER ACTION** | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US13/62842 | International filing date *(day/month/year)* 01 October 2013 (01.10.2013) | (Earliest) Priority Date *(day/month/year)* 07 November 2012 (07.11.2012) |
| Applicant Good Start Genetics, Inc. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☐ as suggested by the applicant.

   ☒ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.



Petitioner Illumina, Inc., EX1002, p. 764

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US13/62842

**Box No. I** **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/62842

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G06N 3/12, 7/02; G06F 17/10 (2013.01)
USPC - 706/13, 21; 702/19
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): G06N 3/12, 7/02; G06F 3/00, 17/00, 17/10, 15/18; A61B 5/00, 5/07; C40B 40/10, 40/00, 60/10; A61N 1/30; C12Q 1/68 (2013.01)
USPC Classification(s): 706/13, 21, 52, 15; 702/19; 703/1; 424/422; 600/300, 483; 506/13, 18, 38, 7; 250/282

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-B, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google/Google Scholar; ScienceDirect; ProQuest; Search Terms: genetic, mutation, test, evaluate, computer, sequence, pipeline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | LI, H et al. A New Approach for Detecting Low-level Mutations in Next-generation Seqence Data. Genome Biology. 23 May 2012, Vol. 13, pp. 1-15; page 4, left column, second paragraph; page 12 right column, second paragraph. DOI: 10.1186/gb-2012-13-5-r34 | 1-11 |
| Y | US 2012/0252684 A1 (SELIFONOV, S et al.) October 4, 2012; abstract; paragraphs [0027], [0080], [0138], [0139], [0147]. | 1-11 |
| Y | US 8209130 B1 (KENNEDY, C et al.) June 26, 2012; abstract; column 2, lines 20-21; column 2, lines 44-45; column 3, lines 18-24; column 3, lines 63-64; column 4, lines 1-5; column 5, lines 48-52 | 6-8, 10, 11 |
| Y | EP 1321477 B1 (GRAWUNDER, U et al.) October 13, 2004; page 5, lines 24-27; page 17, lines 32-33 | 4 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 30 December 2013 (30.12.2013) | 04 FEB 2014 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Shane Thomas<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Thomas C Meyers
Brown Rudnick LLP
One Financial Center
Boston, MA 02111
United States of America

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 04 FEB 2014 |
| Applicant's or agent's file reference<br>GSGE-014/01 WO 29258/64 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US13/62842 | 01 October 2013 (01.10.2013) | 07 November 2012 (07.11.2012) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06N 3/12, 7/02; G06F 17/10 (2013.01)
USPC - 706/13, 21; 702/19

Applicant Good Start Genetics, Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 30 December 2013 (30.12.2013) | Shane Thomas<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/62842

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☒ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/62842

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-11 | YES |
| | Claims | NONE | NO |
| Inventive step (IS) | Claims | NONE | YES |
| | Claims | 1-11 | NO |
| Industrial applicability (IA) | Claims | 1-11 | YES |
| | Claims | NONE | NO |

2. Citations and explanations:

Claims 1-3, 5, and 9 lack an inventive step under PCT Article 33(3) as being obvious over the article entitled 'A new approach for detecting low-level mutations in next-generation sequence data' by Li, et al. (hereinafter 'Li') in view of US 2012/0252684 A1 to Selifonov, et al. (hereinafter 'Selifonov').

As per claim 1, Li discloses a method of evaluating a genetic test (we developed a new method that distinguishes real mutations (genetic test) from sequencing errors and test it using three different distributions to evaluate the performance of our method compared to two others; page 4, left column, second paragraph), the method comprising: obtaining, using a computer (here we provided a novel computation method (on a computer system), all common variation positions were flagged in the output file; page 12, right column, second paragraph; supplemental page 5, second paragraph), a plurality of sequence reads (for each bin at a specific position we counted the number of reads with major and minor alleles for the individual (individual sequence) in question as well as the same numbers for the reference individuals (plurality of sequences); supplemental page 3, second paragraph); analyzing the sequence reads using a genetic pipeline (all samples (sequence reads) are analyzed following the same pipeline; page 11, left column, second paragraph); determining if the genetic pipeline identified the mutation (figure 4 shows the low level mutations and sequencing errors detected by the genetic pipeline; page 5, figure 4), thereby validating the genetic pipeline (our method (genetic pipeline) showed a better performance because the empirical error rate is low, making it easier to distinguish real low-level mutations from sequencing errors (simulated mutations); page 8, right column, third paragraph). Li does not disclose a computer comprising a non-transitory memory; introducing, using the computer, a simulated mutation into at least one of the plurality of sequence reads. Selifonov discloses a computer comprising a non-transitory memory (the data structure is designed for a computer can be formed in computer memory such read-only memory (non-transitory memory); paragraph [0147]); introducing, using the computer (the computer program comprises computer code that performs the operations described herein; paragraph [0020]), a simulated mutation into at least one of the plurality of sequence reads (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) the mutations can be random or can be placed into a specific subunit; paragraphs [0027], [0138], [0139]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

As per claim 2, Li and Selifonov, in combination, disclose the method of claim 1, and Li further discloses introducing artificial DNA into about half of the plurality of sequence reads (artificially mixing of 2 DNA samples was done at a ratio of 1:1; page 13, left column, second paragraph). Li does not disclose introducing the simulation sequence. Selifonov discloses introducing the simulation sequence (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) the mutations can be random or can be placed into a specific subunit; paragraphs [0027], [0138], [0139]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

As per claim 3, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose wherein introducing the simulated mutation comprises manipulating a data field for base sequence or quality. Selifonov discloses wherein introducing the simulated mutation comprises manipulating a data field for base sequence (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) the mutations can be random or can be placed into a specific subunit, the data structure can be manipulated by an appropriate computer; paragraphs [0027], [0138], [0139], [0147]) or quality. It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

-***-Continued Within the Next Supplemental Box-***-

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

As per claim 5, Li and Selifonov, in combination, disclose the method of claim 1, and Li further discloses assembling the sequence reads (page 2, Figure 1) Li does not disclose the method comprising assembling the sequence reads prior to introducing the simulated mutation. Selifonov discloses the method comprising assembling the sequence reads prior to introducing the simulated mutation (the variation (mutation) can be introduced in to the concatenated string output (after assembling has occurred); paragraphs [0138], [0182], [0183]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

As per claim 9, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose using the computer to automatically introduce a number of different simulated mutations into different ones of the plurality of sequence reads. Selifonov discloses using the computer (the computer program comprises computer code that performs the operations described herein; paragraph [0020]) to automatically introduce a number of different simulated mutations into different ones of the plurality of sequence reads (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) automatically using a computer, the mutations can be random or can be placed into a specific subunit; paragraphs [0027], [0080], [0138], [0139]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

Claims 6-8, 10, and 11 lack an inventive step under PCT Article 33(3) as being obvious over Li in view of Selifonov, in further view of US 8,209,130 B1 to Kennedy, et al. (hereinafter 'Kennedy').

As per claim 6, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose wherein the analyzing step comprises assembling the plurality of sequence reads to produce a contig. Kennedy discloses wherein the analyzing step comprises assembling the plurality of sequence reads to produce a contig (nucleic acid obtained from a subject is sequenced to produce a plurality of reads and the methods of the invention are accomplished by assembling contigs from sequence reads; column 2, lines 20-21; column 2, lines 44-45). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would have produced a contig that could be compared to a reference sequence.

As per claim 7, Li, Selifonov, and Kennedy, in combination, disclose the method of claim 6, but Li does not disclose wherein the analyzing step further comprises aligning the contig to a reference. Kennedy discloses wherein the analyzing step further comprises aligning the contig to a reference (mutations are identified based on the alignments to the reference and to the contig; abstract). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would have produced a contig that could be compared to a reference sequence.

As per claim 8, Li, Selifonov, and Kennedy, in combination, disclose the method of claim 7, but Li does not disclose wherein the analyzing step further comprises aligning one or more of the plurality of sequence reads to the contig. Kennedy discloses wherein the analyzing step further comprises aligning one or more of the plurality of sequence reads to the contig (with the contigs aligned, each raw read is aligned to a contig in a local alignment, all reads (plurality) can align as a perfect match; figures 1, 2; column 4, lines 1-2; column 4, lines 4-5). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the alignment method, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would be capable of identifying mutations.

As per claim 10, Li and Selifonov, in combination, disclose the method of claim 1, and Li further discloses the genetic pipeline (our method (genetic pipeline) showed a better performance because the empirical error rate is low, making it easier to distinguish real low-level mutations from sequencing errors (simulated mutations); page 8, right column, third paragraph). Li does not disclose comprising instructions in the memory operable to cause the computer to assemble the plurality of sequence reads. Kennedy discloses instructions in the memory operable to cause the computer to assemble the plurality of sequence reads (using barcodes reads (plurality) can be sorted using a computer processor (memory), then assembled into contigs based on the barcodes; figures 1, 2, 3; column 3, lines 18-24). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would be capable of identifying mutations.

-***-Continued Within the Next Supplemental Box-***-

WR[illegible]TEN OPINION OF THE
INTERNAT. AL SEARCHING AUTHORITY

International application No.
PCT/US13/62842

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
-***-Continued from Previous Supplemental Box-***-

As per claim 11, Li, Selifonov, and Kennedy, in combination, disclose the method of claim 10, but Li does not disclose wherein the computer is operable to detect a mutation and report the mutation in relation to a reference. Kennedy discloses wherein the computer is operable to detect a mutation and report the mutation in relation to a reference (each mutation identified here can be described with reference to the reference genome using a computer; figures 1, 2, 3; column 3, lines 63-64). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would be capable of identifying mutations.

Claim 4 lacks an inventive step under PCT Article 33(3) as being obvious over Li and Selifonov and in further view of EP 1,321,477 B1 to Grawunder, et al. (hereinafter 'Grawunder').

As per claim 4, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose wherein the simulated mutation comprises two mutations in cis. Grawunder discloses wherein the simulated mutation comprises two mutations in cis (in order to use the selected cells for antibody production it must be prevented that further productive endogenous Ig gene rearrangements can be performed in these cells, and this can be done by mutating cis on both alleles of the IgH and IgL (two mutations); page 5 lines 24-27). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for evaluating a genetic test, as previously disclosed by Li, with the cis mutations, as previously described by Grawunder, as the previous disclosure by Li includes the testing of a DNA library (Li; page, 10, right column, third paragraph) and the Grawunder reference includes the use of exons encoding variable domains derived from a library of diverse arrangements (Grawunder; page 17, lines 32-33), and the combination of these references would have resulted in the use of a simulated mutation comprising two mutations in cis that would allow for better detection of low-frequency mutations such as cis mutations.

Claims 1-11 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

**(PCT Article 18 and Rules 43 and 44)**

| Applicant's or agent's file reference GSGE-010/01WO | **FOR FURTHER ACTION** | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. **PCT/US2013/032885** | International filing date *(day/month/year)* **19 March 2013 (19.03.2013)** | (Earliest) Priority Date *(day/month/year)* 09 April 2012 (09.04.2012) |

Applicant

**GOOD START GENETICS, INC.**

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **3** sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of :
      ☒ the international application in the language in which it was filed
      ☐ a translation of the international application into _____ , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☐ **Certain claims were found unsearchable** (See Box No. II)
3. ☐ **Unity of invention is lacking** (See Box No. III)
4. With regard to the **title,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. With regard to the drawings,
   a. the figure of the **drawings** to be published with the abstract is Figure No. **9**
      ☐ as suggested by the applicant.
      ☒ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☐ none of the figure is to be published with the abstract.

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/US2013/032885**

**A. CLASSIFICATION OF SUBJECT MATTER**

**G06F 19/28(2011.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
G06F 19/28; C40B 30/00; G06F 19/00; C40B 50/02; G06F 19/18; G06F 17/30; G06F 19/22; C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords:genetic, test, variant, data, mutation, information

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 2011-0034342 A1 (RICHARD FOX) 10 February 2011<br>See paragraphs [0015], [0035], [0040], [0119], [0165], [0173], [0178]; and fig. 1. | 1,3,11,13 |
| A | | 2,4-10,12,14-40 |
| Y | US 2002-0187496 A1 (LEIF ANDERSSON et al.) 12 December 2002<br>See paragraphs [0003]-[0004], [0006], [0023], [0030], [0032], [0041], [0047], [0052], [0060], [0062], [0075], [0078], [0080]; and figs. 1-2, 4-6, 10-11. | 1,3,11,13 |
| A | EP 2437191 A2 (GENE SECURITY NETWORK LLC) 04 April 2012<br>See paragraphs [0002], [0024]-[0050]; and fig. 19. | 1-40 |
| A | WO 2002-093453 A2 (X-MINE, INC.) 21 November 2002<br>See paragraphs [0002], [0024]-[0027]; and figs. 3A-3B. | 1-40 |
| A | WO 2010-024894 A1 (23ANDME, INC.) 04 March 2010<br>See paragraph [0002]; and fig. 1. | 1-40 |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 June 2013 (25.06.2013) | **28 June 2013 (28.06.2013)** |
| Name and mailing address of the ISA/KR<br>Korean Intellectual Property Office<br>189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea<br>Facsimile No. 82-42-472-7140 | Authorized officer<br>BYUN, Sung Cheal<br>Telephone No. 82-42-481-8262 |

Form PCT/ISA/210 (second sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.
**PCT/US2013/032885**

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 2011-0034342 A1 | 10.02.2011 | EP 2250594 A1 | 17.11.2010 |
| | | WO 2009-102901 A1 | 20.08.2009 |
| US 2002-0187496 A1 | 12.12.2002 | WO 2002-17207 A2 | 28.02.2002 |
| | | WO 2002-17207 A3 | 12.12.2002 |
| EP 2437191 A2 | 04.04.2012 | AU 2006-318425 A1 | 31.05.2007 |
| | | AU 2006-318425 A2 | 21.08.2008 |
| | | CA 2632230 A1 | 31.05.2007 |
| | | CN 101346724 A | 14.01.2009 |
| | | EP 1960929 A2 | 27.08.2008 |
| | | EP 1960929 A4 | 28.01.2009 |
| | | JP 2009-517050 A | 30.04.2009 |
| | | US 2007-0027636 A1 | 01.02.2007 |
| | | WO 2007-062164 A2 | 31.05.2007 |
| | | WO 2007-062164 A3 | 29.11.2007 |
| WO 02-093453 A2 | 21.11.2002 | AU 2002-308662 A1 | 25.11.2002 |
| | | AU 2002-308662 A8 | 25.11.2002 |
| | | WO 2009-3453 A2 | 21.11.2002 |
| | | WO 2009-3453 A3 | 11.03.2004 |
| WO 2010-024894 A1 | 04.03.2010 | US 2010-0057374 A1 | 04.03.2010 |
| | | US 2010-0057807 A1 | 04.03.2010 |
| | | US 8428886 B2 | 23.04.2013 |

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
TOSTI ROBERT J.

BROWN RUDNICK LLP ONE FINANCIAL CENTER
BOSTON MA 02111 USA

# PCT

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

**(PCT Rule 43bis.1)**

| | |
|---|---|
| Date of mailing *(day/month/year)* | **28 June 2013 (28.06.2013)** |
| Applicant's or agent's file reference<br>GSGE-010/01WO | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| **PCT/US2013/032885** | **19 March 2013 (19.03.2013)** | 09 April 2012 (09.04.2012) |

International Patent Classification (IPC) or both national classification and IPC

***G06F 19/28(2011.01)i***

Applicant

**GOOD START GENETICS, INC.**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.
For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/KR | Date of completion of this opinion | Authorized officer |
|---|---|---|
| Korean Intellectual Property Office<br>189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea<br>Facsimile No. 82-42-472-7140 | 25 June 2013 (25.06.2013) | BYUN, Sung Cheal<br>Telephone No. 82-42-481-8262 |

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
**PCT/US2013/032885**

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of :

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

   a. a sequence listing filed or furnished
      ☐ on paper
      ☐ in electronic form

   b. time of filing or furnishing
      ☐ contained in the international application as filed.
      ☐ filed together with the international application in electronic form.
      ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additioanl copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
**PCT/US2013/032885**

**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-40 | YES |
| | Claims | NONE | NO |
| Inventive step (IS) | Claims | 2,4-10,12,14-40 | YES |
| | Claims | 1,3,11,13 | NO |
| Industrial applicability (IA) | Claims | 1-40 | YES |
| | Claims | NONE | NO |

2. Citations and explanations :

Reference is made to the following documents:

D1: US 2011-0034342 A1 (RICHARD FOX) 10 February 2011
D2: US 2002-0187496 A1 (LEIF ANDERSSON et al.) 12 December 2002
D3: EP 2437191 A2 (GENE SECURITY NETWORK LLC) 04 April 2012
D4: WO 2002-093453 A2 (X-MINE, INC.) 21 November 2002
D5: WO 2010-024894 A1 (23ANDME, INC.) 04 March 2010

2.1 Novelty and Inventive Step

2.1.1 Independent Claim 1 and Dependent Claims 2-10

D1, which is considered to be the closest prior art to the subject matter of claim 1, discloses a method comprising:

retrieving from one or more computer-readable storage devices a first variant object comprising a description of a first mutation as a first variant of a reference and a second variant object comprising a description of a second mutation as a second variant of the reference, each of the first and second variant objects being an instance of an abstract class of a genomic feature (retrieving from a library each variant which describe properties of molecular variants, see paragraphs [0119], [0165] and fig. 1 in D1);

determining a relationship between the first and second mutations (determining mutations and establishing relationships between the mutations, see paragraphs [0050], [0118] and fig. 1 in D1);

retrieving a third object comprising a description of the relationship, the third object also being an instance of the abstract class (receiving a data set of mutations which established relationships between the various members of the data, see paragraphs [0050], [0165] and fig. 1

Continued on Supplemental Box



Petitioner Illumina, Inc., EX1002, p. 777

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/032885

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

In claim 4, the term "the memory" has not been previously defined. Therefore, claim 4 is not clear, contrary to the requirement of PCT Article 6.

In claims 8 and 18, the term "the information" has not been previously defined. Therefore, claims 8 and 18 are not clear, contrary to the requirement of PCT Article 6.

In claim 24, the term "the one or more computer-readable storage devices" has not been previously defined. Therefore, claim 24 is not clear, contrary to the requirement of PCT Article 6.



Petitioner Illumina, Inc., EX1002, p. 778

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box No. V

in D1); and

providing a description of the variant based on the third object (describing properties of the molecular variants of objective data, see paragraph [0119] and fig. 1 in D1).

The subject matter of claim 1 differs from that of D1 in that claim 1 comprises the feature of receiving genetic data relating to an individual, the data representing at least a first mutation and a second mutation. However, this feature can readily be predicted from D2 that discloses the feature of obtaining genetic information about sequence variants from the sampled individuals' information (see paragraphs [0006], [0030], [0041] and figs. 1-2, 4 in D2). Accordingly, claim 1 would have been obvious over D1 in view of D2. Therefore, claim 1 lacks an inventive step under PCT Article 33(3).

The additional feature (the one or more computer-readable storage devices comprise a relational database) of claim 3 dependent on claim 1 can readily be predicted from D1 that discloses the feature of one computer comprising a database capable of storing relationships between the data (see paragraphs [0050], [0199] and fig. 1 in D1). Accordingly, claim 3 would have been obvious over D1 in view of D2. Therefore, claim 3 lacks an inventive step under PCT Article 33(3).

The additional features of claims 2 and 4-10 directly or indirectly dependent on claim 1 are not disclosed in any of the prior art documents, nor are they obvious to a person skilled in the art over the documents. Accordingly, claims 2 and 4-10 are novel and involve an inventive step under PCT Article 33(2) and (3).

2.1.2 Independent Claim 11 and Dependent Claims 12-20

The features of independent claim 11 essentially correspond to those of claim 1, except for the category of the invention and an added feature of a processor (a processor, see paragraph [0078] and fig. 11 in D2). Therefore, for the same reasons as those of claim 1, claim 11 lacks an inventive step under PCT Article 33(3).

The additional feature (the database is a relational database) of claim 13 dependent on claim 11, can readily be predicted from D2 that discloses the feature of a relationship between database objects (see paragraph [0023] and fig. 2 in D2). Accordingly, claim 13 would have been obvious over D1 in view of D2. Therefore, claim 13 lacks an inventive step under PCT

Continued on The Next Page

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**
Continuation of: Previous Page

Article 33(3).

The additional features of claims 12 and 14-20 directly or indirectly dependent on claim 11 are not disclosed in any of the prior art documents, nor are they obvious to a person skilled in the art over the documents. Accordingly, claims 12 and 14-20 are novel and involve an inventive step under PCT Article 33(2) and (3).

2.1.3 Independent Claim 21 and Dependent Claims 22-30

The subject matter of claim 21 differs from these prior art documents in that a method in claim 21 comprises: providing, using a computer comprising a memory coupled to a processor, code describing an abstract class of genomic feature object and creating relation objects, each relation object being an instance of the genomic feature object and comprising one or more genomic feature objects and description of a relationship among the one or more genomic feature objects. And it is not obvious to a person skilled in the art over the documents taken alone or in combination. Therefore, claim 21 meets the requirements of PCT Article 33(2) and (3) with respect to novelty and inventive step.

Claims 22-30 are dependent on claim 21 and therefore meet the requirements of PCT Article 33(2) and (3).

2.1.4 Independent Claim 31 and Dependent Claims 32-40

The features of independent claim 31 essentially correspond to those of claim 21, except for the category of the invention and an added feature of a processor (see paragraph [0078] and fig. 11 in D2). Therefore, for the same reasons as those of claim 21, claim 31 meets the requirements of PCT Article 33(2) and (3) with respect to novelty and inventive step.

Claims 32-40 are dependent on claim 31 and therefore meet the requirements of PCT Article 33(2) and (3).

2.2 Industrial Applicability

Claims 1-40 are industrially applicable under PCT Article 33(4).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 November 2010 (04.11.2010)

PCT

(10) International Publication Number
WO 2010/126614 A3

(51) International Patent Classification:
*G01N 33/48* (2006.01)

(21) International Application Number:
PCT/US2010/001293

(22) International Filing Date:
30 April 2010 (30.04.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

| | | |
|---|---|---|
| 61/174,470 | 30 April 2009 (30.04.2009) | US |
| 61/178,923 | 15 May 2009 (15.05.2009) | US |
| 61/179,358 | 18 May 2009 (18.05.2009) | US |
| 61/182,089 | 28 May 2009 (28.05.2009) | US |


(71) Applicant *(for all designated States except US)*: **GOOD START GENETICS, INC.** [US/US]; 8 St. Mary's Street, Suite 621, Boston, MA 02215 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PORRECA, Gregory** [US/US]; 911 Garden Parkway, Ocean City, NJ 08226 (US). **LASERSON, Uri** [US/US]; 645 Beacon Street #215, Boston, MA 02215 (US). **LI, Jin, Billy** [CN/US]; 100 Station Landing, Unit 204, Medford, MA 02155 (US). **WASSMAN, E., Robert** [US/US]; 90 West Shore Drive, Marblehead, MA 01945 (US).

(74) Agent: **WALLER, Patrick, R., H.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

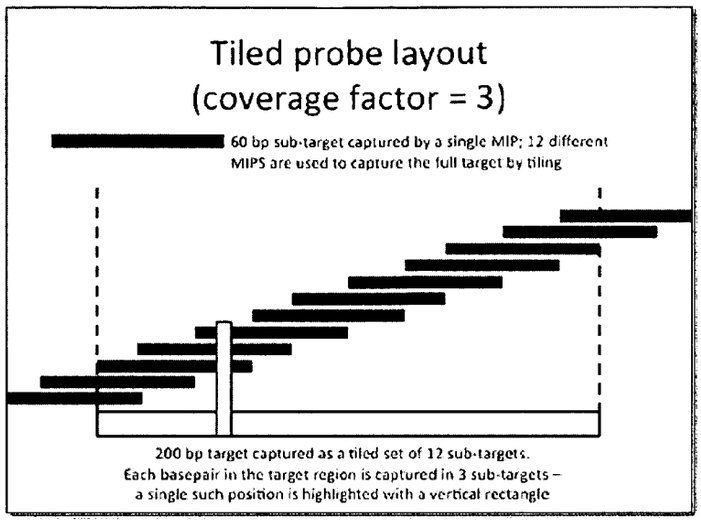
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

*[Continued on next page]*

(54) Title: METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

Figure 1




(57) Abstract: Aspects of the invention relates to methods and compositions that are useful to reduce bias and increase the reproducibility of multiplex analysis of genetic loci. In some configurations, predetermined preparative steps and/or nucleic acid sequence analysis techniques are used in multiplex analyses for a plurality of genetic loci in a plurality of samples.

WO 2010/126614 A3

WO 2010/126614 A3

**Published**:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

**(88) Date of publication of the international search report**:
17 March 2011

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/01293

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/94
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 436/94

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, Google Scholar
Search Terms:molecular inversion probe, multiplex, overlap, tile, stack, probe, DNA, bias, reduce, circular, padlock, genotyping, probe set

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y<br>---<br>A | Hardenbol et al., 'Multiplexed genotyping with sequence-tagged molecular inversion probes' Nature Biotechnology, 2003, Vol. 21, No. 6, pages 673-678; abstract, Fig. 2; pg 673, col 2, para 2pg 674, col 1, para 2-3; pg 674, col 2, para 1-3; pg 677, col 1, para 3 | 66<br>---<br>1-8, 10-14<br>---<br>17-18, 57 |
| X<br>---<br>Y | US 2003/0203370 A1 (YAKHINI et al.) 30 October 2003 (30.10.2003) abstract, para [0026] | 9<br>---<br>10-14 |
| Y | Mockler et al., 'Applications of DNa tiling arrays for while-genome analysis', Genomics, 2005, Vol. 85, pages 1-15; Fig. 2; pg 2, col 1, para 4 to pg 2, col 2, para 1 | 1-8 |
| Y | US 2008/0280955 A1 (MCCAMISH et al), 13 Nov. 2008 (13.11.2008), Table 9, para [0258], claims 1, 15 | 6 |
| Y | WO 2007/107717 A1 (CHAMBERLAIN et al) 27 Sep. 2007 (27.09.2007), Tables 3, 4; pg 4, ln 16-33 | 8 |
| & | US 2010/0129896 A1 (KNAPP) 27 May 2010 (27.05.2010) | 57 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 15 Oct. 2010 (15.10.2010) | 28 OCT 2010 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 10/01293

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 15, 16, 19, 22-56, 64, 65, 67
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-8, and 57, directed to a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-14, 17, 18, 57 and 66

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 10/01293

Continuation of Box III: Unity of Invention

Group II: claims 9-14, 17, 18, and 66, directed to a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject.

Group III: claims 20-21, directed to a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject.

Group IV: claims 58-63, directed to a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The inventions listed as Groups I - IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different. The special technical feature of the Group II claims is a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject. The special technical feature of the Group III claims is a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject. The special technical feature of the Group IV claims is a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract; para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface). Therefore, the inventions of Groups I - IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK R.H. WALLER
WOLF, GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| Date of mailing *(day/month/year)* | 28 OCT 2010 |
|---|---|
| Applicant's or agent's file reference<br>G0814.70000 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 10/01293 | 30 April 2010 (30.04.2010) | 30 April 2009 (30.04.2009) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/94

Applicant GOOD START GENETICS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 07 Oct. 2010 (07.10.2010) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2009)

PCT/US2010/001293 28.10.2010

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 15, 16, 19, 22-56, 64, 65, 67

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 15, 16, 19, 22-56, 64, 65, 67 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims are not formed according to the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 15, 16, 19, 22-56, 64, 65, 67

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. IV Lack of unity of invention**

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☒ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-8, and 57, directed to a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different.

Group II: claims 9-14, 17, 18, and 66, directed to a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject.

Group III: claims 20-21, directed to a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject.

Group IV: claims 58-63, directed to a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The inventions listed as Groups I - IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different. The special technical feature of the Group II claims is a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject. The special technical feature of the Group III claims is a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject. The special technical feature of the Group IV claims is a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract; para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface). Therefore, the inventions of Groups I - IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-14, 17, 18, 57 and 66

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-14, 17, 18, 57 and 66 | YES |
| | Claims | None | NO |
| Inventive step (IS) | Claims | 17, 18, 57 | YES |
| | Claims | 1-14 and 66 | NO |
| Industrial applicability (IA) | Claims | 1-14, 17, 18, 57 and 66 | YES |
| | Claims | None | NO |

2. Citations and explanations:

Claim 66 lacks an inventive step under PCT Article 33(3) as being obvious over the article entitled 'Multiplexed genotyping with sequence-tagged molecular inversion probes' by Hardenbol et al. (hereinafter 'Hardenbol').

As per claim 66, Hardenbol discloses a method of genotyping a nucleic acid in a biological sample, the method comprising: obtaining a nucleic acid preparation using a preparative method on a biological sample (pg 674, col 2, para 2--25 different individuals were genotyped, pg 677, col 1, para 3), sequencing a target nucleic acid of the nucleic acid preparation (pg 674, col 2, para 3), and performing a molecular inversion probe capture reaction on the biological sample (pg 677, col 2, para 2), genotyping the target nucleic acid based on the results of the capture reaction (pg 674, col 2, para 2). Hardenbol further teaches that the MIPs are engineered such that when annealed to a target nucleic acid, a gap between the flanking regions comprises only one nucleotide for detection of a single nucleotide polymorphism (pg 674, col 1, para 2) and it would have been obvious to one skilled in the art during the polymerization reaction to add only the nucleotide that would have indicated an SNP at this locus if the molecular inversion probe captured the target nucleic acid in the biological sample to save reagents and time. It further would have been obvious to one skilled in the art to utilize the sequence information in addition to the capture reaction information to ensure accurate genotyping.

Claim 9 lacks an inventive step under PCT Article 33(3) as being obvious over US 2003/0203370 A1 to Yakhini et al. (hereinafter "Yakhini").

As per claim 9, although Yakhini does not explicitly teach a method of genotyping a subject, the method comprising: determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject, Yakhini discloses a method of genotyping using mass tags (abstract), wherein "As discussed above, there are generally a much smaller number of mass tags than primer sequences, and many different primers may be therefore identically labeled. Thus, fingerprinting SNPs using the CMT/PEA technique involves extracting a small fingerprint signal from complex mass spectra in which the presence of a particular mass tag may indicate extension of many different oligonucleotide primers. Moreover, even considering only SNP-characteristic primers, there may be a number of different genotypes that map to a particular mass spectrum. For example, the mass spectrum shown in FIG. 10 corresponds to mass tags removed from primers, extended as shown in FIG. 8, characteristic for the genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6. However, the same mass spectrum is produced by the above-illustrated CMT/PEA technique when applied to the example genotype S1/S1', S2/S2', S3/S3', S4'/S4', S5/S5', S6/S6, as can be see noting that, in FIG. 6, the characteristic primers for polymorphism S3 610-613 carry the same mass tags as the primer sequences characteristic for polymorphism S4' 614-617 and the characteristic primers for polymorphism S3' carry the same mass tags as the primer sequences characteristic for polymorphism S4. Therefore, the genotyping assay illustrated in FIGS. 4-10 needs to be carried out in multiple assay steps in order to distinguish genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6 from genotype S1/S1', S2/S2', S3'/S3', S4'/S4', S5/S5', S6/S6. Instead of hybridizing the set of oligonucleotide primers to all chromosome fragments, including fragments produced from all six polymorphisms, the chromosome fragments need to be partitioned into sets that generate unambiguous mask-tag fingerprints under the CMT/PEA assay technique". (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini.

----Continued in Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/01293

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V(2): Citations and Explanations

Claims 1- 5, and 7 lack an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of the article entitled 'Applications of DNA tiling arrays for whole-genome analysis' by Mockler et al. (hereinafter 'Mockler').

As per claim 1, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein each subregion overlaps with at least one other subregion. Mockler, however, discloses wherein each subregion overlaps with at least one other subregion (Fig. 2a). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 2, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other subregion)).

As per claim 3, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

As per claim 4, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

As per claim 5, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising: contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3--1,517 loci were genotyped in a 1,517 probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe. Mockler, however, discloses a tiling array wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe (Fig. 2a, d). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 7, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol further teaches wherein the analyzing comprises genotyping a polymorphism of the target nucleic acid (abstract).

Claims 10-14 lack an inventive step under PCT Article 33(3) as being obvious over Yakhihi in view of Hardenbol.

As to claim 10, although Yakhini does not explicitly teach wherein the isolated nucleic acids are molecular inversion probe capture products, Hardenbol teaches the use of said products for the genotyping of SNP's (abstract). It would have been obvious to a person skilled in the art that the mollecular inversion probe capture products taught by Hardenbol could have substituted for the mass spectrometric tags taught by Yakhini, with a similar type of degeneracy requiring the determination of sets to generate unambiguous sequence-tag fingerprints (para [0026]) in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

------Continued in Subsequent Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2: Citations and Explanations

As to claim 11, Hardenbol discloses a method of genotyping a subject, the method comprising:
determining the sequence of molecular inversion probe capture products (abstract, analyzed using universal sequence tag DNA microarrays), each comprising a molecular inversion probe and a target nucleic acid (Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a--P1,P2,Tag), and wherein the target nucleic acid is a captured genomic locus of a subject (pg 674, col 2, para 2), and genotyping the subject at the captured genomic locus based on the sequence of the captured target nucleic acid (abstract, pg 674, col 2, para 2), but fails to specifically teach genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 12, Hardenbol discloses a method of genotyping a subject, the method comprising: obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid , wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), wherein the target nucleic acid is a captured genomic locus of the subject (abstract, pg 674, col 2, para 2), amplifying the molecular inversion probe capture products (pg 674, col 1, para 2), and genotyping the subject by determining, for each target nucleic acid, the sequence of the target nucleic acid (abstract--analzed using unversal sequence tag DNA microarrays), but fails to specifically disclose genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 13, Hardenbol discloses wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence (Each amplified probe contains a unique tag sequence that is complementary to a sequence on the universal tag array; pg. 270, col 1, para 2).

As to claim 14, although neither Hardenbol nor Yakhini explcitly teaches wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05, Hardenbol teaches wherein repeatability and trio accuracy were greater than 99%, which corresponds to an error rate of less than 0.01. A person skilled in the art would have known that a likelihood of ambiguity (obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences) of the results for a genotyping assay, particularly assays associated with either prognostication of disease or of the potential utility of pharmaceuticals to treat a disease, would have been required to be at least less than 5%.

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of Mockler, further in view of US 2008/0280955 A1 to McCamish et al. (hereinafter 'McCamish').

As per claim 6, the combination of Hardenbol and Mockler discloses the method of claim 1, but fails to teach that which McCamish teaches, that is wherein the target nucleic acids are selected from Table 1 (Table 9- NC_000011.8 (DHCR7), NC_000016.8 (GCSH), NC_000019.8 (BCKDH), NC_000021.7 (HLCS)). As McCamish teaches that the SNPs in Table 9 are related to drug response to blood glucose regulation (para [0258], claims 1, 15), it would have been obvious to one skilled in the art to analyze the loci taught by McCamish using the combined method of Hardenbol and Mockler in order to ascertain whether a particular subject would be susceptible to drug therapy for blood glucose regulation.

Claim 8 lacks inventive step under PCT Article 33(3) as being obvious over Hardenbol, in view of Mockler, in further view of WO 2007/107717 A1 to Chamberlain et al. (hereinafter 'Chamberlain').

As per claim 8, the combination of Hardenbol and Mockler teaches the method of claim 7, but fails to specifically teach that which Chamberlain teaches, that is wherein the polymorphism is selected from Table 2 (Table 3, 4). As Chamberlain teaches that the listed SNP's are related to responsiveness to treatment with bisphosphonate (pg 4, ln 16-33), it would have been obvious to one skilled in the art to analyze the loci and polymorphisms as taught by Chamberlain using the combined method of Hardenbol and Mockler in order to determine who would be responsive to treatment with bisphosphonate.

-------Continued in Subsequent Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2: Citations and Explanations

Claims 17-18 meet the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Hardenbol teaches a a method of analyzing a plurality of genetic loci, the method comprising:
obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid (pg 674, col 2, para 2; Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), amplifying the plurality of molecular inversion probe capture products (pg 674, col 1, para 2), but fails to teach: determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Therefore, claim 17 and claim 18, which depends therefrom, meet the aforementioned requirements for the above reasons.

Claim 57 meets the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Hardenbol discloses a method comprising:
(i) contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid (pg 674, col 1, para 2), wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid, wherein the first and second regions are on a common strand of the target nucleic acid, and wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid (Fig. 2a, b); and
(ii) capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes (pg 674, col 1, para 3 to pg 674, col 2, para 1), but fails to specifically teach (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Therefore claim 57 meets the aforementioned requirements for the above reasons.

Claims 1-14, 17, 18, 57, 66, have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

**OR**

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2014-05-27 |
|---|---|---|---|
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

## Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

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**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

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| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2014-05-27 |
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

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| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
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| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
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| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

**PATENT COOPERATION TREATY**

# PCT

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

| | | |
|---|---|---|
| Applicant's or agent's file reference<br>G0814.70000 | **FOR FURTHER ACTION** | See item 4 below |
| International application No.<br>PCT/US2010/001293 | International filing date *(day/month/year)*<br>30 April 2010 (30.04.2010) | Priority date *(day/month/year)*<br>30 April 2009 (30.04.2009) |
| International Patent Classification (8th edition unless older edition indicated)<br>See relevant information in Form PCT/ISA/237 | | |
| Applicant<br>GOOD START GENETICS, INC. | | |

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the report |
| ☐ | Box No. II | Priority |
| ☒ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☒ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☐ | Box No. VIII | Certain observations on the international application |

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

| | |
|---|---|
| | Date of issuance of this report<br>01 November 2011 (01.11.2011) |
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No. +41 22 338 82 70 | Authorized officer<br>Philippe Bécamel<br>e-mail: pt01.pct@wipo.int |

Form PCT/IB/373 (January 2004)

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK R.H. WALLER
WOLF, GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| Date of mailing *(day/month/year)* | 28 OCT 2010 |
|---|---|
| Applicant's or agent's file reference<br>G0814.70000 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 10/01293 | 30 April 2010 (30.04.2010) | 30 April 2009 (30.04.2009) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/94

Applicant GOOD START GENETICS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 07 Oct. 2010 (07.10.2010) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2009)

PCT/US2010/001293 28.10.2010

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/01293

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 15, 16, 19, 22-56, 64, 65, 67

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 15, 16, 19, 22-56, 64, 65, 67 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims are not formed according to the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 15, 16, 19, 22-56, 64, 65, 67

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. IV Lack of unity of invention**

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☒ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-8, and 57, directed to a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different.

Group II: claims 9-14, 17, 18, and 66, directed to a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject.

Group III: claims 20-21, directed to a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject.

Group IV: claims 58-63, directed to a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The inventions listed as Groups I - IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different. The special technical feature of the Group II claims is a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject. The special technical feature of the Group III claims is a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject. The special technical feature of the Group IV claims is a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract; para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface). Therefore, the inventions of Groups I - IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-14, 17, 18, 57 and 66

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 10/01293

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-14, 17, 18, 57 and 66 | YES |
| | Claims | None | NO |
| Inventive step (IS) | Claims | 17, 18, 57 | YES |
| | Claims | 1-14 and 66 | NO |
| Industrial applicability (IA) | Claims | 1-14, 17, 18, 57 and 66 | YES |
| | Claims | None | NO |

2. Citations and explanations:

Claim 66 lacks an inventive step under PCT Article 33(3) as being obvious over the article entitled 'Multiplexed genotyping with sequence-tagged molecular inversion probes' by Hardenbol et al. (hereinafter 'Hardenbol').

As per claim 66, Hardenbol discloses a method of genotyping a nucleic acid in a biological sample, the method comprising: obtaining a nucleic acid preparation using a preparative method on a biological sample (pg 674, col 2, para 2--25 different individuals were genotyped, pg 677, col 1, para 3), sequencing a target nucleic acid of the nucleic acid preparation (pg 674, col 2, para 3), and performing a molecular inversion probe capture reaction on the biological sample (pg 677, col 2, para 2), genotyping the target nucleic acid based on the results of the capture reaction (pg 674, col 2, para 2). Hardenbol further teaches that the MIPs are engineered such that when annealed to a target nucleic acid, a gap between the flanking regions comprises only one nucleotide for detection of a single nucleotide polymorphism (pg 674, col 1, para 2) and it would have been obvious to one skilled in the art during the polymerization reaction to add only the nucleotide that would have indicated an SNP at this locus if the molecular inversion probe captured the target nucleic acid in the biological sample to save reagents and time. It further would have been obvious to one skilled in the art to utilize the sequence information in addition to the capture reaction information to ensure accurate genotyping.

Claim 9 lacks an inventive step under PCT Article 33(3) as being obvious over US 2003/0203370 A1 to Yakhini et al. (hereinafter "Yakhini").

As per claim 9, although Yakhini does not explicitly teach a method of genotyping a subject, the method comprising: determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject, Yakhini discloses a method of genotyping using mass tags (abstract), wherein "As discussed above, there are generally a much smaller number of mass tags than primer sequences, and many different primers may be therefore identically labeled. Thus, fingerprinting SNPs using the CMT/PEA technique involves extracting a small fingerprint signal from complex mass spectra in which the presence of a particular mass tag may indicate extension of many different oligonucleotide primers. Moreover, even considering only SNP-characteristic primers, there may be a number of different genotypes that map to a particular mass spectrum. For example, the mass spectrum shown in FIG. 10 corresponds to mass tags removed from primers, extended as shown in FIG. 8, characteristic for the genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6. However, the same mass spectrum is produced by the above-illustrated CMT/PEA technique when applied to the example genotype S1/S1', S2/S2', S3/S3', S4'/S4', S5/S5', S6/S6, as can be see noting that, in FIG. 6, the characteristic primers for polymorphism S3 610-613 carry the same mass tags as the primer sequences characteristic for polymorphism S4' 614-617 and the characteristic primers for polymorphism S3' carry the same mass tags as the primer sequences characteristic for polymorphism S4. Therefore, the genotyping assay illustrated in FIGS. 4-10 needs to be carried out in multiple assay steps in order to distinguish genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6 from genotype S1/S1', S2/S2', S3'/S3', S4'/S4', S5/S5', S6/S6. Instead of hybridizing the set of oligonucleotide primers to all chromosome fragments, including fragments produced from all six polymorphisms, the chromosome fragments need to be partitioned into sets that generate unambiguous mask-tag fingerprints under the CMT/PEA assay technique". (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini.

----Continued in Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/01293

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V(2): Citations and Explanations

Claims 1- 5, and 7 lack an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of the article entitled 'Applications of DNA tiling arrays for whole-genome analysis' by Mockler et al. (hereinafter 'Mockler').

As per claim 1, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein each subregion overlaps with at least one other subregion. Mockler, however, discloses wherein each subregion overlaps with at least one other subregion (Fig. 2a). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 2, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other subregion)).

As per claim 3, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

As per claim 4, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

As per claim 5, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising: contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3--1,517 loci were genotyped in a 1,517 probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe. Mockler, however, discloses a tiling array wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe (Fig. 2a, d). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 7, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol further teaches wherein the analyzing comprises genotyping a polymorphism of the target nucleic acid (abstract).

Claims 10-14 lack an inventive step under PCT Article 33(3) as being obvious over Yakhihi in view of Hardenbol.

As to claim 10, although Yakhini does not explicitly teach wherein the isolated nucleic acids are molecular inversion probe capture products, Hardenbol teaches the use of said products for the genotyping of SNP's (abstract). It would have been obvious to a person skilled in the art that the mollecular inversion probe capture products taught by Hardenbol could have substituted for the mass spectrometric tags taught by Yakhini, with a similar type of degeneracy requiring the determination of sets to generate unambiguous sequence-tag fingerprints (para [0026]) in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

------Continued in Subsequent Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2: Citations and Explanations

As to claim 11, Hardenbol discloses a method of genotyping a subject, the method comprising:
determining the sequence of molecular inversion probe capture products (abstract, analyzed using universal sequence tag DNA microarrays), each comprising a molecular inversion probe and a target nucleic acid (Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a--P1,P2,Tag), and wherein the target nucleic acid is a captured genomic locus of a subject (pg 674, col 2, para 2), and genotyping the subject at the captured genomic locus based on the sequence of the captured target nucleic acid (abstract, pg 674, col 2, para 2), but fails to specifically teach genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 12, Hardenbol discloses a method of genotyping a subject, the method comprising: obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid , wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), wherein the target nucleic acid is a captured genomic locus of the subject (abstract, pg 674, col 2, para 2), amplifying the molecular inversion probe capture products (pg 674, col 1, para 2), and genotyping the subject by determining, for each target nucleic acid, the sequence of the target nucleic acid (abstract--analzed using unversal sequence tag DNA microarrays), but fails to specifically disclose genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 13, Hardenbol discloses wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence (Each amplified probe contains a unique tag sequence that is complementary to a sequence on the universal tag array; pg. 270, col 1, para 2).

As to claim 14, although neither Hardenbol nor Yakhini explcitly teaches wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05, Hardenbol teaches wherein repeatability and trio accuracy were greater than 99%, which corresponds to an error rate of less than 0.01. A person skilled in the art would have known that a likelihood of ambiguity (obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences) of the results for a genotyping assay, particularly assays associated with either prognostication of disease or of the potential utility of pharmaceuticals to treat a disease, would have been required to be at least less than 5%.

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of Mockler, further in view of US 2008/0280955 A1 to McCamish et al. (hereinafter 'McCamish').

As per claim 6, the combination of Hardenbol and Mockler discloses the method of claim 1, but fails to teach that which McCamish teaches, that is wherein the target nucleic acids are selected from Table 1 (Table 9- NC_000011.8 (DHCR7), NC_000016.8 (GCSH), NC_000019.8 (BCKDH), NC_000021.7 (HLCS)). As McCamish teaches that the SNPs in Table 9 are related to drug response to blood glucose regulation (para [0258], claims 1, 15), it would have been obvious to one skilled in the art to analyze the loci taught by McCamish using the combined method of Hardenbol and Mockler in order to ascertain whether a particular subject would be susceptible to drug therapy for blood glucose regulation.

Claim 8 lacks inventive step under PCT Article 33(3) as being obvious over Hardenbol, in view of Mockler, in further view of WO 2007/107717 A1 to Chamberlain et al. (hereinafter 'Chamberlain').

As per claim 8, the combination of Hardenbol and Mockler teaches the method of claim 7, but fails to specifically teach that which Chamberlain teaches, that is wherein the polymorphism is selected from Table 2 (Table 3, 4). As Chamberlain teaches that the listed SNP's are related to responsiveness to treatment with bisphosphonate (pg 4, ln 16-33), it would have been obvious to one skilled in the art to analyze the loci and polymorphisms as taught by Chamberlain using the combined method of Hardenbol and Mockler in order to determine who would be responsive to treatment with bisphosphonate.

-------Continued in Subsequent Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

**Supplemental Box**

**In case the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2: Citations and Explanations

Claims 17-18 meet the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Hardenbol teaches a a method of analyzing a plurality of genetic loci, the method comprising:
obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid (pg 674, col 2, para 2; Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), amplifying the plurality of molecular inversion probe capture products (pg 674, col 1, para 2), but fails to teach: determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Therefore, claim 17 and claim 18, which depends therefrom, meet the aforementioned requirements for the above reasons.

Claim 57 meets the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Hardenbol discloses a method comprising:
(i) contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid (pg 674, col 1, para 2), wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid, wherein the first and second regions are on a common strand of the target nucleic acid, and wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid (Fig. 2a, b); and
(ii) capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes (pg 674, col 1, para 3 to pg 674, col 2, para 1), but fails to specifically teach (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Therefore claim 57 meets the aforementioned requirements for the above reasons.

Claims 1-14, 17, 18, 57, 66, have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference 29258/28WO | **FOR FURTHER ACTION** | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US 11/65098 | International filing date *(day/month/year)* 15 December 2011 (15.12.2011) | (Earliest) Priority Date *(day/month/year)* 23 December 2010 (23.12.2010) |
| Applicant GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

# INTERNATIONAL SEARCH REPORT

PCT/US2011/065098 03.04.2012

International application No.
PCT/US 11/65098

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/00; C12Q 1/68 (2012.01)
USPC - 536/23.1, 435/6.11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C07H 21/00; C12Q 1/68 (2012.01)
USPC: 536/23.1, 435/6.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 536/24.3, 435/6.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, Google Scholar: Sequence identifier$2, 5', 3', sequenc$6, template$2, nucleic acid$2, barcode, validat$4, amplif$4, target polynucleotide$2, sequence identif$6, tag$2, detect$4, label$2, validate$4, identif$4, detect$4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | US 2010/0297626 A1 (MCKERNAN et al.) 25 November 2010 (25.11.2010); abstract; para [0026], [0027], [0028], [0029], [0075], [0087], [0096], [0110], [0116], [0117], [0127], [0141], [0142], [0144], [0163], [0215], [0323], [0332], [0336], [0337], [0342], | 1-2, 4-15<br>---<br>3, 16-46 |
| Y | US 2008/0269068 A1 (CHURCH et al.) 30 October 2008 (30.10.2008); para [0007], [0022] | 3, 16-41 |
| Y | US 2010/0035243 A1 (MULLER et al.) 11 February 2010 (11.02.2010); para [0012], [0017] | 42-46 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 March 2012 (14.03.2012) | 03 APR 2012 |

| Name and mailing address of the ISA/US | Authorized officer: |
|---|---|
| Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| Date of mailing *(day/month/year)* | 03 APR 2012 |
|---|---|
| Applicant's or agent's file reference<br>29258/28WO | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 11/65098 | 15 December 2011 (15.12.2011) | 23 December 2010 (23.12.2010) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C07H 21/00; C12Q 1/68 (2012.01)
USPC - 536/23.1, 435/6.11

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 14 March 2012 (14.03.2012) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 11/65098

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
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**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 3, 11-12, 16-46 | YES |
| | Claims | 1-2, 4-10, 13-15 | NO |
| Inventive step (IS) | Claims | None | YES |
| | Claims | 1-46 | NO |
| Industrial applicability (IA) | Claims | 1-46 | YES |
| | Claims | None | NO |

2. Citations and explanations:

Claims 1-2, 4-10 and 13-15 lack novelty under PCT Article 33(2) as being anticipated by US 2010/0297626 A1 to McKernan et al. (hereinafter 'McKernan').

Regarding claim 1, McKernan teaches a method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction (abstract, para [0087]), the method comprising:
obtaining a template nucleic acid (abstract);
incorporating at least two sequence identifiers into the template (abstract); and
sequencing the template (abstract).

Regarding claim 2 McKernan further teaches the method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template (para [0026], [0027]).

Regarding claim 4, McKernan further teaches the method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template (para [0026]).

Regarding claim 5, McKernan further teaches the method according to claim 2, wherein the first and second identifiers are the same (para [0144]- paired tags have the same sequence).

Regarding claim 6, McKernan further teaches the method according to claim 2, wherein the first and second identifiers are different (para [0163]).

Regarding claim 7, McKernan further teaches the method according to claim 1, wherein prior to sequencing, the template is attached to e substrate (para [0332]).

Regarding claim 8, McKernan further teaches the method according to claim 2, wherein the template is directly attached to the substrate (para [0336]).

Regarding claim 9, McKernan further teaches the method according to claim 2, wherein the template is indirectly attached to the substrate (para [0336]).

Regarding claim 10, McKernan further teaches the method according to claim 7, further comprising, amplifying the template (para [0075]).

Regarding claim 13, McKernan further teaches the method according to claim 1, wherein sequencing comprises:
hybridizing a primer to the template to form a template/primer duplex (para [0215]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0215], [0116]);
detecting a signal from the incorporated labeled nucleotide (para [0197]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid (para [0323]).

Regarding claim 14, McKernan further teaches the method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0029]).

Regarding claim 15, McKernan further teaches the method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides (para [0029]).

Claims 11-12 and lacks inventive step under PCT Article 33(3) as being obvious over McKernan.

Regarding claim 11, McKernan teaches the method according to claim 1, and further makes obvious wherein sequencing is sequencing by synthesis (para [0127], sequencing by synthesis is found to lead to errors, however the possibility of sequencing by synthesis is identified and made obvious as a possible sequencing method).

*****Continued in Supplemental Box*****

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/65098

**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V(2):

Regarding claim 12, McKernan makes obvious the method according to claim 11, and further teaches wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0117]).

Claims 3, 16-41 lack inventive step under PCT Article 33(3) as being obvious over McKernan in view of US 2008/0269068 A1 to Church et al. (hereinafter 'Church').

Regarding claim 3, McKernan teaches the method according to claim 2, however fails to teach wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence. McKernan does teach wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first identifier sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second identifier sequence; and a fourth known sequence (para [0027], [0096], [0110]). McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 16, McKernan teaches the method according to claim 1, however fails to teach wherein the pair of identifiers are first and second barcode sequences that are attached to the template. McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 17, the combination of McKernan and Church makes obvious the method according to claim 16, and McKernan further makes obvious wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template (para [0141]-[0142], if barcodes are being utilized as the identifies as made obvious in claim 16, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 18, the combination of McKernan and Church makes obvious the method according to claim 17, and McKernan further teaches wherein the first and second barcode sequences are the same (para [0144], same sequence).

Regarding claim 19, the combination of McKernan and Church makes obvious the method according to claim 17, and McKernan further teaches wherein the first and second barcode sequences are different (para [0163]).

Regarding claim 20, McKernan teaches a method for analyzing a nucleic acid (abstract), the method comprising:
obtaining a nucleic acid template (abstract);however fails to teach
attaching a pair of barcode sequences to the template; and McKernan further teaches
sequencing the template (abstract).
McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 21, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template (para [0026]).

Regarding claim 22, the combination of McKernan and Church makes obvious the method according to claim 21, and McKernan makes obvious wherein after attaching of the pair of barcode sequences,
the template has the following configuration from 5' to 3': a first known sequence; a first
barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence (para [0026]-[0027], if barcodes are being utilized as the identifies as made obvious in claim 16, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 23, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template (para [0026]-[0027]).

Regarding claim 24, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein prior to sequencing, the template is attached to a substrate (para [0332]).

Regarding claim 25, the combination of McKernan and Church makes obvious the method according to claim 24, and McKernan further teaches wherein the template is directly attached to the substrate (para [0336]).

Regarding claim 26, the combination of McKernan and Church makes obvious the method according to claim 24, and McKernan further teaches wherein the template is indirectly attached to the substrate (para [0336]).

******Continued in Supplemental Box*****

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Previous Supplemental Box:

Regarding claim 27, the combination of McKernan and Church makes obvious the method according to claim 24, and McKernan further teaches the method further comprising, amplifying the template (para [0075]).

Regarding claim 28, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further makes obvious wherein sequencing is sequencing by synthesis (para [0127], sequencing by synthesis is found to lead to errors, however the possibility of sequencing by synthesis is identified and made obvious as a possible sequencing method).

Regarding claim 29, the combination of McKernan and Church makes obvious the method according to claim 28, and McKernan further makes obvious wherein the sequencing by synthesis is single molecule sequencing by synthesis.

Regarding claim 30, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein sequencing comprises:
hybridizing a primer to the template to form a template/primer duplex (para [0215]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably
labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0215], [0116]);
detecting a signal from the incorporated labeled nucleotide (para [0323]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid (para [0117]).

Regarding claim 31, the combination of McKernan and Church makes obvious the method according to claim 29, and McKernan further teaches wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0029]).

Regarding claim 32, the combination of McKernan and Church makes obvious the method according to claim 31, and McKernan further teaches wherein the optically labeled nucleotide is a fluorescently labeled nucleotides (para [0029]).

Regarding claim 33, the combination of McKernan and Church makes obvious the method according to claim 23, and McKernan further teaches wherein the first and second barcode sequences are the same (para [0144]- have the same sequence. If barcodes are being utilized as the identifies as made obvious in claim 23, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 34, the combination of McKernan and Church makes obvious the method according to claim 23, and McKernan further teaches wherein the first and second barcode sequences are different (para [0163]).

Regarding claim 35, McKernan teaches a method for analyzing a nucleic acid (abstract), the method comprising:
obtaining a nucleic acid template (abstract); however fails to teach
attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template;
McKernan goes on to teach
amplifying the template (para [0075]); and
sequencing the template (abstract).
McKernan does teach attaching an identifier sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (para [0141]-[0142]).
McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 36, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further makes obvious wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence (para [0026]-[0027], If barcodes are being utilized as the identifies as made obvious in claim 23, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 37, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template (para [0141]-[0142]).

Regarding claim 38, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein prior to amplifying, the template is immobilized to a solid support (para [0332])

Regarding claim 39, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein the first and second barcode sequences are the same (para [0144], tags have the same sequence).

Regarding claim 40, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein the first and second barcode sequences are different (para [0163]).
*****Continued in Supplemental Box*****

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/65098

**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Previous Supplemental Box:

Regarding claim 41, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein sequencing comprises:
hybridizing a primer to the template to form a template/primer duplex (para [0215]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably
labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0215], [0116]);
detecting a signal from the incorporated labeled nucleotide (para [0323]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential
detection of incorporated labeled nucleotide determines the sequence of the nucleic acid (para [0323]).

Claims 42-46 lack inventive step under PCT Article 33(3) as being obvious over McKernan in view of US 2010/0035243 A1 to Muller et al. (hereinafter 'Muller').

Regarding claim 42, concerning a method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte. McKernan does teach a method for validating detection of a nucleic acid sequence, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said sequence (abstract). McKernan further teaches wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]). Muller teaches using a specific barcode sequence for detection of analyte (para [0017]). It would have been thus obvious to one of ordinary skill in the art to utilize the method taught by McKernan to identify an analytein order to increase the accuracy of the method.

Regarding claim 43, the combination of McKernan and Muller makes obvious the method of claim 42, and McKernan further teaches wherein said identifiers are nucleic acid sequence tags (abstract).

Regarding claim 44, the combination of McKernan and Muller makes obvious the method of claim 43, however fails to teach wherein said sequence tags are associated with a moiety that binds said analyte. Muller teaches using a specific barcode sequence for detection of analyte (para [0017]). It would have been thus obvious to one of ordinary skill in the art to utilize the method taught by McKernan to identify an analyte in order to increase the accuracy of the method.

Regarding claim 45, the combination of McKernan and Muller makes obvious the method of claim 42, and Muller further teaches wherein said analyte is a protein (para [0012]).

Regarding claim 46, the combination of McKernan and Muller makes obvious the method of claim 45,and Muller further teaches wherein said identifiers are peptides (para [0047]).

Claims 1-46 have industrial applicability under PCT Article 33(4) because the subject matter can be made or used in industry.

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 19111735 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Diane Riordan |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 27-MAY-2014 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 16:24:37 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

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|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Non Patent Literature | Alazard2005CPNAC.PDF | 413930<br>ae947d0e0d36acdbbc5f975edf65b4515688b0e6 | no | 7 |

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| 2 | Non Patent Literature | Aljanabi-Nucleic-Acids-Research-1997.PDF | 211563<br>6da9399d0e31c38f1db0b21905342337 6f537b96 | no | 2 |
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| 3 | Non Patent Literature | Antonarakis-1998-HumanMutation.PDF | 254596<br>4eb25e1dbb96fc05cdfa1ca46b9f0149e270bbb7 | no | 3 |
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| 4 | Non Patent Literature | Barany-PNAS-1991.PDF | 4636767<br>2d77043dc4553b3b11fd1e4795822641180cc704 | no | 5 |
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| 6 | Non Patent Literature | Benner_TrendsinGenetics_2001.PDF | 530901<br>3557d643d22f6d8724bf107825e29d22f0d29986 | no | 5 |
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| 12 | Non Patent Literature | BrownMethodsEnzym1979.PDF | 3881622<br>9df4e575a38b37dfdf14c06d4fcb4bed60542cba | no | 43 |
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| 13 | Non Patent Literature | BunyanBritishJournalofCancer2004.PDF | 108147<br>21bc3c6d82133f90f84196e9c57de2583b0596d4 | no | 5 |
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| 14 | Non Patent Literature | BurrowWheelerTechnicalReport121994.PDF | 107864<br>aeb130c4ed75137d8b77a87ae32eaadbccf5b83b | no | 24 |
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| 17 | Non Patent Literature | -Chevreux_.PDF | 938737<br>b76bfd8c00bffe37adda70239b69a7da7c85798d | no | 12 |
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| 19 | Non Patent Literature | CiottiJMolDiag2004.PDF | 289994<br>488108dfd11b71fc2e89564ee3bbff710cea5c5d | no | 5 |
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| 21 | Non Patent Literature | delaBastide-2007-CurrProtocinBioinform.PDF | 1148953<br>bd434a8ba82927db3c7730d813e976e2338c5adf | no | 15 |
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| 23 | Non Patent Literature | DongMethodsMolBio2011.PDF | 904922<br>35353e12b241a9cfa6147737d2cb9a35329e0a15 | no | 15 |
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| 24 | Non Patent Literature | DoreBiophysJ1969.PDF | 3607483<br>f2edcc8fc404ba8a912b60d5fdce25c7fcc16a46 | no | 31 |
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| 25 | Non Patent Literature | DudleyLOSComputationalBiology-2009.PDF | 8584977<br>ffc9bfbe380d7d028169b2a5fd1b589a0fd7b430 | no | 7 |
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| 26 | Non Patent Literature | EP_Exam_Report_10770071-8.PDF | 177083<br>a88c6b57c267c7270bdf7102ca1bbf1ca1d5e0d1 | no | 5 |
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| 27 | Non Patent Literature | FaresPrenatalDiagnosis2008.PDF | 714972<br>841fcb94005eda80d448e1abffcb59d212a7a268 | no | 6 |
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| 28 | Non Patent Literature | Faulstich1997AnalChem.PDF | 384743<br>e3d7e9d3056c3f09f31c7eeb1007b62392af2790 | no | 5 |
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| 31 | Non Patent Literature | GemayelAnnRevGen2010.PDF | 5396197<br>4cf2b1ba6573bc01b72af7284c126600f416484f | no | 36 |
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| 32 | Non Patent Literature | Glover-1995-RCMS.PDF | 1217547<br>533f03a47d226d46a1679d87021729b72afc53d2 | no | 5 |
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| 33 | Non Patent Literature | GnirkeNatureBiotech2009.PDF | 842579<br>bebeb548d00724087bb89d9b5a8bcead65faf0b3 | no | 8 |
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| 34 | Non Patent Literature | -Goto.PDF | 249641<br>7673f6c0ace711168211e48929bc29d7b4989906 | no | 3 |
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| 40 | Non Patent Literature | HuangWorldJGastroenterol2008.PDF | 483828<br>27a1de681b818030902d7ff44703f93f766b1ab8 | no | 8 |
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| 49 | Non Patent Literature | ISRandWrittenOpinionforPCTUS1001293.PDF | 772151<br>e817a68f57b03d355048f4b75906332771aec315 | no | 13 |
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| 58 | Non Patent Literature | DiGuistini-PloSOne-2009.pdf | 1983154<br>448ff349e395ab7b70399e7cf199cca92c2141d6 | no | 12 |
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**<u>New Applications Under 35 U.S.C. 111</u>**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**<u>National Stage of an International Application under 35 U.S.C. 371</u>**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**<u>New International Application Filed with the USPTO as a Receiving Office</u>**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

# Electronic Acknowledgement Receipt

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| **EFS ID:** | 19111932 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Diane Riordan |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 27-MAY-2014 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 16:26:19 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

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| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
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| 1 | Non Patent Literature | JonesScience2008.PDF | 754439<br>489e1415151c12481273b606572b013b17d3c8c3 | no | 6 |

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| 9 | Non Patent Literature | --LangmeadGenomeBiology2009.PDF | 585413<br>678dd28e4449ce6c866dd539f2f500f5e20d3e19 | no | 10 |
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| 16 | Non Patent Literature | Lipman-1985-Science.PDF | 704555<br>97841a122574ba0c436b102733636e331b3ab7e7 | no | 7 |
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| 18 | Non Patent Literature | MarguliesNature2005.PDF | 372263<br>51bf4bde9291b1e3cffb7d29f22b55a9101da6f0 | no | 6 |
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| 19 | Non Patent Literature | Marras-1999-GenAnaly.PDF | 364179<br>f03a0ca474e405e8e0d5d678edda316567f5b238 | no | 6 |
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| 24 | Non Patent Literature | Moudrianakis-1965-PNAS.PDF | 1197431<br>d0cd6afbddfba3328d552a257c5ce55f96503672 | no | 8 |
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| 26 | Non Patent Literature | NanChineseMedicalJournal2006.PDF | 1911869<br>056da02d0d2e19909540d279613d3f891835e756 | no | 7 |
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| 27 | Non Patent Literature | NarangMethodsEnzym1979.PDF | 1153971<br>f94dca5a5e5512cc08c073002cfb2a5564267e50 | no | 9 |
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| 29 | Non Patent Literature | NickersonPNAS1990.PDF | 1173715<br>0743acea27f2b9a6144a7641d93f8c236c3d3898 | no | 5 |
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| 30 | Non Patent Literature | NielsenHorizonScientific1999.PDF | 17336331<br>4f0d3084fb27a54285357806b82c1767596a376b | no | 22 |
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| 31 | Non Patent Literature | NingScienceGenomeResearch2001.PDF | 112487<br>81a8606bef710fa84edc9f9fba9bb603e59969a8 | no | 5 |
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| 32 | Non Patent Literature | Nordhoff2003Nucleic_Acids_Research.PDF | 814372<br>93702861b4d162d965e811b59e09f20d3d085d0b | no | 11 |
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| 51 | Non Patent Literature | --Pertea_et_Bioinformatics_2003.pdf | 152710<br>0a311526f7ff774ef650f29fffa7e2158a51db56 | no | 2 |
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| 58 | Non Patent Literature | Nicholas-2002-Biotechniques.pdf | 1062282<br>82603d99199051f8224250f92dfed94a2f08355e | no | 13 |
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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

# Electronic Acknowledgement Receipt

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| **EFS ID:** | 19112013 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Diane Riordan |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 27-MAY-2014 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 16:27:56 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

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| 4 | Non Patent Literature | SchadtHurmMolecularGen2010.PDF | 1623865<br>62396b83a614553549c2e86272739b9c171c894b | no | 14 |
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| 5 | Non Patent Literature | Schatz-GenomeResearch.PDF | 971875<br>bdfde8178ef821b65825cc2924fba1e22d1a7d91 | no | 9 |
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| 6 | Non Patent Literature | SchrijverJMolDiag2005.PDF | 1126745<br>3c58e4dcbdc8ef7631c6551c4ca3abf9d88b6f84 | no | 11 |
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| 7 | Non Patent Literature | Schuette1995JPBA.PDF | 1928851<br>9c08cf35ec4009b8e73b30185282b647344874d7 | no | 9 |
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| 8 | Non Patent Literature | Schwartz-2009-JMolDiag.PDF | 279927<br>4e9da494d964152870db82e7dc9de9bf30882956 | no | 5 |
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| 9 | Non Patent Literature | SchwartzClinicsLabMed2011.PDF | 1957253<br>e5b2fdd196f74ff83863aed8a487e70aaad35206 | no | 14 |
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| 10 | Non Patent Literature | Sequeira-1997-EcoModel.PDF | 716225<br>9251b52f1c855875f89d6f3fe3321c42e843301b | no | 15 |
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| 11 | Non Patent Literature | --Sievers-et-al_Molecular_systems_biology_2011.PDF | 227187<br>6b764ab0b8d655e4ef5c334972fa74d36f3f3890 | no | 6 |
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| 12 | Non Patent Literature | Simpson-2009-GenomeResearch.PDF | 785522<br>a2b1b9b4e00326f3737614ff4619529e9562f700 | no | 7 |
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| 13 | Non Patent Literature | Soni-2007-Clinical.PDF | 1139439<br>caf6963c6a77c9c7a13c76d381e399da6e6177bc | no | 6 |
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| 15 | Non Patent Literature | SummererGenomics2009.PDF | 911387<br>b1a96b530a9a658ea687e553814d2323e22bcdd6 | no | 6 |
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| 16 | Non Patent Literature | SunnucksGenetics1996.PDF | 3399951<br>eb82af121fe9b0279d3b6a3d1bfc0f558ec9a2f1 | no | 10 |
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| 17 | Non Patent Literature | ThauvinRobinetJournalofMedicalGenetics2009.PDF | 1037096<br>044b9931c0687df5e6fd1a16636ec53b7bc7b3b1 | no | 8 |
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| 19 | Non Patent Literature | ThorstensonGenomeResearch1998.PDF | 693961<br>cf91d7633239d718d682d30c236c7566a1a9e607 | no | 8 |

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| 20 | Non Patent Literature | ThorvaldsdottiBriefBioinform2012.PDF | 1960276<br>28acc260a018925de3e0ead51ad746f5687e2299 | no | 15 |
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| 21 | Non Patent Literature | Turner-2009-AnnRevGenomics.PDF | 2025120<br>27d20c419b25838f37a038c8b3f87ccf9dc1c8c1 | no | 25 |
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| 23 | Non Patent Literature | WarnerJMedGenet1996.PDF | 771105<br>dfa8dc92bac8d928c59c6fb02205ad38657cbe5e | no | 5 |
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| 28 | Non Patent Literature | Wu2001AnalBioch.PDF | 3404326<br>e6ede1ea5c52b9d188eee24522dfd5686d86aeb8 | no | 6 |

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| 29 | Non Patent Literature | Wu1998AnalBiochem.PDF | 4725990<br>ee3180d5663dbdbb9e2f6e5ac21c8a519e4ada07 | no | 10 |
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| 33 | Non Patent Literature | YuClinicCancerRes2007.PDF | 1641311<br>d8a85ea262f6f3bff622d5d3f3c3fd410d76c8fb | no | 9 |
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| 37 | Non Patent Literature | Zhao-2009-Genomics.PDF | 200937<br>bc1515fbcc7504a77e6b645b71d7f55224233fbd | no | 3 |

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| 38 | Non Patent Literature | --Zheng-et-al-BMC-bioinformatics-2011.PDF | 177498<br>845c54329347f99fde0f27f82e95154eb7ac9d14 | no | 8 |
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| 39 | Non Patent Literature | ZimmermanetalGeneticsinMedicine2010.PDF | 5749145<br>4de85d5f486a605f1da90fc4a932c537b6a53d3c | no | 11 |
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| 40 | Foreign Reference | EP1321477A1.PDF | 5877566<br>65fc785a8a22f8c2cac9169f13480d5b20fdd268 | no | 113 |
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| 44 | Non Patent Literature | Tokino-Hum-Genet-1996.pdf | 639093<br>beb698f66ccf47448026c8400d4449eab93a2820 | no | 7 |
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| 45 | Non Patent Literature | Husemann-2009-AlgBioinform.pdf | 957759<br>6a7e9e9831e020372fa6806af4fd691a06b6949f | no | 12 |
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| 46 | Non Patent Literature | EP-Search-Report-11-08-2012.pdf | 679410<br>56cf27b03274a26c140845c3cc0d1e67624edc48 | no | 17 |

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| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers |
| **Filer Authorized By:** | |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 01-JUL-2014 |
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| 5 | Non Patent Literature | Kumar-2010-BMCGenomics.PDF | 6195666<br>b67c0ecf1c7dc7c3e04001c7764e72da0bdb1277 | no | 12 |
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| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| | 3 | Husemann, P. & Stoye, 2009, Phylogenetic Comparative Assembly, Algorithms in Bioinformatics: 9th International Workshop, pp. 145-156, Salzberg, S., and Warnow, T., Eds. Springer-Verlag, Berlin Heidelberg. | ☐ |
| | 4 | Kumar, S., et al., 2010, Comparing de novo assemblers for 454 transcriptome data, Genomics 11:571. | ☐ |

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|---|---|
| **EFS ID:** | 20396763 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Steve Whitaker |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 15-OCT-2014 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 09:08:10 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Non Patent Literature | Thompson-2011-GenomeBiol.PDF | 440819<br>e60f684cb72bf8344ebb6e22d2faa8615c42a6dd | no | 10 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 2 | Non Patent Literature | APER-for-AU2010242073.PDF | 106866<br>b75b376bc996e39c7d6f895e7788803c25f5e3e9 | no | 4 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Non Patent Literature | ISRandWOforPCTUS1427324.PDF | 520887<br>0064619892496cd712e0d391236b1aa2da951778 | no | 8 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Non Patent Literature | SESR-for-EP12765217.PDF | 413065<br>5e2b780661a8490ba7321a075c2857d0fa2e1005 | no | 5 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Foreign Reference | WO2004083819A2.PDF | 1574928<br>a7e691af2fa371c6c43b14393d5c4cb7ac841a93 | no | 34 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Foreign Reference | WO1996019586.PDF | 1044274<br>a16821bf77614a932b76010347f497430cef03fa | no | 27 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 7 | Foreign Reference | WO1998014275.PDF | 1382425<br>54ed640163171c240d538335597b8a4b653449dc | no | 27 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 8 | Foreign Reference | WO2005003304.PDF | 8811059<br>a978816da74d7dc1d7bd3c8af0cd0b45d3e08e81 | no | 156 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 9 | Information Disclosure Statement (IDS) Form (SB08) | Information_Disclosure_Statement_.pdf | 1211591<br>4ac2c1a8d61215bff34f468389b3b2620d490ad6 | no | 4 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| | | **Total Files Size (in bytes):** | 15505914 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

**OR**

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2014-10-15 |
|---|---|---|---|
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

## Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (**Not for submission under 37 CFR 1.99**) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**U.S.PATENTS**

| Examiner Initial* | Cite No | Patent Number | Kind Code[1] | Issue Date | Name of Patentee or Applicant of cited Document | Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear |
|---|---|---|---|---|---|---|
| | 1 | | | | | |

If you wish to add additional U.S. Patent citation information please click the Add button.

**U.S.PATENT APPLICATION PUBLICATIONS**

| Examiner Initial* | Cite No | Publication Number | Kind Code[1] | Publication Date | Name of Patentee or Applicant of cited Document | Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear |
|---|---|---|---|---|---|---|
| | 1 | | | | | |

If you wish to add additional U.S. Published Application citation information please click the Add button.

**FOREIGN PATENT DOCUMENTS**

| Examiner Initial* | Cite No | Foreign Document Number[3] | Country Code[2] | Kind Code[4] | Publication Date | Name of Patentee or Applicant of cited Document | Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear | T[5] |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2004/083819 | WO | A2 | 2004-09-30 | Trace Genetics, Inc | | ☐ |
| | 2 | 1996/019586 | WO | A1 | 1996-06-27 | Visible Genetics Inc | | ☐ |
| | 3 | 1998/014275 | WO | A1 | 1998-04-09 | Intronn Llc | | ☐ |

EFS Web 2.1.17

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 2005/003304 | WO | A2 | 2005-01-13 | Illumina, Inc | | ☐ |

If you wish to add additional Foreign Patent Document citation information please click the Add button

**NON-PATENT LITERATURE DOCUMENTS**

| Examiner Initials* | Cite No | Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published. | T[5] |
|---|---|---|---|
| | 1 | Thompson, et al., 2011, The properties and applications of single-molecule DNA sequencing," Genome Biology 12(2):217, 10 pages | ☐ |
| | 2 | Australian Patent Examination Report No. 1 issued August 12, 2014, for Australian Patent Application No. 2010242073 filed April 30, 2010, 4 pages | ☐ |
| | 3 | International Search Report and Written Opinion mailed on September 3, 2014 for International Patent Application No. PCT/US14/27324, filed March 14, 2014 (8 pages) | ☐ |
| | 4 | Supplementary European Search Report issued August 26, 2014, for European Patent Application No. 12765217.0, filed March 20, 2012, 5 pages | ☐ |

If you wish to add additional non-patent literature document citation information please click the Add button

**EXAMINER SIGNATURE**

| Examiner Signature | | Date Considered | |
|---|---|---|---|

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ENTERED IN CPI

**PATENT COOPERATION TREATY**

From the INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

# PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

| | |
|---|---|
| | Date of mailing *(day/month/year)* 03 SEP 2014 |
| Applicant's or agent's file reference GSGE-016/01WO 29258/76 | **FOR FURTHER ACTION** See paragraphs 1 and 4 below |
| International application No. PCT/US 14/27324 | International filing date *(day/month/year)* 14 March 2014 (14.03.2014) |
| Applicant GOOD START GENETICS, INC. | |

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   **Filing of amendments and statement under Article 19:**
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   **When?** The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   **How?** Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   **For more detailed instructions**, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

| | |
|---|---|
| Name and mailing address of the ISA/<br>Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>Telephone No. PCT OSP: 571-272-7774 |

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference<br>GSGE-016/01WO 29258/76 | **FOR FURTHER ACTION** | see Form PCT/ISA/220<br>as well as, where applicable, item 5 below. |
|---|---|---|
| International application No.<br>PCT/US 14/27324 | International filing date *(day/month/year)*<br>14 March 2014 (14.03.2014) | (Earliest) Priority Date *(day/month/year)*<br>14 March 2013 (14.03.2013) |
| Applicant<br>GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. _____

   ☐ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☒ none of the figures is to be published with the abstract.

**INTERNATIONAL SEARCH REPORT** PCT/US2014/027324 03.09.2014

International application No.
PCT/US 14/27324

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, C12P 19/34 (2014.01)
USPC - 435/6.11, 435/91.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 435/6.11, 435/91.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- C12Q1/6883, C12Q1/6837, C12Q1/6827, C12Q1/686, C12Q2525/161, C12Q2537/143, C12Q2549/113, C12Q2561/125, C12Q1/6869; C12Q2521/501; C12Q2533/107; C12Q2535/122 (2014.01); USPC- 435/6.1, 435/6.12, 435/91.2, 506/2, 506/4

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: GOOD START GENETICS, INC.; UMBARGER, Mark; tiling, molecular inversion probes, MIP, adjacent probes, hybridize, opposite, antisense, strand, nucleic acid template, overlapping, filled-in region, Illumina, single molecule sequencing-by synthesis, universal portion, flanked

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>--<br>Y | US 2012/0165202 A1 (PORRECA, et al.) 28 June 2012 (28.06.2012) Abstract, claim 1; Figs 1, 11; para [0014], [0123], [0133], [0149], [0150], [0155], [0171], [0175], [0198], [0220]-[0224], [0235]-[0240], [0276], [0295], [0339]-[0354], [0376]-[0381] | 1-3, 6-13<br>----------<br>4, 5, 14, 15 |
| Y | THOMPSON, et al. The properties and applications of single-molecule DNA sequencing. Genome Biol. 2011, 12(2):217; Abstract, pg 3, col 2; pg 5, Fig 2 and its legend | 4, 5 |
| Y | US 2008/0269068 A1 (CHURCH, et al.) 30 October 2008 (30.10.2008) Abstract, claims 1, 6, 7; para [0081]-[0083] | 14, 15 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 July 2014 (28.07.2014) | 03 SEP 2014 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| Date of mailing *(day/month/year)* | 03 SEP 2014 |
|---|---|
| Applicant's or agent's file reference<br>GSGE-016/01WO 29258/76 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 14/27324 | 14 March 2014 (14.03.2014) | 14 March 2013 (14.03.2013) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68, C12P 19/34 (2014.01)
USPC - 435/6.11, 435/91.1

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 28 July 2014 (28.07.2014) | Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 14/27324

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

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**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-15 | YES |
| | Claims | NONE | NO |
| Inventive step (IS) | Claims | NONE | YES |
| | Claims | 1-15 | NO |
| Industrial applicability (IA) | Claims | 1-15 | YES |
| | Claims | NONE | NO |

2. Citations and explanations:

Claims 1-3 and 6-13 lack an inventive step under PCT Article 33(3) as being obvious over US 2012/0165202 A1 to Porreca et al. (hereinafter "Porreca").

As to claim 1, Porreca discloses a method for analyzing a nucleic acid template, the method comprising:
obtaining a sample comprising a nucleic acid template (para [0014], "analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample)", wherein "target nucleic acid" is synonymous with "nucleic acid template");
tiling a plurality of molecular inversion probes across a portion of the template (para [0123], "FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout showing ten captured sub-targets tiled across a single target. Each position in the target is covered by three sub-targets such that MIP [molecular inversion probe] performance per base pair is averaged across three probes"),
wherein immediately adjacent probes hybridize to the same strand of the nucleic acid template and probes on the same strand hybridize to the template in an overlapping manner (claim 1, "each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion"; para [0123], "FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout showing ten captured sub-targets tiled across a single target. Each position in the target is covered by three sub-targets such that MIP performance per base pair is averaged across three probes");
filling-in a region between targeting arms of a plurality of the molecular inversion probes with nucleotides (para [0339]-[0354], Example 6, "[0347] Convert Hybridized Probes into Covalently-Closed Circular Products Containing Target Nucleic Acids [0348] Prepare fill-in/ligation reaction mixture: ... [0350] 2.5 ul 10.times. Ampligase buffer (Epicentre) [0351] 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems) [0352] 12.5 ul 5 U/ul Ampligase (Epicentre) [0353] 4.75 ul dH2O [0354] Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60 C for 10 hours"); and
analyzing the filled-in region of a plurality of the probes to obtain sequence information about the nucleic acid template (para [0376]-[0381], "[0376] Prepare a Shotgun Next-Generation Sequencing Library for Analysis... [0379] Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length. [0380] Ligate adapters required for amplification by the sequencing platform used... [0381] Perform Sequencing of Library According to Manufacturer's Directions (e.g. Illumina, ABI, etc), Reading the Target Sequence to Determine Abundance of the Target Nucleic Acid").
Porreca does not specifically disclose that immediately adjacent probes hybridize to opposite strands of the nucleic acid template. However, Porreca does disclose selecting molecular inversion probes targeting opposing strands of the DNA target (para [0171], "In some embodiments, targeting arms that can be used for capturing the repeat region of the Fragile X locus can have the following sequences or complementary to these sequences depending on the strand that is captured"). Thus, while Porreca does not specifically disclose the avoidance of probe interference by selecting alternate inversion probes so as to target opposing strands of the DNA target in alternation, it would have been obvious to one of ordinary skill in the art that genetic information is present in two equivalent complementary copies, which can be separately detected by MIP probes. It would have been further obvious to one of ordinary skill in the art that by utilizing MIPs hybridizing to both, sense and anti-sense, strands of the target nucleic acid the density of accessible for hybridization DNA target sequences would increase by up to a 100%.

As to claim 2, Porreca discloses that analyzing is by sequencing (para [0295], "Perform Sequencing of Library According to Manufacturer's Directions (e.g. Illumina, ABI, etc), Reading Both the Target Sequence and the Differentiator Tag Sequence").

As to claim 3, Porreca discloses amplifying the filled-in region of the plurality of the probes prior to sequencing (para [0276], "Amplify Circular Material by PCR Using Primers Specific to the 'Constant Region' of the Probes").

********** See Supplemental Sheet to continue **********

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
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**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V(2). Citations and Explanations:

As to claims 6 and 7, Porreca discloses sequencing information comprises a mutation in the nucleic acid sequence, wherein the mutation is selected from the group consisting of: a single nucleotide polymorphism (SNP), and insertion, a deletion, an inversion, a translocation, a substitution, and a combination thereof (para [0133], "Multiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs, or preparatively, often for next-generation sequencing or other sequencing techniques" para [0155], "The mutations listed in Table 2 are documented polymorphisms in several disease-associated genes... The polymorphisms are of several types: insertion/deletion polymorphisms which will cause frameshifts (and thus generally interrupt protein function) unless the insertion/deletion length is a multiple of 3 bp, and substitutions which can alter the amino acid sequence of the protein and in some cases cause complete inactivation by introduction of a stop codon").

As to claim 8, Porreca discloses that the filling-in step uses a ligase (para [0220], "Prepare fill-in/ligation reaction mixture:... [0222] 2.5 ul 10.times. Ampligase buffer (Epicentre) [0223] 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems) [0224] 12.5 ul 5 U/ul Ampligase (Epicentre)").

As to claim 9, Porreca discloses that the filling-in step uses a polymerase (para [0235] "Prepare PCR mixture: ... [0240] 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)").

As to claim 10, Porreca discloses that a ligase is used to covalently link the filled-in region to one of the targeting arms (para [0219], "Convert Hybridized Probes into Covalently-Closed Circular Products Containing Subtargets [0220] Prepare fill-in/ligation reaction mixture:... [0222] 2.5 ul 10.times. Ampligase buffer (Epicentre)... [0224] 12.5 ul 5 U/ul Ampligase (Epicentre)").

As to claim 11, Porreca discloses that prior to sequencing, a barcode is attached to the molecular inversion probe (para [0149], "barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together"; para [0198], "In some embodiments, a sequencing technique (e.g., a next-generation sequencing technique) is used to sequence part of one or more captured targets (e.g., or amplicons thereof) and the sequences are used to count the number of different barcodes that are present. Accordingly, in some embodiments, aspects of the invention relate to a highly-multiplexed qPCR reaction", para [0175] "When a precise measurement of the length of both alleles from a diploid sample is desired, further manipulations may be required. This is because the capture efficiency measured will actually be the average efficiency of the two alleles. To effectively achieve separate measurements for each allele, barcodes (e.g., sequence tags) can be used that allow the efficiency of individual capture events (from individual genomic loci) to be followed. FIG. 11A-C shows the approach... The allele lengths can therefore be read directly off this graph, after absolute length calibration using known standards. In some embodiments, a sequencing technique (e.g., a next-generation sequencing technique) is used to sequence part of one or more captured targets (e.g., or amplicons thereof) and the sequences are used to count the number of different barcodes that are present. Accordingly, in some embodiments, aspects of the invention relate to a highly-multiplexed qPCR reaction").

As to claim 12, Porreca discloses that prior to sequencing, a sequencing adaptor is attached to the molecular inversion probe (para [0150], "The sequencing primers must be incorporated subsequently such that they sequence both the differentiator tag sequence and the target sequence. Alternatively, the sequencing adaptors can be synthesized with the random differentiator tag sequences at their 3' end (as degenerate bases), so that only one ligation must be performed. Another method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags").

As to claim 13, Porreca discloses the method according to claim 2, wherein prior to sequencing, a barcode and a sequencing adaptor are attached to the molecular inversion probe (para [0150], "The actual incorporation of the random differentiator tag sequences can be performed through various methods known in the art. For example, nucleic acids comprising differentiator tag sequences may be incorporated by ligation. This is a flexible method, because molecules having differentiator tag sequence can be ligated to any blunt-ended nucleic acids. The sequencing primers must be incorporated subsequently such that they sequence both the differentiator tag sequence and the target sequence. Alternatively, the sequencing adaptors can be synthesized with the random differentiator tag sequences at their 3' end (as degenerate bases), so that only one ligation must be performed. Another method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags").

Claims 4 and 5 lack an inventive step under PCT Article 33(3) as being obvious over Porreca, as above, in view of an article titled "The properties and applications of single-molecule DNA sequencing" by Thompson, et al. (hereinafter "Thompson").

As to claims 4 and 5, Porreca discloses analyzing of the nucleic acid template by sequencing (para [0295], "Perform Sequencing of Library According to Manufacturer's Directions (e.g. Illumina, ABI, etc), Reading Both the Target Sequence and the Differentiator Tag Sequence"), but does not specifically disclose that sequencing is done by sequencing-by-synthesis. Thompson discloses using single molecule sequencing-by-synthesis for high throughput analysis of nucleic acids (pg 5, Fig 2 and its legend, "Overview of single-molecule sequencers. The three most advanced single-molecule sequencing systems all carry out sequencing-by-synthesis using laser excitation to generate a fluorescent signal from labeled nucleotides, which is then detected using a camera") and further discloses that sequencing-by-synthesis technique is advantageous because it avoids "the many artifacts associated with reverse transcriptase and providing unparalleled quantitative accuracy" (pg 3, col 2). It would have been obvious to one of ordinary skill in the art at the time of the invention to combine Porreca and Thompson by incorporating, in the course of routine experimentation and with a reasonable expectation of success, single molecule sequencing-by-synthesis disclosed by Thompson into the method of Porreca, to improve sensitivity and accuracy of the method of analyzing of the nucleic acid template disclosed by Porreca.

********** See the Following Supplemental Sheet to continue **********

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 14/27324

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V(2). Citations and Explanations and the Preceding Supplemental Sheet:

Claims 14 and 15 lack an inventive step under PCT Article 33(3) as being obvious over Porreca, as above, in view of US 2008/0269068 A1 to Church, et al. (hereinafter "Church").

As to claim 14, Porreca does not specifically disclose that prior to sequencing, the molecular inversion probe is attached to a solid surface. Church, in the context of a "method of analyzing an array of nucleic acid sequences" (Church, claim 1), discloses that prior to sequencing, the molecular inversion probe is attached to a solid surface (Church, claim 1, "b) providing a plurality of molecular inversion probes, each molecular inversion probe having a tag sequence, a barcode sequence, and two guide sequences; c) hybridizing the molecular inversion probes to the immobilized query oligonucleotide sequences; d) performing rolling circle amplification such that the barcode sequence of one molecular inversion probe is transferred to one immobilized query oligonucleotide sequence; e) arraying the immobilized query oligonucleotide sequences; and f) identifying barcodes present on an immobilized query oligonucleotide sequence"; claims 6, 7, "the plurality of immobilized query oligonucleotide sequences are immobilized on beads...the beads are arranged on a solid support"). It would have been obvious to one of ordinary skill in the art at the time of the invention to combine Porreca and Church by incorporating, in the course of routine experimentation and with a reasonable expectation of success, immobilization of the molecular inversion probe on a solid surface prior to sequencing, to facilitate analysis of the resulting sequencing reads.

As to claim 15, Porreca in view of Church discloses that the surface is a surface of a flow cell (Church, para [0081]-[0083], Example III, "[0083] 16 query adaptors will be ligated with all possible two nucleotide 3' overhangs, with 4 different 16-mer sequences associable with the identity of the 5'-most query base (magenta) (FIG. 7, Step 1). 160 different possible pairings of 40 EcoP15I and 4 query 16-mers, separated by 17-26 base pairs, should be present on every bead. Non-biotinylated strands will be denatured and washed away. The remaining strands will be immobilized and loaded into a flow cell (FIG. 7, Step 2). No further enzymology is necessary after this point. 40 four-color hybridization-imaging cycles will be performed to read four contiguous 10-mer tag sequences").

Claims 1-15 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 | 6082 |

21710 7590 10/29/2014

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| EXAMINER | |
|---|---|
| POHNERT, STEVEN C | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1634 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 10/29/2014 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

| Office Action Summary | Application No. 13/266,862 | Applicant(s) PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | AIA (First Inventor to File) Status No |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *9/9/2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims***

5)☒ Claim(s) *1-4,12-16,20-30,33,36-38,40-47 and 50-56* is/are pending in the application.

5a) Of the above claim(s) *12-16,20-30,33,36-38,40-47 and 50-56* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-4* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *4/8/2013* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.



Petitioner Illumina, Inc., EX1002, p. 896

## DETAILED ACTION

### *Notice of Pre-AIA or AIA Status*

1. The present application is being examined under the pre-AIA first to invent provisions.

### *Continued Examination Under 37 CFR 1.114*

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/9/2013 has been entered.

### *Claim Status and Formal matters*

This action is in response to papers filed 9/9/2013.

Claim 1 has been amended.

Claims 1-4, 12-16, 20-30, 33, 36-38, 40-47 and 50-56 are pending.

Claims 5-11, 17-19,31-32, 34-35, 39-38, 48-49 and 57-67 have been canceled.

Claims 12-16, 20-30, 33, 36-38, 40-47 and 50-56 withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention , there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 12/3/2012.

Claims 1-4 are being examined.

### *Priority*

The instant application was filed 3/13/2012 and is a national stage entry of PCT/US10/01293 filed 4/30/2010 and claims priority to provisional application 61/147,470 filed 4/30/2009; provisional application 61/147923 filed 5/1/2009; provisional application 61179358 filed 5/18/2009; and 61/182,089 filed 5/28/2009.

3. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) or the first paragraph of pre-AIA 35 U.S.C. 112, except for the best mode requirement. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994)

The disclosure of the prior-filed application, Application Nos. 61/174,470, 61/174,923, 61/179,358, 61/182, 089, fails to provide adequate support or enablement in the manner provided by 35 U.S.C. 112(a) or pre-AIA 35 U.S.C. 112, first paragraph for one or more claims of this application. Application Nos. 61/174,470, 61/174,923, 61/179,358, 61/182, 089, fail to provide support for, "wherein at least two of the plurality of molecular inventions probes are present at different concentrations. " Thus the

instant claims are being given priority to 4/30/2010. The filing date of the instant application as a PCT.

***Claim Rejections - 35 USC § 112***

4. The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

***5.*** Claims 1-4 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 1 has been amended to recite, “wherein at least two of the plurality of molecular inversion probes are present at different concentrations. “ The response asserts the amendment is supported on pages 69 line 28 to page 70 line 23 which states:

According to aspects of the invention, such biases can be detected or overcome by systematically combining different capture and/or analytical techniques in an assay that interrogates a plurality of loci in a plurality of subject samples.

Accordingly, it should be appreciated that in any of the embodiments described herein (e.g., tiling/staggering, tagging, size-detection, sensitivity enhancing algorithms,or any combination thereof), aspects of the invention involve preparing genomic nucleic acid and/or contacting them with one or more different probes (e.g., capture probes, hybridization probes, MIPs, others etc.). In some embodiments, the amount ofgenomic nucleic acid used per subject ranges from 1 ng to 10 micrograms (e.g., 500 ng to 5 micrograms). However, higher or lower amounts (e.g., less than 1 ng, more than 10 micrograms, 10-50 micrograms, 50-100 micrograms or more) may be used. In some embodiments, for each locus of interest, the amount of probe used per assay may be optimized for a particular application. In some embodiments, the ratio (molar ratio, for example measured as a concentration ratio) of probe to genome equivalent (e.g., haploid or diploid genome equivalent, for example for each allele or for both alleles of a nucleic acid target or locus of interest)ranges from 1/100, 1/10, 1/1, 10/1, 100/1, 1000/1. However, lower, higher, or intermediate ratios may be used.

In some embodiments, the amount of target nucleic acid and probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios. In some embodiments, in order to normalize genomic DNA and probe, the genomic DNA concentration is read using a standard spectrophotometer or by fluorescence (e.g., using a fluorescent intercalating dye). The probe concentration may be determined experimentally or using information specified by the probe manufacturer.

Similarly, once a locus has been captured (e.g., on a MIP or other probe or in another form), it may be amplified and/or sequenced in a reaction involving one or more primers. The amount of primer added for each reaction can range from 0.1 pmol to 1 nmol, 0.15 pmol to 1.5 nmol (for example around 1.5 pmol). However, other amounts (e.g., lower, higher, or intermediate amounts) may be used.

The specification teaches on page 3, “In some embodiments, sources of error and ambiguity in one or more steps can be addressed by capturing and/or interrogating each target locus of interest with at least one set of probes.”

Thus the specification teaches varying the concentration of probes between different loci, but does not clearly envision the use of probes of different concentration for a single loci as the active steps of the claims suggest. Thus the amendment has introduced new matter.

**Response to Arguments**

This is a new ground of rejection.

6. The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 1-4 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The Board has held, “If a claim is amenable to two or more plausible constructions, applicant is required to amend the claim to more precisely define the metes and bounds of the claimed invention or the claim is indefinite under §112, ¶2.” Ex parte Miyazaki, 89 USPQ2d 1207 (BPAI 2008) (expanded panel).

The US Supreme Court in NAUTILUS, INC. v. BIOSIG INSTRUMENTS, INC. (2014) held, “we hold that a patent is invalid for indefiniteness if its claims, read in light of the specification delineating the patent, and the prosecution history, fail to inform, with reasonable certainty, those skilled in the art about the scope of the invention.”

The preamble of the instant claim is drawn to a method of analyzing a plurality of genetic loci. The contacting step requires, “contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region that comprises a 5' terminal nucleotide and a 3' region that comprises a 3' terminal nucleotide, the 5' region and 3' region being

complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion, wherein when hybridized to the target nucleic acid in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide of each probe are separated by a plurality of nucleotides on the target nucleic acid, and wherein at least two of the plurality of molecular inversion probes are present at different concentrations." Thus all probes of contacting step of the claim and thus the rest of the active steps require the probes overlap and thus it is unclear if the intent of the claim is to require analyzing a plurality of probes to a single loci as the active steps of the claims suggest or require analyzing a plurality of loci and how this relates to the claims as they require overlapping probes and thus are target to a single loci.

Claim 1 further recites, "the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid." The claim appears to require a single target nucleic acid, but sequences to "nucleic acids flanking one of a plurality of subregions." Thus the metes and bounds of the claim are unclear if there is a single target nucleic acid to which the molecular inversion probe is being hybridized or if the intent is the 3' region and 5' region of each probe is hybridizing to a different nucleic acid. The claims should be amended to clarify the relationship between the 3' region and 5' region of the probes as to whether they are hybridizing a single target polynucleotide or different nucleic acids.

Thus claims 2-4 are rejected as they depend from claim 1.

Claim 2 requires, “wherein at least one subregion entirely overlaps at least one other subregion.” Claim 1 requires, “the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion,.” Thus it is unclear how one subregion can entirely overlap as required by claim 2 and yet be different as required in claim 1. Thus the metes and bounds of the claim are unclear.

**Response to Arguments**

This is a new ground of rejection.

***Claim Rejections - 35 USC § 103***

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhras et al (PLOS one (2007) volume 2 (9) e915) (IDS 3/28/2013), Nilsson et al (Trends in Biotechnology (2006 ) volume 24, pages 83-88), Hardenbohl et al (Nature Biotechnology(2003) volume 21, pages 673-678), Mockler et al (Genomics (2005) volume 85, pages 1-15), Porecca et al ( Nature methods (2007) volume 4, pages 931-936), Hardenbohl et al (USPGPUB 2006/0019304) and Browne et al (US PGPUB 20060246500).

MPEP 2111 states:

The court held that the PTO is not required, in the course of prosecution, to interpret claims in applications in the same manner as a court would interpret claims in an infringement suit. Rather, the “PTO applies to verbiage of the proposed claims the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in applicant’s specification.”

MPEP 2111.01 states

Though understanding the claim language may be aided by explanations contained in the written description, it is important not to import into a claim limitations that are not part of the claim.

It is noted that the specification lacks a limiting definition of a molecular inversion probe. Thus "molecular inversion probe" is being given the broadest reasonable interpretation in view of the claims and art as a probe that allows for formation of a circular probe upon hybridization to a target nucleic acid.

It is further noted the 112-2$^{nd}$ issues of claim 3 are considered in rendering this rejection.

Akhras teaches a connector inversion probe method (CIPer) that is a combination of Gap-LCR and padlock probes. Akhras teaches, “The Connector Inversion Probe (CIPer) reaction (Figure 1) shares some basic design features with a recently described assay, the PathogenMip assay [17], which includes: i) probe hybridization, single “G” base gap fill and probe circularization, ii) exonuclease based circular DNA selection, iii) probe inversion by re-linearization, and iv) universal PCR amplification. The distinguishing and key feature of the CIPer method is a modified first reaction step involving a complete filling of an extensive gap formed by the probe and target.” Akhaus further teaches, " Connector Inversion Probe (CIPer) technology is a

powerful method for multiplex amplification, and has been validated here in genetic pathogen diagnostics, typing, and antibiotic resistance detection arenas as a proof-of-concept. This technology can be designed and optimized for use in numerous other scientific areas and applications. Other areas suitable for use of the CIPer method include cancer genomics [32], human SNP detection [15], and selective exon amplification [7], among numerous other promising applications. Modified CIPers may also in the future offer an alternative strategy for gateway cloning [33]. In this paper, we focused on “lowplex” systems amplifying shorter stretches of DNA of up to 100 base pair lengths. To achieve higher degrees of multiplexing and longer clone reads, one must take into account i) clone GC content, ii) clone length variability, and iii) steric effects for longer clones. Analog amplification and downstream validation are delimiting factors for multiplex detection methods. We believe that the future of CIPer technology will rely on digital amplification [34] and high throughput sequencing validation (Figure S5). The CIPers universal primer sites can be formatted to fit any amplification method, e.g. RCA [12], [13], emulsion PCR [34], SPIA [35] LAMP [36] and SMAP 2 [37]; universal adaptor ligation, a tedious and sample-consuming procedure [38], is therefore optional. Proposed digital amplification methods include emulsion PCR, RCA, or a combination of the two [39]. Suitable upcoming high throughput validation methods include next-generation sequence systems such as the 454 Life Sciences platform [38] (http://www.454.com), the Solexa platform [40] (http://www.illumina.com), polony sequencing [41] (http://www.agentcourt.com), Helicos Biosciences [42] (http://www.helicosbio.com), or use of a resequencing array (GeneChip CustomSeq,

www.affymetrix.com). Recent digital quantification methods [43] open the possibility for quantitative CIPer technology. With the addition of a molecular barcode to the padlocks [14], MIPs were used for ultra high-throughput SNP detection based on microarray barcode hybridization [15]. Because CIPers can also include a barcode, they could be used for microarray validation, or amplicon tracing with sequencing methods [17], [44] allowing for longer read lengths [38]. With the introduction of a third universal sequence flanking the barcode and the universal primer sites, one can begin to envision a two-dimension informational barcode, a concept we call “multiplexing multiplex padlocks” (MMP) (Figure S6). Probe construction based on ligation of three smaller constructs [45] would allow for variability in fragment assembly. By combining the one-genotype detection pair of AS/ES constructs with multiple unique barcodes, we can create patient and genotype specific probes with one barcode per probe. This will allow for analysis of multiple parallel samples, essentially lowering the cost of microarray chips and sequencing reactions.”

Nilsson teaches the use of padlock probes and “selector” probes that form circles with gaps of several nucleotides between the 5’ terminal nucleotide and the 3’ terminal nucleotide.

Akhras and Nilsson demonstrate probes were known that result in a circular probe upon hybridization to a target sequence with a gap between the 3' terminal nucleotide and 5' terminal nucleotide were known. Akhras suggests the use of CIPer methods similar to the use of MIPs, including the use of “barcodes to allow detection by microarrays.”

Akhras and Nilsson do not specifically teach, “contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion.”

However, Hardenbol discloses a method of analyzing a plurality of genetic loci by contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1). Hardenbohl teaches these probes are MIP or padlock probes.

Hardenbohl teaches in figure 2b the joining of the molecular inversion probes which results in a circular type configuration. Hardenbohl exemplifies in fig 2b a probe that has a 5’ region and a 5’ terminal nucleotide, a 3’ region and a 3’ terminal

nucleotide, and a single base gap. Thus the 5' terminal nucleotide, 3' terminal nucleotide and single base gap are a plurality of nucleotides between the 3' and 5' region of the probe.

Hardenbohl does not specifically teach wherein each subregion overlaps with at least one other subregion. Hardenbohl does not specifically teach, "wherein at least one subregion entirely overlaps at least one subregion."

Mockler, teaches each subregion overlaps with at least one other subregion (Fig. 2a).

With regards to claim 2, Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other Subregion)).

With regards to claim 3, Mockler teaches the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

With regards to claim 4, Mockler teaches sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the CIPer or selector probes taught by Akhras and Nilsson with a gap of a plurality of nucleotides between the 5' terminal

nucleotide and 3' terminal of the padlock probes with tiling probes of Mockler in the method of Hardenbol. The artisan would be motivated as Akhras suggests the use of CIPer methods in MIP methods. The teachings of Hardenbohl set forth one embodiment of the use of MIP or padlock probes. The artisan further would be motivated as Mockler teaches the probe tiling approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1). The artisan would have a reasonable expectation of success as the artisan is merely increasing the number of nucleotides between the 3' terminal nucleotide and 5' terminal nucleotide of the probes of Mockler, which Akhras demonstrates were known and worked. Thus the combination of references suggests all the limitations of claims and suggests the use of CIPer in place of the similar MIP methods.

Akhras, Nilsson, Hardenbohl, and Mockler do not specifically teach the use of probes at two or more cencentrations.

However, Porecca teaches a method of amplifying human exons using MIPS. Porecca teaches, "It is both concerning and interesting that the resequencing data show skewing of base-calls at heterozygous positions away from the expected ratio of 0.5 (Fig. 4). This skewing might be expected if stochastic events (for example, a finite number of capture events during themultiplex capture reaction) are a primary source of bias. Given that the concentration of individual targeting oligos in the capture reaction is very low (B0.36 pM per species), it is likely that their hybridization to genomic DNA is inefficient. This problem might therefore be mitigated simply by increasing the concentration of reactants. If either the targeting oligos or genomic DNA can be defined

as limiting, one could potentially hybridize with an excess of the complement, that is, to saturation, such that small differences in capture efficiency would be 'normalized' away." Porecca further teaches, "After characterizing the efficiency of individual capture oligos in each reaction, a second iteration could be carried out, in which capture oligos performing similarly to one another are grouped together within the same oligo mixture (thereby maximizing uniformity within any single reaction)."

Further, Hardenbohl(PGPUB) teaches, "Genomic material is complex and thus individual sequences exist at relatively low concentrations in a mixture. As a result of this, at a given probe concentration there is a finite rate at which the probe sequences will hybridize to the genomic templates. Probe concentrations can be adjusted to some extent to vary this rate, but as probe complexity is increased, for example, when highly multiplexed assays are used, such flexibility is greatly reduced" (003). Hardenbohl continues, "Multiplexing occurs at two levels in the invention. First, hybridization-based assays are designed to measure characteristics of one or more target polynucleotides, such as genomic fragments, at multiple loci. The number of loci measured in each hybridization-based assay can vary widely, and its upper limit depends on well-known factors, including the capacity of the readout device being employed, trade-offs selected between probe concentrations and reaction times, the genetic characteristic being measured (for example, trait association studies require very large numbers of measurements; genetic identification requires relative few measurements; testing for adverse drug reactions may require a medium number of measurements, e.g. several tens to hundreds), and the like. In one aspect, the number of loci analyzed in each

hybridization-based assay is in the range of from a few tens, e.g. 10 to 20, to many thousands, e.g. fifty to a hundred thousand. In another aspect, the number of loci per hybridization-based assay is in the range of from 10 to 40,000; or it is in the range of from 100 to 30,000; or it is in the range of from 100 to 20,000. Second, multiplexing occurs from the combination of assay products from multiple hybridization-based assays for determining multiple signals on a single readout device. Usually, there is a one-to-one correspondence between the number of hybridization-based assay products combined in accordance with the invention and the number of different genomic samples being analyzed; however, the invention comprehends situations where different numbers of samples from different individuals are analyzed by varying numbers of hybridization-based assays, the products of which are combined. The level of such multiplexing depends of the capacity of the readout device and the number of loci measured by each hybridization-based assay. In one aspect, the number of hybridization-based assays that may be multiplexed is simply the capacity of a readout device, e.g. the number of features on a microarray, divided by the number of loci being measured in the hybridization-based assays. For example, in one aspect, a 100 thousand-feature microarray can serve as a readout device for fifty hybridization based assays that each measure the zygosity of one thousand loci, when two features are required for each determination (e.g. homozygous in allele 1, homozygous in allele 2, or heterozygous in allele 1 and 2). As discussed more fully below, particular trade-offs between the use of microarray features, different colored fluorescent dyes, number of

loci measured per hybridization-based assay, capacity of a readout device, and the like, are routine design choices for those of ordinary skill in the art."

Hardenbohl (PGPUB) continues, "Operation of one embodiment of the invention is illustrated in FIGS. 1A and 1B for a particular hybridization-based assay (one based on the template-driven ligation of two oligonucleotide components). Hybridization-based assays are carried out separately on a sample of DNA from genome G.sub.1 and a sample of DNA from genome G.sub.2. Probes p.sub.1 to p.sub.K (100) containing oligonucleotide tags t.sub.1 to t.sub.K, respectively, and probes p.sub.1 to p.sub.K (102) containing oligonucleotide tags t.sub.K+1 to t.sub.2K, respectively, are combined in separate reaction mixtures with genome G.sub.1 DNA and genome G.sub.2 DNA, respectively, under conditions that permit specific hybridization of such probes to their respective DNA stands (104) and (106) containing target loci, L.sub.1 through L.sub.K ((108) to (110) for genome G.sub.1 and (112) to (114) for genome G.sub.2). After an incubation time depending on parameters well-known to those of ordinary skill, e.g. target complexity, probe concentration, temperature, and the like, discussed more fully below, probe-genome complexes form ((118) and (116), respectively) that include recognition structures for enzyme(s) of subsequent step(s)."

Finally, Browne demonstrates the use of dual inversion probes at different concentrations in figure 6.

As stated in the MPEP, 2144.05 II, "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges

by routine experimentation.” In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)."

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of Akhras, Nilsson, Hardenbohl, and Mockler to include at probes at different concentrations. The artisan would be motivated as Porecca, Hardenbohl(PGPUB), and Brown recognize that probe concentration is a variable in the speed and performance of hybridization assays including molecular inversion probe assays. Thus it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to include additional probes at different concentrations so as to test for efficiency of hybridization as well as speed of hybridization to optimize the reaction. The artisan would have a reasonable expectation of success as the artisan is merely using known methods to optimize conditions.

**Response to Arguments**

The response traverses the instant rejection asserting the amendment has overcome the issues of the claim. This argument is not persuasive in view of the teachings of Porecca, Hardenbohl(PGPUB), and Brown.

**Summary**

No claims are allowed.

The following prior art is made of record but not relied upon herein, however it is considered pertinent to applicants' disclosure: Thyiyagarajan et al (BMC Bioinformatics (2006) volume 7, 700)

***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:00-5:00, every second Thursday and Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/STEVEN POHNERT/
Primary Examiner, Art Unit 1634

| Notice of References Cited | Application/Control No. 13/266,862 | Applicant(s)/Patent Under Reexamination PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | Classification |
|---|---|---|---|---|---|
| * | A | US-2006/0019304 | 01-2006 | Hardenbol et al. | 435/006 |
| * | B | US-2006/0246500 | 11-2006 | Browne, Kenneth A. | 435/006 |
| | C | US- | | | |
| | D | US- | | | |
| | E | US- | | | |
| | F | US- | | | |
| | G | US- | | | |
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|---|---|---|
| | U | Thyiyagarajan et al (BMC Bioinformatics (2006) volume 7, 700) |
| | V | Porecca et al ( Nature methods (2007) volume 4, pages 931-936) |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.



Petitioner Illumina, Inc., EX1002, p. 915

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

**OR**

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| | | | |
|---|---|---|---|
| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2014-05-27 |
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
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| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2014-07-01 |
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| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

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| /S.P./ | 3 | Husemann, P. & Stoye, 2009, Phylogenetic Comparative Assembly, Algorithms in Bioinformatics: 9th International Workshop, pp. 145-156, Salzberg, S., and Warnow, T., Eds. Springer-Verlag, Berlin Heidelberg. | ☐ |
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Petitioner Illumina, Inc., EX1002, p. 965

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| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

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|---|---|---|---|
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| Examiner Signature | /Steven Pohnert/ (10/23/2014) | Date Considered | |
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|---|---|
| **EFS ID:** | 20959512 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Catherine Heath |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 15-DEC-2014 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 15:54:24 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| Submitted with Payment | no |
|---|---|

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Power of Attorney | GSGE002012925820POA.pdf | 215515<br>87ccab44ba9189c32340fc873d6b6ebbbadd9666 | no | 1 |

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| 2 | Assignee showing of ownership per 37 CFR 3.73. | GSGE0020129258 20Statement.pdf | 18721<br>0918664d54a373863a3d50a9cd81802caf729735 | no | 1 |

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| **Total Files Size (in bytes):** | 234236 |

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# POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number: 21710

**OR**

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

| Name | Registration Number | Name | Registration Number |
|---|---|---|---|
| | | | |
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| | | | |
| | | | |

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☒ The address associated with Customer Number: 21710

**OR**

| | | | | |
|---|---|---|---|---|
| ☐ | Firm or Individual Name | | | |
| | Address | | | |
| | City | | State | Zip |
| | Country | | | |
| | Telephone | | Email | |

Assignee Name and Address: Good Start Genetics, Inc.
237 Putnam Avenue
Cambridge, MA 02139

**A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.**

**SIGNATURE of Assignee of Record**

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

| | | | |
|---|---|---|---|
| Signature | Gregory J. Porreca | Date | 12-4-2013 |
| Name | Gregory J. Porreca | Telephone | 617-714-0855 |
| Title | Vice President of Research and Techology | | |

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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# STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Gregory Porreca et al.

Application No./Patent No.: 13/266,862 Filed/Issue Date: March 13, 2012

Titled: METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

Good Start Genetics, Inc. , a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. [X] the assignee of the entire right, title, and interest in;

2. [ ] an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or

3. [ ] the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. [X] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 027860 , Frame 0025 , or for which a copy therefore is attached.

**OR**

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

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[ ] Additional documents in the chain of title are listed on a supplemental sheet(s).

[X] As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.,* a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

| /Thomas C. Meyers/ | December 15, 2014 |
|---|---|
| Signature | Date |
| Thomas C. Meyers | 36989 |
| Printed or Typed Name | Title or Registration No. |

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| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|---|---|---|---|
| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 |

**CONFIRMATION NO. 6082**

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

**POA ACCEPTANCE LETTER**

*OC000000072492927*

Date Mailed: 12/22/2014

## NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 12/15/2014.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/ttkim/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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|---|---|---|---|
| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 |

**CONFIRMATION NO. 6082**

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

**POWER OF ATTORNEY NOTICE**

*OC000000072492920*

Date Mailed: 12/22/2014

## NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 12/15/2014.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

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Petitioner Illumina, Inc., EX1002, p. 977

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 21165381 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers |
| **Filer Authorized By:** | |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 09-JAN-2015 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 11:16:30 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

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## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Non Patent Literature | Thiyagarajan-2006-BMC.PDF | 1617312<br>574bd7353aed83d637acb0a578c2f37c8f46c6ec | no | 11 |

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| 2 | Information Disclosure Statement (IDS) Form (SB08) | Information_Disclosure_Statement_Fillable_PDF.PDF | 612459<br>34a934128eb33c9d0ad468ec14fd2977cddd8d11 | no | 4 |
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| **Information:** | | | | | |
| 3 | Non Patent Literature | ISRWO-PCT-US14-28212.PDF | 641193<br>7c43e60ddccee0f2e195384c89ec1a3f88dc92c4 | no | 11 |
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| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( **Not for submission under 37 CFR 1.99)** | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US |

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☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

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**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2015-01-09 |
|---|---|---|---|
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

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| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US |

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| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US |

| | | | |
|---|---|---|---|
| | 1 | International Search Report and Written Opinion mailed December 9, 2014, for International Patent Application No. PCT/US14/28212, filed March 14, 2014 (17 pages) | ☐ |
| | 2 | Thiyagarajan, 2006, PathogenMIPer: a tool for the design of molecular inversion probes to detect multiple pathogens, BMC Bioinformatics, 7:500 (10 pages). | ☐ |

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## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference GSGE-002/03WO 29258/77 | FOR FURTHER ACTION | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| International application No. PCT/US14/28212 | International filing date *(day/month/year)* 14 March 2014 (14.03.2014) | (Earliest) Priority Date *(day/month/year)* 15 March 2013 (15.03.2013) |
| Applicant GOOD START GENETICS, INC. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☐ as suggested by the applicant.

☒ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US14/28212

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)
   - ☐ on paper
   - ☐ in electronic form

   b. (time)
   - ☒ in the international application as filed
   - ☒ together with the international application in electronic form
   - ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US14/28212

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Groups I+: Claims 1-9, SEQ ID NOs: 185 (synthetic probe nucleic acid sequence), 186 (synthetic probe nucleic acid sequence)
Exemplary Election: SEQ ID NO: 187 (synthetic probe nucleic acid sequence)

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

Groups I+: Claims 1, 2 (in-part), 3-9, SEQ ID NOs: 185, 186

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/28212

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, 1/02; G01N 33/50
CPC - C12Q 1/6827, 1/6869, 2600/158
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/68, 1/02; A61K 45/00, G01N 37/00, 33/50, 33/15, 33/53, 33/58; C12N 15/01, 15/09; C07H 21/04; A61P 17/16 (2014.01)
CPC: C12Q 1/6827, 1/6869, 2600/158

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; ScienceDirect; ProQuest; target, 'nucleic acid,' screen, probe, plurality, complementary, hybridize, target, arm, region, inversion, coding, 'non-coding'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y<br>---<br>A | US 2012/0165202 A1 (PORRECA, G et al.); June 28, 2012; abstract; paragraphs [0014], [0015], [0020], [0021], [0042], [0104], [0105], [0113], [0212], [0253]; Table 1 | 1, 3, 5-8<br>-----<br>4, 9<br>-----<br>2 |
| Y | THIYAGARAJAN, S et al. PathogenMIPer: A Tool For The Design Of Molecular Inversion Probes To Detect Multiple Pathogens. 14 November 2006; page 2, page 3, figure 1. | 4 |
| Y | US 2007/0162983 A1 (HERSTERKAMP, T et al.); July 12, 2007; paragrpah [0013] | 9 |
| A | WO 2010/126614 A2 (PORRECA, G et al.); November 4, 2010; SEQ ID NOs: 297, 458 | 2 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 21 November 2014 (21.11.2014) | 09 DEC 2014 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Shane Thomas<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US14/28212

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Claims 1-9 are directed toward a method of analyzing a target nucleic acid.

The method of analyzing a target nucleic acid will be searched to the extent that the combination of molecular inversion probes encompasses SEQ ID NOs: 185 (synthetic probe nucleic acid sequence) and 186 (synthetic probe nucleic acid sequence). It is believed that Claims 1, 2 (in-part) and 3-9 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 185 (synhetic probe nucleic acid sequence) and 186 (synthetic probe nucleic acid sequence). Applicant is invited to elect additional molecular inversion probe sequence(s) to be searched. Additional molecular inversion probe sequences will searched upon the payment of additional fees. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: SEQ ID NO: 187 (synthetic probe nucleic acid sequence).

Groups I+ share the technical features including: a method of analyzing a target nucleic acid, the method comprising: introducing a plurality of molecular inversion probes to a sample derived from a human and suspected of containing a target nucleic acid, wherein each probe is complementary to nucleotides flanking a sub-region of the target nucleic acid and comprises a first targeting arm and a second targeting arm, and wherein each sub-region is different and overlapping with at least one other sub-region; capturing a plurality of the sub-regions of the target nucleic acid with at least a portion of the molecular inversion probes, wherein the plurality of sub-regions of the target nucleic acid are from a combination of genes comprising ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8), aspartoacylase (ASPA), branched chain keto acid dehydrogenase EI, alpha polypeptide (BCKDHA), branched chain keto acid dehydrogenase EI, beta polypeptide (BCKDHB), Bloom Syndrome, RecQ Helicase-Like (BLM), cystic fibrosis transmembrane conductance regulator (CFTR), clarin 1 (CLRN1), dihydrolipoamide dehydrogenase (DLD), Fanconi anemia, complementation group C (FANCC), glucose-6-phosphatase, catalytic subunit (G6PC), hexosaminidase A alpha polypeptide (HEXA), inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP), mucolipin 1 (MCOLN1), protocadherin-related 15 (PCDH15), and sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1); and analyzing the captured sub-regions for one or more genetic disorders, thereby analyzing the target nucleic acid.

However, these shared technical features are previously disclosed by US 2012/0165202 A1 to Porreca, et al. (hereinafter 'Porreca'). Porreca discloses a method of analyzing a target nucleic acid (a method of analyzing genetic loci (a target nucleic acid); abstract), the method comprising: introducing a plurality of molecular inversion probes to a sample (contacting a plurality of target nucleic acids from a patient sample with a set of molecular inversion probes; paragraphs [0014], [0020], [0021]) derived from a human (paragraph [0015]) and suspected of containing a target nucleic acid (a target region suspected of containing an insertion or deletion; paragraph [0042]), wherein each probe is complementary to nucleotides flanking a sub-region of the target nucleic acid (paragraph [0021]) and comprises a first targeting arm (comprises a 5' region (a first targeting arm); paragraph [0021]) and a second targeting arm (and a 3' region (a second targeting arm); paragraph [0021]), and wherein each sub-region is different and overlapping with at least one other sub-region (paragraph [0021]); capturing a plurality of the sub-regions of the target nucleic acid (capturing a pool of target nucleic acids (capturing a plurality of the sub-regions of the target nucleic acid); paragraph [0020]) with at least a portion of the molecular inversion probes (paragraph [0020]), wherein the plurality of sub-regions of the target nucleic acid are from a combination of genes (wherein a plurality of genetic loci from a combination of genes are captured in parallel; paragraph [0105]) comprising ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8) (ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8); Table 1), aspartoacylase (ASPA) (aspartoacylase (ASPA); Table 1), branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA) (branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA); Table 1), branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB) (branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB); Table 1), Bloom Syndrome, RecQ Helicase-Like (BLM) (Bloom Syndrome, RecQ Helicase-Like (BLM); Table 1), cystic fibrosis transmembrane conductance regulator (CFTR) (cystic fibrosis transmembrane conductance regulator (CFTR); Table 1), clarin 1 (CLRN1), dihydrolipoamide dehydrogenase (DLD) (dihydrolipoamide dehydrogenase (DLD); Table 1), Fanconi anemia, complementation group C (FANCC)(Fanconi anemia, complementation group C (FANCC); Table 1), glucose-6-phosphatase, catalytic subunit (G6PC) (glucose-6-phosphatase, catalytic subunit (G6PC); Table 1), hexosaminidase A alpha polypeptide (HEXA) (hexosaminidase A alpha polypeptide (HEXA); Table 1), inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP) (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP); Table 1), mucolipin 1 (MCOLN1) (mucolipin 1 (MCOLN1); Table 1), protocadherin-related 15 (PCDH15) (protocadherin-related 15 (PCDH15); Table 1), and sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1) (sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1); Table 1); and analyzing the captured sub-regions for one or more genetic disorders (paragraphs [0014], [0104], [0105]), thereby analyzing the target nucleic acid (paragraphs [0020], [0021], [0105]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Porreca reference, unity of invention is lacking.

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Thomas C. Meyers
Brown Rudnick LLP
One Financial Center
Boston, MA 02111
United States of America

# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 09 DEC 2014 |
| Applicant's or agent's file reference<br>GSGE-002/03WO 29258/77 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US14/28212 | 14 March 2014 (14.03.2014) | 15 March 2013 (15.03.2013) |

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68, 1/02; G01N 33/50 (2014.01)
CPC - C12Q 1/6827, 1/6869, 2600/158

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer: |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | 21 November 2014 (21.11.2014) | Shane Thomas<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US14/28212

**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:
   - ☒ the international application in the language in which it was filed.
   - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)
   - ☐ on paper
   - ☐ in electronic form

   b. (time)
   - ☒ in the international application as filed
   - ☒ together with the international application in electronic form
   - ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US14/28212

**Box No. IV Lack of unity of invention**

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

-***-Please See Supplemental Page-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. Claims 1, 2 (in-part), 3-9, SEQ ID NOs: 185, 186

Form PCT/ISA/237 (Box No. IV) (July 2011)

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US14/28212

**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 2, 4, 9 | YES |
| | Claims | 1, 3, 5-8 | NO |
| Inventive step (IS) | Claims | 2 | YES |
| | Claims | 1, 3-9 | NO |
| Industrial applicability (IA) | Claims | 1-9 | YES |
| | Claims | NONE | NO |

2. Citations and explanations:

Claims 1, 3 and 5-8 lack novelty under PCT Article 33(2) as being anticipated by US 2012/0165202 A1 to Porreca, et al. (hereinafter 'Porreca').

As per claim 1, Porreca discloses a method of analyzing a target nucleic acid (a method of analyzing genetic loci (a target nucleic acid); abstract) for carrier screening (the analysis of the nucleic acid may indicate the subject is a carrier of a disease or condition; paragraph [0047]), the method comprising: introducing a plurality of molecular inversion probes to a sample (contacting a plurality of target nucleic acids from a patient sample with a set of molecular inversion probes; paragraphs [0014], [0020], [0021]) derived from a human (samples may include tumor tissue for a human subject; paragraph [0015]) and suspected of containing a target nucleic acid (a target region suspected of containing an insertion or deletion; paragraph [0042]), wherein each probe is complementary to nucleotides flanking a sub-region of the target nucleic acid (target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of a target nucleic acid; paragraph [0021]) and comprises a first targeting arm (comprises a 5' region (a first targeting arm); paragraphs [0021], [0113]) and a second targeting arm (and a 3' region (a second targeting arm); paragraphs [0021], [0113]), and wherein each sub-region is different and overlapping with at least one other sub-region (each subregion overlaps with at least one other subregion; paragraph [0021]); capturing a plurality of the sub-regions of the target nucleic acid (capturing a pool of target nucleic acids (capturing a plurality of the sub-regions of the target nucleic acid); paragraph [0020]) with at least a portion of the molecular inversion probes (methods for capturing target nucleic acids involve the use of multiple sets of probes; paragraph [0020]), wherein the plurality of sub-regions of the target nucleic acid are from a combination of genes (wherein a plurality of genetic loci from a combination of genes are captured in parallel; paragraph [0105]) comprising ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8) (ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8); Table 1), aspartoacylase (ASPA) (aspartoacylase (ASPA); Table 1), branched chain keto acid dehydrogenase EI, alpha polypeptide (BCKDHA) (branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA); Table 1), branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB) (branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB); Table 1), Bloom Syndrome, RecQ Helicase-Like (BLM) (Bloom Syndrome, RecQ Helicase-Like (BLM); Table 1), cystic fibrosis transmembrane conductance regulator (CFTR) (cystic fibrosis transmembrane conductance regulator (CFTR); Table 1), clarin 1 (CLRN1), dihydrolipoamide dehydrogenase (DLD) (dihydrolipoamide dehydrogenase (DLD); Table 1), Fanconi anemia, complementation group C (FANCC) (Fanconi anemia, complementation group C (FANCC); Table 1), glucose-6-phosphatase, catalytic subunit (G6PC) (glucose-6-phosphatase, catalytic subunit (G6PC); Table 1), hexosaminidase A alpha polypeptide (HEXA) (hexosaminidase A alpha polypeptide (HEXA); Table 1), inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP) (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP); Table 1), mucolipin 1 (MCOLN1) (mucolipin 1 (MCOLN1); Table 1), protocadherin-related 15 (PCDH15) (protocadherin-related 15 (PCDH15); Table 1), and sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1) (sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1); Table 1); and analyzing the captured sub-regions for one or more genetic disorders (the invention allows for assessing multiple heritable (genetic) disorders in parallel; paragraphs [0014], [0104], [0105]), thereby analyzing the target nucleic acid (the disclosure provides methods that reduce variability in the detection of target nucleic acids for diagnostic purposes (analyzing); paragraphs [0020], [0021], [0105]).

As per claim 3, Porreca discloses the method of claim 1, and Porreca further discloses wherein each of the plurality of the molecular inversion probes includes a central region flanked by the first targeting arm and the second targeting arm (in the plurality of different inversion probes each probe contains a central region flanked by a 5' region (first targeting arm) and a 3' region (second targeting arm) that are complementary to one of a plurality of different subregions of the target nucleic acids; paragraphs [0021], [0113]).

As per claim 5, Porreca discloses the method of claim 3, and Porreca further discloses wherein the central region is the same for each of the plurality of the molecular inversion probes (targeting arm sequences will be different in each capture probe, while constant region (central region) will be the same for all probes in the set; paragraph [0212]).

-***-Continued on Next Supplemental Page-***-

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US14/28212

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. IV: Lack of Unity of Invention:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Claims 1-9 are directed toward a method of analyzing a target nucleic acid.

The method of analyzing a target nucleic acid will be searched to the extent that the combination of molecular inversion probes encompasses SEQ ID NOs: 185 (synthetic probe nucleic acid sequence) and 186 (synthetic probe nucleic acid sequence). It is believed that Claims 1, 2 (in-part) and 3-9 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 185 (synthetic probe nucleic acid sequence) and 186 (synthetic probe nucleic acid sequence). Applicant is invited to elect additional molecular inversion probe sequence(s) to be searched. Additional molecular inversion probe sequences will searched upon the payment of additional fees. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: SEQ ID NO: 187 (synthetic probe nucleic acid sequence).

Groups I+ share the technical features including: a method of analyzing a target nucleic acid, the method comprising: introducing a plurality of molecular inversion probes to a sample derived from a human and suspected of containing a target nucleic acid, wherein each probe is complementary to nucleotides flanking a sub-region of the target nucleic acid and comprises a first targeting arm and a second targeting arm, and wherein each sub-region is different and overlapping with at least one other sub-region; capturing a plurality of the sub-regions of the target nucleic acid with at least a portion of the molecular inversion probes, wherein the plurality of sub-regions of the target nucleic acid are from a combination of genes comprising ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8), aspartoacylase (ASPA), branched chain keto acid dehydrogenase EI, alpha polypeptide (BCKDHA), branched chain keto acid dehydrogenase EI, beta polypeptide (BCKDHB), Bloom.Syndrome, RecQ Helicase-Like (BLM), cystic fibrosis transmembrane conductance regulator (CFTR), clarin 1 (CLRN1), dihydrolipoamide dehydrogenase (DLD), Fanconi anemia, complementation group C (FANCC), glucose-6-phosphatase, catalytic subunit (G6PC), hexosaminidase A alpha polypeptide (HEXA), inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP), mucolipin 1 (MCOLN1), protocadherin-related 15 (PCDH15), and sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1); and analyzing the captured sub-regions for one or more genetic disorders, thereby analyzing the target nucleic acid.

However, these shared technical features are previously disclosed by US 2012/0165202 A1 to Porreca, et al. (hereinafter 'Porreca'). Porreca discloses a method of analyzing a target nucleic acid (a method of analyzing genetic loci (a target nucleic acid); abstract), the method comprising: introducing a plurality of molecular inversion probes to a sample (contacting a plurality of target nucleic acids from a patient sample with a set of molecular inversion probes; paragraphs [0014], [0020], [0021]) derived from a human (paragraph [0015]) and suspected of containing a target nucleic acid (a target region suspected of containing an insertion or deletion; paragraph [0042]), wherein each probe is complementary to nucleotides flanking a sub-region of the target nucleic acid (paragraph [0021]) and comprises a first targeting arm (comprises a 5' region (a first targeting arm); paragraph [0021]) and a second targeting arm (and a 3' region (a second targeting arm); paragraph [0021]), and wherein each sub-region is different and overlapping with at least one other sub-region (paragraph [0021]); capturing a plurality of the sub-regions of the target nucleic acid (capturing a pool of target nucleic acids (capturing a plurality of the sub-regions of the target nucleic acid); paragraph [0020]) with at least a portion of the molecular inversion probes (paragraph [0020]), wherein the plurality of sub-regions of the target nucleic acid are from a combination of genes (wherein a plurality of genetic loci from a combination of genes are captured in parallel; paragraph [0105]) comprising ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8) (ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8); Table 1), aspartoacylase (ASPA) (aspartoacylase (ASPA); Table 1), branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA) (branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA); Table 1), branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB) (branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB); Table 1), Bloom Syndrome, RecQ Helicase-Like (BLM) (Bloom Syndrome, RecQ Helicase-Like (BLM); Table 1), cystic fibrosis transmembrane conductance regulator (CFTR) (cystic fibrosis transmembrane conductance regulator (CFTR); Table 1), clarin 1 (CLRN1), dihydrolipoamide dehydrogenase (DLD) (dihydrolipoamide dehydrogenase (DLD); Table 1), Fanconi anemia, complementation group C (FANCC)(Fanconi anemia, complementation group C (FANCC); Table 1), glucose-6-phosphatase, catalytic subunit (G6PC) (glucose-6-phosphatase, catalytic subunit (G6PC); Table 1), hexosaminidase A alpha polypeptide (HEXA) (hexosaminidase A alpha polypeptide (HEXA); Table 1), inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP) (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP); Table 1), mucolipin 1 (MCOLN1) (mucolipin 1 (MCOLN1); Table 1), protocadherin-related 15 (PCDH15) (protocadherin-related 15 (PCDH15); Table 1), and sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1) (sphingomyelin phosphodiesterase 1, acid lysosomal (SMPD1); Table 1); and analyzing the captured sub-regions for one or more genetic disorders (paragraphs [0014], [0104], [0105]), thereby analyzing the target nucleic acid (paragraphs [0020], [0021], [0105]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Porreca reference, unity of invention is lacking.

**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US14/28212

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V:
-Continued from Citations and Explanations:

As per claim 6, Porreca discloses the method of claim 1, and Porreca further discloses wherein the capturing step comprises: hybridizing at least a portion of the molecular inversion probes to the plurality of sub regions (the plurality of probes are allowed to hybridize to the genomic target; paragraphs [0020], [0028]); converting the hybridized probes into circularized probes containing a copy of the sub regions (the isolated nucleic acids are products of a circularization preparative method after binding and amplification of the target nucleic acids; paragraphs [0027], [0028]); and amplifying the circularized probe/sub-region products (the nucleic acids are amplified using oligonucleotide primers, or other methods; paragraphs [0029]).

As per claim 7, Porreca discloses the method of claim 6, and Porreca further discloses wherein the amplification step requires a single set of primers (one oligonucleotide primer may be used in amplification; paragraph [0029]).

As per claim 8, Porreca discloses the method of claim 1, and Porreca further discloses wherein the one or more genetic disorders (the methods may be used to diagnose heritable disorders (genetic disorders); paragraph [0104]) comprise the combination of Familial hyperinsulinism, Canavan disease (paragraph [0104]), Maple Syrup Urine disease (paragraph [0104]), Bloom syndrome (paragraph [0104]), Cystic fibrosis (paragraph [0104]), Dihydrolipoamide dehydrogenase deficiency, Fanconi anemia (paragraph [0104]), Glycogen Storage disease(paragraph [0104]), Tay-Sachs diseases (paragraph [0104]), Familial dysautonomia (paragraph [0104]), Mucolipidosis (paragraph [0104]), Usher syndrome (paragraph [0104]), and Neimann-Pick disease.

Claim 9 lacks an inventive step under PCT Article 33(3) as being obvious over Porreca in view of US 2007/0162983 A1 to Hersterkamp, et al. (hereinafter 'Hesterkamp').

As per claim 9, Porreca discloses the method of claim 1, and Porreca further discloses the plurality of sub-regions comprising regions of the target nucleic acid (target nucleic acids with a probe set, wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of a target nucleic acid; paragraph [0021]). Porreca does not disclose wherein the plurality of sub-regions comprises coding and non-coding regions of the target nucleic acid. Hesterkamp discloses wherein the plurality of sub-regions comprises coding and non-coding regions of the target nucleic acid (the probes of the invention may be 10 base pairs (non-coding) up to the entire coding sequence of a target nucleic acid (coding region); paragraph [0013]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified method of analyzing a target nucleic acid, in order to have allowed the sub-regions of the probes to comprise both coding and non-coding regions of the target nucleic acids, as previously disclosed by Hesterkamp, for providing a superior method of targeting nucleic acids in a sample.

Claim 4 lacks an inventive step under PCT Article 33(3) as being obvious over Porreca in view of the publication entitled 'PathogenMIPer: A Tool For The Design Of Molecular Inversion Probes To Detect Multiple Pathogens' by Thiyagarajan, et al. (hereinafter 'Thiyagarajan').

As per claim 4, Porreca discloses the method of claim 3, but Porreca does not disclose wherein the central region is different for at least two of the plurality of the molecular inversion probes. Thiyagarajan discloses wherein the central region is different for at least two of the plurality of the molecular inversion probes (the molecular inversion probe includes a unique tag sequence (in the central region) which detects and identifies each of the probes; page 2, right column, third paragraph; page 3, figure 1). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the molecular inversion probes of the invention, for providing a different central region in at least two of the plurality of probes, as previously disclosed by Thiyagarajan, for implementing a superior method of differentiating the probes after hybridizing to the target nucleic acids.

Claim 2 meets the criteria set-out in PCT Articles 33(2)-(3), because the prior art does not teach or fairly suggest the method of claim 1, wherein the plurality of molecular inversion probes are a combination of nucleic acid fragments comprising SEQ ID NO: 185 and 186.

Porreca discloses the plurality of molecular inversion probes (contacting a plurality of target nucleic acids from a patient sample with a set of molecular inversion probes; paragraphs [0014], [0020], [0021]). Porreca does not disclose wherein the plurality of molecular inversion probes are a combination of nucleic acid fragments comprising SEQ ID NO: 185 and 186.

WO 2010/126614 A2 to Porreca, et al. (hereinafter 'Porreca 2010') discloses molecular inversion probes are a combination of nucleic acid fragments comprising SEQ ID NO: 185 (SEQ ID NO: 185 of the instant PCT application is 59.7% identical to SEQ ID NO: 458 of the Porreca'2010 reference; SEQ ID NO: 458) and 186 (SEQ ID NO: 186 of the instant PCT application is 57.4% identical to SEQ ID NO: 297 of the Porreca'2010 reference; SEQ ID NO: 297).

The prior art does not teach or fairly suggest the plurality of molecular inversion probes are a combination of nucleic acid fragments comprising SEQ ID NO: 185 and 186, and sufficient motivation, based upon the previous disclosures of the Porreca and Porreca'2010 references does not exist to have implemented that a plurality of molecular inversion probes are a combination of nucleic acid fragments comprising SEQ ID NO: 185 and 186.

Claims 1-9 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

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| **EFS ID:** | 21342733 |
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| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
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| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Christian Dolores |
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| 1 | | GSGE-002-01US-Response-to-OA-102914.pdf | 105449<br>e8ef93e139ebfe002a6a262b8b980d9001c17d5a | yes | 13 |

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| | **Document Description** | **Start** | **End** |
| | Amendment/Req. Reconsideration-After Non-Final Reject | 1 | 1 |
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| | Applicant Arguments/Remarks Made in an Amendment | 10 | 13 |
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**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANT: | Porreca et al. | ART UNIT: | 1634 |
| SERIAL NUMBER: | 13/266,862 | CONFIRMATION NO.: | 6082 |
| FILING DATE: | March 13, 2012 | EXAMINER: | Pohnert, Steven C. |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

**FILED ELECTRONICALLY**

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**RESPONSE**

This paper is in response to a non-final Office Action mailed October 29, 2014 from the United States Patent and Trademark Office. In the event that the fees are required, Applicants authorize the Commissioner to charge any required fees due with this response to Deposit Account No. 500369.

**Amendments to the specification** begin on page 2.

**Amendments to the claims** begin on page 3.

**Remarks** begin on page 10.

Amendments to the specification

No amendments to the specification are made herein.



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Amendment to the claims

This list of claims replaces all prior versions and listings of claims in this application.

1. (Currently amended) A method of analyzing a plurality of genetic loci, the method comprising:

contacting each of a plurality of target nucleic acids in a sample with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region that comprises a 5' terminal nucleotide and a 3' region that comprises a 3' terminal nucleotide, the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion, wherein when hybridized to the target nucleic acid in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide of each probe are separated by a plurality of nucleotides on the target nucleic acid, and wherein at least two of the plurality of molecular inversion probes are present at different concentrations in the sample,

converting hybridized probes into closed circularized probes,

isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and

analyzing the isolated nucleic acids.

2. (Original) The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion.

3. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.



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4. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5-11. (Canceled)

12. (Withdrawn) A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. (Withdrawn) The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.

14. (Withdrawn-Previously presented) The method of claim 12, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.



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15. (Withdrawn-Previously presented) The method of claim 12, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. (Withdrawn-Previously presented) The method of claim 12, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

17-19. (Canceled)

20. (Withdrawn) A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. (Withdrawn) The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is lower than the reference capture efficiency.

23. (Withdrawn) The method of claim 22, wherein the subject is identified as having an insertion in the target region.



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24. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is higher than the reference capture efficiency.

25. (Withdrawn) The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. (Withdrawn) The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.

27. (Withdrawn) The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. (Withdrawn-Previously presented) The method of claim 20, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. (Withdrawn) The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.

30. (Withdrawn) The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31-32. (Canceled)



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33. (Withdrawn-Previously presented) The method of claim 29, wherein the first and second targeting arms have the same length.

34-35. (Canceled)

36. (Withdrawn-Previously presented) The method of claim 29, wherein the hybridization Tms of the first and second targeting arms are identical.

37. (Withdrawn-Previously presented) The method of claim 28, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. (Withdrawn) The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. (Canceled)

40. (Withdrawn) The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.

42. (Withdrawn-Previously presented) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.



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43. (Withdrawn-Previously presented) The method of claim 29, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. (Withdrawn-Previously presented) The method of claim 20, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. (Withdrawn) The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. (Withdrawn) The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. (Withdrawn) The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48-49. (Canceled)

50. (Withdrawn-Previously presented) The method of claim 20, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. (Withdrawn) The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.

52. (Withdrawn-Previously presented) The method of claim 50, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.



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53. (Withdrawn) The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.

55. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. (Withdrawn-Previously presented) The method of claim 20, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57-67 (Canceled)



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Remarks

Claims 1-4 are pending in the application. Claim 1 is amended to recite contacting each of a plurality of target nucleic acids in a sample. Support for the amendment can be found in the as-filed application on at least page 69 line 28 through page 70 line 23. No new matter is added.

Rejections under 35 U.S.C. §112

The Office Action rejects claim 1 under 35 U.S.C. §112 ¶1, alleging that "at least two of the plurality of molecular inversion probes are present at different concentrations" is not supported by the as-filed application. Applicants respectfully disagree.

Claim 1 has been amended to clarify that the different probes are in a single sample at different concentrations. The amendment is supported by the as-filed application. For example, the as-filed application teaches varying the concentration of probes at a locus. For example, at page 70, lines 6-8, the application teaches that "for each locus of interest, the amount of probe used per assay may be optimized for a particular application." The application continues, at page 70, lines 13-15, stating that "the amount of target nucleic acid and probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios." Therefore, the as-filed application supports the recitation of "at least two of the plurality of molecular inversion probes are present at different concentrations in the sample".

Based on the foregoing, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112.

Rejections under 35 U.S.C. §103

The Office rejects claims 1-4 under 35 U.S.C. §103(a) in view of the combination of Akhras (PLOS one, 2007, 2(9):e915), Nilsson (Trends in Biotechnology, 2006, 24:83-88), Hardenbol (Nature Biotechnology, 2003, 21:673-78), Mockler (Genomics, 2005, 85:1-15), Porreca (Nature Methods, 2007, 4:931-36), Hardenbol (2006/0019304), and Browne (2006/0246500). Applicants respectfully disagree.



Petitioner Illumina, Inc., EX1002, p. 1006

In order to establish a prima facie case of obviousness, three basic criteria must be established by the Patent Office. First, there must be a suggestion in the cited art to modify a reference or to combine multiple references in a manner that would lead one of ordinary skill in the art to an applicant's claimed invention. Second, the cited references themselves must convey a reasonable expectation of success in arriving at the claimed invention based upon the proposed modification or combination. Finally, the teaching or suggestion to make the claimed invention must be found in the prior art and not in the applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). In addition to the requirements for making a combination, an obviousness rejection cannot be established in the first place unless each claim element is disclosed in the cited art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

The pending claims require that at least two among a plurality of molecular inversion probes be present at different concentrations in the sample. The as-filed application, at page 67, line 28, through page 69, line 2, addresses the problem that bias results when alleles are present in a sample at ratios other than 50:50. As explained at page 69, line 28, through page 70, line 23, such bias can be normalized by using probes at different concentrations in the sample, thereby enhancing under-represented targets and suppressing over-represented targets. None of the cited references disclose or suggest normalizing bias by using two different concentrations of probes in a single sample.

Akhras, Nilsson, Hardenbol (2003), and Mockler are all limited to reporting the use of padlock probes or general tiling strategies. Akhras reports a connector inversion probe method. Nilsson reports padlock probes that form circles with gaps between the 5' and 3' terminal nucleotides. Hardenbol (2003) reports padlock probes that hybridize to a target in a manner such that a 5' terminal nucleotide and a 3' terminal nucleotide are separated from each other by only a single nucleotide. Mockler reports general tiling strategies using linear probes. None of those references disclose or suggest varying concentrations of probes in a sample to account for biases associated with alleles that do not have 50:50 ratios.

The three references that the Examiner alleges report using probes at two or more

concentrations in a single sample are Porreca, Hardenbol (2006), and Browne. Applicants respectfully disagree and show below that none of those references disclose or suggest using two different concentrations of probes in a single sample.

Porreca reports a method for gene amplification using molecular inversion probes. See Porreca, page 932, col 1, lines 11-25. Porreca notes that some base calls were skewed away from the expected 50:50 ratio. Porreca suggests solving that problem by saturating the reaction mixture with an excess of all reactants. *Id.* at 934, col 2, lines 30-59. Porreca does not disclose or suggest using different probe concentrations in a single sample.

Hardenbol (2006) addresses concentration as a factor in hybridization assay design. See, for example, *id.* at paragraphs [0038] and [0048]. Hardenbol (2006) mentions probe concentration as a standard parameter in a hybridization assay, but never discloses or suggests using two different probe concentrations in an assay. The protocols described do not contemplate varying probe concentration to account for biases due to alleles bring present at ratios other than 50:50. Accordingly, Hardenbol (2006) cannot properly be read to disclose or suggest using different probe concentrations in a sampleto address the problem of bias occurring at loci with alleles having ratios that deviate from 50:50.

The Office Action alleges that Browne demonstrates dual inversion probes at different concentrations in FIG. 6. However, Browne FIG. 6 merely shows the results of a series of separate and discrete assays, each using different probe concentrations. See Browne at paragraph [0114]. For any single assay in Browne, the probe concentrations are the same. Browne does not disclose or suggest an assay using different probe concentrations in the same sample. Browne would have no reason to report more than one probe concentration in a single assay because Browne is not concerned with controlling for bias. Instead, Browne is interested in probe concentration merely to minimize signal-to-noise ratio in fluorescent probe hybridization assays. Browne is attempting to control background fluorescence, not accounting for biases resulting from allele fractions that are not 50:50.

The pending claims are not obvious in view of any combination of the cited prior art

because none of those references teaches normalizing probe concentrations to account for allelic ratios that are not 50:50.

Applicants respectfully request withdrawal of the rejection of claims 1-4 under 35 U.S.C. §103(a).

Summary

On the basis of the foregoing reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: January 29, 2015

Respectfully submitted,
**BROWN RUDNICK LLP**

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

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BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

| Office Action Summary | Application No. 13/266,862 | Applicant(s) PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | AIA (First Inventor to File) Status No |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *1/29/2015*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims***

5)☒ Claim(s) *1-4,12-16,20-30,33,36-38,40-47 and 50-56* is/are pending in the application.

5a) Of the above claim(s) *12-16,20-30,33,36-38,40-47 and 50-56* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-4* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *4/8/2013* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.



Petitioner Illumina, Inc., EX1002, p. 1011

## DETAILED ACTION

### *Notice of Pre-AIA or AIA Status*

1. The present application is being examined under the pre-AIA first to invent provisions.

### *Claim Status and Formal matters*

This action is in response to papers filed 1/29/2015.

Claim 1 has been amended.

Claims 1-4, 12-16, 20-30, 33, 36-38, 40-47 and 50-56 are pending.

Claims 5-11, 17-19,31-32, 34-35, 39-38, 48-49 and 57-67 have been canceled.

Claims 12-16, 20-30, 33, 36-38, 40-47 and 50-56 withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention , there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 12/3/2012.

Claims 1-4 are being examined.

### *Priority*

The instant application was filed 3/13/2012 and is a national stage entry of PCT/US10/01293 filed 4/30/2010 and claims priority to provisional application 61/147,470 filed 4/30/2009; provisional application 61/147923 filed 5/1/2009; provisional application 61179358 filed 5/18/2009; and 61/182,089 filed 5/28/2009.

2. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied

with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) or the first paragraph of pre-AIA 35 U.S.C. 112, except for the best mode requirement. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994)

The disclosure of the prior-filed application, Application Nos. 61/174,470, 61/174,923, 61/179,358, 61/182, 089, fails to provide adequate support or enablement in the manner provided by 35 U.S.C. 112(a) or pre-AIA 35 U.S.C. 112, first paragraph for one or more claims of this application. Application Nos. 61/174,470, 61/174,923, 61/179,358, 61/182, 089, fail to provide support for, “wherein at least two of the plurality of molecular inventions probes are present at different concentrations. “Thus the instant claims are being given priority to 4/30/2010. The filing date of the instant application as a PCT.

***Claim Rejections - 35 USC § 112***

3. The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

***4.*** Claims 1-4 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 1 has been amended to recite, "wherein at least two of the plurality of molecular inversion probes are present at different concentrations. " The response asserts the amendment is supported on pages 69 line 28 to page 70 line 23 which states:

According to aspects of the invention, such biases can be detected or overcome by systematically combining different capture and/or analytical techniques in an assay that interrogates a plurality of loci in a plurality of subject samples.

Accordingly, it should be appreciated that in any of the embodiments described herein (e.g., tiling/staggering, tagging, size-detection, sensitivity enhancing algorithms,or any combination thereof), aspects of the invention involve preparing genomic nucleic acid and/or contacting them with one or more different probes (e.g., capture probes, hybridization probes, MIPs, others etc.). In some embodiments, the amount of genomic nucleic acid used per subject ranges from 1 ng to 10 micrograms (e.g., 500 ng to 5 micrograms). However, higher or lower amounts (e.g., less than 1 ng, more than 10 micrograms, 10-50 micrograms, 50-100 micrograms or more) may be used. In some embodiments, for each locus of interest, the amount of probe used per assay may be optimized for a particular application. In some embodiments, the ratio (molar ratio, for example measured as a concentration ratio) of probe to genome equivalent (e.g., haploid or diploid genome equivalent, for example for each allele or for

both alleles of a nucleic acid target or locus of interest)ranges from 1/100, 1/10, 1/1, 10/1, 100/1, 1000/1. However, lower, higher, or intermediate ratios may be used.

In some embodiments, the amount of target nucleic acid and probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios. In some embodiments, in order to normalize genomic DNA and probe, the genomic DNA concentration is read using a standard spectrophotometer or by fluorescence (e.g., using a fluorescent intercalating dye). The probe concentration may be determined experimentally or using information specified by the probe manufacturer.

Similarly, once a locus has been captured (e.g., on a MIP or other probe or in another form), it may be amplified and/or sequenced in a reaction involving one or more primers. The amount of primer added for each reaction can range from 0.1 pmol to 1 nmol, 0.15 pmol to 1.5 nmol (for example around 1.5 pmol). However, other amounts (e.g., lower, higher, or intermediate amounts) may be used.

The specification teaches on page 3, “In some embodiments, sources of error and ambiguity in one or more steps can be addressed by capturing and/or interrogating each target locus of interest with at least one set of probes.”

Thus the specification teaches varying the concentration of probes between different loci, but does not clearly envision the use of probes of different concentration for a single loci as the active steps of the claims suggest. Thus the amendment has introduced new matter.

**Response to Arguments**

The response traverses the rejection asserting, “Claim 1 has been amended to clarify that the different probes are in a single sample at different concentrations. The amendment is supported by the as-filed application. For example, the as-filed application teaches varying the concentration of probes at a locus. For example, at page 70, lines 6-8, the application teaches that "for each locus of interest, the amount of probe used per assay may be optimized for a particular application." The application continues, at page 70, lines 13-15, stating that "the amount of target nucleic acid and

probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios." Therefore, the as-filed application supports the recitation of "at least two of the plurality of molecular inversion probes are present at different concentrations in the sample"." This argument has been thoroughly reviewed but is not considered persuasive as the instant claims require that each subregion of a probe overlaps with another subregion of the probe and thus is to a single loci. The specification does not envision the use of different probes at a single loci.

5. The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 1-4 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The Board has held, "If a claim is amenable to two or more plausible constructions, applicant is required to amend the claim to more precisely define the metes and bounds of the claimed invention or the claim is indefinite under §112, ¶2." Ex parte Miyazaki, 89 USPQ2d 1207 (BPAI 2008) (expanded panel).

The US Supreme Court in NAUTILUS, INC. v. BIOSIG INSTRUMENTS, INC. (2014) held, "we hold that a patent is invalid for indefiniteness if its claims, read in light

of the specification delineating the patent, and the prosecution history, fail to inform, with reasonable certainty, those skilled in the art about the scope of the invention."

The preamble of the instant claim is drawn to a method of analyzing a plurality of genetic loci. The contacting step requires, "contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region that comprises a 5' terminal nucleotide and a 3' region that comprises a 3' terminal nucleotide, the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion, wherein when hybridized to the target nucleic acid in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide of each probe are separated by a plurality of nucleotides on the target nucleic acid, and wherein at least two of the plurality of molecular inversion probes are present at different concentrations." Thus all probes of contacting step of the claim and thus the rest of the active steps require the probes overlap and thus it is unclear if the intent of the claim is to require analyzing a plurality of probes to a single loci as the active steps of the claims suggest or require analyzing a plurality of loci and how this relates to the claims as they require overlapping probes and thus are target to a single loci.

Claim 1 further recites, "the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid." The claim appears to require a single target nucleic acid, but sequences to "nucleic acids

flanking one of a plurality of subregions." Thus the metes and bounds of the claim are unclear if there is a single target nucleic acid to which the molecular inversion probe is being hybridized or if the intent is the 3' region and 5' region of each probe is hybridizing to a different nucleic acid. The claims should be amended to clarify the relationship between the 3' region and 5' region of the probes as to whether they are hybridizing a single target polynucleotide or different nucleic acids.

Claim 1 was amended in the response of 1/29/2015 to recite, "contacting each of a plurality of target nucleic acids in a sample with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region that comprises a 5' terminal nucleotide and a 3' region that comprises a 3' terminal nucleotide, the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion, wherein when hybridized to the target nucleic acid in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide of each probe are separated by a plurality of nucleotides on the target nucleic acid, and wherein at least two of the plurality of molecular inversion probes are present at different concentrations in the sample." It is first unclear if the intent is to require "a plurality of nucleic acids in a sample" is defining the sample as a plurality of target nucleic acids or if "in a sample with a plurality of different molecular inversion probes" is defining the sample. Further the recitation of " wherein at least two of the plurality of molecular inversion probes are present at different concentrations in the sample" is unclear as the literal reading is that

that at least of the plurality of molecular inversion probes is in the sample prior to the contacting step, which does not logically make sense. If the intent is to require probes at different concentration to be mixed with the sample, the claim should be amended to require the contacting step results in a reaction mixture or hybridization reaction and amending the wherein clause to be consistent with the contacting step.

Thus claims 2-4 are rejected as they depend from claim 1.

Claim 2 requires, "wherein at least one subregion entirely overlaps at least one other subregion." Claim 1 requires, "the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion,." Thus it is unclear how one subregion can entirely overlap as required by claim 2 and yet be different as required in claim 1. Thus the metes and bounds of the claim are unclear.

**Response to Arguments**

The response provides no specific arguments with respect to the 112-2nd paragraph rejections. Further the amendment has raised new issues.

***Claim Rejections - 35 USC § 103***

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhras et al (PLOS one (2007) volume 2 (9) e915) (IDS 3/28/2013), Nilsson et al (Trends in Biotechnology (2006 ) volume 24, pages 83-88), Hardenbohl et al (Nature Biotechnology(2003) volume 21, pages 673-678), Mockler et al (Genomics (2005) volume 85, pages 1-15), Porecca et al ( Nature methods (2007) volume 4, pages 931-936), Hardenbohl et al (USPGPUB 2006/0019304) and Browne et al (US PGPUB 20060246500).

MPEP 2111 states:

> The court held that the PTO is not required, in the course of prosecution, to interpret claims in applications in the same manner as a court would interpret claims in an infringement suit. Rather, the "PTO applies to verbiage of the proposed claims the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in applicant's specification."

MPEP 2111.01 states

> Though understanding the claim language may be aided by explanations contained in the written description, it is important not to import into a claim limitations that are not part of the claim.

It is noted that the specification lacks a limiting definition of a molecular inversion probe. Thus "molecular inversion probe" is being given the broadest reasonable interpretation in view of the claims and art as a probe that allows for formation of a circular probe upon hybridization to a target nucleic acid.

It is further noted the 112-2$^{nd}$ issues are considered in rendering this rejection.

Akhras teaches a connector inversion probe method (CIPer) that is a combination of Gap-LCR and padlock probes. Akhras teaches, "The Connector

Inversion Probe (CIPer) reaction (Figure 1) shares some basic design features with a recently described assay, the PathogenMip assay [17], which includes: i) probe hybridization, single “G” base gap fill and probe circularization, ii) exonuclease based circular DNA selection, iii) probe inversion by re-linearization, and iv) universal PCR amplification. The distinguishing and key feature of the CIPer method is a modified first reaction step involving a complete filling of an extensive gap formed by the probe and target.” Akhaus further teaches, " Connector Inversion Probe (CIPer) technology is a powerful method for multiplex amplification, and has been validated here in genetic pathogen diagnostics, typing, and antibiotic resistance detection arenas as a proof-of-concept. This technology can be designed and optimized for use in numerous other scientific areas and applications. Other areas suitable for use of the CIPer method include cancer genomics [32], human SNP detection [15], and selective exon amplification [7], among numerous other promising applications. Modified CIPers may also in the future offer an alternative strategy for gateway cloning [33]. In this paper, we focused on “lowplex” systems amplifying shorter stretches of DNA of up to 100 base pair lengths. To achieve higher degrees of multiplexing and longer clone reads, one must take into account i) clone GC content, ii) clone length variability, and iii) steric effects for longer clones. Analog amplification and downstream validation are delimiting factors for multiplex detection methods. We believe that the future of CIPer technology will rely on digital amplification [34] and high throughput sequencing validation (Figure S5). The CIPers universal primer sites can be formatted to fit any amplification method, e.g. RCA [12], [13], emulsion PCR [34], SPIA [35] LAMP [36] and SMAP 2 [37];

universal adaptor ligation, a tedious and sample-consuming procedure [38], is therefore optional. Proposed digital amplification methods include emulsion PCR, RCA, or a combination of the two [39]. Suitable upcoming high throughput validation methods include next-generation sequence systems such as the 454 Life Sciences platform [38] (http://www.454.com), the Solexa platform [40] (http://www.illumina.com), polony sequencing [41] (http://www.agentcourt.com), Helicos Biosciences [42] (http://www.helicosbio.com), or use of a resequencing array (GeneChip CustomSeq, www.affymetrix.com). Recent digital quantification methods [43] open the possibility for quantitative CIPer technology. With the addition of a molecular barcode to the padlocks [14], MIPs were used for ultra high-throughput SNP detection based on microarray barcode hybridization [15]. Because CIPers can also include a barcode, they could be used for microarray validation, or amplicon tracing with sequencing methods [17], [44] allowing for longer read lengths [38]. With the introduction of a third universal sequence flanking the barcode and the universal primer sites, one can begin to envision a two-dimension informational barcode, a concept we call “multiplexing multiplex padlocks” (MMP) (Figure S6). Probe construction based on ligation of three smaller constructs [45] would allow for variability in fragment assembly. By combining the one-genotype detection pair of AS/ES constructs with multiple unique barcodes, we can create patient and genotype specific probes with one barcode per probe. This will allow for analysis of multiple parallel samples, essentially lowering the cost of microarray chips and sequencing reactions.”

Nilsson teaches the use of padlock probes and “selector” probes that form circles with gaps of several nucleotides between the 5’ terminal nucleotide and the 3’ terminal nucleotide.

Akhras and Nilsson demonstrate probes were known that result in a circular probe upon hybridization to a target sequence with a gap between the 3' terminal nucleotide and 5' terminal nucleotide were known. Akhras suggests the use of CIPer methods similar to the use of MIPs, including the use of “barcodes to allow detection by microarrays.”

Akhras and Nilsson do not specifically teach, “contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion.”

However, Hardenbol discloses a method of analyzing a plurality of genetic loci by contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were

generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1). Hardenbohl teaches these probes are MIP or padlock probes.

Hardenbohl teaches in figure 2b the joining of the molecular inversion probes which results in a circular type configuration. Hardenbohl exemplifies in fig 2b a probe that has a 5' region and a 5' terminal nucleotide, a 3' region and a 3' terminal nucleotide, and a single base gap. Thus the 5' terminal nucleotide, 3' terminal nucleotide and single base gap are a plurality of nucleotides between the 3' and 5' region of the probe.

Hardenbohl does not specifically teach wherein each subregion overlaps with at least one other subregion. Hardenbohl does not specifically teach, "wherein at least one subregion entirely overlaps at least one subregion."

Mockler, teaches each subregion overlaps with at least one other subregion (Fig. 2a).

With regards to claim 2, Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other Subregion)).

With regards to claim 3, Mockler teaches the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

With regards to claim 4, Mockler teaches sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the CIPer or selector probes taught by Akhras and Nilsson with a gap of a plurality of nucleotides between the 5' terminal nucleotide and 3' terminal of the padlock probes with tiling probes of Mockler in the method of Hardenbol. The artisan would be motivated as Akhras suggests the use of CIPer methods in MIP methods. The teachings of Hardenbohl set forth one embodiment of the use of MIP or padlock probes. The artisan further would be motivated as Mockler teaches the probe tiling approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1). The artisan would have a reasonable expectation of success as the artisan is merely increasing the number of nucleotides between the 3' terminal nucleotide and 5' terminal nucleotide of the probes of Mockler, which Akhras demonstrates were known and worked. Thus the combination of references suggests all the limitations of claims and suggests the use of CIPer in place of the similar MIP methods.

Akhras, Nilsson, Hardenbohl, and Mockler do not specifically teach the use of probes at two or more concentrations.

However, Porecca teaches a method of amplifying human exons using MIPS. Porecca teaches, “It is both concerning and interesting that the resequencing data show skewing of base-calls at heterozygous positions away from the expected ratio of 0.5 (Fig. 4). This skewing might be expected if stochastic events (for example, a finite number of capture events during the multiplex capture reaction) are a primary source of bias. Given that the concentration of individual targeting oligos in the capture reaction is very low (B0.36 pM per species), it is likely that their hybridization to genomic DNA is inefficient. This problem might therefore be mitigated simply by increasing the concentration of reactants. If either the targeting oligos or genomic DNA can be defined as limiting, one could potentially hybridize with an excess of the complement, that is, to saturation, such that small differences in capture efficiency would be ‘normalized’ away.” Porecca further teaches, “After characterizing the efficiency of individual capture oligos in each reaction, a second iteration could be carried out, in which capture oligos performing similarly to one another are grouped together within the same oligo mixture (thereby maximizing uniformity within any single reaction).”

Further, Hardenbohl(PGPUB) teaches, “Genomic material is complex and thus individual sequences exist at relatively low concentrations in a mixture. As a result of this, at a given probe concentration there is a finite rate at which the probe sequences will hybridize to the genomic templates. Probe concentrations can be adjusted to some extent to vary this rate, but as probe complexity is increased, for example, when highly multiplexed assays are used, such flexibility is greatly reduced” (003). Hardenbohl continues, “Multiplexing occurs at two levels in the invention. First, hybridization-based

assays are designed to measure characteristics of one or more target polynucleotides, such as genomic fragments, at multiple loci. The number of loci measured in each hybridization-based assay can vary widely, and its upper limit depends on well-known factors, including the capacity of the readout device being employed, trade-offs selected between probe concentrations and reaction times, the genetic characteristic being measured (for example, trait association studies require very large numbers of measurements; genetic identification requires relative few measurements; testing for adverse drug reactions may require a medium number of measurements, e.g. several tens to hundreds), and the like. In one aspect, the number of loci analyzed in each hybridization-based assay is in the range of from a few tens, e.g. 10 to 20, to many thousands, e.g. fifty to a hundred thousand. In another aspect, the number of loci per hybridization-based assay is in the range of from 10 to 40,000; or it is in the range of from 100 to 30,000; or it is in the range of from 100 to 20,000. Second, multiplexing occurs from the combination of assay products from multiple hybridization-based assays for determining multiple signals on a single readout device. Usually, there is a one-to-one correspondence between the number of hybridization-based assay products combined in accordance with the invention and the number of different genomic samples being analyzed; however, the invention comprehends situations where different numbers of samples from different individuals are analyzed by varying numbers of hybridization-based assays, the products of which are combined. The level of such multiplexing depends of the capacity of the readout device and the number of loci measured by each hybridization-based assay. In one aspect, the number of

hybridization-based assays that may be multiplexed is simply the capacity of a readout device, e.g. the number of features on a microarray, divided by the number of loci being measured in the hybridization-based assays. For example, in one aspect, a 100 thousand-feature microarray can serve as a readout device for fifty hybridization based assays that each measure the zygosity of one thousand loci, when two features are required for each determination (e.g. homozygous in allele 1, homozygous in allele 2, or heterozygous in allele 1 and 2). As discussed more fully below, particular trade-offs between the use of microarray features, different colored fluorescent dyes, number of loci measured per hybridization-based assay, capacity of a readout device, and the like, are routine design choices for those of ordinary skill in the art."

Hardenbohl (PGPUB) continues, "Operation of one embodiment of the invention is illustrated in FIGS. 1A and 1B for a particular hybridization-based assay (one based on the template-driven ligation of two oligonucleotide components). Hybridization-based assays are carried out separately on a sample of DNA from genome G.sub.1 and a sample of DNA from genome G.sub.2. Probes p.sub.1 to p.sub.K (100) containing oligonucleotide tags t.sub.1 to t.sub.K, respectively, and probes p.sub.1 to p.sub.K (102) containing oligonucleotide tags t.sub.K+1 to t.sub.2K, respectively, are combined in separate reaction mixtures with genome G.sub.1 DNA and genome G.sub.2 DNA, respectively, under conditions that permit specific hybridization of such probes to their respective DNA stands (104) and (106) containing target loci, L.sub.1 through L.sub.K ((108) to (110) for genome G.sub.1 and (112) to (114) for genome G.sub.2). After an incubation time depending on parameters well-known to those of ordinary skill, e.g.

target complexity, probe concentration, temperature, and the like, discussed more fully below, probe-genome complexes form ((118) and (116), respectively) that include recognition structures for enzyme(s) of subsequent step(s)."

Finally, Browne demonstrates the use of dual inversion probes at different concentrations in figure 6.

As stated in the MPEP, 2144.05 II, "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)."

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of Akhras, Nilsson, Hardenbohl, and Mockler to include at probes at different concentrations in a sample to optimize hybridization and assay conditions in a sample. The artisan would be motivated as Porecca, Hardenbohl(PGPUB), and Brown recognize that probe concentration is an optimizable variable in the speed and performance of hybridization assays including molecular inversion probe assays. Thus it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to include additional probes at different concentrations so as to optimize efficiency of hybridization as well as speed of hybridization in a sample. The artisan would have a reasonable expectation of success as the artisan is merely using known methods to optimize conditions.

**Response to Arguments**

The response traverses the instant rejection asserting, “Porreca reports a method for gene amplification using molecular inversion probes. See Porreca, page 932, col 1, lines 11-25. Porreca notes that some base calls were skewed away from the expected 50:50 ratio. Porreca suggests solving that problem by saturating the reaction mixture with an excess of all reactants. Id. at 934, col 2, lines 30-59. Porreca does not disclose or suggest using different probe concentrations in a single sample.” This argument has been thoroughly reviewed but is not considered persuasive as Porreca teaches, “Given that the concentration of individual targeting oligos in the capture reaction is very low ( ~ 0.36 pM per species), it is likely that their hybridization to genomic DNA is inefficient. This problem might therefore be mitigated simply by increasing the concentration of reactants. If either the targeting oligos or genomic DNA can be defined as limiting, one could potentially hybridize with an excess of the complement, that is, to saturation, such that small differences in capture efficiency would be 'normalized' away”.(emphasis added by the examiner). Thus Porrecca suggest that individual targeting oligos may be rate limiting and can be increased. In the context of the paragraph rendering obvious varying conditions of probes. Further this argument is arguing the teachings of Porrecca alone. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The response continues by asserting Hardenbol does not specifically disclose or suggest probes of different concentration in a sample. This argument has been thoroughly reviewed but is not considered persuasive as Hardenbohl clearly envisions that probe concentration is a hybridization parameter that can be optimized. Further this argument is arguing the teachings of Hardenbohl alone. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The response continues by asserting Browne does not teach probe concentration is a single sample. This argument has been thoroughly reviewed but is not considered persuasive as the response is arguing the teachings of Browne alone. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The rejection is maintained as the prior art as a whole demonstrates that varying probe concentrations is an optimizable factor in hybridization and specifically in inversion probes. Thus the art recognizes the problem of probe concentration on hybridization and recognizes that varying the probe concentration can be done to improve the results of the assay.

**Summary**

No claims are allowed.

***Conclusion***

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:00-5:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/STEVEN POHNERT/
Primary Examiner, Art Unit 1634

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

**OR**

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2015-01-09 |
|---|---|---|---|
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
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| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** ( **Not for submission under 37 CFR 1.99)** | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US |

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| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US |

| | | | |
|---|---|---|---|
| /S.P./ | 1 | International Search Report and Written Opinion mailed December 9, 2014, for International Patent Application No. PCT/US14/28212, filed March 14, 2014 (17 pages) | ☐ |
| /S.P./ | 2 | Thiyagarajan, 2006, PathogenMIPer: a tool for the design of molecular inversion probes to detect multiple pathogens, BMC Bioinformatics, 7:500 (10 pages). | ☐ |

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**EXAMINER SIGNATURE**

| Examiner Signature | /Steven Pohnert/ (03/06/2015) | Date Considered | |
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|---|---|
| **EFS ID:** | 23469249 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Lauren Poon |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 14-SEP-2015 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 09:33:28 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

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| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $1550 |
| RAM confirmation Number | 8697 |
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| 1 | | GSGE-002-01US-Response-to-OA-031215.pdf | 47125<br>9b0067486fcea667430436ca3cff1eea7ff29e15 | yes | 14 |
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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANT: | Porreca et al. | ART UNIT: | 1634 |
| SERIAL NUMBER: | 13/266,862 | CONFIRMATION NO.: | 6082 |
| FILING DATE: | March 13, 2012 | EXAMINER: | Pohnert, Steven C. |
| TITLE: | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | | |

**FILED ELECTRONICALLY**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

**RESPONSE AND REQUEST FOR CONTINUED EXAMINATION**

This paper is in response to the Office Action mailed March 12, 2015, from the United States Patent and Trademark Office. Applicants submit herewith a Request for Continued Examination (RCE) and petition for a three-month extension of time, to bring the response period up to and including September 14, 2015. Applicants pay herewith the RCE fee and the extension of time fee. Applicants believe no other fee is due. Nevertheless, Applicants authorize the Commissioner to charge any required fees due with this response to Deposit Account No. 500369.

**Amendments to the claims** begin on page 2.

**Remarks** begin on page 9.

Amendment to the claims

This list of claims replaces all prior versions and listings of claims in this application.

1. (Currently Amended) A method ~~of~~ for analyzing a ~~plurality of genetic loci~~ target nucleic acid, the method comprising:

providing a sample comprising a target nucleic acid;

contacting ~~each of a plurality of target nucleic acids in a~~ the sample with a probe set to generate hybridized probes, the probe set comprising a plurality of different molecular inversion probes, each different molecular inversion probe ~~having~~ comprising:

~~a central region flanked by~~ a 5' region ~~that comprises~~ comprising a 5' terminal nucleotide; ~~and~~

a 3' region ~~that comprises~~ comprising a 3' terminal nucleotide; and

a central region flanked by the 5' region and the 3' region; [[,]]

wherein the 5' region and 3' region ~~being~~ of each probe are complementary to nucleic acid[[s]] residues flanking the same strand of one of a plurality of subregions of the target nucleic acid; [[,]]

wherein ~~the~~ each subregion[[s]] ~~of the target nucleic acid are~~ is different~~, wherein each subregion~~ and overlaps with at least one other subregion; [[,]]

wherein when hybridized to the target nucleic acid in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide of each probe are separated by a plurality of nucleotides on the target nucleic acid; [[,]] and

wherein at least two of the plurality of molecular inversion probes are present at different concentrations in the ~~sample~~ probe set; [[,]]

converting hybridized probes into closed circularized probes; [[,]]

isolating a plurality of ~~nucleic acids each~~ circularized probes having [[a]] nucleic acid sequences of [[a]] different subregions; ~~for each of the plurality of target nucleic acids,~~ and

analyzing the isolated ~~nucleic acids~~ subregions.



Petitioner Illumina, Inc., EX1002, p. 1042

2. (Currently Amended) The method of claim 1, wherein at least one subregion entirely overlaps at least one other subregion and captures additional flanking sequence.

3. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe.

4. (Original) The method of claim 1, wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe.

5-11. (Canceled)

12. (Withdrawn) A method of genotyping a subject, the method comprising:

obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid, wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence, wherein the target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products, and

genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products.

13. (Withdrawn) The method of claim 12, wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence.



Petitioner Illumina, Inc., EX1002, p. 1043

14. (Withdrawn) The method of claim 12, wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05.

15. (Withdrawn) The method of claim 12, wherein the threshold number for a specific target nucleic acid sequence is selected based on a desired statistical confidence for the genotype.

16. (Withdrawn) The method of claim 12, further comprising determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.

17-19. (Canceled)

20. (Withdrawn) A method for determining whether a target nucleic acid has an abnormal length, the method comprising evaluating the capture efficiency of a target nucleic acid in a biological sample from a subject, wherein a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.

21. (Withdrawn) The method of claim 20, comprising determining the capture efficiency of a target region suspected of having a deletion or insertion and comparing the capture efficiency to a reference indicative of a normal capture efficiency.

22. (Withdrawn) The method of claim 20, wherein the capture efficiency is lower than the reference capture efficiency.

23. (Withdrawn) The method of claim 22, wherein the subject is identified as having an insertion in the target region.

24. (Withdrawn) The method of claim 20, wherein the capture efficiency is higher than the reference capture efficiency.

25. (Withdrawn) The method of claim 24, wherein the subject is identified as having a deletion in the target region.

26. (Withdrawn) The method of claim 23, wherein the subject is identified as being heterozygous for the insertion.

27. (Withdrawn) The method of claim 25, wherein the subject is identified as being heterozygous for the deletion.

28. (Withdrawn) The method of claim 20, wherein a sub-target nucleic acid is captured from the target nucleic acid using a molecular inversion probe.

29. (Withdrawn) The method of claim 28, wherein the molecular inversion probe comprises a first targeting arm at its 5' end and a second targeting arm at its 3' end, wherein the first targeting arm is capable of specifically hybridizing to a first region flanking one end of the sub-target nucleic acid, and wherein the second targeting arm is capable of specifically hybridizing to a second region flanking the other end of the sub-target nucleic acid on the same strand of the target nucleic acid.



Petitioner Illumina, Inc., EX1002, p. 1045

30. (Withdrawn) The method of claim 29, wherein the first and second targeting arms are between about 10 and about 100 nucleotides long.

31-32. (Canceled)

33. (Withdrawn) The method of claim 29, wherein the first and second targeting arms have the same length.

34-35. (Canceled)

36. (Withdrawn) The method of claim 29, wherein the hybridization Tms of the first and second targeting arms are identical.

37. (Withdrawn) The method of claim 28, wherein the sub-target nucleic acid contains a nucleic acid repeat.

38. (Withdrawn) The method of claim 37, wherein the nucleic acid repeat is a dinucleotide or trinucleotide repeat.

39. (Canceled)

40. (Withdrawn) The method of claim 37, wherein the sub-target nucleic acid is a region of the Fragile-X locus that contains a nucleic acid repeat.

41. (Withdrawn) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is immediately adjacent to a region of nucleic acid repeats.



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42. (Withdrawn) The method of claim 29, wherein one or both targeting arms hybridize to a region on the target nucleic acid that is separated from a region of nucleic acid repeats by a region that does not contain any nucleic acid repeats.

43. (Withdrawn) The method of claim 29, wherein the molecular inversion probe further comprises a primer-binding region that can be used to sequence the captured sub-target nucleic acid and optionally the first and/or second targeting arm.

44. (Withdrawn) The method of claim 20, wherein a plurality of different target nucleic acids are analyzed in a biological sample.

45. (Withdrawn) The method of claim 44, wherein the plurality of target nucleic acids are analyzed using a plurality of different molecular inversion probes.

46. (Withdrawn) The method of claim 45, wherein each different molecular inversion probe comprises a different pair of first and second targeting arms at each of the 3' and 5' ends.

47. (Withdrawn) The method of claim 46, wherein each different molecular inversion probe comprises the same primer-binding sequence.

48-49. (Canceled)

50. (Withdrawn) The method of claim 20, wherein the capture efficiency is evaluated by determining an amount of target nucleic acid that is captured.

51. (Withdrawn) The method of claim 50, wherein the amount of target nucleic acid that is captured is determined by determining a number of independently captured target nucleic acid molecules.

52. (Withdrawn) The method of claim 50, wherein the amount of target nucleic acid that is captured is compared to a reference amount of captured nucleic acid.

53. (Withdrawn) The method of claim 52, wherein the reference amount is determined by determining a number of independently captured molecules of a reference nucleic acid.

54. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of a different locus in the biological sample that is not suspected of containing a deletion or insertion.

55. (Withdrawn) The method of claim 53, wherein the reference nucleic acid is a nucleic acid of known size and amount that is added to the capture reaction.

56. (Withdrawn) The method of claim 20, wherein a subject is identified as having an insertion or deletion in one or more alleles of the genetic locus if the capture efficiency is statistically significantly different that the reference capture efficiency.

57-67. (Canceled)

Remarks

Claims 1-4 are pending in the application. Claims 1 and 2 are amended to address several indefiniteness rejections. Support for the amendments can be found in the claims as-filed, and in the application at page 32, line 24, through page 33, line 29, and page 69, line 32, through page 70, line 12. No new matter is added.

Rejections under 35 U.S.C. §112

The Office Action rejects claims 1-4 under 35 U.S.C. §112, first paragraph, for introducing new matter. The Examiner alleges that the specification "does not clearly envision the use of probes of different concentration for a single loc[us]." Applicants respectfully submit that the present claims are fully supported by the specification.

The claims do not recite "probes of different concentration for a single loc[us]," as alleged by the Examiner. However, claim 1 does recite contacting a target nucleic acid with "a plurality of different molecular inversion probes . . . wherein at least two of the plurality of molecular inversion probes are present at different concentrations."

The Application states, "the amount of probe used per assay may be optimized for a particular application." Application, page 70, lines 7-8. That "optimization" can include adjusting the "ratio (molar ratio, for example measured as a concentration ratio) of probe." *Id.* at 70, lines 8-9. Therefore, the as-filed application supports the recitation of "at least two of the plurality of molecular inversion probes are present at different concentrations."

The Office Action rejects claims 1-4 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention. The Office Action states on page 7 that "it is unclear if the intent of the claim is to require analyzing a plurality of probes to a single loc[us] . . . or require analyzing a plurality of loci." The Office Action states on page 8 that it is "unclear if there is a single target nucleic acid to which the molecular inversion probe is being hybridized or if the intent is the 3' region and 5' region of each probe is hybridizing to a different nucleic acid." The Office Action states on page

8 that it is "unclear if . . . 'a plurality of nucleic acids in a sample' is defining the sample as a plurality of target nucleic acids or if 'in a sample with a plurality of different molecular inversion probes' is defining the sample." The Office Action states on page 9 that "the plurality of molecular inversion probes is in the sample prior to the contacting step . . . does not logically make sense." Regarding claim 2, the Office Action states on page 9 that "it is unclear how one subregion can entirely overlap . . . and yet be different."

Claims 1 and 2 have been amended to remove the allegedly indefinite language. Accordingly, Applicants submit that the 35 U.S.C. §112 rejections are overcome.

Rejections under 35 U.S.C. §103

The Office Action maintains the rejections of claims 1-4 under 35 U.S.C. §103(a) over the combination of Akhras (PLOS one, 2007, 2(9):e915), Nilsson (Trends in Biotechnology, 2006, 24:83-88), Hardenbol (Nature Biotechnology, 2003, 21:673-78), Mockler (Genomics, 2005, 85:1-15), Porreca (Nature Methods, 2007, 4:931-36), Hardenbol (2006/0019304), and Browne (2006/0246500). Applicants submit that the present claims are not made obvious by any combination of those references.

The present claims require that at least two of the plurality of molecular inversion probes are present at different concentrations in the probe set. The as-filed application, at page 67, line 28, through page 69, line 2, addresses the problem that bias results when alleles are present in a sample at ratios other than 50:50. As explained at page 69, line 28, through page 70, line 23, such bias can be normalized by using probes at different concentrations in the sample, thereby enhancing under-represented targets and suppressing over-represented targets. None of the cited references disclose or suggest normalizing bias by using two different concentrations of probes in a single sample.

Akhras, Nilsson, Hardenbol (2003), and Mockler are all limited to reporting the use of padlock probes or general tiling strategies. Akhras reports a connector inversion probe method. Nilsson reports padlock probes that form circles with gaps between the 5' and 3' terminal



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nucleotides. Hardenbol (2003) reports padlock probes that hybridize to a target in a manner such that a 5' terminal nucleotide and a 3' terminal nucleotide are separated from each other by only a single nucleotide. Mockler reports general tiling strategies using linear probes. None of those references disclose or suggest varying concentrations of probes in a sample to account for biases associated with alleles that do not have 50:50 ratios.

The three references that the Examiner alleges report "probes at two or more concentrations" are Porreca, Hardenbol (2006), and Browne. Applicants disagree with the Examiner's interpretation of those references. None of those references reports probes at two or more concentrations in the same reaction.

Porreca reports a method for gene amplification using molecular inversion probes. *See* Porreca, page 932, col 1, lines 11-25. Porreca notes that some base calls were skewed away from the expected 50:50 ratio. Porreca suggests solving that problem by saturating the reaction mixture with an excess of all reactants and not by using different probe concentrations. *Id.* at 934, col 2, lines 30-59. At page 16, the Examiner refers to Porreca's statement that "[i]f either the targeting oligos or genomic DNA can be defined as limiting, one could potentially hybridize with an excess of the complement, that is, to saturation, such that small differences in capture efficiency would be 'normalized' away." Again Porreca describes saturating the reaction mixture with either excess probe or excess genomic DNA rather than having two different probe concentrations.

Hardenbol (2006) addresses concentration as a factor in hybridization assay design. *See, e.g.,* Hardenbol (2006), paragraphs [0038] and [0048]. Hardenbol (2006) mentions probe concentration as a standard parameter in a hybridization assay, but never discloses or suggests using two different probe concentrations in an assay. The protocols described do not contemplate varying probe concentration to account for biases due to alleles bring present at ratios other than 50:50. Accordingly, Hardenbol (2006) cannot properly be read to disclose or suggest using different probe concentrations in a reaction to address the problem of bias occurring at loci with alleles having ratios that deviate from 50:50.

On page 16, the Examiner points to a passage in Hardenbol (2006) related to probe concentration: "at a given probe concentration there is a finite rate at which the probe sequences will hybridize to the genomic templates. Probe concentrations can be adjusted to some extent to vary this rate." However, that passage merely reports adjusting the concentration of probe to vary the hybridization rate. It fails to say anything about varying concentration between two probes in a reaction.

On page 17, the Examiner points to a passage in Hardenbol that states: "[t]he number of loci in each hybridization-based assay can vary widely, and its upper limit depends on well-known factors, including . . . probe concentrations." Again, that passage fails to disclose varying concentrations between two probes in a reaction. Instead, that passage is about varying concentrations between different assays. The Examiner fails to distinguish adjusting concentrations among different assays from varying probe concentrations within an assay. The Examiner at pages 17-18 further points to a passage of Hardenbol that states:

> Usually there is a one-to-one correspondence between the number of hybridization-based assay products combined in accordance with the invention and the number of different genomic samples being analyzed; however, the invention comprehends situations where different numbers of samples from different individuals are analyzed by varying numbers of hybridization-based assays, the products of which are combined. The level of such multiplexing depends on the capacity of the readout device and the number of loci measured by each hybridization-based assay.

Once again, that passage fails to disclose two or more probe concentrations in a single assay. Instead, that passage indicates that often each sample being analyzed in a multiplex assay has a set of assay products associated with it. Hardenbol merely notes that an assay can be performed that combines a different number of samples and hybridization-based assay products (i.e., not the same number as each other). In other words, a set of assay products can analyze two or more different samples and therefore the assay can have a different number of samples and hybridization-based assay products. But that passage in no way reports different concentrations between two probes in an assay.

The Office Action alleges that Browne demonstrates dual inversion probes at different concentrations in FIG. 6. However, Browne FIG. 6 merely shows the results of a series of separate and discrete assays, each using different probe concentrations. *See* Browne, paragraph [0114]. For any single assay in Browne, the probe concentrations are the same. Browne does not disclose or suggest an assay using different probe concentrations in the same assay. Browne would have no reason to report more than one probe concentration in a single assay because Browne is not concerned with controlling for bias. Instead, Browne is interested in probe concentration merely to minimize signal-to-noise ratio in fluorescent probe hybridization assays. Browne is attempting to control background fluorescence, not accounting for biases resulting from allele fractions that are not 50:50.

The Examiner cites Porreca, Hardenbol (2006), and Browne as containing the element of two or more probe concentrations in a single assay, but as explained above, all of those references in fact fail to disclose or suggest that element. The Examiner alleges that using two or more probe concentrations is "merely using known methods to optimize conditions," and yet fails to cite a single reference where that element is disclosed. Because the cited art cannot be combined to achieve the present claims, the proposed combination does not make the claims obvious.

Applicants respectfully request withdrawal of the rejections under 35 U.S.C. §103(a).



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Summary

On the basis of the foregoing reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: September 14, 2015

Respectfully submitted,
**BROWN RUDNICK LLP**

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com



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# Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
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Alexandria, VA 22313-1450

| | |
|---|---|
| Application Number | 13/266862 |
| Filing Date | March 13, 2012 |
| First Named Inventor | Porreca, Gregory |
| Art Unit | 1634 |
| Examiner Name | Pohnert, Steven C. |
| Attorney Docket Number | GSGE-002/01US 29258/20 |

**This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.**
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, to any international application that does not compy with the requirements of 35 U.S.C 371, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO on page 2.)

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

   a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

      i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

      ii. ☐ Other _____

   b. ☑ Enclosed

      i. ☑ Amendment/Reply

      ii. ☐ Affidavit(s)/ Declaration(s)

      iii. ☐ Information Disclosure Statement (IDS)

      iv. ☐ Other _____

2. Miscellaneous

   a. ☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

   b. ☐ Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

   a. ☑ The Director is hereby authorized to charge the following fees, any underpayment of fees, or credit any overpayments, to Deposit Account No. 50-0369.

      i. ☑ RCE fee required under 37 CFR 1.17(e)

      ii. ☑ Extension of time fee (37 CFR 1.136 and 1.17)

      iii. ☐ Other _____

   b. ☐ Check in the amount of $ _____ enclosed

   c. ☐ Payment by credit card (Form PTO-2038 enclosed)

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***SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED***

| | | | |
|---|---|---|---|
| Signature | /Thomas C. Meyers/ | Date | September 14, 2015 |
| Name (Print/Type) | Thomas C. Meyers | Registration No. | 36989 |

***CERTIFICATE OF MAILING OR TRANSMISSION***

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below.

| | | | |
|---|---|---|---|
| Signature | | | |
| Name (Print/Type) | | Date | |

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

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# Instruction Sheet for RCEs

(not to be submitted to the USPTO)

## *NOTES:*

An RCE is not a new application, and filing an RCE will not result in an application being accorded a new filing date.

**Filing Qualifications:**

The application must be a utility or plant application filed on or after June 8, 1995. The application cannot be a provisional application, a utility or plant application filed before June 8, 1995, an international application that does not comply with the requirements of 35 U.S.C. 371, a design application, or a patent under reexamination. See 37 CFR 1.114(e). An international application does not comply with the requirements of 35 U.S.C. 371 until the requirements under 35 U.S.C. 371(c), including the requirement for the inventor's oath or declaration under 35 U.S.C. 371(c)(4), have been complied with.

**Filing Requirements:**

***Prosecution in the application must be closed.*** Prosecution is closed if the application is under appeal, or the last Office action is a final action, a notice of allowance, or an action that otherwise closes prosecution in the application (e.g., an Office action under *Ex parte Quayle* ). See 37 CFR 1.114(b).

***A submission and a fee are required at the time the RCE is filed.*** If reply to an Office action under 35 U.S.C. 132 is outstanding (e.g., the application is under final rejection), the submission must meet the reply requirements of 37 CFR 1.111. If there is no outstanding Office action, the submission can be an information disclosure statement, an amendment, new arguments, or new evidence. See 37 CFR 1.114(c). The submission may be a previously filed amendment ( e.g., an amendment after final rejection).

## WARNINGS:

**Request for Suspension of Action:**

All RCE filing requirements must be met before suspension of action is granted. A request for a suspension of action under 37 C FR 1.103(c) does not satisfy the submission requirement and does not permit the filing of the required submission to be suspended.

**Improper RCE will NOT toll Any Time Period:**

***Before Appeal*** **-** If the RCE is improper (e.g., prosecution in the application is not closed or the submission or fee has not been filed) and the application is not under appeal, the time period set forth in the last Office action will continue to run and the application will be abandoned after the statutory time period has expired if a reply to the Office action is not timely filed. No additional time will be given to correct the improper RCE.

***Under Appeal*** **-** If the RCE is improper (e.g., the submission or the fee has not been filed) and the application is under appeal, the improper RCE is effective to withdraw the appeal. Withdrawal of the appeal results in the allowance or abandonment of the application depending on the status of the claims. If there are no allowed claims, the application is abandoned. If there is at least one allowed claim, the application will be passed to issue on the allowed claim(s). See MPEP 1215.01.

**See MPEP 706.07(h) for further information on the RCE practice.**



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The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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| **PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)** | Docket Number (Optional) GSGE-002/01US 29258/20 |
|---|---|
| Application Number 13/266,862 | Filed March 13, 2012 |
| For METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS | |
| Art Unit 1634 | Examiner Pohnert, Steven C. |

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

| | | Fee | Small Entity Fee | Micro Entity Fee | |
|---|---|---|---|---|---|
| ☐ | One month (37 CFR 1.17(a)(1)) | $200 | $100 | $50 | $ |
| ☐ | Two months (37 CFR 1.17(a)(2)) | $600 | $300 | $150 | $ |
| ☑ | Three months (37 CFR 1.17(a)(3)) | $1,400 | $700 | $350 | $ 700.00 |
| ☐ | Four months (37 CFR 1.17(a)(4)) | $2,200 | $1,100 | $550 | $ |
| ☐ | Five months (37 CFR 1.17(a)(5)) | $3,000 | $1,500 | $750 | $ |

☑ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-0369.

☑ Payment made via EFS-Web.

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I am the

☐ applicant.

☑ attorney or agent of record. Registration number 36989.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

| /Thomas C. Meyers/ | September 14, 2015 |
|---|---|
| Signature | Date |
| Thomas C. Meyers | (617) 856-8483 |
| Typed or printed name | Telephone Number |

**NOTE:** This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☑ * Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

| Electronic Patent Application Fee Transmittal | |
|---|---|
| **Application Number:** | 13266862 |
| **Filing Date:** | 13-Mar-2012 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Filer:** | Thomas C. Meyers/Lauren Poon |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| Filed as Small Entity | |
| **Filing Fees for U.S. National Stage under 35 USC 371** | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| Extension - 3 months with $0 paid | 2253 | 1 | 700 | 700 |
| **Miscellaneous:** | | | | |
| RCE- 2nd and Subsequent Request | 2820 | 1 | 850 | 850 |
| | | **Total in USD ($)** | | **1550** |

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 23592190 |
| **Application Number:** | 13266862 |
| **International Application Number:** | |
| **Confirmation Number:** | 6082 |
| **Title of Invention:** | METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS |
| **First Named Inventor/Applicant Name:** | Gregory Porreca |
| **Customer Number:** | 21710 |
| **Filer:** | Thomas C. Meyers/Lauren Poon |
| **Filer Authorized By:** | Thomas C. Meyers |
| **Attorney Docket Number:** | GSGE-002/01US 29258/20 |
| **Receipt Date:** | 24-SEP-2015 |
| **Filing Date:** | 13-MAR-2012 |
| **Time Stamp:** | 15:57:18 |
| **Application Type:** | U.S. National Stage under 35 USC 371 |

## Payment information:

| Submitted with Payment | no |
|---|---|

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Non Patent Literature | Diep-2012-NM.pdf | 3494246<br>8a1b2ab1fbf1ffbcbe4e754d6fba7241ed841587 | no | 29 |

| Warnings: |
|---|
| Information: |

| | | | | | |
|---|---|---|---|---|---|
| 2 | Non Patent Literature | Flaschker-2007-JIMD.pdf | 1349465<br>7091062611b80f90faf1367fac7b7d7ca71631e5 | no | 7 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Non Patent Literature | Saihan-2009-COiN.pdf | 1801272<br>4de21ade17b8ea391cea6060f4a2a9e434e76eb9 | no | 9 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Information Disclosure Statement (IDS) Form (SB08) | GSGE-002-01US-SuppIDS.pdf | 1125940<br>f5cde2ef6e266555e0f69c65e3627bf4b08f8e87 | no | 4 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| | | **Total Files Size (in bytes):** | 7770923 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**<u>New Applications Under 35 U.S.C. 111</u>**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**<u>National Stage of an International Application under 35 U.S.C. 371</u>**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**<u>New International Application Filed with the USPTO as a Receiving Office</u>**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

**CERTIFICATION STATEMENT**

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

**OR**

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

**SIGNATURE**

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

| | | | |
|---|---|---|---|
| Signature | /Thomas C. Meyers/ | Date (YYYY-MM-DD) | 2015-09-24 |
| Name/Print | Thomas C. Meyers | Registration Number | 36989 |

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

## Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
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# INFORMATION DISCLOSURE STATEMENT BY APPLICANT

**( Not for submission under 37 CFR 1.99)**

| | |
|---|---|
| Application Number | 13266862 |
| Filing Date | 2012-03-13 |
| First Named Inventor | Gregory Porreca |
| Art Unit | 1634 |
| Examiner Name | S. C. Pohnert |
| Attorney Docket Number | GSGE-002/01US 29258/20 |

**U.S.PATENTS**

| Examiner Initial* | Cite No | Patent Number | Kind Code[1] | Issue Date | Name of Patentee or Applicant of cited Document | Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear |
|---|---|---|---|---|---|---|
| | 1 | | | | | |

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**U.S.PATENT APPLICATION PUBLICATIONS**

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|---|---|---|---|---|---|---|
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**FOREIGN PATENT DOCUMENTS**

| Examiner Initial* | Cite No | Foreign Document Number[3] | Country Code[2] | Kind Code[4] | Publication Date | Name of Patentee or Applicant of cited Document | Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear | T[5] |
|---|---|---|---|---|---|---|---|---|
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**NON-PATENT LITERATURE DOCUMENTS**

| Examiner Initials* | Cite No | Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published. | T[5] |
|---|---|---|---|

| INFORMATION DISCLOSURE STATEMENT BY APPLICANT ( Not for submission under 37 CFR 1.99) | | |
|---|---|---|
| | Application Number | 13266862 |
| | Filing Date | 2012-03-13 |
| | First Named Inventor | Gregory Porreca |
| | Art Unit | 1634 |
| | Examiner Name | S. C. Pohnert |
| | Attorney Docket Number | GSGE-002/01US 29258/20 |

| | | | |
|---|---|---|---|
| | 1 | Diep et al., 2012, Library-free methylation sequencing with bisulfite padlock probes, Nature Methods 9:270-272 (and supplemental information) | ☐ |
| | 2 | Flaschker et al., 2007, Description of the mutations in 15 subjects with variant forms of maple syrup urine disease, J Inherit Metab Dis 30:903-909 | ☐ |
| | 3 | Saihan et all., 2009, Update on Usher syndrome, Current Opinion in Neurology 22:19-27 | ☐ |

If you wish to add additional non-patent literature document citation information please click the Add button

**EXAMINER SIGNATURE**

| Examiner Signature | | Date Considered | |
|---|---|---|---|

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 13/266,862 | 03/13/2012 | Gregory Porreca | GSGE-002/01US 29258/20 | 6082 |

21710 7590 01/22/2016

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

| EXAMINER | |
|---|---|
| POHNERT, STEVEN C | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1634 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 01/22/2016 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

| Office Action Summary | Application No. 13/266,862 | Applicant(s) PORRECA ET AL. | |
|---|---|---|---|
| | Examiner STEVEN POHNERT | Art Unit 1634 | AIA (First Inventor to File) Status No |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *9/24/2015*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims***

5)☒ Claim(s) *1-4,12-16,20-30,33,36-38,40-47 and 50-56* is/are pending in the application.

5a) Of the above claim(s) *5-11, 17-19,31-32, 34-35, 39-38, 48-49 and 57-67* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-4* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *4/8/2013* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.



Petitioner Illumina, Inc., EX1002, p. 1069

## DETAILED ACTION

### *Notice of Pre-AIA or AIA Status*

1. The present application is being examined under the pre-AIA first to invent provisions.

### *Continued Examination Under 37 CFR 1.114*

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/14/2015 has been entered.

### *Claim Status and Formal matters*

This action is in response to papers filed 1/29/2015.

Claim 1 has been amended.

Claims 1-4, 12-16, 20-30, 33, 36-38, 40-47 and 50-56 are pending.

Claims 5-11, 17-19,31-32, 34-35, 39-38, 48-49 and 57-67 have been canceled.

Claims 12-16, 20-30, 33, 36-38, 40-47 and 50-56 withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention , there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 12/3/2012.

Claims 1-4 are being examined.

***Priority***

The instant application was filed 3/13/2012 and is a national stage entry of PCT/US10/01293 filed 4/30/2010 and claims priority to provisional application 61/147,470 filed 4/30/2009; provisional application 61/147923 filed 5/1/2009; provisional application 61179358 filed 5/18/2009; and 61/182,089 filed 5/28/2009.

3. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) or the first paragraph of pre-AIA 35 U.S.C. 112, except for the best mode requirement. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994)

The disclosure of the prior-filed application, Application Nos. 61/174,470, 61/174,923, 61/179,358, 61/182, 089, fails to provide adequate support or enablement in the manner provided by 35 U.S.C. 112(a) or pre-AIA 35 U.S.C. 112, first paragraph for one or more claims of this application. Application Nos. 61/174,470, 61/174,923, 61/179,358, 61/182, 089, fail to provide support for, "wherein at least two of the plurality of molecular inventions probes are present at different concentrations. "Thus the instant

claims are being given priority to 4/30/2010. The filing date of the instant application as a PCT.

***Claim Rejections - 35 USC § 112***

4. The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

***5.*** Claims 1-4 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 1 has been amended to recite, “wherein at least two of the plurality of molecular inversion probes are present at different concentrations. “ The response asserts the amendment is supported on pages 69 line 28 to page 70 line 23 which states:

According to aspects of the invention, such biases can be detected or overcome by systematically combining different capture and/or analytical techniques in an assay that interrogates a plurality of loci in a plurality of subject samples.

Accordingly, it should be appreciated that in any of the embodiments described herein (e.g., tiling/staggering, tagging, size-detection, sensitivity enhancing algorithms, or any combination thereof), aspects of the invention involve preparing genomic nucleic acid and/or contacting them with one or more different probes (e.g., capture probes, hybridization probes, MIPs, others etc.). In some embodiments, the amount of genomic nucleic acid used per subject ranges from 1 ng to 10 micrograms (e.g., 500 ng to 5 micrograms). However, higher or lower amounts (e.g., less than 1 ng, more than 10 micrograms, 10-50 micrograms, 50-100 micrograms or more) may be used. In some embodiments, for each locus of interest, the amount of probe used per assay may be optimized for a particular application. In some embodiments, the ratio (molar ratio, for example measured as a concentration ratio) of probe to genome equivalent (e.g., haploid or diploid genome equivalent, for example for each allele or for both alleles of a nucleic acid target or locus of interest)ranges from 1/100, 1/10, 1/1, 10/1, 100/1, 1000/1. However, lower, higher, or intermediate ratios may be used.

In some embodiments, the amount of target nucleic acid and probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios. In some embodiments, in order to normalize genomic DNA and probe, the genomic DNA concentration is read using a standard spectrophotometer or by fluorescence (e.g., using a fluorescent intercalating dye). The probe concentration may be determined experimentally or using information specified by the probe manufacturer.

Similarly, once a locus has been captured (e.g., on a MIP or other probe or in another form), it may be amplified and/or sequenced in a reaction involving one or more primers. The amount of primer added for each reaction can range from 0.1 pmol to 1 nmol, 0.15 pmol to 1.5 nmol (for example around 1.5 pmol). However, other amounts (e.g., lower, higher, or intermediate amounts) may be used.

The specification teaches on page 3, “In some embodiments, sources of error and ambiguity in one or more steps can be addressed by capturing and/or interrogating each target locus of interest with at least one set of probes.”

Thus the specification teaches varying the concentration of probes between different loci, but does not clearly envision the use of probes of different concentration for a single loci as the active steps of the claims suggest. Thus the amendment has introduced new matter.

**Response to Arguments**

The response traverses the rejection asserting, “Claim 1 has been amended to clarify that the different probes are in a single sample at different concentrations. The amendment is supported by the as-filed application. For example, the as-filed application teaches varying the concentration of probes at a locus. For example, at page 70, lines 6-8, the application teaches that "for each locus of interest, the amount of probe used per assay may be optimized for a particular application." The application continues, at page 70, lines 13-15, stating that "the amount of target nucleic acid and probe used for each reaction is normalized to avoid any observed differences being caused by differences in concentrations or ratios." Therefore, the as-filed application supports the recitation of "at least two of the plurality of molecular inversion probes are present at different concentrations in the sample".” This argument has been thoroughly reviewed but is not considered persuasive as the instant claims require that each subregion of a probe overlaps with another subregion of the probe and thus is to a single loci. The specification does not envision the use of different probes at a single loci.

6. The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 1-4 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly

claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The Board has held, “If a claim is amenable to two or more plausible constructions, applicant is required to amend the claim to more precisely define the metes and bounds of the claimed invention or the claim is indefinite under §112, ¶2.” Ex parte Miyazaki, 89 USPQ2d 1207 (BPAI 2008) (expanded panel).

The US Supreme Court in NAUTILUS, INC. v. BIOSIG INSTRUMENTS, INC. (2014) held, “we hold that a patent is invalid for indefiniteness if its claims, read in light of the specification delineating the patent, and the prosecution history, fail to inform, with reasonable certainty, those skilled in the art about the scope of the invention.”

The preamble of the instant claim is drawn to a method of analyzing a plurality of genetic loci. The contacting step requires, “contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region that comprises a 5' terminal nucleotide and a 3' region that comprises a 3' terminal nucleotide, the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion, wherein when hybridized to the target nucleic acid in a circular configuration, the 5' terminal nucleotide and the 3' terminal nucleotide of each probe are separated by a plurality of nucleotides on the target nucleic acid, and wherein at least two of the plurality of molecular inversion probes are present at different concentrations.” Thus all probes of contacting step of

the claim and thus the rest of the active steps require the probes overlap and thus it is unclear if the intent of the claim is to require analyzing a plurality of probes to a single loci as the active steps of the claims suggest or require analyzing a plurality of loci and how this relates to the claims as they require overlapping probes and thus are target to a single loci.

Claim 1 further recites, “the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid.” The claim appears to require a single target nucleic acid, but sequences to “nucleic acids flanking one of a plurality of subregions.” Thus the metes and bounds of the claim are unclear if there is a single target nucleic acid to which the molecular inversion probe is being hybridized or if the intent is the 3’ region and 5’ region of each probe is hybridizing to a different nucleic acid. The claims should be amended to clarify the relationship between the 3’ region and 5’ region of the probes as to whether they are hybridizing a single target polynucleotide or different nucleic acids.

Claim 1 was amended in the response of 1/29/2015 to recite, “contacting each of a plurality of target nucleic acids in a sample with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region that comprises a 5' terminal nucleotide and a 3' region that comprises a 3' terminal nucleotide, the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion, wherein when hybridized to the target nucleic acid in a circular configuration,

the 5' terminal nucleotide and the 3' terminal nucleotide of each probe are separated by a plurality of nucleotides on the target nucleic acid, and wherein at least two of the plurality of molecular inversion probes are present at different concentrations in the sample." It is first unclear if the intent is to require "a plurality of nucleic acids in a sample" is defining the sample as a plurality of target nucleic acids or if "in a sample with a plurality of different molecular inversion probes" is defining the sample. Further the recitation of " wherein at least two of the plurality of molecular inversion probes are present at different concentrations in the sample" is unclear as the literal reading is that that at least of the plurality of molecular inversion probes is in the sample prior to the contacting step, which does not logically make sense. If the intent is to require probes at different concentration to be mixed with the sample, the claim should be amended to require the contacting step results in a reaction mixture or hybridization reaction and amending the wherein clause to be consistent with the contacting step.

Thus claims 2-4 are rejected as they depend from claim 1.

Claim 2 requires, "wherein at least one subregion entirely overlaps at least one other subregion." Claim 1 requires, "the 5' region and 3' region being complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion,." Thus it is unclear how one subregion can entirely overlap as required by claim 2 and yet be different as required in claim 1. Thus the metes and bounds of the claim are unclear.

**Response to Arguments**

The response provides no specific arguments with respect to the 112-2nd paragraph rejections. Further the amendment has raised new issues.

***Claim Rejections - 35 USC § 103***

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhras et al (PLOS one (2007) volume 2 (9) e915) (IDS 3/28/2013), Nilsson et al (Trends in Biotechnology (2006 ) volume 24, pages 83-88), Hardenbohl et al (Nature Biotechnology(2003) volume 21, pages 673-678), Mockler et al (Genomics (2005) volume 85, pages 1-15), Porecca et al ( Nature methods (2007) volume 4, pages 931-936), Hardenbohl et al (USPGPUB 2006/0019304) and Browne et al (US PGPUB 20060246500).

MPEP 2111 states:

> The court held that the PTO is not required, in the course of prosecution, to interpret claims in applications in the same manner as a court would interpret claims in an infringement suit. Rather, the "PTO applies to verbiage of the proposed claims the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in applicant's specification."

MPEP 2111.01 states

> Though understanding the claim language may be aided by explanations contained in the written description, it is important not to import into a claim limitations that are not part of the claim.

It is noted that the specification lacks a limiting definition of a molecular inversion probe. Thus "molecular inversion probe" is being given the broadest reasonable interpretation in view of the claims and art as a probe that allows for formation of a circular probe upon hybridization to a target nucleic acid.

It is further noted the 112-2nd issues are considered in rendering this rejection.

Akhras teaches a connector inversion probe method (CIPer) that is a combination of Gap-LCR and padlock probes. Akhras teaches, “The Connector Inversion Probe (CIPer) reaction (Figure 1) shares some basic design features with a recently described assay, the PathogenMip assay [17], which includes: i) probe hybridization, single “G” base gap fill and probe circularization, ii) exonuclease based circular DNA selection, iii) probe inversion by re-linearization, and iv) universal PCR amplification. The distinguishing and key feature of the CIPer method is a modified first reaction step involving a complete filling of an extensive gap formed by the probe and target.” Akhaus further teaches, " Connector Inversion Probe (CIPer) technology is a powerful method for multiplex amplification, and has been validated here in genetic pathogen diagnostics, typing, and antibiotic resistance detection arenas as a proof-of-concept. This technology can be designed and optimized for use in numerous other scientific areas and applications. Other areas suitable for use of the CIPer method include cancer genomics [32], human SNP detection [15], and selective exon amplification [7], among numerous other promising applications. Modified CIPers may also in the future offer an alternative strategy for gateway cloning [33]. In this paper, we focused on “lowplex” systems amplifying shorter stretches of DNA of up to 100 base

pair lengths. To achieve higher degrees of multiplexing and longer clone reads, one must take into account i) clone GC content, ii) clone length variability, and iii) steric effects for longer clones. Analog amplification and downstream validation are delimiting factors for multiplex detection methods. We believe that the future of CIPer technology will rely on digital amplification [34] and high throughput sequencing validation (Figure S5). The CIPers universal primer sites can be formatted to fit any amplification method, e.g. RCA [12], [13], emulsion PCR [34], SPIA [35] LAMP [36] and SMAP 2 [37]; universal adaptor ligation, a tedious and sample-consuming procedure [38], is therefore optional. Proposed digital amplification methods include emulsion PCR, RCA, or a combination of the two [39]. Suitable upcoming high throughput validation methods include next-generation sequence systems such as the 454 Life Sciences platform [38] (http://www.454.com), the Solexa platform [40] (http://www.illumina.com), polony sequencing [41] (http://www.agentcourt.com), Helicos Biosciences [42] (http://www.helicosbio.com), or use of a resequencing array (GeneChip CustomSeq, www.affymetrix.com). Recent digital quantification methods [43] open the possibility for quantitative CIPer technology. With the addition of a molecular barcode to the padlocks [14], MIPs were used for ultra high-throughput SNP detection based on microarray barcode hybridization [15]. Because CIPers can also include a barcode, they could be used for microarray validation, or amplicon tracing with sequencing methods [17], [44] allowing for longer read lengths [38]. With the introduction of a third universal sequence flanking the barcode and the universal primer sites, one can begin to envision a two-dimension informational barcode, a concept we call “multiplexing multiplex padlocks”

(MMP) (Figure S6). Probe construction based on ligation of three smaller constructs [45] would allow for variability in fragment assembly. By combining the one-genotype detection pair of AS/ES constructs with multiple unique barcodes, we can create patient and genotype specific probes with one barcode per probe. This will allow for analysis of multiple parallel samples, essentially lowering the cost of microarray chips and sequencing reactions."

Nilsson teaches the use of padlock probes and "selector" probes that form circles with gaps of several nucleotides between the 5' terminal nucleotide and the 3' terminal nucleotide.

Akhras and Nilsson demonstrate probes were known that result in a circular probe upon hybridization to a target sequence with a gap between the 3' terminal nucleotide and 5' terminal nucleotide were known. Akhras suggests the use of CIPer methods similar to the use of MIPs, including the use of "barcodes to allow detection by microarrays."

Akhras and Nilsson do not specifically teach, "contacting each of a plurality of target nucleic acids with a plurality of different molecular inversion probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, wherein each subregion overlaps with at least one other subregion."

However, Hardenbol discloses a method of analyzing a plurality of genetic loci by contacting each of a plurality of target nucleic acids with a probe set (abstract, pg

673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1). Hardenbohl teaches these probes are MIP or padlock probes.

Hardenbohl teaches in figure 2b the joining of the molecular inversion probes which results in a circular type configuration. Hardenbohl exemplifies in fig 2b a probe that has a 5' region and a 5' terminal nucleotide, a 3' region and a 3' terminal nucleotide, and a single base gap. Thus the 5' terminal nucleotide, 3' terminal nucleotide and single base gap are a plurality of nucleotides between the 3' and 5' region of the probe.

Hardenbohl does not specifically teach wherein each subregion overlaps with at least one other subregion. Hardenbohl does not specifically teach, "wherein at least one subregion entirely overlaps at least one subregion."

Mockler, teaches each subregion overlaps with at least one other subregion (Fig. 2a).

With regards to claim 2, Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other Subregion)).

With regards to claim 3, Mockler teaches the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

With regards to claim 4, Mockler teaches sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the CIPer or selector probes taught by Akhras and Nilsson with a gap of a plurality of nucleotides between the 5' terminal nucleotide and 3' terminal of the padlock probes with tiling probes of Mockler in the method of Hardenbol. The artisan would be motivated as Akhras suggests the use of CIPer methods in MIP methods. The teachings of Hardenbohl set forth one embodiment of the use of MIP or padlock probes. The artisan further would be motivated as Mockler teaches the probe tiling approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1). The artisan would have a reasonable expectation of success as the artisan is merely increasing the number of nucleotides between the 3' terminal nucleotide and 5' terminal nucleotide of the probes of Mockler, which Akhras

demonstrates were known and worked. Thus the combination of references suggests all the limitations of claims and suggests the use of CIPer in place of the similar MIP methods.

Akhras, Nilsson, Hardenbohl, and Mockler do not specifically teach the use of probes at two or more concentrations.

However, Porecca teaches a method of amplifying human exons using MIPS. Porecca teaches, “It is both concerning and interesting that the resequencing data show skewing of base-calls at heterozygous positions away from the expected ratio of 0.5 (Fig. 4). This skewing might be expected if stochastic events (for example, a finite number of capture events during the multiplex capture reaction) are a primary source of bias. Given that the concentration of individual targeting oligos in the capture reaction is very low (B0.36 pM per species), it is likely that their hybridization to genomic DNA is inefficient. This problem might therefore be mitigated simply by increasing the concentration of reactants. If either the targeting oligos or genomic DNA can be defined as limiting, one could potentially hybridize with an excess of the complement, that is, to saturation, such that small differences in capture efficiency would be ‘normalized’ away.” Porecca further teaches, “After characterizing the efficiency of individual capture oligos in each reaction, a second iteration could be carried out, in which capture oligos performing similarly to one another are grouped together within the same oligo mixture (thereby maximizing uniformity within any single reaction).”

Further, Hardenbohl(PGPUB) teaches, “Genomic material is complex and thus individual sequences exist at relatively low concentrations in a mixture. As a result of

this, at a given probe concentration there is a finite rate at which the probe sequences will hybridize to the genomic templates. Probe concentrations can be adjusted to some extent to vary this rate, but as probe complexity is increased, for example, when highly multiplexed assays are used, such flexibility is greatly reduced" (003). Hardenbohl continues, "Multiplexing occurs at two levels in the invention. First, hybridization-based assays are designed to measure characteristics of one or more target polynucleotides, such as genomic fragments, at multiple loci. The number of loci measured in each hybridization-based assay can vary widely, and its upper limit depends on well-known factors, including the capacity of the readout device being employed, trade-offs selected between probe concentrations and reaction times, the genetic characteristic being measured (for example, trait association studies require very large numbers of measurements; genetic identification requires relative few measurements; testing for adverse drug reactions may require a medium number of measurements, e.g. several tens to hundreds), and the like. In one aspect, the number of loci analyzed in each hybridization-based assay is in the range of from a few tens, e.g. 10 to 20, to many thousands, e.g. fifty to a hundred thousand. In another aspect, the number of loci per hybridization-based assay is in the range of from 10 to 40,000; or it is in the range of from 100 to 30,000; or it is in the range of from 100 to 20,000. Second, multiplexing occurs from the combination of assay products from multiple hybridization-based assays for determining multiple signals on a single readout device. Usually, there is a one-to-one correspondence between the number of hybridization-based assay products combined in accordance with the invention and the number of different genomic

samples being analyzed; however, the invention comprehends situations where different numbers of samples from different individuals are analyzed by varying numbers of hybridization-based assays, the products of which are combined. The level of such multiplexing depends of the capacity of the readout device and the number of loci measured by each hybridization-based assay. In one aspect, the number of hybridization-based assays that may be multiplexed is simply the capacity of a readout device, e.g. the number of features on a microarray, divided by the number of loci being measured in the hybridization-based assays. For example, in one aspect, a 100 thousand-feature microarray can serve as a readout device for fifty hybridization based assays that each measure the zygosity of one thousand loci, when two features are required for each determination (e.g. homozygous in allele 1, homozygous in allele 2, or heterozygous in allele 1 and 2). As discussed more fully below, particular trade-offs between the use of microarray features, different colored fluorescent dyes, number of loci measured per hybridization-based assay, capacity of a readout device, and the like, are routine design choices for those of ordinary skill in the art."

Hardenbohl (PGPUB) continues, "Operation of one embodiment of the invention is illustrated in FIGS. 1A and 1B for a particular hybridization-based assay (one based on the template-driven ligation of two oligonucleotide components). Hybridization-based assays are carried out separately on a sample of DNA from genome G.sub.1 and a sample of DNA from genome G.sub.2. Probes p.sub.1 to p.sub.K (100) containing oligonucleotide tags t.sub.1 to t.sub.K, respectively, and probes p.sub.1 to p.sub.K (102) containing oligonucleotide tags t.sub.K+1 to t.sub.2K, respectively, are combined in

separate reaction mixtures with genome G.sub.1 DNA and genome G.sub.2 DNA, respectively, under conditions that permit specific hybridization of such probes to their respective DNA stands (104) and (106) containing target loci, L.sub.1 through L.sub.K ((108) to (110) for genome G.sub.1 and (112) to (114) for genome G.sub.2). After an incubation time depending on parameters well-known to those of ordinary skill, e.g. target complexity, probe concentration, temperature, and the like, discussed more fully below, probe-genome complexes form ((118) and (116), respectively) that include recognition structures for enzyme(s) of subsequent step(s)."

Finally, Browne demonstrates the use of dual inversion probes at different concentrations in figure 6.

As stated in the MPEP, 2144.05 II, "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)."

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of Akhras, Nilsson, Hardenbohl, and Mockler to include at probes at different concentrations in a sample to optimize hybridization and assay conditions in a sample. The artisan would be motivated as Porecca, Hardenbohl(PGPUB), and Brown recognize that probe concentration is an optimizable variable in the speed and performance of hybridization assays including molecular inversion probe assays. Thus it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to

include additional probes at different concentrations so as to optimize efficiency of hybridization as well as speed of hybridization in a sample. The artisan would have a reasonable expectation of success as the artisan is merely using known methods to optimize conditions.

**Response to Arguments**

The response traverses the instant rejection asserting, "Porreca reports a method for gene amplification using molecular inversion probes. See Porreca, page 932, col 1, lines 11-25. Porreca notes that some base calls were skewed away from the expected 50:50 ratio. Porreca suggests solving that problem by saturating the reaction mixture with an excess of all reactants. Id. at 934, col 2, lines 30-59. Porreca does not disclose or suggest using different probe concentrations in a single sample." This argument has been thoroughly reviewed but is not considered persuasive as Porreca teaches, "Given that the concentration of individual targeting oligos in the capture reaction is very low ( ~ 0.36 pM per species), it is likely that their hybridization to genomic DNA is inefficient. This problem might therefore be mitigated simply by increasing the concentration of reactants. If either the targeting oligos or genomic DNA can be defined as limiting, one could potentially hybridize with an excess of the complement, that is, to saturation, such that small differences in capture efficiency would be 'normalized' away".(emphasis added by the examiner). Thus Porrecca suggest that individual targeting oligos may be rate limiting and can be increased. In the context of the paragraph rendering obvious varying conditions of probes. Further this argument is arguing the teachings of Porrecca alone. In response to applicant's

arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The response continues by asserting Hardenbol does not specifically disclose or suggest probes of different concentration in a sample. This argument has been thoroughly reviewed but is not considered persuasive as Hardenbohl clearly envisions that probe concentration is a hybridization parameter that can be optimized. Further this argument is arguing the teachings of Hardenbohl alone. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The response continues by asserting Browne does not teach probe concentration is a single sample. This argument has been thoroughly reviewed but is not considered persuasive as the response is arguing the teachings of Browne alone. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The rejection is maintained as the prior art as a whole demonstrates that varying probe concentrations is an optimizable factor in hybridization and specifically in

inversion probes. Thus the art recognizes the problem of probe concentration on hybridization and recognizes that varying the probe concentration can be done to improve the results of the assay.

**Summary**

No claims are allowed.

***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:00-5:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/STEVEN POHNERT/
Primary Examiner, Art Unit 1634