Electronic Acknowledgement Receipt

EFS ID:	37483420
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
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Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
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Application Type:	Utility under 35 USC 111(a)

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.)
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National Stage of an International Application under 35 U.S.C. 371

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Electronic Acknowledgement Receipt

EFS ID:	37483742
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
Filing Date:	28-FEB-2019
Time Stamp:	13:06:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

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Information:					
		Total Files Size (in bytes):	92851839		

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.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Scott Bortner
201 Industrial Road
Suite 410
San Carlos, California 94070
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 W19	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US14/51926	20 August 2014 (20.08.2014)	20 August 2013 (20.08.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2014.01)
CPC - C12Q 1/6883, 1/6827, 1/6816

Applicant NATERA, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [X] 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	17 November 2014 (17.11.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/51926

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/US14/51926

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. 5-14

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. 5-14 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 5-14 are dependent claims whch are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ 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. 5-14

☐ 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 mannerceptable 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 mannerceptable 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/US14/51926

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	YES
	Claims	1	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-4	NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by US 2013/0196862 A1 to Rabinowitz, et al. (hereinafter 'Rabinowitz'862').

Regarding claim 1, Rabinowitz'862 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), determining if the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is within the lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]), and generating a report (generating a ploidy status report; paragraphs [0232], [0510]) indicating that an invasive genetic analysis procedure should be performed (report with results for determining need for amniocentesis; paragraph [0034]).

Claim 2 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz'862.

Regarding claim 2, Rabinowitz'862 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), determining if the ratio (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample to maternal cell free DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is in lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]), and performing an invasive genetic test (performing amniocentesis; paragraph [0034]). Rabinowitz'862 does not disclose, in a single, concise embodiment, wherein the invasive genetic test is performed if the fetal fraction is in lowest 1 percentile of a reference population. However, since Rabinowitz'862 discloses determining a fetal fraction in lowest 1 percentile of a reference and performing confirmative, invasive studies, such as amniocentesis, both as disclosed above, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have implemented the use of the separate disclosures of Rabinowitz'862, for including the invasive test step if warranted by results indicative of a fetal DNA fraction, which is in lowest 1 percentile of a reference population, through routine experimentation and testing.

-***-Continued on Next Supplemental Page-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/51926

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:

Claim 3 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz'682 in view of US 2012/0270212 A1 to Rabinowitz et al. (hereinafter 'Rabinowitz'212').

Regarding claim 3, Rabinowitz'862 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), determining if the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is within the lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]), and performing an invasive genetic test (performing amniocentesis; paragraph [0034]) if the fetal fraction is in lowest 1 percentile of a reference population (report with results for determining need for amniocentesis; paragraph [0034]; if fetal DNA is low in a mixed sample; paragraph [0105]). Rabinowitz'682 does not disclose the steps of: obtaining a plurality blood samples from a test population of pregnant human subjects, measuring the fetal fraction of the cell free fetal DNA in the blood samples, determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing an invasive genetic test on at least one member of the population subset. Rabinowitz'212 discloses obtaining a plurality blood samples from a test population of pregnant human subjects (blood samples derived from four euploid and two trisomy-indicated pregnancies; paragraph [0508]), and measuring the fetal fraction of the cell free fetal DNA in the blood samples (estimating fetal DNA fractions; paragraph [0062]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Rabinowitz'682, for implementing the step of determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing an invasive genetic test on at least one member of the population subset, since the data on the cell-free fetal fraction of DNA obtained from analysis of a plurality of blood samples from a test population of pregnant human subjects, as previously disclosed by Rabinowitz'212, would have allowed individuals exhibiting fetal fractions in the lowest 1 percentile to be selected for performing an invasive genetic test, as previously disclose by Rabinowitz'682 (paragraph [0034]).

Claim 4 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz'682 and further in view of Rabinowitz'212 and US 2013/0190653 A1 (ALVAREZ-RAMOS).

Regarding claim 4, Rabinowitz'682 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), and determining if the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is within the lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]). Rabinowitz'682 does not disclose obtaining a plurality blood samples from a test population of pregnant human subjects, measuring the fetal fraction of the cell free fetal DNA in the blood sample to maternal cell free DNA in the blood samples, determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing a test for a detached placenta on at least one member of the population subset. Rabinowitz'212 discloses obtaining a plurality blood samples from a test population of pregnant human subjects (blood samples derived from four euploid and two trisomy-indicated pregnancies; paragraph [0508]), and measuring the fetal fraction of the cell free fetal DNA in the blood samples (estimating fetal DNA fractions; paragraph [0062]). Alvarez-Ramos discloses blood sample collections appropriate for pregnancies susceptible to placental detachment (blood sampling to prevent placental detachment; abstract; paragraphs [0033], [0036]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Rabinowitz'682, for implementing the steps of determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing a test for a detached placenta on at least one member of the population subset, since the data on the cell-free fetal fraction of DNA obtained from analysis of a plurality of blood samples from a test population of pregnant human subjects, as disclosed by Rabinowitz'212, would have allowed individuals exhibiting fetal fractions in the lowest 1 percentile to be selected for testing for a detached placenta, as disclosed by Alvarez-Ramos, on at least one member of the population subset, as determined by the analysis for low fetal DNA fractions, as previously disclosed by Rabinowitz'682.

Claims 1-4 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
VICKI L. HEALY
NATERA, INC.
201 INDUSTRIAL ROAD
SUITE 410
SAN CARLOS, CA 94070

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)* 18 FEB 2014	
Applicant's or agent's file reference W16	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 13/57924	International filing date *(day/month/year)* 04 September 2013 (04.09.2013)
Applicant NATERA, 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.

   Where? Directly to the International Bureau of WIPO, 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. 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. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   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.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference W16	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 13/57924	International filing date *(day/month/year)* 04 September 2013 (04.09.2013)	(Earliest) Priority Date *(day/month/year)* 04 September 2012 (04.09.2012)
Applicant NATERA, 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/US 13/57924

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.: 5-29
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:

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.:

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 13/57924

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C40B 20/00 (2013.01)
USPC - 506/2,12; 702/1,19; 435/6.1,7.2
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) -C40B 20/00 (2013.01)
USPC - 506/2,12; 702/1,19; 435/6.1,7.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Patents, non-patent literature, search term limited

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase (pgpb, uspt, usoc, epab, jpab, dwpi, tdbd), Dialog Proquest (npl), Google Patents (pl, npl), Google scholar (pl, npl); WIPO (npl); Search Terms: enrich, fetal, increase, fraction, dna, pregnant, prenatal, maternal, blood, serum, plasma, noninvasive, non-invasive, carbohydrate, diet, drink, food, stimulant, natera, nutritious, testing, conc

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 5,716,776 A (BOGART) 10 February 1998 (10.02.1998) entire document, especially pg 3, ln 64-pg 4, ln 4, 34-38	31 --- 1-4, 30-34
A	US 2011/0251149 A1 (PERRINE, et al.) 13 October 2011 (13.10.2011) entire document, especially para [0008]	1-4, 30-34
A	US 2008/0138809 A1 (KAPUR, et al.) 12 June 2008 (12.06.2008) entire document, para [0005], [0009]	1-4, 30-34
A	Ashoor, Ghalia, et al. "Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: effect of maternal and fetal factors." Fetal diagnosis and therapy May 4, 2012, 237-243.	1-4, 30-34

☐ 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 January 2014 (23.01.2014)	18 FEB 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: VICKI L. HEALY
NATERA, INC.
201 INDUSTRIAL ROAD
SUITE 410
SAN CARLOS, CA 94070

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	18 FEB 2014

Applicant's or agent's file reference	FOR FURTHER ACTION
W16	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 13/57924	04 September 2013 (04.09.2013)	04 September 2012 (04.09.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 20/00 (2013.01)
USPC - 506/2,12; 702/1,19; 435/6.1,7.2

Applicant NATERA, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [x] 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	23 January 2014 (23.01.2014)	Lee W. Young PCT Helpdesk: 571-272-4300 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 13/57924

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 13/57924

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. 5-29

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. 5-29 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 5-29 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ 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. 5-29

☐ 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 13/57924

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	1-4, 30, 32-34	YES
	Claims	31	NO
Inventive step (IS)	Claims	1-4, 30, 32-34	YES
	Claims	31	NO
Industrial applicability (IA)	Claims	1-4, 30-34	YES
	Claims	None	NO

2. Citations and explanations:

Claim 31 lacks novelty under PCT Article 33(2) as being anticipated by US 5,716,776 A (Bogart).

As to claim 31, Bogart discloses the use of (ii) a stimulant (col 4, ln 34-38) for increasing the fetal fraction in the blood, plasma, or serum of a pregnant woman (col 3, ln 64 to pg 4, ln 4).

Claims 1-4, 30 and 32-34 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the claimed subject matter, specifically increasing the fetal fraction in the blood, plasma, serum of a pregnant woman by administering a nutritious composition or stimulant prior to obtaining the sample.

The best prior art on record that discloses increased fetal fractions in prenatal testing are below:

US 5,716,776 A (Bogart) discloses the use of a stimulant (pg 4, ln 34-38) to increase the fetal fraction in the blood of a pregnant woman (col 3, ln 64 to pg 4, ln 4), but does not disclose wherein the administration of the stimulant occurs before the blood is taken from the pregnant woman.

US 2011/0251149 A1 to Perrine, et al. (hereinafter 'Perrine') discloses increasing the percentage of fetal hemoglobin in the blood of subject by administering a substance to the subject (para [0008]), but does not disclose wherein the substance is a stimulant or a nutritious composition or wherein the increase is used in prenatal testing.

US 2008/0138809 A1 to Kapur, et al. (hereinafter 'Kapur') discloses the enrichment of fetal cells from blood samples that are used in prenatal testing (para [0005] and [0009]), but does not disclose wherein the increase occurs before the blood is taken from the pregnant woman.

The article titled "Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: Effect of maternal and fetal factors" by Ashoor, et al. (hereinafter 'Ashoor') discloses wherein the fetal fraction in maternal plasma cfDNA increases with serum PAPP-A and free B-hCG and decreases with maternal weight and wherein (pg 1, 1st paragraph) and wherein the fetal fraction is used in prenatal testing (pg 1, 2nd paragraph), but does not disclose administering any substance to a pregnant woman to increase the fetal fraction before the sample is taken. There is no prior art on record that discloses the claimed subject matter, specifically increasing the fetal fraction in the blood, plasma, serum of a pregnant woman by administering a nutritious composition or stimulant prior to obtaining the sample. Thus, claims 1-4, 30 and 32-34 meet the criteria set out in PCT Article 33(2)-(3).

Claims 1-4 and 30-34 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in the industry.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Ivor R. Elrifi
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo PC
One Financial Center
Boston, Massachusetts 02111

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 SEP 2007

Applicant's or agent's file reference	FOR FURTHER ACTION
35506-501-061	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 06/45281	22 November 2006 (22.11.2006)	26 November 2005 (26.11.2005)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06F 19/00 (2007.01)
USPC - 702/20

Applicant Gene Security Network, LLC

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.

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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	02 May 2007 (02.05.2007)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 06/45261

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. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/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

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto 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.

4. Additional comments:

NeoGenomics
Exhibit 1003 Page 942

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 06/45281

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	5, 15, 18, 26-27, 29, 32-35, 43	YES
	Claims	1-4, 6-14, 16-17, 19-25, 28, 30-31, 36-42, 44-61	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-61	NO
Industrial applicability (IA)	Claims	1-61	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4, 6-14, 16-17, 19-25, 28, 30-31, 36-42 and 44 lack novelty under PCT Article 33(2) as being anticipated by WO 03/031646 A1 to Findlay et al. (hereinafter 'Findlay).

As to claims 1 and 28, Findlay teaches a method for determining genetic data of a target individual based on the imperfect knowledge of the target individual's genetic data, and knowledge of the genetic data of one or more individuals genetically related to the target (see pg 14, ln 1 - pg 16, ln 27), the method comprising:
(i) creating a set of one or more hypotheses concerning which segments of which chromosomes from the related individual(s) correspond to those segments found in the target individual's genome (appropriate chromosome) (see pg 8, ln 16-19).
(ii) determining the probability of each of the hypotheses (genetic identification, human embryo identification, forensics, genetic identification by DNA fingerprinting) given the measurements of the target individual's genetic data and of the related individual's genetic data (see pg 9, ln 1-4 and pg 12, ln 26-28); and
(iii) using the probabilities associated with each hypothesis to determine the most likely state of the actual genetic material (heterozygosity index, genetic marker selection) of the target individual (see pg 7, ln 25 pg 8, in4).

As to claim 2, Findlay teaches the method of claim 1, wherein the method involves determining which regions of the parental chromosomes have the maximum likelihood of having contributed to the formation of the gametes that contributed to the target individual, based on the measurements of genetic data of the target and determination of the likelihood of those particular measurements given the genetic data of the parents (see pg 7, ln 25 - pg 8, ln 4).

As to claim 3, Findlay teaches the haplotypes of at least one of the parents have been determined by using genetic data measured from the parent's diploid sample, and the genetic data measured from a haploid sample from the parent which is used to determine which alleles measured from the diploid sample belong to which haplotype (see table 6b).

As to claim 4, Findlay teaches the genetic data from genetically related individuals are taken from a group comprising genetic data from a diploid maternal sample, a haploid maternal sample, a diploid paternal sample, and a haploid paternal sample (see pg 20, ln 11-24).

As to claim 6-7, Findlay teaches the method of claim 1, wherein the genetic data from the genetically related individual are taken from a group comprising genetic data from diploid maternal cell, diploid paternal cells, and diploid cells from a related individual known to be a carrier of the phenotype in question (see Fig 4-7 and pg 6, ln 23 - pg 7, ln 3).

As to claim 8-11, Findlay teaches:
the genetically related individuals are taken from a group consisting of the father, mother, twin1, twin2 (see table 6a, 6b and pg 6, ln 8-12);
the target individual is a human embryo (see pg 9, ln 1-2);
one or more of the individual's genetic data has been amplified using tools and/or techniques taken from the group comprising Polymerase Chain Reaction (PCR) (see pg 9, ln 6-8);
the individual's genetic data has been measured using fluorescent in-situ hybridization (FISH) (see pg 17, ln 1-2).

As to claim 12, Findlay teaches the method of claim 1, where one or more of the individual's genetic data has been measured by analyzing substances taken from the group comprising the individual's bulk diploid tissue, one or more diploid cells taken from the individual, one or more blastocysts taken from the individual, the individual's semen, the individual's egg, extra cellular genetic material from the individual found in maternal blood, genetic material known to have originated from the individual and combinations thereof (nucleic acids isolated from fetal cells) (see pg 14, ln 1-7).

As to claims 13-14 Findlay teaches one or more of the related individual's genetic data is partly or wholly known in silico, or is provided by parties other than those determining the target individual's genetic data; and where the haploid genetic data of one or more of the individuals is partly or wholly generated in silico by means of a computer algorithm that simulates haploid data from diploid data (forensic, pathology, genetic diagnosis, and evolutionary genetics) (see pg 22, ln 18-19).

(See Supplemental Box - Reasoned Statement)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 06/45281

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. V.2 - Citations and explanations

As to claim 16-17, Findlay teaches the method of claim 1, wherein the determination of the target's genetic data is used for the purpose of embryo selection in the context of in-vitro fertilization and wherein the determination of the target's genetic data is used for the purpose of prenatal genetic diagnosis (see pg 19, ln 15-30).

As to claim 19-23, Findlay teaches the determination of the target's genetic data is used for the purpose of making phenotypic predictions (sex diagnosis, trisomy diagnosis), and
where the likelihood of displaying some or all of the phenotypes is affected by other previously known phenotypic information; and wherein the determination of the target's genetic data is used for the purpose of making phenotypic predictions;
where the predictions are made by comparing the target's genetic data to known genetic markers found in the public domain;
wherein the determination of the target's genetic data is used for the purpose of making clinical decisions;
wherein the determination of the target's genetic data is used, in combination with phenotypic markers for the purpose of making clinical decisions;
wherein the determination of the target's genetic data is used for the purpose of screening for susceptibly to one or more diseases, where no family history of the disease is present (see pg 19, ln 16-30).

As to claim 24, Findlay teaches the determination of the target's genetic data is used for the purpose of screening for susceptibility to one or more phenotypes, where some or all of the phenotypes are multigenic (trialielic) (see pg 8, ln 1-2).

As to claim 25, Findlay teaches the knowledge of the target's genetic data is known to or suspected to contain spurious data from contaminating DNA or RNA (semen from multiple sources are present) (see pg 18, ln 29 - pg 19, ln 7).

As to claims 30 and 42, Findlay teaches a system and method wherein the measurement of multiple loci on a given segment of a given chromosome of a target individual is used to determine the number of instances of the given segment in the genome of the target individual, the method comprising:
(i) creating a set of one or more hypotheses about the number of instances of the given segment present in the genome of the target individual,
(ii) measuring the amount of genetic data for some of all of the possible alleles (number of alleles)at a plurality of loci (number of polymorphic loci) on the given segment,
(iii) determining the relative probability of each of the hypotheses given the measurements of the target individual's genetic data and possibly also the related individual's genetic data, and
(iv) using the relative probability (frequency distribution in a population) associated with each hypothesis to determine the most likely state of the actual genetic material of the target individual (see pg 22, ln 20-26).

As to claim 31, Findlay teaches the method of claim 30, where the determination of the number of instances of segments of chromosomes that are present in the target's genome is done in the context of screening for trisomy (see Fig 14 and pg 7, ln 16-17).

As to claim 36, Findlay teaches the method of claim30, where the target individual is a human embryo (see pg 9, ln 1-2).

As to claim 37, Findlay teaches the method of claim 30, wherein the target individual's genetic data has been amplified using Polymerase Chain Reaction (PCR) (see pg 9, ln 6-8).

As to claim 38, Findlay teaches the method of claim 30, wherein the target individual's genetic data has been measured using florescent in-situ hybridization (FISH), and combinations thereof (see pg 17, ln 1-2).

As to claim 39, Findlay teaches the method of claim 30, wherein the target individual's genetic data has been measured by analyzing substances taken from the extra-cellular genetic material found in maternal blood, cells (see pg 36, ln 26).

As to claims 40-41, Findlay teaches the method of claim 30,
wherein the determination of the number of chromosomes or chromosome segments in the target is used for the purpose of embryo selection in the context of in-vitro fertilization (preimplantation genetic diagnosis);
wherein the determination of the number of chromosomes or chromosome segments of the target is used for the purpose of prenatal genetic diagnosis (see pg 19, ln 15-30).

As to claim 44, Findlay teaches a method for determining the genetic data for a target individual, and also the number of instances of chromosomes or segments of chromosomes that are present in the target's genome,base on the imperfect knowledge of the target individual's genetic data, and knowledge of the genetic data of one or more individuals genetically related to the target (see pg 14, ln 1 - pg 16, ln 27), the method comprising:
(i) creating a set of one or more hypotheses about which segments of which chromosomes from the related individuals correspond to those segments found in the target individual's genome (appropriate chromosome) (see pg 8, ln 16-19),
(ii) creating a set of one or more hypotheses about the number of a given chromosomal segment present in the genome of the target,
(iii) measuring the amount of genetic data for each of the possible alleles at a plurality of loci on the given segment,
(iv) determining the relative probability of each of the hypotheses (genetic identification, human embryo identification, forensics, genetic identification by DNA fingerprinting) given the measurements of the target individual's genetic data and of the related individual's genetic data (see pg 9, ln 1-4 and pg 12, ln 26-28); and
(v) using the relative probabilities associated with each hypothesis to determine the most likely state (heterozygosity index, genetic marker selection)of the actual genetic material of the target individual (see pg 7, ln 25 - pg 8, ln 4).

(See Supplemental Box - Reasoned Statement)

NeoGenomics
Exhibit 1003 Page 944

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 06/45281

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. V Citations and explanations

Claims 45-61 lack novelty under PCT Article 33(2) as being anticipated by US 2004/0137470 A1 (Dhallan).

As to claims 45 and 60, Dhallan teaches a system and method for making predictions regarding an individual, comprising:
(i) constructing models based on contingency tables built from publicly available information about gene-disease associations (the sequence of multiple loci of interest that cause or presuppose to a disease phenotype can be determined) (para [0591]); and (ii) applying models to make the predictions by operating on data pertaining to the individual (the number of mutant cells in the sample is used to determine patient prognosis, the degree and severity of the disease) (see para [0879]).

As to claims 46-48, Dhallan teaches an accuracy of the contingency tables that employ multiple independent variables can be refined using outcome data where only a subset of those independent variables was measured (the sequence of multiple loci of interest that cause or predispose to a disease phenotype can be determined) (see para [0591]);
wherein an accuracy of the contingency tables that employ multiple independent variables can be refined using data about the association of the independent variables (wild type DNA sequence and mutant DNA sequence) (see para [0879]);
wherein an accuracy of the contingency tables that employ multiple independent variable (independently varying) can be refined using data about the frequency of occurrence of certain values of the independent variables (see para [2645]).

As to claims 50, 52, 54, 56 and 58, Dhallan teaches the type of data pertaining to the individual comprises data taken from a group consisting of the individual's genotypic data (the number of mutant cells in the sample), the individual's phenotypic data, the individual's clinical data, and the individual's laboratory data (see para [0879]);
where the type of data also consists of data of a pathogen infecting the individual (detect an allele from a non-pathogenic bacterial strain amongst alleles from a pathogenic bacterial strain) (see para [0350]);
where said predictions concern topics selected from the group consisting of the individual's phenotypes, phenotype susceptibilities, possible clinical outcomes, lifestyle decisions, physical exercise, dietary habits, hormonal supplements, nutritional supplements, treatments for a disease, treatments for a pathogen, treatments for an undesirable condition, treatments with pharmaceuticals, and combinations thereof (see para [0828]);
where said predictions are used to generate a report for the individual or for an agent of the individual (see para [0226]); and
wherein the act of operating comprises operating on new data to update the individual's predictions where the data is taken from a group comprising new research data or new aggregated data on other subjects (loci of interest can be combined and treated as a single sample) (see para [0591]).

As to claims 49 and 61 Dhallan teaches a system and method for making predictions (detection of genetic disorders) regarding a first individual, comprising:
(i) creating and testing a plurality of models and using aggregated data from a second set of individuals for whom the outcome to be predicted is known;
(ii) calculating the relative accuracies of the various models for making the prediction given the data available to the first individual (quantitating a ratio for the alleles at a locus of interest) ; and
(iii) using the model that is identified as the most accurate to make a prediction for the first individual (see Abstract).

As to claims 51, 53, 55, 57 and 59, Dhallan teaches where the type data pertaining to the individual comprises data taken from a group consisting of the individual's genotypic data, the individual's phenotypic data, and the individual's clinical data, and the individual's laboratory data;
where the type of data also consists of data of a pathogen infecting the individual (see para [0350]);
where said predictions concern topics selected from the group consisting of the individual's phenotypes, phenotype susceptibilities, possible clinical outcomes, lifestyle decisions, physical exercise, mental exercise, dietary habits, hormonal supplements, nutritional supplements, treatments for a disease, treatments for a pathogen, treatments for an undesirable condition, treatments with pharmaceuticals, and combinations thereof (see para [0006]-[0029]);
where said predictions are used to generate a report for the individual or for an agent of the individual (see para [2260]);
wherein the act of operating comprises operating on new data to update the individual's predictions where the data is taken from a group comprising new research data or new aggregated data on other subjects (see table XV, Diagnostic Applications).

Claims 5 and 26-27 lack an inventive step under PCT Article 33(3) as being obvious over Findlay in view of Dhallan.

As to claims 5 and 26-27, Findlay teaches a confidence (prenatal diagnosis) is computed for each of the individual cells in the the cleaned embryonic genetic data (cells isolated from amniotic fluid) (see pg 14, ln 1-9 and pg 22, ln 4-28). Findlay does not specifically teach the prenatal diagnosis cells are SNP cells, or that the genetic data of one or more of the individuals include a plurality of SNPs. Dhallan teaches
fetal chromosomal abnormalities are determined by analyzing SNPs (see para [2693]);
the genetic data of one or more of the individuals includes the allele calls for a plurality of SNPs and the confidence with which each SNP is known (see fig 3A-3C and para [0101]); and
wherein the confidence in the SNP calls of the target individual is determined by computing the odds ratio of the probabilities that the SNP is correctly versus incorrectly called (SNP sites analyzed on chromosome 13 are present in the expected 50:50 ratio) (see Fig 12 and para [0117]).
It would have been obvious to one of ordinary skill in the art to combine the gene marker and amplification process as taught by Findlay with the methods for detection of genetic disorders as taught by Dhallan, because Dhallan teaches clinical techniques and practical applications to use a gene marker and amplification system, such as the system taught by Findlay.

(See Supplemental Box)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 06/45261

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. V Citations and explanations

Claims 15, 18, 29, 32-35 and 43 lack an inventive step under PCT Article 33(3) as being obvious over Findlay in view of US 2004/0236518 A1 to Pavlovic et al. (hereinafter 'Pavlovic').

As to claim 15, 18, 29, 32-35 and 43, Findlay teaches a method for determining the genetic data of a target individual. Findlay does not specifically teach the method as part of a computer algorithm, where the method comprises a hidden Markov model, making predictions using statistical models or using matched filtering.
Pavlovic teaches the computer algorithm comprises a hidden Markov model (see Fig 4, 5 and para [0060]); wherein the determination of the target's genetic data is used for the purpose of making predictions of phenotype susceptibility using statistical models and/or expert rules (combination of experts) (see para [013]);
including a computer implemented system configured to accomplish the method of claim 1 (see Fig 1A, 1B and para [0036]-[0037]);
where the determination of the relative probability of each hypothesis is made using the concept of matched filtering and where the determination of the relative probability of each hypothesis is made using quantitative techniques (probability equation) that do not make allele calls and where the mean and standard deviation of the measurement of each locus is either known, unknown, or uniform (see table 7 and para [0031]);
where the determination of the relative probability of each hypothesis is made using qualitative techniques that make use of allele calls; or where the determination of the relative probability of each hypothesis is made by making use of known alleles of reference sequence,and quantitative allele measurements (see para [0042]-[0044]); and a computer-implemented system configured to accomplish the method of claim 30 (see Fig 1A, 1B and para [0036]-[0037]).
It would have been obvious for one of ordinary skill in the art to combine the method for determining the genetic data of an individual as taught by Findlay with the expert system for gene prediction as taught by Pavlovic because a computer modeling and statistical analysis system, such as that taught by Pavlovic would be a required element in a system that fully utilized the genetic data identification system taught by Findlay.

Claims 1-61 have industrial applicability under PCT Article 33(4) as the subject matter claimed can be made or used in industry.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 06/45281

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8): G06F 19/00 (2007.01)
USPC: 702/20
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)
USPC: 702/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 702/1, 19; 705/2

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic Databases Searched: PubWEST (USPT,PGPB,EPAB,JPAB
Search Terms Used: DNA fingerprinting, sparse data, Markov, genetically related, haplotype, genome, alleles, relationship

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 03/031646 A1 (FINDLAY et al) 17 April 2003 (07.04.2003), entire document, especially Fig 4-7, pg 6, ln 8 - pg 36, ln 26, table 6a, 6b	1-4, 6-14, 16-17, 19-25, 28, 30-31, 36-42, 44 --- 5, 15, 18, 26-27, 29, 32-35, 43
X --- Y	US 2004/0137470 A1 (DHALLAN) 15 July 2004 (15.07.2004), entire document, especially Abstract, Fig 3A-3C, 12, para [0006] - [2693]	45-61 --- 5, 26-27
Y	US 2004/0236518 A1 (PAVLOVIC et al.) 25 November 2004 (25.11.2004), entire document, especially Fig 4, 5 and Fig 1A-1B, para [0036] - [0060	15, 18, 29, 32-35, 43

☐ 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
2 May 2007 (02.05.2007)	28 SEP 2007
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2005)

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 35506-501-061	**FOR FURTHER ACTION** See item 4 below	
International application No. PCT/US2006/045281	International filing date *(day/month/year)* 22 November 2006 (22.11.2006)	Priority date *(day/month/year)* 26 November 2005 (26.11.2005)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GENE SECURITY NETWORK LLC		

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 7 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 27 May 2008 (27.05.2008)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Nora Lindner e-mail: pt11.pct@wipo.int

Form PCT/IB/373 (January 2004)

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 35506-503001WO	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2008/003547	International filing date *(day/month/year)* 17 March 2008 (17.03.2008)	Priority date *(day/month/year)* 16 March 2007 (16.03.2007)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GENE SECURITY NETWORK		

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 22 September 2009 (22.09.2009)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pt04.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2008/003547	17.03.2008	16.03.2007

International Patent Classification (IPC) or both national classification and IPC
INV. G06F19/00

Applicant
GENE SECURITY NETWORK

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 usually 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 notifed 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:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Swarén, Peter Telephone No. +31 70 340-4875

Form PCT/ISA/237 (Cover Sheet) (April 2005)

Box No. I Basis of the 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 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☐ on paper

      ☐ in electronic form

   c. time of filing/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 and/or table relating thereto 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:

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:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-6, 8-33

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)	Yes:	Claims	1-6, 8-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6, 8-33
Industrial applicability (IA)	Yes:	Claims	1-6, 8-33
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item IV.

1. The separate inventions/groups of inventions are:

Invention 1: claims 1, 2, 4-6 and 8-16
Implementation details of a method for determining the number of copies of a segment of a chromosome in the genome of a target individual by measurement of genetic data at a plurality of loci on the chromosome segment for the purpose of screening for a chromosomal condition in the context of prenatal genetic diagnosis during in-vitro fertilization embryo selection;

Invention 2: claims 17-33
An allele calling method;

Invention 3: claim 3
Probability determination of genetic measurements by integration of the probabilities over the possible set of parameters that vary across the measurement experiment;

Invention 4: claim 7
Amplification of genetic material.

1.1 They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The common concept linking and shared by the independent claims 1 and 18 is the method steps of incorporating into a dataset genetic data from a target individual and from one or more related individuals and estimating the characteristics of the platform response associated with the dataset, where the platform response may vary from one experiment to another. However, the dataset referred to is solely defined by the content of its information. Therefore, said method step does not go beyond subject-matter covered by the provisions of Rule 67.1(v) PCT and cannot be considered for an inventive step. Estimation of the characteristics of platform responses associated with genetic datasets is commonplace, see the document "QuantiSNP: an objective

Bayes hidden-Markov model to detect and accurately map copy number variation using SNP genotyping data", p.2019, left-hand col., §4. Consequently, said common concept lacks an inventive step and therefore there is no single general inventive concept linking the independent claims, which leads to that lack of unity arises. Furthermore, the method steps of independent claim 1 only specify vague, abstract functions such as creating, incorporating, estimating, computing, determining, etc., which lack any recognised precise technical meanings. Apart from specifying that characteristics of a platform response are estimated, which is already known from the prior art, no further instruction is given as to a technological procedure to be followed to put the method into practice. In fact, the method steps of "creating a set of copy number hypotheses", "incorporating genetic information", "computing the conditional probabilities" and "determining the copy number ... based on the most probable copy number hypothesis" do not go beyond subject-matter covered by the provisions of Rule 67.1(i), (iii) and (v) PCT. Hence, they cannot be considered for an inventive step. Consequently, the subject-matter of claim 1 lacks an inventive step. Therefore, there is no single general inventive concept linking those claims that are dependent on claim 1. In other words, inventions 1-4 are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, and lack of unity arises.

2. Since the appropriate fees were paid only for inventions 1-3, this opinion will be directed only to that subject-matter.

Re Item V.

3. With respect to invention 1 (claims 1, 2, 4-6 and 8-16), reference is made to the following document:

D1: Colella, S. *et al.* QuantiSNP: an objective Bayes hidden-Markov model to detect and accurately map copy number variation using SNP genotyping data. *Nucleic Acids Res.* 35:2013-2025, 2007 (XP002506459).

4. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1, 2, 4-6 and 8-16 does not involve an inventive step in the sense of Article 33(3) PCT.

4.1 The method steps of independent claim 1 only specify vague, abstract functions such as creating, incorporating, estimating, computing, determining, etc., which lack any recognised precise technical meaning. Apart from specifying that the characteristics of a platform response are estimated, which is already known from the document D1 (see p.2019, left-hand col., §4) no further instruction is given as to a technological procedure to be followed to put the method into practice. In fact, the method steps of "creating a set of copy number hypotheses", "incorporating genetic information", "computing the conditional probabilities" and "determining the copy number ... based on the most probable copy number hypothesis" do not go beyond subject-matter covered by the provisions of Rule 67.1(i), (iii) and (v) PCT. Hence, they cannot be considered for an inventive step. Consequently, the subject-matter of claim 1 lacks an inventive step.

4.2 Dependent claims 2, 4-6 and 8-16 do not seem to contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with regard to an inventive step. The reasons are as follows:

Method steps (i), (iii) and (iv) of claim 2 relate to subject-matter covered by the provisions of Rule 67.1(i) and (iii) PCT and cannot therefore be considered for an inventive step. Method step (ii) is already known from D1 (see p.2019, left-hand col., §2).

The additional features of claims 4-6 and 8 are also already known from D1 (see p.2019, left-hand col. §3 - right-hand col., §1).

The additional features of claims 9-12 and 16 relate to obvious implementation choices within the capabilities of the ordinarily skilled person.

The additional features of claims 13-15 appear solely defined by the data and do not

therefore go beyond subject-matter covered by the provisions of Rule 67.1(v) PCT and which cannot be considered for an inventive step.

5. With respect to invention 2 (claims 17-33), reference is in addition made to the following document:

D2: US-A-2006 134 662.

6. The present application does not meet the criteria of Article 33(1) PCT, because also the subject-matter of claims 17-33 does not involve an inventive step in the sense of Article 33(3) PCT.

6.1 The method steps of the independent claim 18 only specify vague, abstract functions such as incorporating, estimating and calculating, which lack any recognised precise technical meaning. Apart from specifying a method to call one or more alleles and that characteristics of a platform response are estimated, which is already known from the document D2 (see §[0017]) no further instruction is given as to a technological procedure to be followed to put the method into practice. In fact, the method steps of "incorporating into a dataset measured genetic data" and "calculating the most likely correct allele calls of the target individual given the genetic data and the platform response characteristics" do not go beyond subject-matter covered by the provisions of Rule 67.1(i), (iii) and (v) PCT. Hence, they cannot be considered for an inventive step. Consequently, the subject-matter of claim 18 lacks an inventive step.

6.2 Dependent claims 19-33 and 17 do not seem to contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with regard to an inventive step. The reasons are as follows:

Method steps (i)-(iii) of claim 19 and steps (ii)-(vi) of claim 20 relate to subject-matter covered by the provisions of Rule 67.1(i) and (iii) PCT and cannot therefore be

NeoGenomics
Exhibit 1003 Page 956

considered for an inventive step.

Step (i) of claim 20 (measurement at a plurality of loci) as well as the additional features of claims 21-27, 30-33 and 17 relate to obvious implementation choices within the capabilities of the ordinarily skilled person.

The additional features of claims 28 and 29 appear solely defined by the data and do not therefore go beyond subject-matter covered by the provisions of Rule 67.1(v) PCT, which cannot be considered for an inventive step.

7. With respect to invention 3 (claim 3), the present application does not meet the criteria of Article 33(1) PCT, because also the subject-matter of claim 3 does not involve an inventive step in the sense of Article 33(3) PCT.

7.1 The additional method steps of claim 3 only specify vague, abstract functions such as creating, determining, integrating and selecting, which lack any recognised precise technical meaning. In fact, the method steps of "creating a set of hypotheses...", "determining the probability...", "integrating the probabilities over the possible set of parameters..." and "selecting those hypotheses that have the highest probabilities" do not go beyond subject-matter covered by the provisions of Rule 67.1(i) and (iii) PCT. Hence, they cannot be considered for an inventive step. Consequently, the subject-matter of claim 3 lacks an inventive step.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/003547

A. CLASSIFICATION OF SUBJECT MATTER
INV. G06F19/00

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

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 practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2007/062164 A (GENE SECURITY NETWORK LLC [US]; RABINOWITZ MATTHEW [US]; BANJEVIC MILE) 31 May 2007 (2007-05-31) the whole document -/--	1-6,8-16

[X] Further documents are listed in the continuation of Box C.

[X] 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 document 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
31 March 2009	15/04/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Swarén, Peter

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/003547

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLELLA STEFANO ET AL: "QuantiSNP: an Objective Bayes Hidden-Markov Model to detect and accurately map copy number variation using SNP genotyping data." NUCLEIC ACIDS RESEARCH 2007, vol. 35, no. 6, 6 March 2007 (2007-03-06), pages 2013-2025, XP002506459 ISSN: 1362-4962	1-6,8-16
A	abstract page 2014, left-hand column, last paragraph - page 2017, left-hand column, last paragraph page 2018, left-hand column, last paragraph - page 2019, right-hand column, paragraph 1 page 2023, left-hand column, paragraph 1	17-33
A	FREEMAN JENNIFER L ET AL: "Copy number variation: new insights in genome diversity." GENOME RESEARCH AUG 2006, vol. 16, no. 8, August 2006 (2006-08), pages 949-961, XP002506460 ISSN: 1088-9051 figure 5	1,2,4-6, 8-16
X	US 2006/134662 A1 (PRATT MARK R [US] ET AL) 22 June 2006 (2006-06-22) abstract; claims 1-4; figures 1,2,5,6 paragraphs [0017], [0022], [0023], [0075], [0078], [0081] - [0092]	17-33
A	BEAUMONT MARK A ET AL: "The Bayesian revolution in genetics." NATURE REVIEWS. GENETICS APR 2004, vol. 5, no. 4, April 2004 (2004-04), pages 251-261, XP002521825 ISSN: 1471-0056 the whole document	1-6,8-33
A	OGINO SHUJI ET AL: "Bayesian analysis and risk assessment in genetic counseling and testing." THE JOURNAL OF MOLECULAR DIAGNOSTICS : JMD FEB 2004, vol. 6, no. 1, February 2004 (2004-02), pages 1-9, XP002521826 ISSN: 1525-1578 the whole document	1-6,8-33

NeoGenomics
Exhibit 1003 Page 959

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/003547

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:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an 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 reportcovers only those claims for which fees were paid, specifically claims Nos.:

1-6,8-33

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2, 4-6 and 8-16

implementation details of a method for determining the number of copies of a segment of a chromosome in the genome of a target individual by measurement of genetic data at a plurality of loci on the chromosome segment for the purpose of screening for a chromosomal condition in the context of prenatal genetic diagnosis during in-vitro fertilization embryo selection;

---

2. claims: 17-33

an allele calling method;

---

3. claim: 3

probability determination of genetic measurements by integration of the probabilities over the possible set of parameters that vary across the measurement experiment;

---

4. claim: 7

amplification of genetic material.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/003547

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007062164 A	31-05-2007	AU 2006318425 A1	31-05-2007
		CA 2632230 A1	31-05-2007
		EP 1960929 A2	27-08-2008
US 2006134662 A1	22-06-2006	NONE	

Form PCT/ISA/210 (patent family annex) (April 2005)

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 118103-010200/PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2009/034506	International filing date *(day/month/year)* 19 February 2009 (19.02.2009)	Priority date *(day/month/year)* 19 February 2008 (19.02.2008)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GENE SECURITY NETWORK, 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 6 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 24 August 2010 (24.08.2010)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Gijsbertus Beijer e-mail: pt02.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	08 JUL 2009
Applicant's or agent's file reference 118103-010200/PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 09/34506	19 February 2009 (19.02.2009)	19 February 2008 (19.02.2008)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 33/48; C12Q 1/68 (2009.01)
USPC - 702/20 435/6

Applicant GENE SECURITY NETWORK, 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.

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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	30 June 2009 (30.06.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/34506

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. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/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 and/or table(s) relating thereto 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 09/34506

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. 4-9, 13-19, 23-32

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. 4-9, 13-19, 23-32 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 4-9, 13-19, 23-32 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ 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. 4-9, 13-19, 23-32

☐ 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).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/34506

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	1-3, 10-12, 20-22	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-3, 10-12, 20-22	NO
Industrial applicability (IA)	Claims	1-3, 10-12, 20-22	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-3 lack an inventive step under PCT Article 33(3) as being obvious over US 2004/0259100 A1 to Gunderson et al. (hereinafter 'Gunderson') in view of US 2003/0228613 A1 to Bornarth et al. (hereinafter 'Bornarth').

Regarding claim 1, Gunderson discloses a method of performing genome amplification on a nucleic acid sample from a subject (abstract), wherein the method comprises:

adding one or more spike-in primers to a nucleic acid sample that targets one or more loci of interest (para [0138]; "Spike-in primers" are not explicitly taught, and are believed to be coined by applicant, where the primers are interpreted to comprise locus-specific primers (see applicant's specification, pg 9, ln 18-19; pg 16, ln 9-10)), and

amplifying the nucleic acid sample using a method for whole genome amplification (para [0164]).

Gunderson does not disclose the decrease in the likelihood of allele drop out at the one or more loci of interest. Bornarth discloses the decrease in the likelihood of allele drop out at the one or more loci of interest (para [0012], [0033]). It would have been obvious to a person skilled in the art to combine the method of Gunderson and the decrease in the likelihood of allele drop out at the one or more loci of interest as taught by Bornarth to provide the claimed method, since Gunderson and Bornarth (abstract) contemplate the amplification of a nucleic acid with the use of primers, wherein Bornarth discloses the decrease in the likelihood of allele drop out at the one or more loci of interest. A person skilled in the art would have readily appreciated the decrease in the likelihood of allele drop out at the one or more loci of interest, because the method of Gunderson as combined with the decrease in likelihood of allele drop out as taught by Bornath would have created a better product (Bornath, para [0257]).

Regarding claim 2, Gunderson further discloses wherein the nucleic acid sample is a single cell (para [0051]).

Regarding claim 3, Gunderson further discloses wherein the amplification is performed using a Whole Genome Amplification (WGA) kit or a Multiple Displacement Amplification (MDA) kit (para [0117], [0164], [0282]).

Claims 10-12 lack an inventive step under PCT Article 33(3) as being obvious over US 2006/0040300 A1 to Dapprich et al. (hereinafter 'Dapprich') in view of the article entitled "A Comparison of Bayesian Methods for Haplotype Reconstruction from Population Genotype Data" by Stephens et al. (hereinafter 'Stephens').

Regarding claim 10, Dapprich discloses a method for determining the haplotype of a DNA sample (para [0030]), wherein the method comprises:

dividing a DNA sample from one or more cells into a plurality of fractions (para [0057] [0111]);

genotyping the DNA sample in each fraction individually (para [0198], [0204]).

Dapprich does not disclose the step of reconstructing the haplotype of the one or more cells based on the genotyping. Stephens discloses reconstructing the haplotype based on the genotyping (pg. 1162, abstract - "inferring haplotypes from genotype data"). It would have been obvious to a person skilled in the art to combine the method of Dapprich and the reconstructing step of Stephens to provide the claimed method, since Dapprich and Stephens contemplate determining the haplotype of a sample, wherein Stephens discloses reconstructing the haplotype from the genotype. A person skilled in the art would have readily appreciated the reconstructing step, because the method of Dapprich as combined with the reconstructing step of Stephens would have provided a more accurate haplotype (Stephens, pg 1167, left column, first paragraph, "PHASE-estimated haplotypes tend to be more similar to the true haplotypes...").

Regarding claim 11, Dapprich further discloses wherein the DNA sample contains DNA from a single cell (para [0057]).

Regarding claim 12, Dapprich further discloses wherein the DNA sample is divided into from two to five fractions (para [0111]).

--- Please see continuation on Supplemental Box ---

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/34506

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V. (2). Citations and Explanations

Claims 20-22 lack an inventive step under PCT Article 33(3) as being obvious over Dapprich in view of US 2005/0272073 A1 to Vaisberg et al. (hereinafter 'Vaisberg').

Regarding claim 20, Dapprich discloses a given chromosome is associated with a known set of alleles (para [0064]), and a method which comprises:
dividing a DNA sample into a plurality of fractions (para [0057], [0111]);
genotyping the DNA sample in each of the fractions (para [0198], [0204]);
determining the number of fractions in which one or more alleles associated with a given chromosome is detected (para [0203]).
Dapprich does not disclose determining the ploidy state of one or more chromosomes of a single cell containing nuclear DNA and the step of using data from the determining step to determine a ploidy state of the given chromosome. Vaisberg discloses determining the ploidy state of one or more chromosomes in a single cell containing DNA (abstract; para [0008]). Vaisberg discloses using data to determine the a ploidy state of a chromosome (para [0006], [0047]). It would have been obvious to a person skilled in the art to combine the method of Dapprich and the determination of a ploidy state as taught by Vaisberg to provide the claimed method, since Dapprich (para [0191]) and Vaisberg (para [0053]) contemplate the creation of a haploid, wherein Vaisberg discloses the determination of a ploidy state and the step of using data to determine the ploidy state of a chromosome. A person skilled in the art would have readily appreciated that determining the ploidy state as combined by Dapprich and Vaisberg would have provided insight into the impact of a stimulus on system of cells (Vaisberg, para [0030]), and therefore it would have been obvious to combine the teachings of Dapprich and Vaisberg to achieve the claimed method.

Regarding claim 21, Dapprich further discloses wherein the DNA sample is divided into from two to five fractions (para [0111]).

Regarding claim 22, Dapprich further discloses wherein a lysing preparation (para [0061]). Neither Dapprich nor Vaisberg teach a recently lysed cell. It would have been obvious to a person skilled in the art to modify the lysing preparation of Dapprich to provide the claimed recently lysed cells, since a lysing preparation involving cells would have created newly and/or recently lysed cells. A person skilled in the art would have readily appreciated recently lysed cells because the sample would have been fresh which would allow for better results when determining the ploidy state.

Claims 1-3, 10-12, 20-22 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/34506

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48; C12Q 1/68 (2009.01)
USPC - 702/20 435/6
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): G01N 33/48; C12Q 1/68 (2009.01)
USPC: 702/20 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Amer. Journal of Human Genetics, Vol 73

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,EPAB,JPAB): amplification, genotyping, fraction, locus specfic primer, ploidy, lysed, haplotype
esp@cenet: gene security, rabinowitz, johnson, locus specific, haplotype, genotyping, ploidy
Google Scholar: amplification, whole genome, multiple displacement, locus specific primers reconstruct haplotype geno

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0259100 A1 (GUNDERSON et al.) 23 December 2004 (23.12.2004), abstract; para [0051], [0117], [0138], [0164], [0282].	1-3
Y	US 2003/0228613 A1 (BORNARTH et al.) 11 December 2003 (11.12.2003), abstract; para [0012], [0033].	1-3
Y	US 2006/0040300 A1 (DAPPRICH et al.) 23 February 2006 (23.02.2006), para [0030], [0057], [0064], [0111], [0191], [0198], [0203], [0204].	10-12, 20-22
Y	STEPHENS et al., A Comparison of Bayesian Methods for Haplotype Reconstruction from Population Genotype Data. Am J Human Genetics, November 2003, Vol 73, No 5, pages 1162-1169. especially page 1162, abstract.	10-12
Y	US 2005/0272073 A1 (VAISBERG et al.) 8 December 2005 (08.12.2005), abstract; para [0006], [0008], [0030], [0047], [0053].	20-22

☐ 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 June 2009 (30.06.2009)	08 JUL 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Fo (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/34506

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.: 4-9, 13-19, 23-32
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:

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.:

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.

NeoGenomics
Exhibit 1003 Page 970

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 118103010301	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2009/045335	International filing date *(day/month/year)* 27 May 2009 (27.05.2009)	Priority date *(day/month/year)* 27 May 2008 (27.05.2008)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GENE SECURITY NETWORK, 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 5 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 30 November 2010 (30.11.2010)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pt04.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
DAVID J. DYKEMAN
GREENBERG TRAURIG
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	27 JUL 2009
Applicant's or agent's file reference 118103010301	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 09/45335	27 May 2009 (27.05.2009)	27 May 2008 (27.05.2008)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61B 17/435 (2009.01)
USPC - 600/34

Applicant GENE SECURITY NETWORK, 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.

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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	16 July 2009 (16.07.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/45335

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [X] 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. type of material
      - [ ] a sequence listing
      - [ ] table(s) related to the sequence listing
   b. format of material
      - [ ] on paper
      - [ ] in electronic form
   c. time of filing/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 and/or table(s) relating thereto 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 09/45335

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. 6-15, 20-32

because:

☒ the said international application, or the said claims Nos. 6-15, 20-32 relate to the following subject matter which does not require an international search *(specify)*:

Claims 6-15 and 20-32 are unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ 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. 6-15, 20-32

☐ 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).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/45335

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	None	YES
	Claims	1-5, 16-19, 33	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-5, 16-19, 33	NO
Industrial applicability (IA)	Claims	1-5, 16-19, 33	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-5, 16-19, and 33 lack novelty under PCT Article 33(2) as being anticipated by the article entitled ' Preimplantation genetic diagnosis and chromosome analysis of blastomeres using comparative genomic hybridization" by Wilton.

As to claim 1, Wilton teaches a method for comparing embryos (Fig. 2; pg 35, para 7; pg 38, para 4-6), the method comprising:
obtaining one or more cells from each embryo in a set of embryos (Fig. 2; pg 38, para 4);
determining one or more subcharacteristics of one or more characteristics of each obtained cell (determination of chromosome length, a subcharacteristic of ploidy; pg 35, para 8); and
estimating the likelihood that each embryo will develop as desired, based on the one or more subcharacteristics of the one or more cells which were obtained from that embryo (pg 38, para 4).

As to claim 2, Wilton teaches further grouping the embryos into groups, wherein each group is defined as having at least one subcharacteristic, wherein groups may contain zero, one or more embryos, and wherein the likelihood that each embryo in a particular group will develop as desired is estimated based on the at least one subcharacteristic (pg 38, para 4-5).

As to claims 3-4, Wilton teaches further relatively characterizing the embryos based on the estimated likelihood of that embryo developing as desired (pg 38, para 4).

As to claim 5, Wilton teaches that the ranking is performed to select at least one embryo for insertion into the uterus (pg 38, para 4).

As to claim 16, Wilton teaches a method of characterizing an embryo for insertion into a uterus (Fig. 2; pg 35, para 7; pg 38, para 4-6), the method comprising:
selecting at least one characteristic (ploidy; pg 35, para 8; pg 38, para 4);
determining a first subcharacteristic of the at least one characteristic of at least one cell from an embryo (chromosome length; Fig. 2; pg 35, para 8);
using the determined first subcharacteristic, predicting a probability of a second cell from the embryo having a second subcharacteristic (predicting probability of chromosomes of remaining cells being normal based on determination of normal chromosomes in analyzed cell; pg 38, para 4); and
characterizing the embryo based on the predicted probability (pg 38, para 4).

As to claim 17, Wilton teaches that the determination step is performed on more than one cells from the embryo (pg 38, para 2).

As to claim 18, Wilton teaches that the predicting step further includes using the determined first subcharacteristic, predicting probabilities of a plurality of cells from the embryo having a plurality of subcharacteristics (predicting probability of remaining cells being normal, i.e., having a plurality of normal chromosomes; pg 38, para 4).

As to claim 19, Wilton teaches that characterizing the embryo includes characterizing the embryo based on all of the predicted probabilities (pg 38, para 4-5).

As to claim 33, Wilton teaches that the first subcharacteristic is the same as the second subcharacteristic (normal chromosomes; pg 38, para 4).

Claims 1-5, 16-19, and 33 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/45335

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61B 17/435 (2009.01)
USPC - 600/34
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: 600/34

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 600/33, 34, 435/325
(text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed
Search terms: preimplantation genetic diagnosis, IVF, in vitro fertilization, embryo, diagnose, probability, ploidy, characteristic, subcharacteristic, single cell

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WILTON. Preimplantation genetic diagnosis and chromosome analysis of blastomeres using comparative genomic hybridization. Hum. Reprod. Update. January-February 2005 (01-02.2005), Vol. 11, No. 1, pages 33-41; Fig. 2; pg 35, para 7-8; pg 38, para 2, 4-6	1-5, 16-19, 33
A	DONOSO et al. Current value of preimplantation genetic aneuploidy screening in IVF. Hum. Reprod. Update January-February 2007 (01-02.2007), Vol. 13, No. 1, pages 15-25	1-5, 16-19, 33
A	STROM et al. Neonatal outcome of preimplantation genetic diagnosis by polar body removal: the first 109 infants. Pediatrics. October 2000 (10.2000), Vol. 106, No. 4, pages 650-653	1-5, 16-19, 33
A	US 2006/0141499 A1 (SHER et al.) 29 June 2006 (29.06.2006)	1-5, 16-19, 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
16 July 2009 (16.07.2009)	27 JUL 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

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 118103010501	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2009/052730	International filing date *(day/month/year)* 04 August 2009 (04.08.2009)	Priority date *(day/month/year)* 04 August 2008 (04.08.2008)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GENE SECURITY NETWORK, 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 6 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 08 February 2011 (08.02.2011)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Yolaine Cussac e-mail: pt05.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 90404

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 SEP 2009
Applicant's or agent's file reference 118103010501	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 09/52730	04 August 2009 (04.08.2009)	04 August 2008 (04.08.2008)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 33/483, C12Q 1/68 (2009.01)
USPC - 435/6; 702/19

Applicant GENE SECURITY NETWORK, 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.

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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	21 September 2009 (21.09.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 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 09/52730

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 09/52730

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	23-26	YES
	Claims	1-22	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-26	NO
Industrial applicability (IA)	Claims	1-26	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-22 lack novelty under PCT Article 33(2) as being anticipated by US 2007/0184467 A1 to Rabinowitz et al. (hereinafter "Rabinowitz").

As to claim 1, Rabinowitz teaches a method for determining a ploidy state of at least one chromosome in a target individual (detect abnormal numbers of chromosomes, sections of chromosomes, and origins of chromosomes. In genetic samples that are aneuploid, have unbalanced translocations, uniparental disomy, or other gross chromosomal abnormalities; para [0068]), the method comprising:
obtaining genetic data from the target individual (genetic data from human subjects, and more specifically on as-yet not implanted embryos or implanted fetuses; para [0029], target cells are isolated, the genetic data contained in those cells are amplified; para [0035]), and from one or more related individuals (the genetic data of an individual can be cleaned using diploid and haploid data from both parents; para [0036]);
creating a set of at least one ploidy state hypothesis (para [0085]) for each of the chromosomes of the target individual (para [0072]);
using one or more expert techniques to determine a statistical probability for each ploidy state hypothesis (para [0085]) in the set, for each expert technique used, given the obtained genetic data (para [0075] - [0084]);
combining, for each ploidy state hypothesis, the statistical probabilities as determined by the one or more expert techniques; and
determining the ploidy state for each of the chromosomes in the target individual based on the combined statistical probabilities of each of the ploidy state hypotheses (para [0074]).

As to claim 2, Rabinowitz teaches the method of claim 1, wherein the related individuals include both parents of the target individual (abstract).

As to claim 3, Rabinowitz teaches the method of claim 1, wherein the related individuals include siblings of the target individual (para [0036]).

As to claim 4, Rabinowitz teaches the method of claim 1, wherein the ploidy state determination is performed in the context of in vitro fertilization, and where the target individual is an embryo (para [0029]).

As to claim 5, Rabinowitz teaches the method of claim 1, wherein the ploidy state determination is performed in the context of non-invasive prenatal diagnosis, and where the target individual is a fetus (para [0029]).

As to claim 6, Rabinowitz teache the method of claim 1, wherein a clinical decision is made after determining the ploidy state of each of the chromosomes in the target individual (para [0029]).

As to claim 7, Rabinowitz teaches the method of claim 1, wherein the ploidy state determination is carried out for at least one embryo, and is used to determine which, if any, embryos to insert into a uterus (para [0119], [0120]).

As to claim 8, Rabinowitz teaches the method of claim 1, wherein, for at least one of the expert techniques, determining the statistical probability for each of the ploidy state hypothesis involves comparing relationships between observed distributions of allele measurement data for a plurality of parental contexts (para [0033]).

As to claim 9, Rabinowitz teaches the method of claim 1, wherein, for at least one of the expert techniques, determining the statistical probability for each of the ploidy state hypothesis involves comparing intensities of genotyping output data, averaged over a set of alleles, to expected intensities (para [0140], [0171]).

As to claim 10, Rabinowitz teaches the method of claim 1, wherein at least one of the expert techniques uses phased parental allele call data (same chromosome; para [0192] - [0197]).

As to claim 11, Rabinowitz teaches the method of claim 1, wherein at least one of the expert techniques is specific to a sex chromosome (para [0128]).

- Please see first continuation sheet -

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/52730

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 12, Rabinowitz teaches the method of claim 1, wherein determining the ploidy state of each of the chromosomes in the target individual is performed in the context of screening for a chromosomal condition selected from the group consisting of euploidy, nullsomy, monosomy, uniparental disomy, trisomy, matched copy error, unmatched copy error, tetrasomy, other aneuploidy, unbalanced translocation, deletions, insertions, mosaicism, and combinations thereof (abstract, para [0032]).

As to claim 13, Rabinowitz teaches a method for determining an allelic state (the a-posteriori probability of a set of alleles is computed given a particular measurement; para [0073]) in a set of alleles, in a target individual and from one or both parents of the target individual (measurements of the genotype at the locus i for the embryonic, paternal and maternal chromosomes may be characterized respectively by random variables representing the pairs of SNP measurements; para [0073]), and optionally from one or more related individuals, the method comprising:
obtaining genetic data from the target individual, and from the one or both parents, and from any related individuals (genetic data from human subjects, and more specifically on as-yet not implanted embryos or implanted fetuses; para [0029], target cells are isolated, the genetic data contained in those cells are amplified; para [0035], the genetic data of an individual can be cleaned using diploid and haploid data from both parents; para [0036]);
creating a set of at least one allelic hypothesis for the target individual (para [0072], [0085]), and for the one or both parents (para [0070]), and optionally for the one or more related individuals, where the hypotheses describe possible allelic states in the set of alleles;
determining a statistical probability for each allelic hypothesis in the set of hypotheses given the obtained genetic data (para [0075] - [0084]); and
determining the allelic state for each of the alleles in the set of alleles for the target individual, and for the one or both parents, and optionally for the one or more related individuals, based on the statistical probabilities of each of the allelic hypotheses (para [0073]).

As to claim 14, Rabinowitz teaches the method of claim 13, wherein the related individuals are siblings of the target individual (para [0036]).

As to claim 15, Rabinowitz teaches the method of claim 13, wherein the allelic state determination is performed in the context of in vitro fertilization, and where the target individual is an embryo (para [0029]).

As to claim 16, Rabinowitz teaches the method of claim 13, wherein the allelic state determination is performed in the context of non-invasive prenatal diagnosis, and where the target individual is a fetus (abstract).

As to claim 17, Rabinowitz teaches the method of claim 13, wherein a clinical decision is made after determining the allelic state in the set of alleles of the target individual (para [0029]).

As to claim 18, Rabinowitz teaches he method of claim 13, wherein determining the allelic state for each of the alleles in the set of alleles in an individual includes determining a phased genotype at a set of alleles for that individual (para [0151], [0194] - [0197]).

As to claim 19, Rabinowitz teaches the method of claim 13, wherein the obtained genetic data includes single nucleotide polymorphisms measured from a genotyping array and also DNA sequence data (para [0035]).

As to claim 20, Rabinowitz teaches the method of claim 13, wherein platform response models are used to determine a characteristic measurement bias of a genotyping technique (para [0185]).

As to claim 21, Rabinowitz teaches the method of claim 13, wherein the method takes into account a possibility of DNA crossovers that may occur during meiosis (para [0067]).

As to claim 22, Rabinowitz teaches the method of claim 13, wherein the target individual is an embryo, and wherein determining the allelic state in the set of alleles of the target individual is performed to select at least one embryo for transfer in the context of IVF, and where the related individuals are selected from the group consisting of one or more embryos that are from the same parents, one or more sperm from the father, and combinations thereof (abstract, para [0032]).

- Please see second continuation sheet -

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/52730

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
- First Continuation Sheet -

Claims 24-26 lack an inventive step under PCT Article 33(3) as being obvious over Rabinowitz.

As to claim 24, Rabinowitz teaches a method for determining a ploidy state of at least one chromosome in a target individual (detect abnormal numbers of chromosomes, sections of chromosomes, and origins of chromosomes. In genetic samples that are aneuploid, have unbalanced translocations, uniparental disomy, or other gross chromosomal abnormalities; para [0068]), the method comprising:
obtaining genetic data from the target individual (genetic data from human subjects, and more specifically on as-yet not implanted embryos or implanted fetuses; para [0029], target cells are isolated, the genetic data contained in those cells are amplified; para [0035]), and from both parent of the target individual the genetic data of an individual can be cleaned using diploid and haploid data from both parents; para [0036]), and from one or more siblings of the target individual (In another aspect, genetic data from any person of a known genetic relation to the individual can be used to clean the data of the individual, including parents, siblings, grandparents, offspring, cousins, uncles, aunts etc.; para [0036]), wherein the genetic data includes data relating to at least one chromosome (para [0032]);
determining a ploidy state of the at least one chromosome in the target individual and in the one or more parents of the target individual by using one or more expert techniques (para [0074]), wherein none of the expert techniques requires phased genetic data as input (SNPs are measured for pairs of chromosomes, rather than for a single chromosome at a time; para [0073]);
determining phased genetic data of the target individual, and of the parents of the target individual, using an informatics based method (para [0194] - [0197]), and the obtained genetic data from the target individual, and from the parents of the target individual, that were determined to be euploid at that chromosome.
Although Rabinowitz does not explicitly teach the use of, or calculation of sibling date, nor redetermining the ploidy state of the at least one chromosome of the target individual, using one or more expert techniques, at least one of which requires phased genetic data as input, and the determined phased genetic data of the target individual, it would have been obvious to a person skilled in the art to apply the analysis taught by Rabinowitz for parental samples to a sample from any other known relative, as taught by Rabinowitz (para [0036]) for siblings. Further, although Rabinowitz teaches the use of data from non-phased genetic data (para [0073]), Rabinowitz also teaches the use, in practice, of phased data (para [0194] - [0197]). It would have been obvious to a person skilled in the art to recalculate the results of a ploidy, chromosomal, or allelic determination using unphased data, as taught by Rabinowitz, with phased data, due to the greater degree of information provided by the phased data regarding linkage or position of the alleles relative to one another, and the probabilities of multiple recombination events from one chromosome to another.

As to claim 25, Rabinowitz obviates the method of claim 24, and further teaches wherein the determined ploidy state of the chromosome on the target individual is used to make a clinical decision about the target individual (para [0029]).

As to claim 26, Rabinowitz obviates the method of claim 24, and further teaches wherein the ploidy state determination is performed in the context of in vitro fertilization, and where the target individual is an embryo (para [0029]).

Claim 23 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz in view of 2008/0138809 A1 to Kapur et al. (hereinafter "Kapur").

As to claim 23, Rabinowitz anticipates the method of claim 13. Although Rabinowitz does not explicitly teach wherein the method is performed alongside or in conjunction with a method that determines a number of copies of a given chromosome segment present in the target individual, and where both methods use a same cell, or group of cells, from the target individual as a source of genetic data, Rabinowitz teaches a model wherein it is desirable to know the number of copoies of a given chromosome segment (para [0131]). Further, Kapur teaches a comparative genomic hybridization analysis used to determine copy numbers of genes and chromosomes (para [0217]). It would have been obvious to a person skilled in the art to use CGH to determine the copy number of a chromosome or region of interest, as taught by Kapur, in a method, as taught by Rabinowitz, in order to enable appropriate probability determinations.

Claims 1-26 have industrial applicability under PCT Article 33(4) because the subject matter may be made or used in industry

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/52730

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/483, C12Q 1/68 (2009.01)
USPC - 435/6; 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): G01N 33/483, C12Q 1/68 (2009.01)
USPC: 435/6; 702/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): G01N 33/483, C12Q 1/68 (2009.01), USPC: 435/6; 702/, 19 - keyword search, as below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Google Scholar, USPTO PubWest (databases: PGPB,USPT,USOC,EPAB,JPAB) - Search Terms: ploidy, ploid, determine, evaluate, assess, measure, ascertain, hypothesis, parent, offspring, related, relative, sibling, maternal, paternal, statistical, probability, combine, sum, add, distribution, allele, phase, gene, gentic, chromosomal, copies, preimplantatio

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2007/0184467 A1 (RABINOWITZ et al.) 9 August 2007 (09.08.07) abstract, para [0068]; [0029]; [0035]; [0036]; [0072]; [0085]; [0075] - [0084]; [0074]; [0119]; [0120]; [0033]; [0140]; [0171]; [0194] - [0197]; [0128]	1-22, 24-26 --- 23
Y	US 2008/0138809 A1 (KAPUR et al.) 12 June 2008 (12.06.2008) para [0217]	23
A	US 2008/0182244 A1 (TAFAS et al.) 31 July 2008 (31.07.2008)	1-26

☐ 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 September 2009 (21.09.2009)	28 SEP 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

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 118103011201	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2010/050824	International filing date *(day/month/year)* 30 September 2010 (30.09.2010)	Priority date *(day/month/year)* 30 September 2009 (30.09.2009)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 5 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 03 April 2012 (03.04.2012)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Nora Lindner e-mail: pt03.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	15 NOV 2010

Applicant's or agent's file reference	FOR FURTHER ACTION
118103011201	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 10/50824	30 September 2010 (30.09.2010)	30 September 2009 (30.09.2009)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68; C07H 21/04; G01N 33/50 (2010.01)
USPC - 435/6, 536/23.1, 702/19

Applicant GENE SECURITY NETWORK, 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.

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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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	03 November 2010 (03.11.2010)	Lee W. Young PCT Helpdesk: 571-272-4300 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 10/50824

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 10/50824

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	1-18	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-18	NO
Industrial applicability (IA)	Claims	1-18	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-18 lack an inventive step under PCT Article 33(3) as being obvious over US 2008/0243398 A1 to Rabinowitz, et al. (hereinafter 'Rabinowitz'), in view of US 2007/0178478 A1 to Dhallan, et al. (hereinafter 'Dhallan').

Regarding Claims 1 and 18, Rabinowitz discloses a method for determining the copy number of a chromosome of interest in a target individual (para [0013]), using genotypic measurements made on genetic material from the target individual (para [0016]; Claims 1 and 2), wherein the genetic material of the target individual is incorporated with genetic material from the mother of the target individual (para [0050], [0107], and [0268]. See - incorporating the genetic data of a target individual and related individuals into a hypothesis, while tissue samples are taken from both parents.), the method comprising:
obtaining genotypic data for a set of SNPs of the parents of the target individual (para [0050] and [0107]);
making genotypic measurements for the set of SNPs on a incorporated sample that comprises DNA from the target individual and also DNA from the mother of the target individual (para [0050], [0107], and [0268]);
creating (para [0045]-[0049]), on a computer (para [0240]), a set of ploidy state hypothesis for the chromosome of interest of the target individual (para [0100]);
determining, on the computer, the probability of each of the hypotheses (Claim 2) given the genetic measurements of the mixed sample and the genetic data of the parents of the target individual (para [0050], [0107], and [0268]); and
using the determined probabilities of each hypothesis to determine the most likely copy number of the chromosome of interest in the target individual (para [0013], [0045], [0049], and [0090]-[0091])).
Rabinowitz does not specifically disclose that the incorporated sample is a mixed sample.
However, Dhallan teaches a mixing maternal and fetal sample DNA (para [0081], See step (d) of the "in some embodiments.") for detecting genetic abnormalities (para [0032]). To a person of ordinary skill in the art, it would have been obvious to mix samples as taught by Dhallan in the method for determining the copy number of a chromosome of interest in a target individual as in Rabinowitz in order to determine the sequence of alleles (Dhallan: para [0081]), because Rabinowitz and Dhallan are directed towards methods of detecting genetic and chromosomal abnormalities using SNP genotyping (Dhallan: para [0233]) and copy numbers (Dhallan: para [0847]).

Regarding Claim 2, Rabinowitz further discloses that the target individual is a fetus (para [0020]).

Regarding Claim 3, Rabinowitz further discloses that the copy number determination is used to make a clinical decision (para [0013]).

Regarding Claim 4, Dhallan further teaches that the target individual is a fetus (para [0024]), and the clinical decision is to terminate a pregnancy where the fetus is found to have a genetic abnormality (para [0023]).

Regarding Claim 5, Rabinowitz further discloses that the set of SNPs comprises a plurality of SNPs from the chromosome of interest (para [0017] and [0050]), and a plurality of SNPs from at least one chromosome that is expected to be disomic on the target individual (para [0241], Claim 4).

Regarding Claim 6, Rabinowitz further discloses that the step of determining, on the computer, the probability of each of the hypotheses comprises:
using the genotypic data of the parents to determine parental contexts for each of the SNPs (para [0032]);
grouping the genotypic measurements of the mixed sample into the parental contexts (para [0032] and [0040]);
using the grouped genotypic measurements from the at least one chromosome that is expected to be disomic to determine a platform response (para [0015], [0016], [0111], and [0241], Claims 1, 4);
using the grouped genotypic measurements from the at least one chromosome that is expected to be disomic to determine a ratio of fetal to maternal DNA in the mixed sample (para [0015]-[0017], [0111], and [0241], Claims 1, 4);
using the determined platform response and the determined ratio to predict an expected distribution of SNP measurements for each set of SNPs in each parental context under each hypothesis (para [0015], [0016], [0111], [0240], and [0241], Claims 1, 4, The distribution of the maximum likelihood is unknown because the specific distribution in question is generated using mean and variance.); and
calculating the probabilities that each of the hypotheses is true given the platform response (para [0022] and [0107]), and given the ratio (para [0017] and [0091]), and given the grouped genotypic measurements of the mixed sample (as discussed above, and in instant Claim 1), and given the predicted expected distributions, for each parental context, for each hypothesis (para [0271]-[0281]; Tables 1-7).

continued on Supplemental Box

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/50824

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V.2., Citations and explanations:

Regarding Claim 7, Rabinowitz further discloses that the chromosome of interest is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, the X chromosome, the Y chromosome, and combinations thereof (para [0108], See chromosome X and 7; para [0226], See chromosome 21.).

Regarding Claim 8, Rabinowitz further discloses that the method is used to determine the copy number of a number of chromosomes in the target individual (Claim 1), where the number is twenty three (para [0083]).

Regarding Claim 9, Rabinowitz further discloses that the mixed sample is maternal blood (para [0013]).

Regarding Claim 10, Rabinowitz further discloses that the target individual's genetic material is free floating DNA found in maternal blood (para [0227], See the DNA fragments.).

Regarding Claim 11, Dhallan further teaches that the target individual's genetic material is nuclear DNA found in one or more cells from the target individual (para [0027]).

Regarding Claim 12, Rabinowitz further discloses a confidence is computed for the chromosome copy number determination (para [0019]).

Regarding Claim 13, Rabinowitz further discloses a ratio of fetal to maternal DNA in the mixed sample is determined for individual chromosomes (para [0017]).

Regarding Claim 14, Rabinowitz further discloses that the step of obtaining of genotypic data, and/or the step of making genetotypic measurements is done by measuring genetic material using techniques of genotyping microarrays (para [0233]), bead based micro arrays (para [0233]), high throughput sequencing (para [0240]), and fluorescent in situ hybridization (FISH) (para [0009]).

Regarding Claim 15, Rabinowitz further discloses that the step of measuring genetic material is done on genetic material that is amplified, prior to being measured, using a technique that is selected from the group consisting of Polymerase Chain Reaction (PCR) (para [0231]), ligand mediated PCR (para [0231]), degenerative oligonucleotide primer PCR (para [0231]), Multiple Displacement Amplification (MDA) and (para [0231]).

Regarding Claim 16, Rabinowitz further discloses that the step of determining the copy number of the chromosome of interest is performed for the purpose of screening for a chromosomal condition, wherein the chromosomal condition is selected from the group consisting of nullsomy, monosomy, disomy, uniparental disomy, euploidy, trisomy (para [0016]) and Claim 4).

Regarding Claim 17, Dhallan further teaches that the method is used for the purpose of paternity testing (para [0439]).

Claims 1-18 have industrial applicability as defined by PCT Article 33(4) because the subject matter could be made or used in industry.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/50824

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C07H 21/04; G01N 33/50 (2010.01)
USPC - 435/6, 536/23.1, 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- C12Q 1/68; C07H 21/04; G01N 33/50 (2010.01);
USPC- 435/6, 536/23.1, 702/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 702/20;
Patents and NPL

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (US Pat, PgPub, EPO, JPO: classification, keyword), GoogleScholar; search terms: copy number, probability, statistical, compute, algorithm, mathematical, hypothesis, fetal, fetus, foetal, chromosome, polymorphism, snp, ploid, disomal, abort, terminate, end, pregnancy, trimester, paternity, mixed sample, father, mother, paternal, maternal, n

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0243398 A1 (RABINOWITZ et al.) 02 October 2008 (02.10.2008), para [0013]-[0020], [0032], [0040], [0045]-[0050], [0083], [0090]-[0091], [0100], [0107]-[0111], [0226], [0227]-[0233], [0240], [0241], [0268]	1-18
Y	US 2007/0178478 A1 (DHALLAN et al.) 02 August 2007 (02.08.2007), para [0023], [0024], [0027], [0032], [0081], [0233], [0439], [0847]	1-18
A	CHU et al. "Statistical model for whole genome sequencing and its application to minimally invasive diagnosis of fetal genetic disease." Bioinformatics [online], 10 May 2009 (10.05.2009) [Retrieved on 2010-11-03], Vol. 25, Iss. 10, pp. 1244-1250, Retrieved from the Internet: <URL: http://bioinformatics.oxfordjournals.org>	1-18
A	PFAFFL et al. "Relative expression software tool (REST?) for group-wise comparison and statistical analysis of relative expression results in real-time PCR." Nucleic Acids Research [online], May 2009 [Retrieved on 2010-11-03], Vol. 30, Iss. 9, p. e36, 10 pages, Retrieved from the Internet: <URL: http://nar.oxfordjournals.org>	1-18
A	US 2008/0070792 A1 (STOUGHTON et al.) 20 March 2008 (20.03.2008), entire document	1-18
A	PERRY et al. "The Fine-Scale and Complex Architecture of Human Copy-Number Variation." American Journal of Human Genetics [online], 03 March 2008 (03.03.2008) [Retrieved on 2010-11-03], Vol. 82, Iss. 3, pp. 685-695, Retrieved from the Internet: <URL: http://www.sciencedirect.com>	1-18
A	US 2008/0020390 A1 (MITCHELL et al.) 24 January 2008 (24.01.2008), entire document	1-18

☒ 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
04 November 2010 (04.11.2010)	15 NOV 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/50824

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 2007/0259351 A1 (CHINITZ et al.) 08 November 2007 (08.11.2007), entire document	1-18
A	US 2007/0212689 A1 (BIANCHI et al.) 13 September 2007 (13.09.2007), entire document	1-18
A	US 2007/0207466 A1 (CANTOR et al.) 06 September 2007 (06.09.2007), entire document	1-18
A	SEBAT et al. "Strong Association of De Novo Copy Number Mutations with Autism." Science [online], 20 April 2007 (20.04.2007) [Retrieved on 2008-03-27], Vol. 316, No. 445, pp. 445-449, Retrieved from the Internet: <URL: http://intramural.nimh.nih.gov/>	1-18
A	US 2006/0229823 A1 (LIU et al.) 12 October 2006 (12.10.2006), entire document	1-18
A	US 2006/0121452 A1 (DALLAN et al.) 08 June 2006 (08.06.2006), entire document	1-18
A	US 2006/0057618 A1 (PIPER et al.) 16 March 2006 (16.03.2006), entire document	1-18
A	NANNYA et al. "A Robust Algorithm for Copy Number Detection Using High-Density Oligonucleotide Single Nucleotide Polymorphism Genotyping Arrays." Cancer Research [online], 15 July 2005 (15.07.2005), Vol. 65, No. 14, pp. 6071-6079, Retrieved from the Internet: <URL: http://cancerres.aacrjournals.org>	1-18
A	US 2005/0049793 A1 (PATERLINI-BRECHOT et al.) 03 March 2005 (03.03.2005), entire document	1-18
A	US 2005/0009069 A1 (LIU et al.) 13 January 2005 (13.01.2005), entire document	1-18
A	DURAWALA et al. "A versatile statistical analysis algorithm to detect genome copy number variation." Proceedings of the National Academy of Science [online], 16 November 2004 (16.11.2004) [Retrieved on 2010-11-03], Vol. 101, No. 46, pp. 16292-16297, Retrieved from the Internet: <URL: http://www.ncbi.nlm.nih.gov>	1-18
A	US 2003/0101000 A1 (BADER et al.) 29 May 2003 (29.05.2003), entire document	1-18
A	US 6,180,349 B1 (GINZINGER et al.) 30 January 2001 (30.01.2001), entire document	1-18

NeoGenomics
Exhibit 1003 Page 990

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 118103011402	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2011/037018	International filing date *(day/month/year)* 18 May 2011 (18.05.2011)	Priority date *(day/month/year)* 18 May 2010 (18.05.2010)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 20 November 2012 (20.11.2012)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Nora Lindner e-mail: pt03.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	27 SEP 2011
Applicant's or agent's file reference 118103011402	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/37018	18 May 2011 (18.05.2011)	18 May 2010 (18.05.2010)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6

Applicant GENE SECURITY NETWORK 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	26 July 2011 (26.07.2011)	Lee W. Young PCT Helpdesk: 571-272-4300 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 11/37018

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 11/37018

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	1-27	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-27	NO
Industrial applicability (IA)	Claims	1-27	YES
	Claims	None	NO

2. Citations and explanations:

Claim 27 lacks an inventive step under PCT Article 33(3) as being obvious over US 2007/0202525 A1 to Quake et al. (hereinafter 'Quake').

Regarding claim 27, Quake discloses a method of determining a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, having target sequences in a mixture of maternal and fetal genetic material (claim 1; para [0026], [0032], [0129]), comprising the steps of:
--a) obtaining maternal tissue comprising both maternal and fetal genetic material (para [0026]);
--b) obtaining a mixture of maternal and fetal genetic material from the maternal tissue (para [0026], [0140]);
--c) distributing the mixture of maternal and fetal genetic material into a plurality of reaction samples, to randomly provide individual reaction samples that contain a target sequence from a target chromosome and individual reaction samples that do not contain a target sequence from a target chromosome (para [0026], [0027]);
--d) analyzing the target sequences of genetic material present or absent in the individual reaction samples to provide a first number of binary results representing presence or absence of a presumably euploid fetal chromosome in the reaction samples and a second number of binary results representing presence or absence of a possibly aneuploid fetal chromosome in the reaction samples (claim 1-2; para [0027], [0028]. [0056]-[0061]); and
--determining whether fetal aneuploidy is present (para [0126], [0127]).
Quake does not expressly disclose that the method further comprises calculating an expected distribution of a number of binary results for a presumably euploid fetal chromosome in the reaction samples using the first number; calculating an expected distribution of a number of binary results for a presumably aneuploid fetal chromosome in the reaction samples using the first number and an estimated fraction of fetal DNA found in the mixture of maternal and fetal genetic material; and using a maximum likelihood approach to determine whether the second number indicates the presence of a fetal aneuploidy, however, this would have been obvious to one of ordinary skill in the art in view of Quake that discloses that the number of said plurality of reaction samples should be large enough to provide statistical confidence and should be based on the estimated fraction of fetal DNA found in the mixture of maternal and fetal genetic material (para [0028], [0029]), because based on this disclosure, one of ordinary skill in the art would have known with such large size of the number of reaction samples, expected distribution of a number of binary results for a presumable euploid fetal chromosome and a presumable aneuploid fetal chromosome could be generated using routine protocols and then the commonly used maximum likelihood approach could be used to determine whether the second number indicates the presence of a fetal aneuploidy, because such distribution of data and maximum likelihood approach were both routinely used in the art.

Claims 25-26 lack an inventive step under PCT Article 33(3) as being obvious over the article entitled "Ploidy status and copy number aberrations in primary glioblastomas defined by integrated analysis of allelic ratios, signal ratios and loss of heterozygosity using 500K SNP Mapping Arrays" by Gardina et al. (hereinafter 'Gardina') in view of the article entitled "Massively parallel exon capture and library-free resequencing across 16 genomes" by Turner et al. (hereinafter 'Turner').

Regarding claim 25, Gardina discloses a sample of preferentially enriched DNA, wherein the sample of preferentially enriched DNA has been preferentially enriched at a plurality of polymorphic loci from a first sample of DNA (abstract; pg 3, in col 1, para 1 to col 2, para 4). Gardina further discloses that said preferential enriching comprises PCR (pg 3, col 1, para 1-4), based on this disclosure, one of ordinary skill in the art would have been able to achieve without undue experimentation desired degree of enrichment by adjusting the number of cycles of the PCR reactions.
Gardina does not expressly disclose that the allelic bias between the first sample and the preferentially enriched sample is, on average, selected from the group consisting of less than 1000%, less than 500%, less than 200%, less than 100%, less than 50%, less than 20%, less than 10%, less than 5%, less than
2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1 %, less than 0.05%, less than 0.02%, and less than 0.01 %. However, Turner discloses a method of preferentially enriching a target DNA with reduced allelic bias (fig 1-2), based on this disclosure, one of ordinary skill in the art would have been able to vary various parameters of said method in order to achieve greater reduction in allelic bias using standard protocols.
One of ordinary skill in the art would have been motivated to modify the sample of preferentially enriched DNA disclosed by Gardina to incorporate the method of preferentially enriching a target DNA disclosed by Turner to design the sample according to claim 25 so as to achieve allelic bias reduction, as disclosed by Turner (fig 1) in order to achieve a more effective and representational sample of preferentially enriched DNA.

**********Continued in Supplemental Boxes**********

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/37018

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:

Claim 8 is objected to because it is self-dependent, for the purpose of this opinion, claim 8 is construed to be a dependent claim of claim 7.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/37018

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V(2) - Citations and Explanations:

Regarding claim 26, as dependent claim of claim 25, Gardina and Turner discloses a sample of preferentially enriched DNA. Further Gardina discloses a method to create a sample of preferentially enriched DNA (pg 3, ln 1, ln 1-4).

Claims 1-4, 11-12 and 14-24 lack an inventive step under PCT Article 33(3) as being obvious over WO 2010/017214 A1 to Rabinowitz et al. (hereinafter 'Rabinowitz') in view of Gardina in further view of US 2005/0255508 A1 to Casey et al. (hereinafter 'Casey').

Regarding claim 1, Rabinowitz discloses a method for determining a ploidy status of a chromosome in a gestating fetus (claim 1, 5; pg 4, ln 12 to pg 5, ln 15; pg 6, ln 3-13), the method comprising:
--obtaining a first sample that contains DNA from the method of fetus and DNA from the fetus (claim 1-2, 5; pg 4, ln 5-11);
--obtaining genotype data from one or both parents of the fetus (claim 1-2, 5);
--processing the first sample by purifying the DNA to obtain a second sample (pg 4, ln 5-11);
--measuring the DNA in the second sample at a set of polymorphic alleles (claim 1, 8-9, 18-19; pg 6, ln 5-25; pg 24, ln 8 to pg 25 ln 32);
--calculating, on a computer, allele ratios at the set of polymorphic alleles from the DNA measurement made on the second sample (claim 8-9, 18-19 - "distributions of allele measurement data", "comparing intensities of genotyping output data, averaged over a set of alleles, to expected intensities", "a phased genotype at a set of alleles"; pg 12, ln 5-10; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25);
--creating, on a computer, a plurality of ploidy hypotheses concerning expected allele ratios at the set of polymorphic alleles on the chromosome for different possible ploidy states of the chromosome (claim 1, 8-9, 18-19 - "distributions of allele measurement data", "comparing intensities of genotyping output data, averaged over a set of alleles, to expected intensities", "a phased genotype at a set of alleles"; pg 12, ln 5-10; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25);
--determining, on a computer, a relative probability of each of the ploidy hypotheses using the allele ratio calculated for the second sample (claim 1, 8-9; pg 12, ln 5-10; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25); and
--calling the ploidy state of the fetus by selecting ploidy state corresponding to hypothesis with the greatest possibility (claim 1, 8-9, 18-19; pg 12, ln 5-10; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25).
Rabinowitz does not expressly disclose that said method further comprises building, on a computer a joint distribution model for heterozygosity rates of each polymorphic allele on the chromosome for each ploidy hypothesis using genotype data from the one or both parents of the fetus and that the relative probability of each of the ploidy hypotheses is also determined by said joint distribution model. However, Rabinowitz discloses that said genotype data comprises haplotype data (pg 12, ln 1-10).
Further Gardina discloses a similar method for determining a ploidy status of a chromosome in a target cell by integrating allele ratio and heterozygosity rates, wherein the method comprises comparing the heterozygosity rate of the target cell and a reference cell to determine loss of heterozygosity (abstract; pg 3, col 2, para 3).
Finally Casey discloses reconstructing two haplotypes for a diploid individual comprising building, on a computer, a joint distribution model for heterozygosity rate of each polymorphic allele on a chromosome, wherein said joint distribution model allow detection and characterization of haplotype (para [0007], [0045]-[0049]).
Based on Gardina and Casey, one of ordinary skill in the art would have been motivated to modify the method disclosed by Rabinowitz so that the method further comprises building, on a computer a joint distribution model for heterozygosity rates of each polymorphic allele on the chromosome for each ploidy hypothesis using genotype data from the one or both parents of the fetus and that the relative probability of each of the ploidy hypotheses is also determined by said joint distribution model in order to achieve more accurate determination of the ploidy state by taking into loss of heterozygosity to enhance interpretation of copy number determination, as disclosed by Gardina (abstract); and to allow haplotype determination by building said joint distribution model, as disclosed by Casey (para [0045]).

Regarding claim 2, Rabinowitz discloses that the first sample has been isolated from maternal blood (pg 4, ln 5-11).

Regarding claim 3, Rabinowitz discloses that processing the first sample further comprises amplifying the DNA (pg 24, ln 12-27).

Regarding claim 4, Gardina discloses processing the first sample further comprises preferentially enriching the DNA at the plurality of polymorphic loci (abstract; pg 3, ln col 1, para 1 to col 2, para 4).

Regarding claim 11, Rabinowitz discloses that the set of polymorphic alleles are SNPs (claim 1, 8-9, 18-19; pg 6, ln 5-25; pg 24, ln 8 to pg 25 ln 32).

Regarding claim 12, Rabinowitz discloses that the measuring the DNA in the second sample is done by sequencing (pg 24, ln 12-35).

**********Continued on Next Page**********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/37018

Supplemental Box

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Regarding claim 14, Rabinowitz discloses for each fetus, the measuring the DNA in the second sample is done by sequencing a fraction of the second sample of DNA to give rise to a first set of measurement (pg 24, ln 8 to pg 25, ln 32), the method further comprises:
--making a first relative probability determination for each of the ploidy hypothesis for the fetus, given the first set of DNA measurement claim 1, 8-9; pg 12, ln 5-10; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25).
Rabinowitz does not expressly disclose that the method is executed for a plurality of gestating fetuses, however, this would have been obvious to one of ordinary skill in the art, said ploidy status determination was routinely practiced in the art on many gestating fetuses.
Rabinowitz does not expressly disclose resequencing a second fraction of the second sample from those fetuses where the first relative probability determination for each of the ploidy hypotheses indicates that a ploidy hypothesis corresponding to an aneuploid fetus has a significant probability, to give a second set of measurement; making a second relative probability determination for ploidy hypotheses for the fetuses using the second set of measurement and optionally also the first set of measurement and calling the ploidy status of the fetuses whose second sample is resequenced by selecting the ploidy state corresponding the hypothesis with the greatest probability as determined by the second relative probability determination, however, this would have been obvious to one of ordinary skill in the art in view of Rabinowitz that discloses determining, on a computer, a relative probability of each of the ploidy hypotheses using the allele ratio calculated for the second sample and calling the ploidy state of the fetus by selecting ploidy state corresponding to hypothesis with the greatest possibility (claim 1, 8-9, 18-19; pg 12, ln 5-10; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25) and that a second fraction of the sample is measured to double check a primary genetic data generated from the first measurement (pg 89, ln 12-25), because double-test when an abnormal condition is diagnosed by a first measurement was routinely practiced in the art in order to confirm the diagnosis.

Regarding claim 15, Rabinowitz discloses that said method further comprises using data about the probability of chromosome crossing over at different crossover locations in a chromosome to model dependence between polymorphic alleles on the chromosome (claim 21; pg 26, ln 7 to pg 27, ln 5), based on this disclosure, one of ordinary skill in the art would have known that the joint distribution model for heterozygosity rate should also take into account said probability of chromosome crossing over in order to achieve better characterizations of the relationships amount different polymorphic locus.

Regarding claim 16, Rabinowitz discloses that determining the relative probability of each hypothesis is done using a method that does not require the use of a reference chromosome (pg 6, ln 3-14 - "the fetal or embryonic genomic data, with or without the use of genetic data from related individuals, can be used to detect if the cell is aneuploid").

Regarding claim 17, Rabinowitz discloses that determining the relative probability of each hypotheses makes use of a fraction of fetal DNA in the measured sample (claim 1, 8-9, 18-19; pg 12, ln 5-10; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25).
Rabinowitz does not expressly disclose that said fraction of fetal DNA is estimated, however, this would have been obvious to one of ordinary skill in the art, because the fraction of fetal DNA present in a sample was routinely measured and estimated before measuring said fetal DNA in order to estimate the proper amount of DNA/sample to be measured and used.

Regarding claim 18, Rabinowitz discloses that the DNA measurement from the second sample used in calculating allele ratios and determining relative probability of each hypotheses comprises primary genetic date (claim 1, 8-9 - "comparing intensities of genotyping output data, averaged over a set of alleles, to expected intensities").

Regarding claim 19, Rabinowitz discloses that selecting the ploidy state corresponding to the hypothesis with the greatest probability is carried out using maximum likelihood estimate (pg 52, ln 1-16).

Regarding claim 20, as dependent claim of claim 1, Rabinowitz, Gardina and Casey obviate determining a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele ratios calculated from the second sample. Rabinowitz further discloses combining the relative probability of each ploidy hypotheses that are calculated using a statistic that is only available when parental genetic information is used (pg 4, ln 12-21; pg 19, ln 28 to pg 20, ln 10; pg 27, ln 28 to pg 28, ln 7).

Regarding claim 21, Rabinowitz discloses that a confidence estimate the calculated for the called ploidy state (pg 29, ln 13-30).

Regarding claim 22, Rabinowitz discloses producing a report stating the called ploidy state of the fetus (pg 100, ln 27 to pg 101, ln 3).

Regarding claim 23, Rabinowitz discloses taking a clinical action based on the called ploidy state of the fetus, wherein the clinical action is selected from one of terminating the pregnancy or maintaining the pregnancy (pg 15, ln 16-21; pg 100, ln 27 to pg 101, ln 3).

Regarding claim 24, Rabinowitz does not expressly disclose that the method can be performed at between 4 and 5 weeks gestation; between 5 and 6 weeks gestation; between 6 and 7 weeks gestation; between 7 and 8 weeks gestation; between 8 and 9 weeks gestation; between 9 and 10 weeks gestation;
between 10 and 12 weeks gestation; between 12 and 14 weeks gestation; between 14 and 20 weeks gestation; between 20 and 40 weeks gestation; in the first trimester; in the second trimester; or in the third trimester, however, this would have been obvious to one of ordinary skill in the art, because one of ordinary skill in the art would have been able to determine the proper time to perform the method according to each individual's needs.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/37018

Supplemental Box

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Claims 5 and 10 lack an inventive step under PCT Article 33(3) as being obvious over Rabinowitz in view of Gardina and Casey, as above, in further view of Turner.

Regarding claim 5, neither Rabinowitz, Gardina, nor Casey expressly discloses that the preferentially enriching the DNA at a plurality of polymorphic loci comprises obtaining a pre-circularized probe such that the 3' and 5' ends are designed to hybridize to a region of DNA that is separated from the polymorphic region of the allele by a 5 small number of bases, where the small number is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or a combination thereof; hybridizing the pre-circularized probe to purified DNA from the first sample; circularizing the pre-circularized probe; and
amplifying some or all of the circularized probe. However, Turner discloses a method of preferentially enriching a target region of a DNA (fig 2);, the method comprising:
--obtaining a pre-circularized probe such that the 3' and 5' ends are designed to hybridize to a region of DNA that is separated by said target region by a small number of basis (fig 2 - "hybridize immediately upstream and downstream of its target");
--hybridizing the pre-circularized probe to purified DNA (fig 2);
--circularizing the pre-circularized probe (fig 2); and
--amplifying some of all of the circularized probe (fig 2).
Based on this disclosure, one of ordinary skill in the art would have been able to determine without undue experimentation the proper number of bases separating the target region and the upstream/downstream hybridization region using standard techniques and protocols.
One of ordinary skill in the art would have been motivated to modify the method for determining a ploidy status of a chromosome in a gestating fetus comprising preferentially enriching DNA at a plurality of polymorphic loci disclosed by Rabinowitz, Gardina and Casey to incorporate the method of preferentially enriching a target DNA disclosed by Turner to design the method according to claim 5 so as to achieve effective amplification of said polymorphic loci with reduced allele bias, as disclosed by Turner (fig 1).

Regarding claim 10, neither Rabinowitz, Gardina, nor Casey expressly discloses that the preferential enrichment results in average degree of allelic bias between the second sample and the first sample of a factor selected from the group consisting of no more than a factor of 2, no more than a factor of 1.5, no more than a factor of 1.2, no more than a factor of 1.1, no more than a factor of 1.05, no more than a factor of 1.02, no more than a factor of 1.01, no more than a factor of 1.005, no more than a factor of 1.002, no more than a factor of 1.001 and no more than a factor of 1.0001. However, Turner discloses a method of preferentially enriching a target DNA with reduced allelic bias (fig 1-2), based on this disclosure, one of ordinary skill in the art would have been able to vary various parameters of said method in order to achieve greater reduction in allelic bias using standard protocols.
One of ordinary skill in the art would have been motivated to modify the method for determining a ploidy status of a chromosome in a gestating fetus comprising preferentially enriching DNA at a plurality of polymorphic loci with reduced bias disclosed by Rabinowitz, Gardina and Casey to incorporate the method of preferentially enriching a target DNA disclosed by Turner to design the method according to claim 10 so as to achieve more accurate determination of a ploidy status.

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz in view of Gardina and Casey, as above, in further view of US 2006/0216738 A1 to Wada et al. (hereinafter 'Wada').

Regarding claim 6, Gardina discloses that the preferentially enriching the DNA at a plurality of polymorphic loci comprises amplifying the DNA using the polymerase chain reaction (pg 3, col 1, para 1-4).
Gardina does not expressly disclose that said preferentially enriching further comprises obtaining a forward probe such that the 3' end of the forward probe is designed to hybridize to the region of DNA immediately upstream from the polymorphic region, and separated from the polymorphic region by a small number of bases, where the small number is selected from the group consisting of 1,2,3,4, 5, 6 to 10, and 11 to 20; obtaining a reverse probe such that the 3' end of the reverse probe is designed to hybridize to the region of DNA immediately downstream from the polymorphic region, and separated from the polymorphic region by a small number of bases, where the small number is selected from the group consisting of 1,2,3,4, 5, 6 to 10, and 11 to 20; hybridizing the two probes to DNA in the first sample of DNA. However, Wada discloses a method of preferentially enriching DNA at a polymorphic loci (para [0022], [0024], [0041])comprising:
--obtaining a forward probe such that the 3' end of the forward probe is designed to hybridize to the region of DNA upstream from the polymorphic region (para [0022], [0024], [0041]);
--obtaining a reverse probe such that 3' end of the reverse probe is designed to hybridize to the region of DNA downstream from the polymorphic region (para [0022], [0024], [0041]); and
--hybridizing the two probes to DNA in a sample (para [0022], [0024], [0041]);and
--amplifying the DNA using the polymerase chain reaction (para [0022], [0024], [0041]).
Based on this disclosure, one of ordinary skill in the art would have been able to determine without undue experimentation the number of bases that separates the polymorphic region from the upstream/downstream hybridization regions using standard protocols because said PCR primers were routinely designed to hybridize to a region of DNA immediately upstream/downstream of a target region.
One of ordinary skill in the art would have been motivated to modify the method for determining a ploidy status of a chromosome in a gestating fetus comprising preferentially enriching DNA at a plurality of polymorphic loci disclosed by Rabinowitz, Gardina and Casey based on the primer designing for polymerase chain reaction used to preferentially enriching a polymorphic loci disclosed by Wada to design the method according to claim 6 so as to achieve effective amplification of said polymorphic loci using proper probes.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/37018

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
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Claims 7-9 lack an inventive step under PCT Article 33(3) as being obvious over Rabinowitz in view of Gardina and Casey, as above, in further view of US 2005/0142577 A1 to Jones et al. (hereinafter 'Jones').

Regarding claim 7, neither Rabinowitz, Gardina, nor Jones expressly discloses that the preferentially enriching the DNA at a plurality of polymorphic loci comprises obtaining a set of hybrid capture probes; hybridizing the hybrid capture probes to the DNA in the first sample; and physically separating the hybridized DNA from the first sample of DNA from the unhybridized DNA from the first sample.
However, Jones discloses preferentially enriching the DNA at a polymorphic loci (para [0058], [0059], [0062], [0063]) comprising:
--obtaining a set of hybrid capture probes (para [0058] - "Capture probes are oligonucleotides that have a 5' common priming sequence and a 3' locus or target specific region or primer"; [0059]);
--hybridizing the hybrid capture probes to the DNA in a sample (para [0058], [0059], [0062], [0063], [0081], [0082]); and
--physically separating the hybridized DNA from the sample of DNA from the unhybridized DNA from the sample (para [0081], [0082]).
One of ordinary skill in the art would have been motivated to modify the method for determining a ploidy status of a chromosome in a gestating fetus comprising preferentially enriching DNA at a plurality of polymorphic loci disclosed by Rabinowitz, Gardina and Casey based on the hybrid capture probe used to preferentially enrich a polymorphic loci disclosed by Jones to design the method according to claim 7 so as to achieve effective amplification of the polymorphic loci.

Regarding claim 8, Jones discloses that the set of hybrid capture probes are designed the hybridize to a region that is flanking but not crossing the polymorphic allele (para [0058], [0059]).

Regarding claim 9, Jones discloses that the set of hybrid capture probes are designed the hybridize to a region that is flanking but not crossing the polymorphic allele (para [0058], [0059]). Further one of ordinary skill in the art would have been able to determine without undue experimentation the proper length of the hybrid capture probes using standard protocols.

Claim 13 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz in view of Gardina and Casey, as above, in further view of US 2010/0112590 A1 to Lo et al. (hereinafter 'Lo').

Regarding claim 13, neither Rabinowitz, Gardina, nor Casey expressly discloses that the method is executed for a plurality of gestating fetuses and that the method further comprises determining the percent of DNA that is fetal in each of the second fractions; and wherein measuring the DNA in the second sample is done by sequencing a number of DNA molecules in each of the second samples, where more molecules of DNA are sequenced from those second samples that have a smaller fraction of fetal DNA than those second samples that have a larger fraction of fetal DNA. However, executing the method for a plurality of gestating fetus would have been obvious to one of ordinary skill in the art, said ploidy status determination was routinely practiced in the art on many gestating fetuses.
Further Lo discloses a method of determining a ploidy status of a chromosome in a gestating fetus (para [0013]) comprising determining the percent of DNA that is fetal in a sample; and and wherein measuring the DNA in the sample is done by sequencing a number of DNA molecules in the sample, where more molecules of DNA are sequenced from those samples that have a smaller fraction of fetal DNA than those samples that have a larger fraction of fetal DNA (para [0112]).
One of ordinary skill in the art would have been motivated to modify the method for determining a ploidy status of a chromosome in a gestating fetus comprising preferentially enriching DNA at a plurality of polymorphic loci disclosed by Rabinowitz, Gardina and Casey based on the percent of fetal DNA used to determine the number of DNA molecules to be sequenced in a method of determining a ploidy status of a chromosome in a gestating fetus disclosed by Lo to design the method according to claim 13 so as to achieve more accurate ploidy status determination by ensuring that enough fetal DNA is sequenced.

Claims 1-27 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made and used in industry.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/37018

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6
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 (2011.01)
USPC - 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/6; 702/19, 435/91.5
(Text Search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, USOC, EPAB, JPAB); Google Scholar.
Search Terms: ploidy calling, copy number calling, joint distribution, ploidy, heterozygo$, rate, copy number, allel$, polymorphism, SNP, enrich, enrich$, amplif$, probe, forward primer, upstream, reverse primer, downstream, hybrid, captur$, probe, hybridiz$, flank, flanking,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0202525 A1 (QUAKE et al.) 30 August 2007 (30.08.2007) claim 1-2; para [0026]-[0028]. [0056]-[0061], [0126], [0127].	27
Y	WO 2010/017214 A1 (RABINOWITZ et al.) 11 February 2010 (11.02.2010) claim 1, 8-9, 18-19; pg 4, ln 5 to pg 5, ln 15; pg 6, ln 5-25; pg 12, ln 1-10; pg 15, ln 16-21; pg 24, ln 8 to pg 25, ln 32; pg 26, ln 20 to pg 27, ln 25 to pg 28, ln 7, pg 29, ln 13-30; pg 52, ln 1-16; pg 100, ln 27 to pg 101, ln 7.	1-24
Y	GARDINA et al., Ploidy status and copy number aberrations in primary glioblastomas defined by integrated analysis of allelic ratios, signal ratios and loss of heterozygosity using 500K SNP Mapping Arrays. BMC Genomics. 2008 (published online 17 October 2008), Vol 9, No 489, pages 1-16: abstract; pg 3, col 1, para 1 to col 2, para 4.	1-26
Y	US 2005/0255508 A1 (CASEY et al.) 17 November 2005 (17.11.2005) para [0007], [0045]-[0049].	1-24
Y	TURNER et al., Massively parallel exon capture and library-free resequencing across 16 genomes. Nature Methods. May 2009 (published online 6 April 2009), Vol 6, No 5, pages 315-316: fig 1-2.	5, 10 and 25-26
Y	US 2006/0216738 A1 (WADA et al.) 28 September 2006 (28.09.2006) para [0022], [0024], [0041].	6
Y	US 2005/0142577 A1 (JONES et al.) 30 June 2005 (30.06.2005) para [0058], [0059], [0062], [0063], [0081], [0082].	7-9

☒ 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
26 July 2011 (26.07.2011)	27 SEP 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/37018

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0112590 A1 (LO et al.) 06 May 2010 (06.05.2010) para [0013], [0112].	13

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

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 W11	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2011/061506	International filing date *(day/month/year)* 18 November 2011 (18.11.2011)	Priority date *(day/month/year)* 09 February 2011 (09.02.2011)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 13 August 2013 (13.08.2013)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Lingfei Bai e-mail: pt02.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02210

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	16 MAR 2012
Applicant's or agent's file reference 118103011702	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/61506	18 November 2011 (18.11.2011)	09 February 2011 (09.02.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06F 19/22; G01N 33/48 (2012.01)
USPC - 702/20; 436/63, 94

Applicant GENE SECURITY NETWORK 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	27 February 2012 (27.02.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 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 11/61506

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 11/61506

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	1-43	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-43	NO
Industrial applicability (IA)	Claims	1-43	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-4 and 22-41 lack an inventive step under PCT Article 33(3) as being obvious over US 2007/0184467 A1 to Rabinowitz, et al. (hereinafter 'Rabinowitz'), in view of US 2007/0178478 A1 to Dhallan, et al. (hereinafter 'Dhallan').

Regarding Claim 1, Rabinowitz discloses a method for determining a ploidy status of a chromosome (para [0032]), the method comprising:
obtaining a first sample of DNA that comprises maternal DNA from the mother of the fetus and fetal DNA from the fetus (para [0029] and [0039]);
preparing the first sample by isolating the DNA so as to obtain a prepared sample (para [0039]);
measuring the DNA in the prepared sample at a plurality of polymorphic loci on the chromosome (para [0033]);
calculating, on a computer (para [0294]), allele counts at the plurality of polymorphic loci from the DNA measurements made on the prepared sample (para [0079]);
creating, on a computer, a plurality of ploidy hypotheses each pertaining to a different possible ploidy state of the chromosome (para [0079]);
building, on a computer (para [0294]), a joint probability distribution model for the expected allele counts at the plurality of polymorphic loci on the chromosome for each ploidy hypothesis (para [0074]-[0077]: see for example the binomial probability distribution, according to SNPs obtained from embryonic, maternal, and paternal DNA);
determining, on a computer (para [0294]), a relative probability of each of the ploidy hypotheses using the joint probability distribution model and the allele counts measured on the prepared sample (para [0126] and [0127]); and
calling the ploidy state of the fetus by selecting the ploidy state corresponding to the hypothesis with the greatest probability (para [0206]);
but does not specifically disclose a method for determining a ploidy status of a chromosome in a gestating fetus.
However, Dhallan discloses a method for calculating allele counts at the plurality of polymorphic loci from the DNA measurements made on the prepared sample (para [0033]); for determining the sequences of alleles and detection of ploidy at a locus of particular interest of a chromosome in a gestating fetus (para [0032], [0081], and [0140]).
To a person of ordinary skill in the art, it would have been obvious to determine a ploidy status of a chromosome in a gestating fetus as in Dhallan in the method for determining a ploidy status of a chromosome as in Rabinowitz in order to detect genetic disorders including mutations and chromosomal abnormalities (Dhallan: para [0032]), because Rabinowitz and Dhallan are directed towards methods of determining ploidy status in a fetus using allele counts.

Regarding Claim 2, Rabinowitz further discloses that the DNA in the first sample originates from maternal plasma (para [0029]; Claim 12).

Regarding Claim 3, Rabinowitz further discloses that the step of preparing the first sample further comprises amplifying the DNA (para [0033]).

Regarding Claim 4, Dhallan further teaches that the step of preparing the first sample further comprises preferentially enriching the DNA in the first sample at a plurality of polymorphic loci (para [0216]).

Regarding Claim 22, Rabinowitz further discloses obtaining genotypic data from one or both parents of the fetus (para [0072]).

Regarding Claim 23, Rabinowitz further discloses that the obtaining genotypic data from one or both parents of the fetus comprises:
preparing the DNA from the parents where the preparing comprises preferentially enriching the DNA at the plurality of polymorphic loci to give prepared parental DNA (para [0033]);
optionally amplifying the prepared parental DNA (para [0035]); and
measuring the parental DNA in the prepared sample at the plurality of polymorphic loci (para [0035]).

Regarding Claim 24, Rabinowitz further discloses that the building a joint distribution model for the expected allele count probabilities of the plurality of polymorphic loci on the chromosome is done using the obtained genetic data from the one or both parents (para [0036]).

Regarding Claim 25, Rabinowitz further discloses that the first sample has been isolated from maternal plasma (para [0029]; Claim 12) and where the obtaining genotypic data from the mother is done by estimating the maternal genotypic data from the DNA measurements made on the prepared sample (para [0032]).

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Box V.2., Citations and explanations:

Regarding Claim 26, Rabinowitz further discloses that the preferential enrichment results in average degree of allelic bias between the prepared sample (para [0171] and [0185]) and the first sample of a factor selected from the group consisting of no more than a factor of 2, no more than a factor of 1.5, no more than a factor of 1.2, no more than a factor of 1.1, no more than a factor of 1.05, no more than a factor of 1.02, no more than a factor of 1.01, no more than a factor of 1.005, no more than a factor of 1.002, no more than a factor of 1.001 and no more than a factor of 1.0001 (para [0323]).

Regarding Claim 27, Rabinowitz further discloses that the plurality of polymorphic loci are SNPs (para [0030]).

Regarding Claim 28, Rabinowitz further discloses that the step of measuring the DNA in the prepared sample is done by sequencing (para [0035]).

Regarding Claim 29, Rabinowitz further discloses a diagnostic box for helping to determine a ploidy status of a chromosome in a gestating fetus where the diagnostic box is capable of executing the preparing and measuring steps of the method of claim 1 (para [0294]).

Regarding Claim 30, Rabinowitz further discloses that the allele counts are probabilistic rather than binary (para [0150]).

Regarding Claim 31, Rabinowitz further discloses that the measurements of the DNA in the prepared sample at the plurality of polymorphic loci are also used to determine whether or not the fetus has inherited one or a plurality of disease linked haplotypes (para [0121]).

Regarding Claim 32, Rabinowitz further discloses that the step of building a joint distribution model for allele count probabilities is done by using data about the probability of chromosomes crossing over at different locations in a chromosome to model dependence between polymorphic alleles on the chromosome (para [0069] and [0070]).

Regarding Claim 33, Rabinowitz further discloses that both the step of building a joint distribution model for allele counts and the step of determining the relative probability of each hypothesis are done using a method that does not require the use of a reference chromosome (para [0142] and [0150]).

Regarding Claim 34, Rabinowitz further discloses that the step of determining the relative probability of each hypothesis makes use of an estimated fraction of fetal DNA in the prepared sample (para [0078], [0126], and [0144]).

Regarding Claim 35, Rabinowitz further discloses that the DNA measurements from the prepared sample used in calculating allele count probabilities and determining the relative probability of each hypothesis comprise primary genetic data (para [0148]-[0150]).

Regarding Claim 36, Rabinowitz further discloses selecting the ploidy state corresponding to the hypothesis with the greatest probability is carried out using maximum likelihood estimates or maximum a posteriori estimates (para [0077]).

Regarding Claim 37, Rabinowitz further discloses that the step of calling the ploidy state of the fetus further comprises:
combining the relative probabilities of each of the ploidy hypotheses determined using the joint distribution model and the allele count probabilities with relative probabilities of each of the ploidy hypotheses that are calculated using statistical techniques taken from a group consisting of a read count analysis, comparing heterozygosity rates (para [0121]), a statistic that is only available when parental genetic information is used, the probability of normalized genotype signals for certain parent contexts (para [0180]), a statistic that is calculated using an estimated fetal fraction of the first sample or the prepared sample, and combinations thereof.

Regarding Claim 38, Rabinowitz further discloses that a confidence estimate is calculated for the called ploidy state (para [0277]).

Regarding Claim 39, Rabinowitz further discloses taking a clinical action based on the called ploidy state of the fetus, wherein the clinical action is selected from one of terminating the pregnancy or maintaining the pregnancy (para [0390]).

Regarding Claim 40, Dhallan further teaches that the method may be performed at between 4 and 5 weeks gestation (para [0081]); between 5 and 6 weeks gestation; between 6 and 7 weeks gestation (para [0081]); between 7 and 8 weeks gestation (para [0081]); between 8 and 9 weeks gestation (para [0081]); between 9 and 10 weeks gestation (para [0081]); between 10 and 12 weeks gestation (para [0081]); between 12 and 14 weeks gestation (para [0081]); between 14 and 20 weeks gestation; between 20 and 40 weeks gestation; in the first trimester; in the second trimester; in the third trimester; or combinations thereof.

Regarding Claim 41, Dhallan further teaches a report displaying a determined ploidy status of a chromosome in a gestating fetus generated using the method of claim 1 (para [0140] and [1964]).

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Box V.2., Citations and explanations:

Claim 43 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz, in view of US 2007/0202525 A1 to Quake, et al. (hereinafter 'Quake').

Regarding Claim 43, Rabinowitz discloses a method for determining presence or absence of fetal aneuploidy in a maternal tissue sample comprising fetal and maternal genomic DNA (para [0029] and [0385], the method comprising:
a) obtaining a mixture of fetal and maternal genomic DNA from said maternal tissue sample (para [0029] and [0039]);
b) conducting massively parallel (para [0378]) of DNA fragments randomly selected from the mixture of fetal and maternal genomic DNA of step a) to determine the sequence of said DNA fragments (para [0295], [0370]);
in h) calculating an expected distribution of the amount of the target chromosome (para [0077]); and
in i) using a maximum likelihood or maximum a posteriori approach to determine whether the chromosome indicating the presence or absence of a fetal aneuploidy (para [0077]);
but does not specifically disclose:
in b) DNA sequencing;
c) identifying chromosomes to which the sequences obtained in step b) belong;
d) using the data of step c) to determine an amount of at least one first chromosome in said mixture of maternal and fetal genomic DNA, wherein said at least one first chromosome is presumed to be euploid in the fetus;
e) using the data of step c) to determine an amount of a second chromosome in said mixture of maternal and fetal genomic DNA, wherein said second chromosome is suspected to be aneuploid in the fetus;
f) calculating the fraction of fetal DNA in the mixture of fetal and maternal DNA;
g) calculating an expected distribution of the amount of the second target chromosome if the second target chromosome is euploid, using the number in step d);
h) calculating an expected distribution of the amount of the second target chromosome if the second target chromosome is aneuploid, using the first number is step d) and the calculated fraction of fetal DNA in the mixture of fetal and maternal DNA in step f); and
i) using a maximum likelihood or maximum a posteriori approach to determine whether the amount of the second chromosome as determined in step e) is more likely to be part of the distribution calculated in step g) or the distribution calculated in step h); thereby indicating the presence or absence of a fetal aneuploidy.
However, Quake discloses a method for determining a ploidy status of a chromosome (para [0027]), comprising
in b) DNA sequencing (para [0033]);
c) identifying chromosomes to which the sequences obtained in step b) belong (para [0082]);
d) using the data of step c) to determine an amount of at least one first chromosome in said mixture of maternal and fetal genomic DNA, wherein said at least one first chromosome is presumed to be euploid in the fetus (para [0125], [0126], and [0129]);
e) using the data of step c) to determine an amount of a second chromosome in said mixture of maternal and fetal genomic DNA, wherein said second chromosome is suspected to be aneuploid in the fetus (para [0125], [0126], and [0129]);
f) calculating the fraction of fetal DNA in the mixture of fetal and maternal DNA (para [0126]);
g) calculating an expected distribution of the amount of the second target chromosome if the second target chromosome is euploid, using the number in step d) (para [0055], [0126], [0140]);
h) calculating an expected distribution of the amount of the second target chromosome if the second target chromosome is aneuploid, using the first number is step d) and the calculated fraction of fetal DNA in the mixture of fetal and maternal DNA in step f) (para [0055], [0126], [0140]).
To a person of ordinary skill in the art, it would have been obvious to determine a euploid status of a chromosome as taught by Quake in the method for determining a ploidy status of a chromosome as in Rabinowitz in order to detect genetic abnormalities that involve quantitative differences between maternal and fetal genetic sequences in aneuploidies (Quake: para [0129]), because Rabinowitz and Quake directed towards methods of determining ploidy status in a fetus using aneuploid measurements.

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Claims 5-21 and 42 lack an inventive step under PCT Article 33(3) as being obvious over Rabinowitz, in view of Dhallan, and in further view of US 2009/0099041 A1 to Church, et al. (hereinafter 'Church').

Regarding Claim 5, the combination of Rabinowitz and Dhallan teach the method of claim 4, but does not specifically disclose that the preferentially enriching the DNA in the first sample at the plurality of polymorphic loci comprises:
obtaining a plurality of pre-circularized probes where each probe targets one of the polymorphic loci, and where the 3' and 5' end of the probes are designed to hybridize to a region of DNA that is separated from the polymorphic site of the locus by a small number of bases, where the small number is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 to 25, 26 to 30, 31 to 60, or a combination thereof;
hybridizing the pre-circularized probes to DNA from the first sample;
filling the gap between the hybridized probe ends using DNA polymerase;
circularizing the pre-circularized probe; and
amplifying the circularized probe.
However, Church discloses preferentially enriching DNA in a sample at a plurality of polymorphic loci (para [0008], [0015]) comprising:
obtaining a plurality of pre-circularized probes where each probe targets one of the polymorphic loci (para [0009]), and where the 3' and 5' end of the probes are designed to hybridize to a region of DNA (para [0012], [0019], and [0038]) that is separated from the polymorphic site of the locus by a small number of bases, where the small number is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (para [0042]), 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,21 to 25, 26 to 30, 31 to 60, or a combination thereof;
hybridizing the pre-circularized probes to DNA from the first sample (para [0041], [0044], and [0051]);
filling the gap between the hybridized probe ends using DNA polymerase (para [0041], [0044], and [0051]);
circularizing the pre-circularized probe (para [0101]; and
amplifying the circularized probe (para [0059] and [0103]).
To a person of ordinary skill in the art, it would have been obvious to enrich DNA as taught by Church in the method for determining a ploidy status of a chromosome in a gestating fetus as in Rabinowitz in view of Dhallan in order to make large numbers of amplifiable oligonucleotide probes using microarray technology (para [0008]), because Rabinowitz, Dhallan, and Church are directed towards amplifying DNA using high throughput microarrays (Rabinowitz: pg 85, ln 15-33; Dhallan: para [0081]).

Regarding Claim 6, the combination of Rabinowitz and Dhallan teach the method of claim 4, but do not specifically disclose that the preferentially enriching the DNA at the plurality of polymorphic loci comprises:
obtaining a plurality of ligation-mediated PCR probes where each PCR probe targets one of the polymorphic loci, and where the upstream and downstream PCR probes are designed to hybridize to a region of DNA, on one strand of DNA, that is separated from the polymorphic site of the locus by a small number of bases, where the small number is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 to 25, 26 to 30, 31 to 60, or a combination thereof;
hybridizing the ligation-mediated PCR probes to the DNA from the first sample;
filling the gap between the ligation-mediated PCR probe ends using DNA polymerase;
ligating the ligation-mediated PCR probes; and
amplifying the ligated ligation-mediated PCR probes.
However, Church discloses preferentially enriching DNA in a sample at a plurality of polymorphic loci (para [0008], [0015]) comprising:
obtaining a plurality of ligation-mediated PCR probes where each PCR probe targets one of the polymorphic loci (para [0009]), and where the upstream and downstream PCR probes are designed to hybridize to a region of DNA (para [0012], [0019], [0038], and [0109]), on one strand of DNA (para [0009]), that is separated from the polymorphic site of the locus by a small number of bases, where the small number is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (para [0042]), 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 to 25, 26 to 30, 31 to 60, or a combination thereof;
hybridizing the ligation-mediated PCR probes to the DNA from the first sample;
filling the gap between the ligation-mediated PCR probe ends using DNA polymerase (para [0041], [0044], and [0051]);
ligating the ligation-mediated PCR probes (para [0039]); and
amplifying the ligated ligation-mediated PCR probes (para [0059] and [0103]).
To a person of ordinary skill in the art, it would have been obvious to enrich DNA as taught by Church in the method for determining a ploidy status of a chromosome in a gestating fetus as in Rabinowitz in view of Dhallan in order to make large numbers of amplifiable oligonucleotide probes using microarray technology (para [0008]), because Rabinowitz, Dhallan, and Church are directed towards amplifying DNA using high throughput microarrays (Rabinowitz: pg 85, ln 15-33; Dhallan: para [0081]).

Regarding Claim 7, the combination of Rabinowitz and Dhallan teach the method of claim 4, but do not specifically disclose that the preferentially enriching the DNA at the plurality of polymorphic loci comprises:
obtaining a plurality of hybrid capture probes that target the polymorphic loci;
hybridizing the hybrid capture probes to the DNA in the first sample; and
physically removing some or all of the unhybridized DNA from the first sample of DNA.
However, Church discloses preferentially enriching DNA in a sample at a plurality of polymorphic loci (para [0008], [0015]) comprising:
obtaining a plurality of hybrid capture probes that target the polymorphic loci (para [0013], [0050], and [0089]);
hybridizing the hybrid capture probes to the DNA in the first sample (para [0041], [0044], and [0051]); and
physically removing some or all of the unhybridized DNA from the first sample of DNA (para [0038]).
To a person of ordinary skill in the art, it would have been obvious to enrich DNA as taught by Church in the method for determining a ploidy status of a chromosome in a gestating fetus as in Rabinowitz in view of Dhallan in order to make large numbers of amplifiable oligonucleotide probes using microarray technology (para [0008]), because Rabinowitz, Dhallan, and Church are directed towards amplifying DNA using high throughput microarrays (Rabinowitz: pg 85, ln 15-33; Dhallan: para [0081]).

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Regarding Claim 8, Church further teaches that the plurality of hybrid capture probes are designed to hybridize to a region that is flanking but not overlapping the polymorphic site (para [0046], [0071], and [0096]).

Regarding Claim 9, Church further teaches that the plurality of hybrid capture probes are designed to hybridize to a region that is flanking but not overlapping the polymorphic site (para [0046], [0071], and [0096]), and where the length of the flanking capture probe may be selected from the group consisting of less than about 120 bases, less than about 110 bases, less than about 100 bases, less than about 90 bases, less than about 80 bases, less than about 70 bases, less than about 60 bases, less than about 50 bases (para [0093]), less than about 40 bases (para [0093]), less than about 30 bases (para [0093]), and less than about 25 bases (para [0093]).

Regarding Claim 10, Church further teaches that the plurality of hybrid capture probes are designed to hybridize to a region that overlaps the polymorphic site (para [0072]), and where the plurality of hybrid capture probes comprise at least two hybrid capture probes for each polymorphic loci (para [0013]), and where each hybrid capture probe is designed to be complementary to a different allele at that polymorphic locus (para [0012]).

Regarding Claim 11, the combination of Rabinowitz and Dhallan teach the method of claim 4, but does not specifically disclose that the preferentially enriching the DNA at a plurality of polymorphic loci comprises:
obtaining a plurality of inner forward primers where each primer targets one of the polymorphic loci, and where the 3' end of the inner forward primers are designed to hybridize to a region of DNA upstream from the polymorphic site, and separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, or 31 to 60 base pairs;
optionally obtaining a plurality of inner reverse primers where each primer targets one of the polymorphic loci, and where the 3' end of the inner reverse primers are designed to hybridize to a region of DNA upstream from the polymorphic site, and separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, or 31 to 60 base pairs;
hybridizing the inner primers to the DNA; and
amplifying the DNA using the polymerase chain reaction to form amplicons.
However, Church discloses preferentially enriching DNA in a sample at a plurality of polymorphic loci (para [0008], [0015]) comprising:
obtaining a plurality of inner forward primers where each primer targets one of the polymorphic loci, and where the 3' end of the inner forward primers are designed to hybridize to a region of DNA upstream from the polymorphic site (para [0113]), and separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10 (para [0042]), 11 to 15, 16 to 20, 21 to 25, 26 to 30, or 31 to 60 base pairs;
optionally obtaining a plurality of inner reverse primers where each primer targets one of the polymorphic loci (para [0109]-[0120]: see the probe production for direct circularization using reverse primers.), and
where the 3' end of the inner reverse primers are designed to hybridize to a region of DNA upstream from the polymorphic site (para [0109]-[0120]: see the probe production for direct circularization using reverse primers.), and separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10 (para [0042]), 11 to 15, 16 to 20, 21 to 25, 26 to 30, or 31 to 60 base pairs;
hybridizing the inner primers to the DNA (para [0041], [0044], and [0051]); and
amplifying the DNA using the polymerase chain reaction to form amplicons (para [0122]).
To a person of ordinary skill in the art, it would have been obvious to determine a ploidy status of a chromosome in a gestating fetus as in Dhallan in the method for determining a ploidy status of a chromosome as in Rabinowitz in order to detect genetic disorders including mutations and chromosomal abnormalities (Dhallan: para [0032]), because Rabinowitz and Dhallan are directed towards methods of determining ploidy status in a fetus using allele counts.

Regarding Claim 12, Church further teaches:
obtaining a plurality of outer forward primers where each primer targets one of the polymorphic loci, and where the outer forward primers are designed to hybridize to the region of DNA upstream from the inner forward primer (para [0109]-[0120]: see the probe production for direct circularization using forward primers.);
optionally obtaining a plurality of outer reverse primers where each primer targets one of the polymorphic loci (para [0109]-[0120]: see the probe production for direct circularization using reverse primers), and
where the outer reverse primers are designed to hybridize to the region of DNA immediately downstream from the inner reverse primer (para [0109]-[0120]: see the probe production for direct circularization using reverse primers.);
hybridizing the first primers to the DNA (para [0041], [0044], and [0051]); and
amplifying the DNA using the polymerase chain reaction (para [0125]).

Regarding Claim 13, Church further teaches:
obtaining a plurality of outer reverse primers where each primer targets one of the polymorphic loci, and where the outer reverse primers are designed to hybridize to the region of DNA immediately downstream from the inner reverse primer (para [0109]-[0120]: see the probe production for direct circularization using reverse primers);
optionally obtaining a plurality of outer forward primers where each primer targets one of the polymorphic loci, and where the outer forward primers are designed to hybridize to the region of DNA upstream from the inner forward primer (para [0109]-[0120]: see the probe production for direct circularization using forward primers.);
hybridizing the first primers to the DNA (para [0041], [0044], and [0051]); and
amplifying the DNA using the polymerase chain reaction (para [0125]).

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Regarding Claim 14, Church further teaches that the preparing the first sample further comprises:
appending universal adapters to the DNA in the first sample (para [0108]; and
amplifying the DNA in the first sample using the polymerase chain reaction (para [0125]).

Regarding Claim 15, Church further teaches that at least a fraction of the amplicons that are amplified are less than 100 bp, less than 90 bp, less than 80 bp, less than 70 bp, less than 65 bp, less than 60 bp, less than 55 bp, less than 50 bp, or less than 45 bp (para [0029]), and where the fraction is 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% (para [0041]).

Regarding Claim 16, Church further teaches that the amplifying the DNA is done in one or a plurality of individual reaction volumes, and where each individual reaction volume contains more than 100 different forward and reverse primer pairs (para [0041], [0093], [0102], and [0111]-[0117], more than 200 different forward and reverse primer pairs, more than 500 different forward and reverse primer pairs, more than 1,000 different forward and reverse primer pairs, more than 2,000 different forward and reverse primer pairs, more than 5,000 different forward and reverse primer pairs, more than 10,000 different forward and reverse primer pairs, more than 20,000 different forward and reverse primer pairs, more than 50,000 different forward and reverse primer pairs, or more than 100,000 different forward and reverse primer pairs.

Regarding Claim 17, Church further teaches that the preparing the first sample further comprises dividing the first sample into a plurality of portions, and where the DNA in each portion is preferentially enriched at a subset of the plurality of polymorphic loci (para [0068]).

Regarding Claim 18, Church further teaches that the inner primers are selected by identifying primer pairs likely to form undesired primer duplexes (para [0063], [0065], and [0079]) and removing from the plurality of primers at least one of the pair of primers indentified as being likely to form undesired primer duplexes (para [0111]).

Regarding Claim 19, Church further teaches that the inner primers contain a region that is designed to hybridize either upstream or downstream of the targeted polymorphic locus (para [0109]), and optionally contain a universal priming sequence designed to allow PCR amplification (para [0046]).

Regarding Claim 20, Church further teaches that at least some of the primers additionally contain a random region that differs for each individual primer molecule (para [0045]).

Regarding Claim 21, Church further teaches that at least some of the primers additionally contain a molecular barcode (para [0037]).

Regarding Claim 42, Dhallan further teaches a kit for determining a ploidy status of a target chromosome in a gestating fetus designed to be used with the method of claim 11 (para [0432]);
but does not specifically disclose that the kit comprises:
a plurality of inner forward primers and optionally the plurality of inner reverse primers, where each of the primers is designed to hybridize to the region of DNA immediately upstream and/or downstream from one of the polymorphic sites on the target chromosome, and optionally additional chromosomes, where the region of hybridization is separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, 31 to 60, and combinations thereof.
However, Church discloses preferentially enriching DNA in a sample at a plurality of polymorphic loci (para [0008], [0015]) comprising:
a plurality of inner forward primers (para [0109]-[0120]: see the probe production for direct circularization using forward primers.) and optionally the plurality of inner reverse primers (para [0109]-[0120]: see the probe production for direct circularization using reverse primers.), where each of the primers is designed to hybridize to the region of DNA immediately upstream and/or downstream from one of the polymorphic sites on the target chromosome (para [0109]), and optionally additional chromosomes, where the region of hybridization is separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1,2,3,4, 5, 6 to 10 (para [0042]), 11 to 15, 16 to 20, 21 to 25, 26 to 30, 31 to 60, and combinations thereof.
To a person of ordinary skill in the art, it would have been obvious to enrich DNA as taught by Church in the method for determining a ploidy status of a chromosome in a gestating fetus as in Rabinowitz in view of Dhallan in order to make large numbers of amplifiable oligonucleotide probes using microarray technology (para [0008]), because Rabinowitz, Dhallan, and Church are directed towards amplifying DNA using high throughput microarrays (Rabinowitz: pg 85, ln 15-33; Dhallan: para [0081]).

Claims 1-43 have industrial applicability as defined by PCT Article 33(4) because the subject matter could be made or used in industry.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G06F 19/22; G01N 33/48 (2012.01)
USPC - 702/20; 436/63, 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)
IPC(8)- G06F 19/22; G01N 33/48 (2012.01);
USPC- 702/20; 436/63, 94

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (US Pat, PgPub, EPO, JPO), GoogleScholar (PL, NPL), FreePatentsOnline (US Pat, PgPub, EPO, JPO, WIPO, NPL); search terms: ploid, fetal, fetus, gestate, state, hypothesis, joint, distribute, probability, likelihood, call, allele, DNA, nucleic, nucleotide, probe, hybrid, ligate, circular, SNP, polymorphism, loci, locus, flank, forward, rever

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2007/0184467 A1 (RABINOWITZ et al.) 09 August 2007 (09.08.2007), para [0029]-[0036], [0039], [0069]-[0079], [0121], [0126], [0127], [0142], [0144], [0148]-[0150], [0171], [0180], [0185], [0206], [0277], [0294], [0323], [0390]	1-43
Y	US 2007/0178478 A1 (DHALLAN et al.) 02 August 2007 (02.08.2007), para [0032], [0033], [0081], [0140], [0216], [1964]	1-42
Y	US 2007/0202525 A1 (QUAKE et al.) 30 August 2007 (30.08.2007), para [0027], [0033], [0055], [0082], [0125], [0126], [0129], [0140]	43
Y	US 2009/0099041 A1 (CHURCH et al.) 16 April 2009 (16.04.2009), para [0008], [0009], [0015], [0019], [0029], [0037]-[0045], [0046], [0051], [0059], [0068], [0071], [0093], [0096], [0101]-[0103], [0109]-[0122], [0125]	5-21, 42
X, P --- Y, P	WO 2010/017214 A1 (RABINOWITZ et al.) 11 February 2010 (11.02.2010), pg 3, ln 31; pg 4, ln 1-8; pg 4, ln1 2-32; pg 5, ln 15-22; pg 6, ln 11-33; pg 10, ln 26-30; pg 15, ln 5-13; pg 19, ln 1 to pg 20, ln 24; pg 22, ln 1-6; pg 23, ln 24-29; pg 24, ln 32 to pg 25, ln 12; pg 27, ln 1-4; pg 46, ln 5-30; pg 49, ln 15-28; pg 50, ln 1 to pg 51, ln 19; pg 73, ln 2-4; pg 79, ln 16 to pg 80, ln 15; pg 85, ln 15-33; pg 89, ln 12-25; pg 91, ln 3-5; pg 92, ln 3-8; pg 97, ln 19-31; pg 98, ln 4 to pg 99, ln 27; pg 101, ln 1-3	1-4, 22, 27-41 --- 5-21, 23-26, 42, 43
Y	US 2005/0255508 A1 (CASEY et al.) 17 November 2005 (17.11.2005), para [0007]-[0128]	1-43

☐ 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
27 February 2012 (27.02.2012)	16 MAR 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

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 W12	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2011/066938	International filing date *(day/month/year)* 22 December 2011 (22.12.2011)	Priority date *(day/month/year)* 22 December 2010 (22.12.2010)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 10 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 10 September 2013 (10.09.2013)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pt04.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	20 JUN 2012

Applicant's or agent's file reference	FOR FURTHER ACTION
118103011602	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/66938	22 December 2011 (22.12.2011)	22 December 2010 (22.12.2010)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2012.02)
USPC - 435/6.1

Applicant GENE SECURITY NETWORK, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	7 June 2012 (07.06.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 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 11/66938

Box No. 1 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 11/66938

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. 14

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. 14 are so unclear that no meaningful opinion could be formed *(specify)*:

Claim 14 is unsearchable because it is a dependent claim and is not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ 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. 14

☐ 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 mannacceptable 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 mannacceptable 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 11/66938

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 1-13, 15-25, drawn to a method for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother, the method comprising:
obtaining genetic material from the alleged father:
obtaining a blood sample from the pregnant mother;
making genotypic measurements, at a plurality of polymorphic loci, on the genetic material from the alleged father:
obtaining genotypic measurements, at the plurality of polymorphic loci, from the genetic material from the pregnant mother:
making genotypic measurements on a mixed sample of DNA originating from the blood sample from the pregnant mother,
where the mixed sample of DNA comprises fetal DNA and maternal DNA;
determining, on a computer, the probability that the alleged father is the biological father of the fetus gestating in the pregnant mother using the genotypic measurements made from the DNA from the alleged father, the genotypic measurements obtained from the pregnant mother, and the genotypic measurements made on the mixed sample of DNA; and
establishing whether the alleged father is the biological father of the fetus using the determined probability that the alleged father is the biological father of the fetus.

Group II: claims 26-42, drawn to a method for determining a fraction of DNA from a target individual present in a mixed sample of DNA that comprises DNA from the target individual and DNA from a second individual, the method comprising:
making genotypic measurements at a plurality of polymorphic loci from the mixed sample of DNA;
obtaining genotypic data at the plurality of polymorphic loci from the second individual; and
determining, on a computer, the fraction of DNA from the target individual present in the mixed sample using the genotypic measurements from the mixed sample of DNA, the genotypic data from the second individual, and probabilistic estimation techniques.

The inventions listed as Groups I-II 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 inventions of Group II do not include the inventive concept of a method for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother, the method comprising: obtaining genetic material from the alleged father: obtaining a blood sample from the pregnant mother; ... determining, on a computer, the probability that the alleged father is the biological father of the fetus gestating in the pregnant mother using the genotypic measurements made from the DNA from the alleged father, the genotypic measurements obtained from the pregnant mother, and the genotypic measurements made on the mixed sample of DNA; and establishing whether the alleged father is the biological father of the fetus using the determined probability that the alleged father is the biological father of the fetus, as required by Group I.

----------(Continued in Supplemental Box)----------

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. 1-13, 15-25

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/66938

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	1-13, 15-25	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-13, 15-25	NO
Industrial applicability (IA)	Claims	1-13, 15-25	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-3, 6-7, and 15-25 lack an inventive step under PCT Article 33(3) as being obvious over US 2008/0071076 A1 to Hahn, et al. (hereinafter "Hahn") in view of the article titled "Resolving relationship tests that show ambiguous STR results using autosomal SNPs as supplementary markers" by Phillips, et al. (hereinafter "Phillips").

As to claim 1, Hahn teaches a method for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother (para [0056]), the method comprising:
obtaining genetic material from the alleged father (para [0038], [0041]);
obtaining a blood sample from the pregnant mother (para [0038]);
making genotypic measurements at a plurality of polymorphic loci in the genetic material from the alleged father (para [0045]-[0054]; Table 2);
obtaining genotypic measurements at a plurality of polymorphic loci in the genetic material from the alleged father (para [0045]-[0054]; Table 2);
making genotypic measurements on a mixed sample of DNA originating from the blood sample from the pregnant mother, where the mixed sample of DNA comprises fetal DNA and maternal DNA (para [0045]-[0054]; Table 2); and
establishing whether the alleged father is the biological father of the fetus (para [0056]).
Hahn does not teach that the method further comprises determining, on a computer, the probability that the alleged father is the biological father of the fetus gestating in the pregnant mother using the genotypic measurements made from the DNA of the alleged father, the genotypic measurements obtained from the pregnant mother, and the genotypic measurements made on the mixed sample of DNA.
Phillips teaches a method for establishing whether an alleged father is the biological father is the biological father of a child (abstract), the method comprising:
obtaining genetic material from the alleged father, mother, and child (pg 200, para 2; Fig. 1A - disputed trio 44p06);
making genotypic measurements at a plurality of polymorphic loci in the genetic material from the alleged father, mother, and child (pg 199, para 3; Table 1);
determining, on a computer, the probability that the alleged father is the biological father of the child using the genotypic measurements made from the DNA of the alleged father, the genotypic measurements obtained from the mother, and the genotypic measurements obtained from the child (pg 199, para 4 - pg 200, para 1); and
establishing whether the alleged father is the biological father of the child using the determined probability that the alleged father is the biological father of the child (pg 201, para 2).
It would have been obvious to one of skill in the art that the method taught by Phillips could be adapted for use in determining paternity prior to the child being born (i.e., for determining paternity of a fetus gestating in a pregnant mother), provided that a method is available for obtaining fetal DNA, because it is often desirable to establish paternity as soon as possible in a pregnancy, rather than waiting until the child is born. The method taught by Hahn is optimal in that it provides a noninvasive method for detecting fetal DNA, which is preferred over riskier invasive methods such as amniocentesis or CVS, and can be performed earlier in a pregnancy than such methods. It further would have been obvious to adapt the method taught by Phillips to the method taught by Hahn, because using a computer to determine statistical probabilities based on the presence or absence of polymorphic markers was routine in the art of genetic analysis in general, and in paternity testing in particular.

As to claim 2, Phillips teaches that the polymorphic loci comprise SNPs (pg 199, para 3; Table 1).

As to claim 3, Hahn teaches that the mixed sample of DNA comprises DNA that was from free floating DNA in a plasma fraction of the blood sample from the pregnant mother (abstract; para [0008], [0045]).

----------(Continued in Supplemental Box)----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/66938

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Box IV.3. Lack of Unity of Invention:

In addition, claim 26 is obvious over a paper titled "Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays" by HOMER, et al. (PLoS Genet. 2008, 4(8):e1000167) (hereinafter "Homer") that teaches a method for resolving a target individual from a mixed sample of DNA comprising DNA from the target individuals and DNA from a second individual (abstract; pg 2, para 5 - pg 3, para 3 - mixtures C1, C2, D1, D2), the method comprising:
making genotypic measurements at a plurality of polymorphic loci from the mixed sample of DNA (pg 3, para 8; pg 5, para 5 - "For the Illumina platform we used the genotypes from the HapMap dataset [18] for both the person of interest and the reference populations"; pg 5, para 8, "To examine empirically the efficacy of our method we formed various known mixtures of DNA from HapMap individuals and genotyped the mixtures on three different platforms");
obtaining genotypic data at the plurality of polymorphic loci from the second individual (pg 3, para 8; pg 5, para 5 - "For the Illumina platform we used the genotypes from the HapMap dataset [18] for both the person of interest and the reference populations"; pg 5, para 8, "To examine empirically the efficacy of our method we formed various known mixtures of DNA from HapMap individuals and genotyped the mixtures on three different platforms"); and
resolving, on a computer, the target individual present in the mixed sample using the genotypic measurement from the mixed sample of DNA, the genotypic data from the second individual, and probalistic estimation techniques (abstract; pg 3, para 9 - pg 4, para 5 - "Theoretical derivation of test statistic"; pg 5, para 3 - "Software"; pg 5, para 4-5 - "Platform specific analysis"; pg 7, para 7 - "Resolving Members within 2 Person Mixtures (f =1% and f = 10%)").
Homer does not teach that the method is used to determing the fraction of DNA from the target individual present in the mixed sample. However, the mixtures taught by Homer comprise known fractions (pg 7, para 7 - "Resolving Members within 2 Person Mixtures (f =1% and f = 10%)"), and it would have been obvious that the same computational algorthims taught by Homer could be worked in reverse to determine an unknown fraction of a known target individual in a mixed sample.

Finally, claim 1 is obvious over US 2008/0071076 A1 to HAHN, et al. (hereinafter "Hahn") in view of the article titled "Resolving relationship tests that show ambiguous STR results using autosomal SNPs as supplementary markers" by PHILLIPS, et al. (Forensic Sci. Int. Genet. 2008, 2(3):198-204) (hereinafter "Phillips") as follows:

Hahn teaches a method for establishing whether an alged father is the biological father of a fetus that is gestating in a pregnant mother (para [0056]), the method comprising:
obtaining genetic material from the alleged father (para [0038], [0041]);
obtaining a blood sample from the pregnant mother (para [0038]);
making genotypic measurements at a plurality of polymorphic loci in the genetic material from the alleged father (para [0045]-[0054]; Table 2);
obtaining genotypic measurements at a plurality of polymorphic loci in the genetic material from the alleged father (para [0045]-[0054]; Table 2);
making genotypic measurements on a mixed sample of DNA originating from the blood sample from the pregnant mother, where the mixed sample of DNA comprises fetal DNA and maternal DNA (para [0045]-[0054]; Table 2); and
establishing whether the alleged father is the biological father of the fetus (para [0056]).
Hahn does not teach that the method further comprises determining, on a computer, the probability that the alleged father is the biological father of the fetus gestating in the pregnant mother using the genotypic measurements made from the DNA of the alleged father, the genotypic measurements obtained from the pregnant mother, and the genotypic measurements made on the mixed sample of DNA.
Phillips teaches a method for establishing whether an altged father is the biological father is the biological father of a child (abstract), the method comprising:
obtaining genetic material from the alleged father, mother, and child (pg 200, para 2; Fig. 1A - disputed trio 44p06);
making genotypic measurements at a plurality of polymorphic loci in the genetic material from the alleged father, mother, and child (pg 199, para 3; Table 1);
determining, on a computer, the probability that the alleged father is the biological father of the child using the genotypic measurements made from the DNA of the alleged father, the genotypic measurements obtained from the mother, and the genotypic measurements obtained from the child (pg 199, para 4 - pg 200, para 1); and
establishing whether the alleged father is the biological father of the child using the determined probability that the alleged father is the biological father of the child (pg 201, para 2).
It would have been obvious to one of skill in the art that the method taught by Phillips could be adapted for use in determining paternity prior to the child being born (i.e., for determining paternity of a fetus gestating in a pregnant mother), provided that a method is available for obtaining fetal DNA, because it is often desirable to establish paternity as soon as possible in a pregnancy, rather than waiting until the child is born. The method taught by Hahn is optimal in that it provides a nonivasive method for detecting fetal DNA, which is preferred over riskier invasive methods such as aminocentesis or CVS, and can be performed earlier in a pregnancy than such methods. It further would have been obvious to adapt the method taught by Phillips to the method taught by Hahn, because using a computer to determine statistical probabilities based on the presence or absence of polymorphic markers was routine in the art of genetic analysis in general, and in paternity testing in particular.

Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/66938

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 6, Phillips teaches that the determining comprises:
calculating a test statistic for the alleged father and the child, wherein the test statistic indicates a degree of genetic similarity between the alleged father and the child, and wherein the test statistic is based on the genotypic measurements from DNA from the alleged father, the genotypic measurements of the child, and the genotypic measurements obtained from DNA from the mother (pg 201, para 2; Table 3);
calculating the distribution of a test statistic for an individual who is genetically unrelated to the fetus, where each calculated test statistic indicates a degree of genetic similarity between the unrelated individual, wherein the test statistic is based on genotypic measurements made from DNA from the unrelated individual, the genotypic measurements made from the DNA from the child, and the genotypic measurements obtained from DNA from the mother (pg 200, para 1 - estimation of paternity index as compared to a random man as true father);
calculating a probability that the test statistic calculated for the alleged father and the child is part of the distribution of the test statistic calculated for the unrelated individual and the child (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man);
determining the probability that the alleged father is the biological father of the child using the probability that the test statistic calculated for the alleged father is part of the distribution of the test statistic calculated for the unrelated individual and the child (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man).
Although Phillips does not teach that the test statistic is determined using a plurality of unrelated individuals rather than a single such individual, it would have been obvious to one of skill in the art that the use of a plurality of unrelated individuals would provide a larger sample size and thus greater statistical accuracy.

As to claim 7, neither Hahn nor Phillips teaches that establishing whether an alleged father is biological father is the biological father of the fetus comprises:
establishing that the alleged father is biological father of the fetus by rejecting a hypothesis that the alleged father is unrelated to the fetus if the probability that the alleged father is the biological father of the fetus is above an upper threshold; or
establishing that the alleged father is not the biological father of the fetus by not rejecting a hypothesis that the alleged father is unrelated to the fetus if the probability that the alleged father is the biological father of the fetus is below a lower threshold; or
not establishing whether an alleged father is the biological father of the fetus if the likelihood is between the lower threshold and the upper threshold, or if the likelihood was not determined with sufficiently high confidence. However, it was routine in the art when performing statistical analysis to establish upper and lower thresholds for determining the outcome of the analysis (i.e., rejecting or not rejecting a hypothesis that the alleged father is the biological father of the fetus), and thus, it would have been obvious to one of skill in the art to perform any of the claimed steps as part of the method taught by Hahn in view of Phillips.

As to claim 15, Hahn teaches that the father's genetic material is obtained from blood (para [0041]).

As to claim 16, Phillips teaches that a confidence is computed for the established determination of whether the alleged father is the biological father of the child (pg 201, para 2).

As to claim 17, Hahn teaches that the fraction of fetal DNA in the mixed sample of DNA has been enriched using size selection (para [0043], [0045]).

As to claim 18, neither Hahn nor Phillips teaches that obtaining genotypic measurements from the genetic material of the pregnant mother comprises making genotypic measurements on a sample of genetic material from the pregnant mother that consists essentially of maternal genetic material. However, it would have been obvious to one of skill in the art that it would be preferable to obtain genotypic measurements from the pregnant mother on a sample of genetic material from the pregnant mother that consists essentially of maternal genetic material, in order to be 100% confident that the data is derived form the mother and not the fetus. Such genetic material can easily be obtained by noninvasive means other than taking a blood sample (e.g., using a buccal swab).

As to claim 19, Hahn teaches that obtaining genotypic measurements from the genetic material from the pregnant mother comprises:
inferring which genotypic measurements from the genotypic measurements made on the mixed sample of DNA are likely attributable to genetic material from the pregnant mother (para [0053]-[0054]; Table 2); and
using those genotypic measurements that were inferred to be attributable to genetic material from the mother as the obtained genotypic measurements (para [0053]-[0054]; Table 2).

As to claims 20-21, neither Hahn nor Phillips teaches that that the method further comprises making a clinical decision based on the established paternity determination, e.g., to terminate the pregnancy. However, it was routine in the art to follow prenatal tests with clinical decisions (in fact, the main purpose of prenatal tests is usually to inform potential subsequent clinical decisions), and it was routine in the art to make the decision to terminate the pregnancy (e.g., if determined biological father is not the desired biological father).

As to claim 22, Phillips teaches that making genotypic measurements is done by measuring genetic material using a SNP genotyping assay (pg 199, para 3).

As to claim 23, Hahn teaches that making genotypic measurements is done on genetic material that is amplified prior to being measured using PCR (para [0045]).

As to claim 24, Phillips teaches further comprising generating a report comprising the established paternity of the child (pg 201, para 2).

As to claim 25, Phillips teaches a report disclosing the established paternity of the child generating using the disclosed method (pg 201, para 2).

----------(Continued in Supplemental Box)----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 11/66938

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V.2. Citations and Explanations (previous Supplemental Box):

Claims 8-13 lack an inventive step under PCT Article 33(3) as being obvious over Hahn in view of Phillips, as above, and further in view of the article titled "Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays" by Homer, et al. (hereinafter "Homer").

As to claim 8, Hahn in view of Phillips teaches a method for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother (see explanation above for claim 1). Phillips further teaches that the determining the probability that the alleged father is the biological father of the child comprises:

obtaining the population frequencies of alleles for each locus in the plurality of polymorphic loci (pg 199, para 4 - "locally derived (NW Spain) allele frequencies");

calculating a probability that the alleged father is the biological father of the child given the genotypic measurements obtained from DNA from the mother, the genotypic measurements from the DNA from the alleged father, and the genotypic measurements made from the DNA from the child (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man);

calculating a probability that the alleged father is not the biological father of the child given the genotypic measurements obtained from DNA from the mother, the genotypic measurements from the DNA from the alleged father, and the genotypic measurements made from the DNA from the child (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man).

It would have been obvious to one of skill in the art to use a mixed sample of DNA in order to perform the disclosed method for determining the paternity of a gestating fetus instead of a child.

Neither Phillips nor Hahn teaches that the method further comprises creating a partition of possible fractions of fetal DNA in the mixed sample of DNA that range from a lower limit of fetal fraction to an upper limit of fetal fraction, performing the calculating steps for each of the possible fetal fractions in the partition, or performing the step of determining the probability that the alleged father is or is not the biological father of the fetus by combining the calculated probabilities that the alleged father is the fetus for each of the possible fetal fractions in the partition.

Homer teaches a method for resolving a target individual from a mixed sample of DNA comprising DNA from the target individual and DNA from a second individual (abstract; pg 2, para 5 - pg 3, para 3 - mixtures C1, C2, D1, D2), wherein the mixtures taught by Homer comprise known fractions (pg 7, para 7 - "Resolving Members within 2 Person Mixtures (f =1% and f = 10%)."). It would have been obvious to one of skill in the art that that the same computational algorithms taught by Homer could be worked in reverse to determine an unknown fraction of a known target individual in a mixed sample. Given a mixed sample of unknown fetal fraction, it thus would have been obvious to create a partition of possible fractions of fetal DNA in the mixed sample of DNA in the method of Hahn in view of Phillips that range from a lower limit of fetal fraction to an upper limit of fetal fraction, performing the calculating steps for each of the possible fetal fractions in the partition, and performing the steps of determining the probability that the alleged father is or is not the biological father of the fetus by combining the calculated probabilities that the alleged father is the fetus for each of the possible fetal fractions in the partition.

As to claim 9, Phillips teaches that wherein calculating the probability that the alleged father is or is not the biological father of the child further comprises:

calculating, for each of the plurality of polymorphic loci, a likelihood of observed sequence data at a particular locus using a plurality of allele ratios for the mother, a plurality of allele ratios for the alleged father, and a plurality of allele ratios for the child (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man);

calculating a likelihood that the alleged father is the biological father by combining the likelihood of the observed sequence data at each polymorphic locus over the mother allele ratios in the set of polymorphic loci, over the alleged father allele ratios in the set of polymorphic loci, and over the child allele ratios in the set of polymorphic loci (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man);

calculating a likelihood that the alleged father is not the biological father by combining the likelihood of the observed sequence data at each polymorphic locus over the mother allele ratios in the set of polymorphic loci, over the alleged father allele ratios in the set of polymorphic loci, and over the child allele ratios in the set of polymorphic loci (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man);

calculating a probability that the alleged father is the biological father based on the likelihood that the alleged father is the biological father (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man); and

calculating a probability that the alleged father is not the biological father based on the likelihood that the alleged father is the biological father (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man).

Philips does not teach that the first calculating step is done for one or a plurality of fractions in the possible fetal fractions partition, or that the second and calculating steps are performed over all fetal fractions in the partition. However, it would have been obvious to one of skill in the art to perform such a calculation for one or a plurality of fractions in the possible fetal fractions and to perform further steps over all fetal fractions in the partition, in order to accurately account for an unknown fetal fraction (see explanation above for claim 8). Phillips further does not teach that the first calculating uses a platform response model. However, it would have been obvious to one of skill in the art to use a platform response model, as such a model was routinely used to calculate statistical likelihoods based on observed allele frequencies.

As to claim 10, Phillips teaches that calculating the probability that the alleged father is the biological father based on the likelihood that the alleged father is the biological father is performed using a maximum likelihood estimation (pg 200, para 1).

As to claim 11, Phillips teaches that establishing whether an alleged father is the biological father of a fetus further comprises:

establishing that the alleged father is the biological father if the calculated probability that the alleged father is the biological father of the child is significantly greater than the calculated probability that the alleged father is not the biological father (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man); or

establishing that the alleged father is not the biological father if the calculated probability that the alleged father is the biological father of the child is significantly greater than the calculated probability that the alleged father is the biological father (pg 200, para 1; pg 201, para 2; Fig. 1A - "RM", random man).

----------(Continued in Supplemental Box)----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/66938

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V.2. Citations and Explanations (previous Supplemental Box):

As to claim 12, neither Hahn, Phillips, nor Homer teaches that that polymorphic loci correspond to chromosomes that that have a high likelihood of being disomic. However, it would have been obvious to one of skill in the art to choose polymorphic loci that correspond to chromosomes that that have a high likelihood of being disomic, in order to increase the statistical accuracy of the method (polymorphic loci that are not disomic would be more likely to result in a match between the alleged father and the fetus even when the alleged father is not the biological father, and therefore, to result in a false positive).

As to claim 13, Phillips teaches that the fetal fraction is determined using an "other" technique for measuring polymorphic alleles (pg 199, para 3 - commercial STR multiplexes and SNPforID assay).

Claims 4-5 lack an inventive step under PCT Article 33(3) as being obvious over Hahn in view of Phillips, as above, and further in view of US 2010/0216153 A1 to Lapidus, et al. (hereinafter "Lapidus").

As to claims 4-5, neither Hahn nor Phillips teaches that the mixed sample of DNA comprises maternal whole blood or a fraction of maternal blood containing nucleated cells, e.g., a fraction of maternal blood containing nucleated cells that has been enriched for cells of fetal origin. Lapidus teaches a method of isolating a fraction of maternal blood containing nucleated cells that has been enriched for cells of fetal origin (para [0039]). It would have been obvious to one of skill in the art that any known method for isolating fetal DNA from maternal blood would be useful in a method for obtaining noninvasive genotypic measurement of fetal DNA from a pregnant mother.

Claims 1-13 and 15-25 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/66938

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2012.02)
USPC - 435/6.1
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: 435/6.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.11, 6.12, 6.1; 702/20 (keywords below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed: paternity, testing, fetus, fetal, maternal, DNA, blood, mixed, SNP, polymorphism, computer, calculate, statistical, noninvasive, cfDNA, cell-free DNA, prenatal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0071076 A1 (HAHN, et al.) 20 March 2008 (20.03.2008) para [0006], [0036], [0041], [0043], [0045]-[0054], [0056]; Table 2	1-13, 15-25
Y	PHILLIPS, et al. Resolving relationship tests that show ambiguous STR results using autosomal SNPs as supplementary markers. Forensic Sci. Int. Genet. 2008, 2(3):198-204; abstract; pg 199, para 3 - pg 200, para 2; pg 201, para 2; Fig. 1A; Tables 1, 3	1-13, 15-25
Y	HOMER, et al. Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays. PLoS Genet. 2008, 4(8):e1000167, pages 1-9; abstract; pg 2, para 5 - pg 3, para 3; pg 7, para 7	8-13
Y	US 2010/0216153 A1 (LAPIDUS, et al.) 26 August 2010 (26.08.2010) para [0039]	4-5
A	WAGNER, et al. Non-invasive prenatal paternity testing from maternal blood. Int. J. Legal Med. 2008, 123:75-79	1-13, 15-25

☐ 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
7 June 2012 (07.06.2012)	20 JUN 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

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	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2012/058578	International filing date *(day/month/year)* 03 October 2012 (03.10.2012)	Priority date *(day/month/year)* 03 October 2011 (03.10.2011)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 6 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 08 April 2014 (08.04.2014)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Philippe Bécamel e-mail: pt03.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	25 MAR 2013
Applicant's or agent's file reference 118103012101	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/58578	03 October 2012 (03.10.2012)	03 October 2011 (03.10.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68, G06F 19/20 (2013.01)
USPC - 435/6.11

Applicant NATERA, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	22 February 2013 (22.02.2013)	Lee W. Young PCT Helpdesk: 571-272-4300 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 12/58578

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 12/58578

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, 7-8, 10, 12, 15, 17-18	YES
	Claims	1, 3-6, 9, 11, 13-14, 16, 19-20	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, 3-6, 9, 11, 13-14, 16, and 19-20 lack novelty under PCT Article 33(2) as being anticipated by US 2011/0201507 A1 to Rava et al. (hereinafter 'Rava').

Regarding claim 1, Rava teaches a method for determining a ploidy state of an embryo at a chromosome of interest (para [0009]-[0011], [0095]-[0103]), the method comprising: obtaining a genetic sample from the embryo (para [0009], [0095]);
preparing the genetic sample for sequencing (para [0011],[0236]);
sequencing the genetic sample to give sequencing data (para [0011], [0236]);
counting the number of sequence reads in the sequence data associated with each of a plurality of loci on the chromosome of interest (para [0081], [0154]-[0155]); and
determining the most likely ploidy state of the chromosome of interest given the sequence read count associated with each allele (para [0210], [0219], [0248], [0311]).

Regarding claim 3, further to the method of claim 1, Rava teaches wherein the step of preparing the genetic sample for sequencing may comprise performing amplification of the DNA in the genetic sample (para [0021], [0093], [0195]).

Regarding claim 4, further to the method of claim 1, Rava teaches wherein the step of preparing the genetic sample for sequencing may comprise performing universal amplification of the DNA in the genetic sample (para [0148], [0267]).

Regarding claim 5, further to the method of claim 1, Rava teaches wherein the step of preparing the genetic sample for sequencing may comprise preferentially enriching the DNA in the genetic sample at a plurality of polymorphic loci (para [0189], [0117]).

Regarding claim 6, further to the method of claim 5, Rava teaches wherein the step of preferentially enriching the DNA comprises performing targeted PCR amplification of the DNA in the genetic sample at a plurality of polymorphic loci (para [0195]).

Regarding claim 9, further to the method of claim 1, Rava teaches wherein the sequencing may be performed using a high throughput sequencer (para [0128]).

Regarding claim 11, further to the method of claim 1, Rava teaches wherein the step of determining the most likely ploidy state of the chromosome may further comprise:
counting the number of sequence reads in the sequence data associated with each of a plurality of loci on one or more reference chromosomes (para [0081], [0154]-[0155]);
and comparing the number of sequence reads associated with each of the plurality of loci on the chromosome of interest to the number of sequence reads associated with each of a plurality of targeted loci at one or a plurality of reference chromosomes where the reference chromosome(s) is assumed to be disomic (i.e. aneuploid) (para [0311]-[0312]; Fig. 19A,B).

Regarding claim 13, further to the method of claim 1, Rava teaches wherein the loci may be single nucleotide polymorphisms (para [0023]).

Regarding claim 14, further to the method of claim 13, Rava teaches wherein the step of determining the most likely ploidy state of the chromosome may comprise comparing the number of sequence reads associated with each of the alleles at the plurality of loci on the chromosome of interest, wherein certain allele ratios may be associated with certain ploidy states (para [0011], [0167]-[0169]).

Regarding claim 16, Rava teaches wherein the method of claim 1 may further comprise calculating a confidence estimate for a called ploidy state (para 0171], [0320]).

----------continued in Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/58578

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V 2. Citations and explanations:

Regarding claim 19, Rava teaches a method for determining a ploidy state of an embryo at a chromosome (para [0009]-[0011], [0095]-[0103]), the method comprising:
obtaining a genetic sample from the embryo (para [0009], [0095]);
amplifying the DNA present in the genetic sample by targeted PCR (para [0021], [0195]);
sequencing the amplified DNA using a high throughput sequencer to give sequencing data (para [0011], [0128]);
counting the number of sequence reads in the sequence data associated with each allele (para [0081], [0154]-[0155]) at a plurality of single nucleotide polymorphisms on the chromosome (para [0190]-[0191]);
calculating the allele ratios between the alleles at the plurality of single nucleotide polymorphisms on the chromosome (para [0011], [0167]-[0169], [0190]-[0191]); and
determining the most likely ploidy state of the chromosome given the calculated allele ratios at each of the polymorphisms on the chromosome (para [0011], [0167]-[0169], [0190]-[0191]).

Regarding claim 20, Rava teaches a method for determining a ploidy state of an embryo at a chromosome of interest (para [0009]-[0011], [0095]-[0103]), the method comprising:
obtaining a genetic sample from the embryo (para [0009], [0095]);
amplifying the DNA present in the genetic sample by targeted PCR where the targeted PCR targets a plurality of loci on the chromosome of interest and on one or more reference chromosomes (para [0021], [0195], [0151]; Fig. 1);
sequencing the amplified DNA using a high throughput sequencer to give sequencing data (para [0011], [0128]);
counting the number of sequence reads in the sequence data associated with each targeted locus on the chromosome of interest and on one or more reference chromosomes (para [0081], [0154]-[0155]);
determining the most likely ploidy state of the chromosome of interest given the ratio between the sequence read count associated with each targeted locus on the target chromosome and the sequence read count associated with each targeted allele on the reference chromosome (para [0011], [0167]-[0169]);
where certain ratios are associated with certain ploidy states (para [0011], [0167]-[0169]).

Claims 7, 12, 15, and 17-18 lack inventive step under PCT Article 33(3) as being obvious over Rava, as above.

Regarding claim 7, further to the method of claim 5, Rava teaches wherein the step of preferentially enriching the DNA may comprise:
obtaining a forward probe (para [0148] - "two amplification primers i.e. primer oligonucleotides ... capable of annealing to a primer-binding sequence in the polynucleotide molecule to be amplified (or the complement thereof if the template is viewed as a single strand) during the annealing step" where primers are synonymous with probes);
obtaining a reverse probe (para [0148]);
hybridizing the two probes to DNA in the first sample of DNA (para [0148] annealing); and
amplifying the DNA using the polymerase chain reaction (para [0148], [0198]).
Rava does not expressly teach wherein the 3' end of the forward probe is designed to hybridize to the region of DNA immediately upstream from the polymorphic region separated from the polymorphic region by a small number of bases, where the small number is selected from the group consisting of 1,2,3,4,5,6 to 10, and 11 to 20. Rava also does not expressly teach wherein the 3' end of the reverse probe is designed to hybridize to the region of DNA immediately downstream from the polymorphic region separated from the polymorphic region by a small number of bases, where the small number
is selected from the group consisting of 1,2,3,4,5,6 to 10, and 11 to 20. However, it would have been obvious to one of ordinary skill in the art to design said probes to hybridize at a region separated from the polymorphic region by a small number of bases, in order to preferentially enrich only regions containing the polymorphic loci (para [0198] primers are designed to discriminate against the amplification of similar genes on other chromosomes).

Regarding claim 12, further to the method of claim 1, Rava teaches counting the number of sequence reads in the sequence data associated with each of a plurality of loci on one or more reference chromosomes (para [0081], [0154]-[0155]). Rava does not expressly teach wherein the ploidy state of the chromosome of interest is determined to be trisomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest is about 50% greater than the number of sequence reads associated with each of a plurality of loci at one or a plurality of reference chromosomes; the ploidy state of the chromosome of interest is determined to be disomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest is about the same as the number of sequence reads associated with each of a plurality of loci at one or a plurality of reference chromosomes; and the ploidy state of the chromosome of interest is determined to be monosomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest is about 50% less than the number of sequence reads associated with each of a plurality of loci at one or a plurality of reference chromosomes.
However, it would have been obvious to one of ordinary skill in the art that the ploidy state of interest may be determined to be trisomy, or disomy, or monosomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest may be the percentage amounts recited in the claim, since a ploidy state of trisomy would have about 50% greater number of sequence reads, corresponding to a 3:2 ratio difference of trisomy to euploidy; and since a ploidy state of disomy would have about the same number of sequence reads, corresponding to a 2:2 ratio difference of diploidy to euploidy; and since a ploidy state of monosomy would have about 50% lesser number of sequence reads, corresponding to a 1:2 ratio difference of monoploidy to euploidy.

----------continued in next Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/58578

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box (Box No. V 2. Citations and explanations:)

Regarding claim 15, further to the method of claim 14, Rava does not expressly teach wherein: the ploidy state of the chromosome of interest is determined to be trisomy when the ratios of the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci on the chromosome of interest are about 100%, 67%, 33% or 0%; the ploidy state of the chromosome of interest is determined to be disomy when the ratios of the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci on the chromosome of interest are about 100%, 50% or 0%; and the ploidy state of the chromosome of interest is determined to be monosomy when the ratios of the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci on the chromosome of interest are about 100% or 0%.
However, it would have been obvious to one of ordinary skill in the art that the ploidy state of the chromosome of interest may be determined to be trisomy, or disomy, or monosomy where the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci at the chromosome of interest may be the percentage amounts recited in the claim, since a ploidy state of trisomy would have sequence reads of about 100%, 67%, 33% or 0% as corresponding to a 3:2 ratio difference of trisomy to euploidy, with determining sequence reads for either one or two or three of the three chromosomes; and since a ploidy state of disomy would have sequence reads of about 100%, 50% or 0% as corresponding to a 2:2 ratio difference of diploidy to euploidy, with determining sequence reads for either one or two of the two chromosomes; and since a ploidy state of monosomy would have sequence reads of about 100% or 0% as corresponding to a 1:2 ratio difference of monoploidy to euploidy, with determining sequence reads for one of the one chromosomes.

Regarding claim 17, Rava teaches wherein the method of claim 1 comprises calling the ploidy state of the embryo at that chromosome (para [0171], [0066]). Although Rava does not expressly teach the step of producing a report, it would have been obvious to one of ordinary skill in the art to generate a report as a way to convey the called ploidy state to the patient or their doctor.

Regarding claim 18, Rava does not expressly teach wherein the method of claim 1 may further comprise taking a clinical action based on the determined ploidy state of the embryo, wherein the clinical action is to transfer or not transfer the embryo into the uterus of the mother. However, as Rava teaches that determining the absence or presence of a clinically-relevant sequence, such as a nucleic acid sequence that is known or is suspected to be associated or implicated with a genetic or disease condition, can be useful in determining a diagnosis or confirming a diagnosis of a medical condition, or providing a prognosis for the development of a disease (para [0089]), it would have been obvious to one of ordinary skill the art that a clinical action based on the determined ploidy state of the embryo may be taken, wherein the clinical action is to transfer or not transfer the embryo into the uterus of the mother, while determining a diagnosis or confirming a diagnosis of a medical condition, or providing a prognosis for the development of a disease (para [0089]).

Claims 2, 8, and 10 lack inventive step under PCT Article 33(3) as being obvious over Rava, as above, in view of WO 2011/041485 A1 to Rabinowitz et al. (hereinafter 'Rabinowitz').

Regarding claim 2, further to the method of claim 1, Rava does not teach the further limitation taught by Rabinowitz, wherein the genetic sample may be one, two, three to five, six to ten, eleven to twenty, twenty one to fifty, or fifty-one to one hundred cells biopsied from an embryo (pg 20, para 3 - pg 21, para 1). It would have been obvious to one of ordinary skill in the art to conduct the method of claim 1, as taught by Rava, with a genetic sample comprising one, two, three to five, six to ten, eleven to twenty, twenty one to fifty, or fifty-one to one hundred cells biopsied from an embryo since a genetic sample comprising cells would enable a doctor to enrich the fraction of enucleated fetal blood cells in a maternal blood sample (Rabinowitz pg 61, para 3 - pg 62, para 1).

Regarding claim 8, further to the method of claim 5, Rava does not teach the further limitation taught by Rabinowitz, wherein the step of preferentially enriching the DNA may result in average degree of allelic bias between the second sample and the first sample of a factor selected from the group consisting of no more than a factor of 2, no more than a factor of 1.5, no more than a factor of 1.2, no more than a factor of 1.1, no more than a factor of 1.05, no more than a factor of 1.02, no more than a factor of 1.01, no more than a factor of 1.005, no more than a factor of 1.002, no more than a factor of 1.001 and no more than a factor of 1.0001 (pg 23, para 4 - "a statistical method may be used to remove the bias in the data due to the tendency for certain maternal or paternal alleles to amplify in a disproportionate manner ... [or]due to the tendency for certain probes to amplify certain SNPs in a manner that is disproportionate to other SNPs"). It would have been obvious to one of ordinary skill in the art to conduct the method of claim 5, as taught by Rava, with enriching the DNA to result in an average degree of allelic bias between the second sample and the first sample to a factor of no more than 1.0001, in order to thereby remove bias in the data that might result from a tendency for certain maternal or paternal alleles to amplify in a disproportionate manner, or from the tendency for certain probes to amplify certain SNPs in a manner that is disproportionate to other SNPs (Rabinowitz; pg 23, para 4).

Regarding claim 10, further to the method of claim 1, Rava does not teach the further limitation taught by Rabinowitz, wherein the step of determining the most likely ploidy state may comprise using maximum likelihood estimates to select the ploidy state corresponding to a hypothesis with the greatest probability (pg 26, para 2-3; pg 27 para 3). It would have been obvious to one of ordinary skill in the art to conduct the method of claim 1, as taught by Rava, to further comprise determining the most likely ploidy state with using maximum likelihood estimates, in order thereby to select a ploidy state for an embryo at a chromosome of interest, that might correspond to a selection hypothesis having the greatest probability.

Claims 1-20 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 118103012101	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/58578	International filing date *(day/month/year)* 03 October 2012 (03.10.2012)	(Earliest) Priority Date *(day/month/year)* 03 October 2011 (03.10.2011)
Applicant NATERA, 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 9 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 12/58578

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, G06F 19/20 (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) - C12Q 1/68, G06F 19/20 (2013.01)
USPC - 435/6.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/6.12, 702/19, 702/20

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubMed
Search terms: ploidy, aneuploidy, euploidy, chromosome, allele, copy number, fetal, embryo, prenatal, sequence reads, trisomy, disomy, monosomy, SNP, amplification, PCR, allelic ratio

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2011/0201507 A1 (RAVA et al.) 18 August 2011 (18.08.2011) para [0009], [0011], [0021], [0023], [0066],[0081], [0083], [0089], [0093], [0095], [0103], [0116]-[0117], [0128], [0140], [0148], [0151], [0154]-[0155], [0171] [0189]-[0191], [0195], [0198], [0219], [0236], [0248], [0254], [0260], [0263], [0267], [0311], [0312], [0320]; Figs 1, 19	1, 3-7, 9, 11-20 ---------- 2, 8, 10
Y	WO 2011/041485 A1 (RABINOWITZ et al.) 07 April 2011 (07.04.2011) pg 20, para 3 - pg 21, para 1; pg 23, para 4; pg 26, para 2-3; pg 27 para 3; pg 61, para 3 - pg 62, para 1	2, 8, 10
A	US 2009/0029377 A1 (LO et al.) 29 January 2009 (29.01.2009) entire document	1-20
A	LIAO et al., Targeted massively parallel sequencing of maternal plasma DNA permits efficient and unbiased detection of fetal alleles. Clin Chem., January 2011 (published online 15 November 2010) vol 57, no 1, pp 92-101; entire document	1-20
A	CHIU et al., Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci USA, 23 December 2008 (published online 10 December 2008) vol 105, no 51, pp 20458-63; 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
22 February 2013 (22.02.2013)	25 MAR 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	25 MAR 2013
Applicant's or agent's file reference 118103012101	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/58578	03 October 2012 (03.10.2012)	03 October 2011 (03.10.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68, G06F 19/20 (2013.01)
USPC - 435/6.11

Applicant NATERA, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	22 February 2013 (22.02.2013)	Lee W. Young PCT Helpdesk: 571-272-4300 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 12/58578

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 12/58578

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, 7-8, 10, 12, 15, 17-18	YES
	Claims	1, 3-6, 9, 11, 13-14, 16, 19-20	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, 3-6, 9, 11, 13-14, 16, and 19-20 lack novelty under PCT Article 33(2) as being anticipated by US 2011/0201507 A1 to Rava et al. (hereinafter 'Rava').

Regarding claim 1, Rava teaches a method for determining a ploidy state of an embryo at a chromosome of interest (para [0009]-[0011], [0095]-[0103]), the method comprising: obtaining a genetic sample from the embryo (para [0009], [0095]);
preparing the genetic sample for sequencing (para [0011],[0236]);
sequencing the genetic sample to give sequencing data (para [0011], [0236]);
counting the number of sequence reads in the sequence data associated with each of a plurality of loci on the chromosome of interest (para [0081], [0154]-[0155]); and
determining the most likely ploidy state of the chromosome of interest given the sequence read count associated with each allele (para [0210], [0219], [0248], [0311]).

Regarding claim 3, further to the method of claim 1, Rava teaches wherein the step of preparing the genetic sample for sequencing may comprise performing amplification of the DNA in the genetic sample (para [0021], [0093], [0195]).

Regarding claim 4, further to the method of claim 1, Rava teaches wherein the step of preparing the genetic sample for sequencing may comprise performing universal amplification of the DNA in the genetic sample (para [0148], [0267]).

Regarding claim 5, further to the method of claim 1, Rava teaches wherein the step of preparing the genetic sample for sequencing may comprise preferentially enriching the DNA in the genetic sample at a plurality of polymorphic loci (para [0189], [0117]).

Regarding claim 6, further to the method of claim 5, Rava teaches wherein the step of preferentially enriching the DNA comprises performing targeted PCR amplification of the DNA in the genetic sample at a plurality of polymorphic loci (para [0195]).

Regarding claim 9, further to the method of claim 1, Rava teaches wherein the sequencing may be performed using a high throughput sequencer (para [0128]).

Regarding claim 11, further to the method of claim 1, Rava teaches wherein the step of determining the most likely ploidy state of the chromosome may further comprise:
counting the number of sequence reads in the sequence data associated with each of a plurality of loci on one or more reference chromosomes (para [0081], [0154]-[0155]);
and comparing the number of sequence reads associated with each of the plurality of loci on the chromosome of interest to the number of sequence reads associated with each of a plurality of targeted loci at one or a plurality of reference chromosomes where the reference chromosome(s) is assumed to be disomic (i.e. aneuploid) (para [0311]-[0312]; Fig. 19A,B).

Regarding claim 13, further to the method of claim 1, Rava teaches wherein the loci may be single nucleotide polymorphisms (para [0023]).

Regarding claim 14, further to the method of claim 13, Rava teaches wherein the step of determining the most likely ploidy state of the chromosome may comprise comparing the number of sequence reads associated with each of the alleles at the plurality of loci on the chromosome of interest, wherein certain allele ratios may be associated with certain ploidy states (para [0011], [0167]-[0169]).

Regarding claim 16, Rava teaches wherein the method of claim 1 may further comprise calculating a confidence estimate for a called ploidy state (para 0171], [0320]).

----------continued in Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/58578

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V 2. Citations and explanations:

Regarding claim 19, Rava teaches a method for determining a ploidy state of an embryo at a chromosome (para [0009]-[0011], [0095]-[0103]), the method comprising:
obtaining a genetic sample from the embryo (para [0009], [0095]);
amplifying the DNA present in the genetic sample by targeted PCR (para [0021], [0195]);
sequencing the amplified DNA using a high throughput sequencer to give sequencing data (para [0011], [0128]);
counting the number of sequence reads in the sequence data associated with each allele (para [0081], [0154]-[0155]) at a plurality of single nucleotide polymorphisms on the chromosome (para [0190]-[0191]);
calculating the allele ratios between the alleles at the plurality of single nucleotide polymorphisms on the chromosome (para [0011], [0167]-[0169], [0190]-[0191]); and
determining the most likely ploidy state of the chromosome given the calculated allele ratios at each of the polymorphisms on the chromosome (para [0011], [0167]-[0169], [0190]-[0191]).

Regarding claim 20, Rava teaches a method for determining a ploidy state of an embryo at a chromosome of interest (para [0009]-[0011], [0095]-[0103]), the method comprising:
obtaining a genetic sample from the embryo (para [0009], [0095]);
amplifying the DNA present in the genetic sample by targeted PCR where the targeted PCR targets a plurality of loci on the chromosome of interest and on one or more reference chromosomes (para [0021], [0195], [0151]; Fig. 1);
sequencing the amplified DNA using a high throughput sequencer to give sequencing data (para [0011], [0128]);
counting the number of sequence reads in the sequence data associated with each targeted locus on the chromosome of interest and on one or more reference chromosomes (para [0081], [0154]-[0155]);
determining the most likely ploidy state of the chromosome of interest given the ratio between the sequence read count associated with each targeted locus on the target chromosome and the sequence read count associated with each targeted allele on the reference chromosome (para [0011], [0167]-[0169]);
where certain ratios are associated with certain ploidy states (para [0011], [0167]-[0169]).

Claims 7, 12, 15, and 17-18 lack inventive step under PCT Article 33(3) as being obvious over Rava, as above.

Regarding claim 7, further to the method of claim 5, Rava teaches wherein the step of preferentially enriching the DNA may comprise:
obtaining a forward probe (para [0148] - "two amplification primers i.e. primer oligonucleotides ... capable of annealing to a primer-binding sequence in the polynucleotide molecule to be amplified (or the complement thereof if the template is viewed as a single strand) during the annealing step" where primers are synonymous with probes);
obtaining a reverse probe (para [0148]);
hybridizing the two probes to DNA in the first sample of DNA (para [0148] annealing); and
amplifying the DNA using the polymerase chain reaction (para [0148], [0198]).
Rava does not expressly teach wherein the 3' end of the forward probe is designed to hybridize to the region of DNA immediately upstream from the polymorphic region separated from the polymorphic region by a small number of bases, where the small number is selected from the group consisting of 1,2,3,4,5,6 to 10, and 11 to 20. Rava also does not expressly teach wherein the 3' end of the reverse probe is designed to hybridize to the region of DNA immediately downstream from the polymorphic region separated from the polymorphic region by a small number of bases, where the small number
is selected from the group consisting of 1,2,3,4,5,6 to 10, and 11 to 20. However, it would have been obvious to one of ordinary skill in the art to design said probes to hybridize at a region separated from the polymorphic region by a small number of bases, in order to preferentially enrich only regions containing the polymorphic loci (para [0198] primers are designed to discriminate against the amplification of similar genes on other chromosomes).

Regarding claim 12, further to the method of claim 1, Rava teaches counting the number of sequence reads in the sequence data associated with each of a plurality of loci on one or more reference chromosomes (para [0081], [0154]-[0155]). Rava does not expressly teach wherein the ploidy state of the chromosome of interest is determined to be trisomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest is about 50% greater than the number of sequence reads associated with each of a plurality of loci at one or a plurality of reference chromosomes; the ploidy state of the chromosome of interest is determined to be disomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest is about the same as the number of sequence reads associated with each of a plurality of loci at one or a plurality of reference chromosomes; and the ploidy state of the chromosome of interest is determined to be monosomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest is about 50% less than the number of sequence reads associated with each of a plurality of loci at one or a plurality of reference chromosomes.
However, it would have been obvious to one of ordinary skill in the art that the ploidy state of interest may be determined to be trisomy, or disomy, or monosomy where the number of sequence reads associated with each of the plurality of loci at the chromosome of interest may be the percentage amounts recited in the claim, since a ploidy state of trisomy would have about 50% greater number of sequence reads, corresponding to a 3:2 ratio difference of trisomy to euploidy; and since a ploidy state of disomy would have about the same number of sequence reads, corresponding to a 2:2 ratio difference of diploidy to euploidy; and since a ploidy state of monosomy would have about 50% lesser number of sequence reads, corresponding to a 1:2 ratio difference of monoploidy to euploidy.

----------continued in next Supplemental Box ----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/58578

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box (Box No. V 2. Citations and explanations:)

Regarding claim 15, further to the method of claim 14, Rava does not expressly teach wherein: the ploidy state of the chromosome of interest is determined to be trisomy when the ratios of the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci on the chromosome of interest are about 100%, 67%, 33% or 0%; the ploidy state of the chromosome of interest is determined to be disomy when the ratios of the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci on the chromosome of interest are about 100%, 50% or 0%; and the ploidy state of the chromosome of interest is determined to be monosomy when the ratios of the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci on the chromosome of interest are about 100% or 0%.
However, it would have been obvious to one of ordinary skill in the art that the ploidy state of the chromosome of interest may be determined to be trisomy, or disomy, or monosomy where the number of sequence reads associated with each of the alleles at a plurality of polymorphic loci at the chromosome of interest may be the percentage amounts recited in the claim, since a ploidy state of trisomy would have sequence reads of about 100%, 67%, 33% or 0% as corresponding to a 3:2 ratio difference of trisomy to euploidy, with determining sequence reads for either one or two or three of the three chromosomes; and since a ploidy state of disomy would have sequence reads of about 100%, 50% or 0% as corresponding to a 2:2 ratio difference of diploidy to euploidy, with determining sequence reads for either one or two of the two chromosomes; and since a ploidy state of monosomy would have sequence reads of about 100% or 0% as corresponding to a 1:2 ratio difference of monoploidy to euploidy, with determining sequence reads for one of the one chromosomes.

Regarding claim 17, Rava teaches wherein the method of claim 1 comprises calling the ploidy state of the embryo at that chromosome (para [0171], [0066]). Although Rava does not expressly teach the step of producing a report, it would have been obvious to one of ordinary skill in the art to generate a report as a way to convey the called ploidy state to the patient or their doctor.

Regarding claim 18, Rava does not expressly teach wherein the method of claim 1 may further comprise taking a clinical action based on the determined ploidy state of the embryo, wherein the clinical action is to transfer or not transfer the embryo into the uterus of the mother. However, as Rava teaches that determining the absence or presence of a clinically-relevant sequence, such as a nucleic acid sequence that is known or is suspected to be associated or implicated with a genetic or disease condition, can be useful in determining a diagnosis or confirming a diagnosis of a medical condition, or providing a prognosis for the development of a disease (para [0089]), it would have been obvious to one of ordinary skill the art that a clinical action based on the determined ploidy state of the embryo may be taken, wherein the clinical action is to transfer or not transfer the embryo into the uterus of the mother, while determining a diagnosis or confirming a diagnosis of a medical condition, or providing a prognosis for the development of a disease (para [0089]).

Claims 2, 8, and 10 lack inventive step under PCT Article 33(3) as being obvious over Rava, as above, in view of WO 2011/041485 A1 to Rabinowitz et al. (hereinafter 'Rabinowitz').

Regarding claim 2, further to the method of claim 1, Rava does not teach the further limitation taught by Rabinowitz, wherein the genetic sample may be one, two, three to five, six to ten, eleven to twenty, twenty one to fifty, or fifty-one to one hundred cells biopsied from an embryo (pg 20, para 3 - pg 21, para 1). It would have been obvious to one of ordinary skill in the art to conduct the method of claim 1, as taught by Rava, with a genetic sample comprising one, two, three to five, six to ten, eleven to twenty, twenty one to fifty, or fifty-one to one hundred cells biopsied from an embryo since a genetic sample comprising cells would enable a doctor to enrich the fraction of enucleated fetal blood cells in a maternal blood sample (Rabinowitz pg 61, para 3 - pg 62, para 1).

Regarding claim 8, further to the method of claim 5, Rava does not teach the further limitation taught by Rabinowitz, wherein the step of preferentially enriching the DNA may result in average degree of allelic bias between the second sample and the first sample of a factor selected from the group consisting of no more than a factor of 2, no more than a factor of 1.5, no more than a factor of 1.2, no more than a factor of 1.1, no more than a factor of 1.05, no more than a factor of 1.02, no more than a factor of 1.01, no more than a factor of 1.005, no more than a factor of 1.002, no more than a factor of 1.001 and no more than a factor of 1.0001 (pg 23, para 4 - "a statistical method may be used to remove the bias in the data due to the tendency for certain maternal or paternal alleles to amplify in a disproportionate manner ... [or]due to the tendency for certain probes to amplify certain SNPs in a manner that is disproportionate to other SNPs"). It would have been obvious to one of ordinary skill in the art to conduct the method of claim 5, as taught by Rava, with enriching the DNA to result in an average degree of allelic bias between the second sample and the first sample to a factor of no more than 1.0001, in order to thereby remove bias in the data that might result from a tendency for certain maternal or paternal alleles to amplify in a disproportionate manner, or from the tendency for certain probes to amplify certain SNPs in a manner that is disproportionate to other SNPs (Rabinowitz; pg 23, para 4).

Regarding claim 10, further to the method of claim 1, Rava does not teach the further limitation taught by Rabinowitz, wherein the step of determining the most likely ploidy state may comprise using maximum likelihood estimates to select the ploidy state corresponding to a hypothesis with the greatest probability (pg 26, para 2-3; pg 27 para 3). It would have been obvious to one of ordinary skill in the art to conduct the method of claim 1, as taught by Rava, to further comprise determining the most likely ploidy state with using maximum likelihood estimates, in order thereby to select a ploidy state for an embryo at a chromosome of interest, that might correspond to a selection hypothesis having the greatest probability.

Claims 1-20 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 PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference W14	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2012/066339	International filing date *(day/month/year)* 21 November 2012 (21.11.2012)	Priority date *(day/month/year)* 24 July 2012 (24.07.2012)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 20 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 27 January 2015 (27.01.2015)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pt04.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	05 MAR 2013
Applicant's or agent's file reference 118103012701	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2012/066339	21 November 2012	24 July 2012

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11

Applicant NATERA, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	21 February 2013	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/066339

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:

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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	15, 20, 21, 25, 28-32, 34-39, 42, 55, 56, 62, 69, 88, 91	YES
	Claims	1-14, 16-19, 22-24, 26, 27, 33, 40, 41, 43-54, 57-61, 63-68, 70-87, 89, 90, 92-102	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-102	NO
Industrial applicability (IA)	Claims	1-102	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-14, 16-19, 22-24, 26, 27, 33, 40, 41, 43-54, 57-61, 63-68, 70-87, 89, 90, and 92-102 lack novelty under PCT Article 33(2) as being anticipated by Ryan et al. (hereafter Ryan).

Regarding claim 1, Ryan discloses a method of amplifying target loci in a nucleic acid sample (Para. [0050], steps may include: amplifying the genetic material; Para. [0139], a technique to enrich a sample of DNA at a set of target loci followed by sequencing), the method comprising: (a) contacting the nucleic acid sample with a library of test primers (Para. [0159], multiplex PCR amplification of the target loci; Para. [0163], a primer library that will result in a greater proportion of amplified DNA that maps to the genome) that simultaneously hybridize to at least 1,000 different target loci to produce a reaction mixture (Para. [0134], The sample is preferentially enriched at a set of loci using a 5,000-plex hemi-nested targeted PCR method (i.e. 5,000 target loci)); and (b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products comprising target amplicons (Para. [0134], The DNA in the sample are transformed through amplification. The sample is preferentially enriched at a set of loci using a 5,000-plex hemi-nested targeted PCR method (PCR comprises primer extension reaction conditions)).

Regarding claim 53, Ryan discloses a method of selecting test primers from a library of candidate primers (Para. [0164], There are a number of ways to choose primers for a library where the amount of non-mapping primer-dimer or other primer mischief products are minimized), the method comprising: (a) calculating on a computer undesirability score (Para. [0164], a small number of 'bad' primers are responsible for a large amount of non-mapping primer dimer side reactions; Para. [0089], associated quality score at each base position of the sequence; Para. [0166], create a thermodynamic model of potentially adverse interactions) for most or all of the possible combinations of two candidate primers from the library (Para. [0137], Any of the embodiments disclosed herein may be implemented in digital electronic circuitry, integrated circuitry, specially designed ASICs (application-specific integrated circuits), computer hardware, firmware, software, or in combinations thereof; Para. [0165], determining which PCR probes are likely to form dimers... analysis may be done and those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed), wherein each undesirability score is based at least in part on the likelihood of dimer formation between the two candidate primers (Para. [0166], create a thermodynamic model of potentially adverse interactions; Para. [0089], associated quality score at each base position of the sequence);(b) removing the candidate primer with the highest undesirability score from the library of candidate primers (Para. [0166], use the model to eliminate designs that are incompatible with other designs in the pool; Para. [0165], determining which PCR probes are likely to form dimers... those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed); (c) if the candidate primer removed in step (b) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers (Para. [0166], a pool of alleged primer pair designs...eliminate designs that are incompatible (i.e. pairs of primers are eliminated)); and (d) optionally repeating steps (b) and (c), thereby selecting a library of test primers (Para. [0166], use the model to eliminate designs that are incompatible with other designs in the pool (the process is repeated to eliminate all incompatible primer pairs)).

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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:

Claim 35 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 35 is indefinite for the following reason(s): With regards to claim 35, the claim depends on itself (claim 35) which is improper. For the purposes of the written opinion purposed, claim 35 is treated to be dependent on claim 34.

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In case **the space in any of the preceding boxes is not sufficient.**
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Regarding claim 54, Ryan discloses a method of selecting test primers from a library of candidate primers (Para. [0164], There are a number of ways to choose primers for a library where the amount of non-mapping primer-dimer or other primer mischief products are minimized), the method comprising: (a) calculating on a computer undesirability score (Para. [0164], a small number of 'bad' primers are responsible for a large amount of non-mapping primer dimer side reactions; Para. [0166], create a thermodynamic model of potentially adverse interactions; Para. [0089], associated quality score at each base position of the sequence) for most or all of the possible combinations of two candidate primers from the library (Para. [0137], Any of the embodiments disclosed herein may be implemented in digital electronic circuitry, integrated circuitry, specially designed ASICs (application-specific integrated circuits), computer hardware, firmware, software, or in combinations thereof; Para. [0165], determining which PCR probes are likely to form dimers... analysis may be done and those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed), wherein each undesirability score is based at least in part on the likelihood of dimer formation between the two candidate primers (Para. [0164], a small number of 'bad' primers are responsible for a large amount of non-mapping primer dimer side reactions; Para. [0166], create a thermodynamic model of potentially adverse interactions); (b) removing from the library of candidate primers the candidate primer that is part of the greatest number of combinations of two candidate primers with an undesirability score above a first minimum threshold (Para. [0166], a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible; Para. [0173], a metric that is directly related to or correlated with the condition is measured, and if the metric is on one side of a given threshold, the condition is determined to be present, while if the metric falls on the other side of the threshold, the condition is determined to be absent); (c) if the candidate primer removed in step (b) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers (Para. [0166], a pool of alleged primer pair designs...eliminate designs that are incompatible (i.e. pairs of primers are eliminated)); and (d) optionally repeating steps (b) and (c), thereby selecting a library of test primers (Para. [0166], use the model to eliminate designs that are incompatible with other designs in the pool (the process is repeated to eliminate all incompatible primer pairs)).

Regarding claim 72, Ryan discloses a library of primers that simultaneously amplifies at least 1,000 different target loci (Para. [0141], The number of genetic target sequences that may be run in parallel can range from one to ten, ten to one hundred, one hundred to one thousand, one thousand to ten thousand, ten thousand to one hundred thousand, one hundred thousand to one million, or one million to ten million. Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000-plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created), such that less than 30% of the amplified products are test primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)).

Regarding claim 73, Ryan discloses a library of primers that simultaneously amplifies at least 1,000 different target loci (Para. [0141], The number of genetic target sequences that may be run in parallel can range from one to ten, ten to one hundred, one hundred to one thousand, one thousand to ten thousand, ten thousand to one hundred thousand, one hundred thousand to one million, or one million to ten million. Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000-plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created)such that at least 80% of the amplified products are target amplicons (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci).

Regarding claim 74, Ryan discloses a library of primers that that simultaneously amplifies target loci such that at least 80% of the targeted loci out of at least 1,000 different target loci are amplified (Para. [0156], Multiplex PCR may involve a single round of PCR in which all targets are amplified; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol; [1200], the total of 1200 targets amplified in the first STA were thus amplified (100% of the targeted loci were amplified)).

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In case **the space in any of the preceding boxes is not sufficient.**
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Regarding claim 92, Ryan discloses a method for determining a ploidy status of a chromosome in a gestating fetus (Para. [0024], In an embodiment, the invention may comprise a report disclosing a ploidy state of the fetus determined using a method disclosed herein; Para. [0220], Determining the fraction of fetal DNA that is present in the mixed fraction of DNA may be an integral part of any method for non-invasive prenatal paternity determination, ploidy calling, or allele calling) , the method comprising: (a) contacting a nucleic acid sample with a library of primers that simultaneously hybridize to at least 1,000 different polymorphic loci to produce a reaction mixture (Para. [0262], 9,600 primers were used in the single well reactions; the primers were designed to target SNPs found on chromosomes 1, 2, 13, 18, 21, X and Y); wherein the nucleic acid sample comprises maternal DNA from the mother of the fetus and fetal DNA from the fetus (Para. [0008], genotypic measurements on a mixed sample of DNA originating from the blood sample from the pregnant mother, where the mixed sample of DNA comprises fetal DNA and maternal DNA); and (b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products (Para. [0261], An aliquot of the STA products was then amplified by standard PCR for 10 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries); (c) measuring the amplified products with a high throughput sequencer to produce sequencing data (Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol, sequenced, and ploidy determinations were made at three chromosomes); (d) calculating, on a computer (Para. [0020], determining, on a computer, the fraction of DNA from the target individual present in the mixed sample using the genotypic measurements from the mixed sample of DNA), allele counts at the polymorphic loci based on the sequencing data (Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol, sequenced, and ploidy determinations were made at three chromosomes); (e) creating on a computer a plurality of ploidy hypotheses each pertaining to a possible ploidy state of the chromosome (Para. [0045], techniques to choose the correct hypothesis from the set of hypotheses that were made using the measurement data); (f) building on a computer a joint distribution model for the expected allele counts at the polymorphic loci on the chromosome for each ploidy hypothesis (Para. [0045], By using the beta-binomial distribution as a model for allele distribution, one can more accurately model likely allele measurements than when using other distributions; this can result in more accurate paternity determinations; Para. [0173],without taking into account the alternate distribution, one cannot estimate the likelihood of each hypothesis given the observed data); (g) determining on a computer a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele counts (Para. [0043], A probability can then be calculated for each of the hypotheses given the data); and (h) calling the ploidy state of the fetus by selecting the ploidy state corresponding to the hypothesis with the greatest probability (Para. [0174], the maximum likelihood estimation method may use the allelic distributions associated with each hypothesis to estimate the likelihood of the data conditioned on each hypothesis. These conditional probabilities can then be converted to a hypothesis call and confidence; Para. [0172], a ploidy status of a target chromosome in a gestating fetus designed to be used with the methods disclosed herein).

Regarding claim 93, Ryan discloses a method of testing for an abnormal distribution of a chromosome in a sample comprising a mixture of maternal and fetal DNA (Para. [0024], In an embodiment, the invention may comprise a report disclosing a ploidy state (distribution of a chromosome) of the fetus determined using a method disclosed herein; Para. [0220], Determining the fraction of fetal DNA that is present in the mixed fraction of DNA may be an integral part of any method for non-invasive prenatal paternity determination, ploidy calling, or allele calling), the method comprising: (a) contacting the sample with a library of primers that simultaneously hybridize to at least 1,000 different target loci to produce a reaction mixture (Para. [0158], 10,000-plex PCR assay pool is created; Para. [0157], a multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes); wherein the target loci are from a plurality of different chromosomes (Para. [0157], a multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes); and wherein the plurality of different chromosomes comprise at least one first chromosome suspected of having an abnormal distribution in the sample and at least one second chromosome presumed to be normally distributed in the sample (Para. [0061], whether the target individual has one or more aneuploid chromosomes); (b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products (Para. [0261], An aliquot of the STA products was then amplified by standard PCR for 10 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries); (c) sequencing the amplified products to obtain a plurality of sequence tags aligning to the target loci (Para. [0261], tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries); wherein the sequence tags are of sufficient length to be assigned to a specific target locus (Para. [0040], primers in a single pool with the resulting amplified DNA comprising a majority of DNA molecules that, when sequenced, will map to targeted loci); (d) assigning on a computer (Para. [0020], determining, on a computer, the fraction of DNA from the target individual present in the mixed sample using the genotypic measurements from the mixed sample of DNA), the plurality of sequence tags to their corresponding target loci (Para. [0044], primers in a single pool with the resulting amplified DNA comprising a majority of DNA molecules that, when sequenced, will map to targeted loci; Para. [0084], the allelic ratio may refer to the ratio of sequence reads that map to each allele at the locus); (e) determining on a computer a number of sequence tags aligning to the target loci of the first chromosome and a number of sequence tags aligning to the target loci of the second chromosome (Para. [0084], when the sample is measured by sequencing, the allelic ratio may refer to the ratio of sequence reads that map to each allele at the locus. When the sample is measured by an intensity based measurement method, the allele ratio may refer to the ratio of the amounts of each allele present at that locus); (f) comparing on a computer the numbers from step (e) to determine the presence or absence of an abnormal distribution of the first chromosome (Para. [0134], This may involve calculating, on a computer, allele counts at the plurality of polymorphic loci from the DNA measurements made; Para. [0085], 'allele count distribution' refers to the relative amount of each allele that is present for each locus in a set of loci. In the context of sequencing, the allelic distribution refers to the number or probable number of reads that map to a particular allele for each allele in a set of polymorphic loci).

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Regarding claim 94, Ryan discloses a method for detecting the presence or absence of a fetal aneuploidy (Para. [0024], In an embodiment, the invention may comprise a report disclosing a ploidy state (distribution of a chromosome) of the fetus determined using a method disclosed herein; Para. [0220], Determining the fraction of fetal DNA that is present in the mixed fraction of DNA may be an integral part of any method for non-invasive prenatal paternity determination, ploidy calling, or allele calling), the method comprising: (a) contacting a sample comprising a mixture of maternal and fetal DNA (Para. [0008], genotypic measurements on a mixed sample of DNA originating from the blood sample from the pregnant mother, where the mixed sample of DNA comprises fetal DNA and maternal DNA) with a library of primers that simultaneously hybridize to at least 1,000 different non-polymorphic target loci to produce a reaction mixture (10,000-plex PCR assay pool is created; Para. [0157], a multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes); wherein the target loci are from a plurality of different chromosomes (Para. [0157], multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes);(b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products comprising target amplicons (Para. [0158], amplification with the first 10,000-plex assay; Para. [0261], An aliquot of the STA products was then amplified by standard PCR for 10 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries); (c) quantifying on a computer a relative frequency of the target amplicons from the first and second chromosomes of interest (Para. [0020], determining, on a computer, the fraction of DNA from the target individual present in the mixed sample using the genotypic measurements from the mixed sample of DNA; Para. [0022], In an embodiment, inferring the genotypic data of the related individual may also include using allele population frequencies at the loci); (d) comparing on a computer the relative frequency of the target amplicons from the first and second chromosomes of interest (Para. [0020], determining, on a computer, the fraction of DNA from the target individual present in the mixed sample using the genotypic measurements from the mixed sample of DNA; Para. [0022], In an embodiment, inferring the genotypic data of the related individual may also include using allele population frequencies at the loci); and (e) identifying the presence or absence of an aneuploidy based on the compared relative frequencies of the first and second chromosome of interest (Para. [0248], the probabilities of child genotypes (i.e. ploidy of chromosomes) may be determined by the known mother genotypes and the population frequencies).

Regarding claim 95, Ryan discloses a method for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother (Para. [0008], a method for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother), the method comprising: (a) simultaneously amplifying a plurality of polymorphic loci comprising at least 1,000 different polymorphic loci on genetic material from the alleged father to produce a first set of amplified products (10,000-plex PCR assay pool is created; Para. [0157], a multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes; Para. [0008], making genotypic measurements, at a plurality of polymorphic loci, on the genetic material from the alleged father; Para. [0253], The genomic DNA in the maternal and alleged paternal buffy coat and the DNA in the maternal serum were prepared for analysis...24 ul DNA was whole genome amplified (all loci were amplified) at 37 C for 20-24 hours followed by fragmentation and precipitation); (b) simultaneously amplifying the corresponding plurality of polymorphic loci on a mixed sample of DNA originating from a blood sample from the pregnant mother to produce a second set of amplified products; wherein the mixed sample of DNA comprises fetal DNA and maternal DNA (Para. [0053], a blood sample is taken from a pregnant mother, and the free floating DNA in the plasma of the mother's blood, which contains a mixture of both DNA of fetal origin, and DNA of fetal origin... enrichment of those DNA sequences in a mixture of DNA that correspond to polymorphic alleles in a way that the allele ratios and/or allele distributions remain reasonably consistent upon enrichment. In an embodiment, the method involves amplifying the isolated DNA using whole genome amplification); (c) determining on a computer the probability that the alleged father is the biological father of the fetus using genotypic measurements based on the first and second sets of amplified products (Para. [0036], the method may include using the genotypic measurements to determine, on a computer, the probability that the alleged father is the biological father of the fetus gestating in the pregnant mother); and (d) establishing whether the alleged father is the biological father of the fetus using the determined probability that the alleged father is the biological father of the fetus (Para. [0010], determining the probability that the alleged father is the biological father of the fetus using the probability that the test statistic calculated for the alleged father is part of the distribution of the test statistic calculated for the plurality of unrelated individuals and the fetus. In an embodiment, establishing whether an alleged father is the biological father of the fetus also includes establishing that the alleged father is the biological father of the fetus by rejecting a hypothesis that the alleged father is unrelated to the fetus).

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Regarding claim 97, Ryan discloses a method of measuring the amount of two or more target loci in a nucleic acid sample (Para. [0014], comparing the measured amount of paternally inherited alleles to the measured amount of maternally inherited alleles), the method comprising: (a) using PCR to amplify a nucleic acid sample (Para. [0018], making genotypic measurements may be done on genetic material that is amplified using a technique or technology that is selected from the group consisting of: Polymerase Chain Reaction (PCR),) comprising a first standard locus, a second standard locus, a first target locus, and a second target locus to form amplified products; wherein the first standard locus and the first target locus have the same number of nucleotides but have a sequence that differs at one or more nucleotides (Para. [0014], measuring polymorphic alleles that are present in the genome of the father but not present in the genome of the mother); and wherein the second standard locus and the second target locus have the same number of nucleotides but have a sequence that differs at one or more nucleotides (Para. [0014], measuring polymorphic alleles that are present in the genome of the father but not present in the genome of the mother, comparing the measured amount of paternally inherited alleles to the measured amount of maternally inherited alleles (a plurality of polymorphic alleles in the fetus (target loci) inherited from the father are determined, the respective loci in the maternal and paternal loci serving as the standard loci)); (b) sequencing the amplified products (Para. [0040], PCR to amplify DNA followed by high throughput sequencing to determine the allele frequencies at each target locus) to determine a standard ratio that compares the relative amount of the amplified first standard locus compared to the amplified second standard locus (Para. [0084], Allelic Ratio refers to the ratio between the amount of each allele at a polymorphic locus that is present in a sample); wherein the standard ratio indicates the difference in PCR efficiency for the amplification of the first standard locus and the second standard locus (Para. [0086], Allelic Bias refers to the degree to which the measured ratio of alleles at a heterozygous locus is different to the ratio that was present in the original sample of DNA. Allelic bias maybe due to amplification bias, purification bias, or some other phenomenon that affects different alleles differently) ; (c) determining a target ratio that compares the relative amount of the amplified first target locus compared to the amplified second target locus (Para. [0084], when the sample is measured by sequencing, the allelic ratio may refer to the ratio of sequence reads that map to each allele at the locus. When the sample is measured by an intensity based measurement method, the allele ratio may refer to the ratio of the amounts of each allele present at that locus) ; and (d) adjusting the target ratio from step (c) based on the standard ratio from step (b) to determine the relative amount of the first target locus and the second target locus in the sample (Para. [0134], This may involve calculating, on a computer, allele counts at the plurality of polymorphic loci from the DNA measurements made; Para. [0085], 'allele count distribution' refers to the relative amount of each allele that is present for each locus in a set of loci. In the context of sequencing, the allelic distribution refers to the number or probable number of reads that map to a particular allele for each allele in a set of polymorphic loci).

Regarding claim 2, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 5,000 different target loci are amplified (Para. [0134], The sample is preferentially enriched at a set of loci using a 5,000-plex hemi-nested targeted PCR method (i.e. 5,000 target loci)).

Regarding claim 3, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 10,000 different target loci are amplified (Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000- plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 4, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 20,000 different target loci are amplified (Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000- plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 5, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 30,000 different target loci are amplified (Para. [0141], The number of genetic target sequences that may be run in parallel can range from one to ten, ten to one hundred, one hundred to one thousand, one thousand to ten thousand, ten thousand to one hundred thousand, one hundred thousand to one million, or one million to ten million. Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000- plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 6, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 90% of the amplified products are target amplicons (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci).

Regarding claim 7, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 95% of the amplified products are target amplicons (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci).

Regarding claim 8, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 99% of the amplified products are target amplicons (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci).

Regarding claim 9, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 90% of the targeted loci are amplified (Para. [0156], Multiplex PCR may involve a single round of PCR in which all targets are amplified; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol; [1200], the total of 1200 targets amplified in the first STA were thus amplified (100% of the targeted loci were amplified)).

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Regarding claim 10, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 95% of the targeted loci are amplified (Para. [0156], Multiplex PCR may involve a single round of PCR in which all targets are amplified; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol; [1200], the total of 1200 targets amplified in the first STA were thus amplified (100% of the targeted loci were amplified)).

Regarding claim 11, Ryan discloses the method of claim 1. Ryan further discloses wherein at least 99% of the targeted loci are amplified (Para. [0156], Multiplex PCR may involve a single round of PCR in which all targets are amplified; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol; [1200], the total of 1200 targets amplified in the first STA were thus amplified (100% of the targeted loci were amplified)).

Regarding claim 12, Ryan discloses the method of claim 1. Ryan further discloses wherein less than 20% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)).

Regarding claim 13, Ryan discloses the method of claim 1. Ryan further discloses wherein less than 10% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)).

Regarding claim 14, Ryan discloses the method of claim 1. Ryan further discloses wherein less than 1% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)).

Regarding claim 16, Ryan discloses the method of claim 1, Ryan further discloses wherein the test primers are selected from a library of candidate primers based on one or more parameters (Para. [0166], one may start with a pool of alleged primer pair designs and create a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible with other the designs in the pool) .

Regarding claim 17, Ryan discloses the method of claim 16. Ryan further discloses wherein the test primers are selected from a library of candidate primers based at least in part on the ability of the candidate primers to form primer dimers (Para. [0165], determining which PCR probes are likely to form dimers. In an embodiment, analysis of a pool of DNA that has been amplified using a non-optimized set of primers may be sufficient to determine problematic primers. For example, analysis may be done using sequencing, and those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed.).

Regarding claim 18, Ryan discloses the method of claim 17. Ryan further discloses wherein the selection comprises (i) calculating on a computer an undesirability score (Para. [0137], Any of the embodiments disclosed herein may be implemented in digital electronic circuitry, integrated circuitry, specially designed ASICs (application-specific integrated circuits), computer hardware, firmware, software, or in combinations thereof; Para. [0166], create a thermodynamic model of potentially adverse interactions) for most or all of the possible combinations of two candidate primers from the library, wherein each undesirability score is based at least in part on the likelihood of dimer formation between the two candidate primers (Para. [0164], publicly available (computer) programs that can calculate the binding energy of various primer combinations, and removing those with the highest binding energy Para. [0166], one may start with a pool of alleged primer pair designs and create a thermodynamic model of potentially adverse interactions between primer pairs (i.e. to form dimers); (ii) removing the candidate primer with the highest undesirability score from the library of candidate primers (Para. [0166], and then use the model to eliminate designs that are incompatible with other the designs in the pool); (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers (Para. [0166], a pool of alleged primer pair designs...eliminate designs that are incompatible (i.e. pairs of primers are eliminated)); and (iv) optionally repeating steps (ii) and (iii) (Para. [0166], use the model to eliminate designs that are incompatible with other designs in the pool (the process is repeated to eliminate all incompatible primer pairs)).

Regarding claim 19, Ryan discloses the method of claim 17. Ryan further discloses wherein the selection comprises (i) calculating on a computer an undesirability score (Para. [0166], create a thermodynamic model of potentially adverse interactions) for most or all of the possible combinations of two candidate primers from the library, wherein each undesirability score is based at least in part on the likelihood of dimer formation between the two candidate primers (Para. [0164], publicly available (computer) programs that can calculate the binding energy of various primer combinations, and removing those with the highest binding energy Para. [0166], one may start with a pool of alleged primer pair designs and create a thermodynamic model of potentially adverse interactions between primer pairs (i.e. to form dimers; Para. [0165], determining which PCR probes are likely to form dimers. In an embodiment, analysis of a pool of DNA that has been amplified using a non-optimized set of primers may be sufficient to determine problematic primers. For example, analysis may be done using sequencing, and those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed); (ii) removing from the library of candidate primers the candidate primer that is part of the greatest number of combinations of two candidate primers (Para. [0165], those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed) with an undesirability score above a first minimum threshold (Para. [0166], a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible; Para. [0173], a metric that is directly related to or correlated with the condition is measured, and if the metric is on one side of a given threshold, the condition is determined to be present, while if the metric falls on the other side of the threshold, the condition is determined to be absent); (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers (Para. [0166], a pool of alleged primer pair designs...eliminate designs that are incompatible (i.e. pairs of primers are eliminated); and (iv) optionally repeating steps (ii) and (iii) (Para. [0166], use the model to eliminate designs that are incompatible with other designs in the pool (the process is repeated to eliminate all incompatible primer pairs)).

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Regarding claim 22, Ryan discloses the method of claim 19. Ryan further discloses wherein a candidate primer is selected out of a group of two or more candidate primers with equal undesirability scores for removal from the library based on one or more parameters (Para. [0165], analysis of a pool of DNA that has been amplified using a non-optimized set of primers may be sufficient to determine problematic primers...analysis may be done using sequencing, and those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed (the pool of non-optimized primers would inherently comprise some primers of roughly equal undesirability, the parameter being the amount of dimer actually formed)).

Regarding claim 23, Ryan discloses the method of claim 1. Ryan further discloses wherein the concentration of each test primer is less than 100 nM (Para. [0263], forward primers and tagged reverse primers at 7.3 nM).

Regarding claim 24, Ryan discloses the method of claim 1. Ryan further discloses wherein the concentration of each test primer is less than 10 nM (Para. [0263], forward primers and tagged reverse primers at 7.3 nM).

Regarding claim 26, Ryan discloses the method of claim 1. Ryan further discloses wherein the library of test primers comprises at least 1,000 test primer pairs that include a forward test primer and a reverse test primer where each pair of test primers hybridize to a target locus (Para. [0263], a semi-nested 9600-plex protocol. The semi-nested protocol is different in that it applies 9,600 outer forward primers and tagged reverse primers).

Regarding claim 27, Ryan discloses the method of claim 1. Ryan further discloses wherein the library of test primers comprises at least 1,000 individual test primers that each hybridize to a different target locus (Para. [0141], The number of genetic target sequences that may be run in parallel can range from one to ten, ten to one hundred, one hundred to one thousand, one thousand to ten thousand, ten thousand to one hundred thousand, one hundred thousand to one million, or one million to ten million).

Regarding claim 33, Ryan discloses the method of claim 1. Ryan further discloses wherein the test primers comprise a tag that is not target specific (Para. [0151], a universal tag that was introduced as part of the target specific forward primers in the first round).

Regarding claim 40, Ryan discloses the method of claim 1. Ryan further discloses wherein range of the length of the target amplicons is less than 15 nucleotides (Para. [0154], a substantial fraction of
the amplicons should be... less than 45 bp).

Regarding claim 41, Ryan discloses the method of claim 1. Ryan further discloses wherein the primer extension reaction conditions are polymerase chain reaction conditions (PCR) (Para. [0018], measurements may be done on genetic material that is amplified and/or preferentially enriched prior to being measured using a technique or technology that is selected from the group consisting of: Polymerase Chain Reaction (PCR); Para. [0153], very short PCR assays, termed mini-PCR assays).

Regarding claim 43, Ryan discloses the method of claim 1. Ryan further discloses determining the presence or absence of at least one target amplicon (Para. [0174], detect the presence or absence of phenotype or genotype, for example, a chromosomal abnormality).

Regarding claim 44, Ryan discloses the method of claim 1. Ryan further discloses determining the sequence of at least one target amplicon (Para. [0134], sample is preferentially enriched at a set of loci using a 5,000-plex hemi-nested targeted PCR method. The mixture of DNA fragments is prepared into a DNA library suitable for sequencing. The DNA is then sequenced).

Regarding claim 45, Ryan discloses the method of claim 1. Ryan further discloses wherein the target loci are present on the same chromosome of interest (Para. [0157], multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes).

Regarding claim 46, Ryan discloses the method of claim 1. Ryan further discloses wherein at least some of the target loci are present on different chromosomes of interest (Para. [0086], alleles at a heterozygous locus).

Regarding claim 47, Ryan discloses the method of claim 1. Ryan further discloses wherein the nucleic acid sample comprises fragmented or digested nucleic acids (Para. [0147], PCR can be used to target specific locations of the genome. In plasma samples, the original DNA is highly fragmented).

Regarding claim 48, Ryan discloses the method of claim 1. Ryan further discloses wherein nucleic acid sample comprises genomic DNA, cDNA, or mRNA (Para. [0147], methods may be used for analysis of any sample of DNA... DNA samples such as... genomic DNA).

Regarding claim 49, Ryan discloses the method of claim 1. Ryan further discloses wherein nucleic acid sample comprises DNA from a single cell (Para. [0147], methods may be used for analysis of any sample of DNA... DNA samples such as a single or small number of cells).

Regarding claim 50, Ryan discloses the method of claim 1. Ryan further discloses wherein the nucleic acid sample comprises or is derived from blood, plasma, saliva, sperm, cell culture supernatant, mucus secretion, dental plaque, gastrointestinal tract tissue, stool, urine, or forensic sample (Para. [0150], In an embodiment, the method may be applied for analysis of single cells, body fluids, mixtures of DNA such as the free floating DNA found in maternal plasma, biopsies, environmental and/or forensic samples).

Regarding claim 51, Ryan discloses the method of claim 1. Ryan further discloses wherein the target loci are segments of human nucleic acids (Para. [0162], It may be used for standard paternity and identity testing of relatives or ancestors, in human).

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Regarding claim 52, Ryan discloses the method of claim 1. Ryan further discloses wherein the target loci comprise single nucleotide polymorphisms (Para. [0157], multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes).

Regarding claim 57, Ryan discloses the method of claim 53. Ryan further discloses wherein a candidate primer is selected out of a group of two or more candidate primers with equal undesirability scores for removal from the library of candidate primers based on one or more other parameters (Para. [0165], analysis of a pool of DNA that has been amplified using a non-optimized set of primers may be sufficient to determine problematic primers...analysis may be done using sequencing, and those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed).

Regarding claim 58, Ryan discloses the method of claim 53. Ryan further discloses wherein the undesirability scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, disease prevalence associated with a polymorphism or mutation at the target locus, disease penetrance associated with a polymorphism or mutation at the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, amplification efficiency of the target amplicon, and size of the target amplicons (Para. [0203], correlation rate measured between the genotypic data of the fetus, as measured on a single cell, and the genotypic data of the assumed parents when zero, one or two of the assumed parents are actually the genetic parents of the fetus).

Regarding claim 59, Ryan discloses the method of claim 58. Ryan further discloses wherein the undesirability scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, amplification efficiency of the target amplicon, and size of the target amplicons (Para. [0203], correlation rate measured between the genotypic data of the fetus, as measured on a single cell, and the genotypic data of the assumed parents when zero, one or two of the assumed parents are actually the genetic parents of the fetus); and wherein the test primers are used to simultaneously amplify at least 1,000 target loci in a sample (Para. [0151], perform about 100-plex, about 1000-plex, or about 10,000-plex amplification of selected targets using one target specific "Forward" primer per target and one tag specific primer) comprising maternal DNA from the pregnant mother of a fetus and fetal DNA to determine the presence or absence of a fetal chromosome abnormality (Para. [0008],genetic material from the pregnant mother, making genotypic measurements on a mixed sample of DNA originating from the blood sample from the pregnant mother, where the mixed sample of DNA comprises fetal DNA and maternal DNA; Para. [0173], for detecting the presence or absence of a phenotype or genotype, for example, a chromosomal abnormality).

Regarding claim 60, Ryan discloses the method of claim 59. Ryan further discloses ligating a universal primer binding site to the DNA molecules in the sample (Para. [0167], The DNA in the sample may have ligation adapters, often referred to as library tags or ligation adaptor tags (LTs), appended, where the ligation adapters contain a universal priming sequence, followed by a universal amplification); amplifying the ligated DNA molecules (Para. [0258], Universal ligation adapters were appended to the end of each molecule) using at least 1,000 specific primers and a universal primer to produce a first set of amplified products (Para. [0151], perform about 100-plex, about 1000-plex, or about 10,000-plex amplification of selected targets using one target specific "Forward" primer per target and one tag specific primer); and amplifying the first set of amplified products using at least 1,000 pairs of specific primers to produce a second set of amplified products (Para. [0158], a 10,000-plex PCR assay pool is created such that reverse primers have tails corresponding to the required reverse sequences required by a high throughput sequencing instmment. After amplification with the first 10,000-plex assay, a subsequent PCR amplification may be performed using a another 10,000-plex pool having partly nested forward primers (e.g. 6-bases nested) for all targets and a reverse primer corresponding to the reverse sequencing tail included in the first round. This subsequent round of partly nested amplification with just one target specific primer and a universal primer).

Regarding claim 61, Ryan discloses the method of claim 58. Ryan further discloses wherein the undesirability scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, amplification efficiency of the target amplicon, and size of the target amplicons (Para. [0203], correlation rate measured between the genotypic data of the fetus, as measured on a single cell, and the genotypic data of the assumed parents when zero, one or two of the assumed parents are actually the genetic parents of the fetus); and wherein the test primers are used to simultaneously amplify at least 1,000 target loci in a sample (Para. [0151], perform about 100-plex, about 1000-plex, or about 10,000-plex amplification of selected targets using one target specific "Forward" primer per target and one tag specific primer) comprising DNA from an alleged father of a fetus (Para. [0008], genetic material from the alleged father) and to simultaneously amplify the target loci in a sample comprising maternal DNA from the pregnant mother of a fetus and fetal DNA (Para. [0008],genetic material from the pregnant mother, making genotypic measurements on a mixed sample of DNA originating from the blood sample from the pregnant mother, where the mixed sample of DNA comprises fetal DNA and maternal DNA) to establish whether the alleged father is the biological father of the fetus (Para. [0008], establishing whether the alleged father is the biological father of the fetus using the determined probability that the alleged father is the biological father of the fetus).

Regarding claim 63, Ryan discloses the method of claim 58. Ryan further discloses using the test primers to simultaneously amplify at least 1,000 target in a control nucleic acid sample loci (Para. [0151], perform about 100-plex, about 1000-plex, or about 10,000-plex amplification of selected targets using one target specific "Forward" primer per target and one tag specific primer; Para. [0202], Genetic measurements made on 200 unrelated males were used as a control) to produce a first set of target amplicons and to simultaneously amplify the target loci in a test nucleic acid sample to produce a second set of target amplicons (Para. [0042], a first individual and a second individual, given genotypic measurements at a plurality of polymorphic loci for the first individual, and genotypic measurements at a plurality of polymorphic loci for a mixture of DNA where the mixture of DNA comprises DNA from the second individual); and comparing the first and second sets of target amplicons to determine whether a target locus is present in one sample but absent in the other, or whether a target locus is present at different levels in the control sample and the test sample (Para. [0056], where the genetic material of the target individual is found in the presence of a quantity of genetic material from another individual; Para. [0173], detecting the presence or absence of a phenotype or genotype, for example, a chromosomal abnormality).

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Regarding claim 64, Ryan discloses the method of claim 63. Ryan further discloses wherein the test sample is from an individual suspected of having a disease or phenotype of interest, or an increased risk for a disease or phenotype of interest; and wherein one or more of the target loci comprise a polymorphism associated with an increased risk for the disease or phenotype of interest, or associated with the disease or phenotype of interest (Para. [0060], DNA from the target individual and DNA from one or a plurality of other individuals, specifically to determine disease-related alleles Para. [0061], In some embodiments the situation in question may include whether the target individual has inherited a disease linked haplotype of interest, whether the target individual has inherited a phenotype linked haplotype of interest,).

Regarding claim 65, Ryan discloses the method of claim 58. Ryan further discloses comprising using the test primers to simultaneously amplify at least 1,000 target loci in a control sample (Para. [0151], perform about 100-plex, about 1000-plex, or about 10,000-plex amplification of selected targets using one target specific "Forward" primer per target and one tag specific primer; Para. [0202], Genetic measurements made on 200 unrelated males were used as a control) comprising RNA (Para. [0059], This method could work equally well with samples of RNA) to produce a first set of target amplicons and to simultaneously amplify the target loci in a test sample comprising RNA to produce a second set of target amplicons (Para. [0042], a first individual and a second individual, given genotypic measurements at a plurality of polymorphic loci for the first individual, and genotypic measurements at a plurality of polymorphic loci for a mixture of DNA where the mixture of DNA comprises DNA from the second individual); and comparing the first and second sets of target amplicons to determine the presence or absence of a difference in the RNA expression levels between the control sample and the test sample (Para. [0056], where the genetic material of the target individual is found in the presence of a quantity of genetic material from another individual; Para. [0173], detecting the presence or absence of a phenotype or genotype, for example, a chromosomal abnormality; Para. [0161], In some embodiments, the methods described herein may be used to amplify and/or detect mRNA levels, or other RNA expression levels).

Regarding claim 66, Ryan discloses the method of claim 65. Ryan further discloses wherein the RNA is mRNA (Para. [0161], detect mRNA levels).

Regarding claim 67, Ryan discloses the method of claim 65. Ryan further discloses wherein the test sample is from an individual suspected of having cancer or an increased risk for cancer (Para. [0160], the target DNA may originate from cancer patient plasma and tumors); and wherein one or more of the target loci comprises a polymorphism or other mutation associated with an increased risk for cancer or associated with cancer (Para. [0060], DNA from the target individual and DNA from one or a plurality of other individuals, specifically to determine disease-related alleles Para. [0061], In some embodiments the situation in question may include whether the target individual has inherited a disease linked haplotype of interest, whether the target individual has inherited a phenotype linked haplotype of interest).

Regarding claim 68, Ryan discloses the method of claim 65. Ryan further discloses wherein the test sample is from an individual diagnosed with cancer (Para. [0160], the target DNA may originate from cancer patient plasma and tumors); and wherein a difference in the RNA expression level between the control sample and test sample (Para. [0059], This method could work equally well with samples of RNA; Para. [0161], In some embodiments, the methods described herein may be used to amplify and/or detect mRNA levels, or other types of RNA expression levels) indicates a target locus comprises a polymorphism or other mutation associated with an increased or decreased risk for cancer (Para. [0060], DNA from the target individual and DNA from one or a plurality of other individuals, specifically to determine disease-related alleles; Para. [0061], In some embodiments the situation in question may include whether the target individual has inherited a disease linked haplotype of interest, whether the target individual has inherited a phenotype linked haplotype of interest).

Regarding claim 70, Ryan discloses the method of claim 53. Ryan further discloses wherein the candidate primers remaining in the library are capable of simultaneously amplifying at least 1,000 different target loci (Para. [0141], The number of genetic target sequences that may be run in parallel can range from one to ten, ten to one hundred, one hundred to one thousand, one thousand to ten thousand, ten thousand to one hundred thousand, one hundred thousand to one million, or one million to ten million. Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000- plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 71, Ryan discloses the method of claim 53. Ryan further discloses (e) contacting a nucleic acid sample comprising target loci with the candidate primers remaining in the library to produce a reaction mixture (Para. [0262], 9,600 primers were used in the single well reactions; the primers were designed to target SNPs found on chromosomes 1, 2, 13, 18, 21, X and Y); and
(f) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products comprising target amplicons (Para. [0261], An aliquot of the STA products was then amplified by standard PCR for 10 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries).

Regarding claim 75, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 5,000 different target loci are amplified (Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000- plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 76, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 10,000 different target loci are amplified (Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000-plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

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Regarding claim 77, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 20,000 different target loci are amplified (Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000-plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 78, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 30,000 different target loci are amplified (Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000-plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 79, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 90% of the amplified products are target amplicons (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci).

Regarding claim 80, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 95% of the amplified products are target amplicons (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci).

Regarding claim 81, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 99% of the amplified products are target amplicons (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci).

Regarding claim 82, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 90% of the targeted loci are amplified (Para. [0156], Multiplex PCR may involve a single round of PCR in which all targets are amplified; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol; [1200], the total of 1200 targets amplified in the first STA were thus amplified (100% of the targeted loci were amplified)).

Regarding claim 83, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 95% of the targeted loci are amplified (Para. [0156], Multiplex PCR may involve a single round of PCR in which all targets are amplified; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol; [1200], the total of 1200 targets amplified in the first STA were thus amplified (100% of the targeted loci were amplified)).

Regarding claim 84, Ryan discloses the library of claim 72. Ryan further discloses wherein at least 99% of the targeted loci are amplified (Para. [0156], Multiplex PCR may involve a single round of PCR in which all targets are amplified; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol; [1200], the total of 1200 targets amplified in the first STA were thus amplified (100% of the targeted loci were amplified)).

Regarding claim 85, Ryan discloses the library of claim 72. Ryan further discloses wherein less than 20% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)).

Regarding claim 86, Ryan discloses the library of claim 72. Ryan further discloses wherein less than 10% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)).

Regarding claim 87, Ryan discloses the library of claim 72. Ryan further discloses wherein less than 1% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)).

Regarding claim 89, Ryan discloses the library of claim 72. Ryan further discloses the library comprising at least 1,000 primer pairs, wherein each primer pair includes a forward primer and a reverse primer that hybridize to a target locus (Para. [0149], forward and reverse primers must anneal to the same fragment to enable amplification).

Regarding claim 90, Ryan discloses the library of claim 72. Ryan further disclose the library comprising at least 1,000 individual primers that hybridize to different target loci (Para. [0040], The PCR probes may be selected by creating a thermodynamic model of potentially adverse interactions, or unintended interactions, between at least 500, at least 1,000, at least 5,000, at least 10,000, at least 20,000, at least 50,000, or at least 100,000 potential primer pairs, or between primers and sample DNA (i.e. 100,000 target loci).

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Regarding claim 96, Ryan discloses the method of claim 95. Ryan further discloses simultaneously amplifying the corresponding plurality of polymorphic loci on genetic material from the mother to produce a third set of amplified products (Para. [0220], genotypic data of the mother, the genotypic data of the father, and the measured genotypic data from the mixed sample that contains both maternal and fetal DNA; Para. [0267],three parallel 400-plea reactions the total of 1200 targets amplified in the first STA were thus amplified); wherein the probability that the alleged father is the biological father of the fetus is determined (Para. [0134], This may involve calculating, on a computer, allele counts at the plurality of polymorphic loci from the DNA measurements made on the enriched sample; and determining the likelihood that the man is the biological father of her fetus) using genotypic measurements based on the first, second, and third sets of amplified products (Para. [0220], the genotypic data of the mother, the genotypic data of the father, and the measured genotypic data from the mixed sample that contains both maternal and fetal DNA; Para. [0267], three parallel400-plea reactions the total of 1200 targets amplified in the first STA were thus amplified).

Regarding claim 98, Ryan discloses the method of claim 97. Ryan further discloses determining the absolute amount of the first target locus and the second target locus in the sample (Para. [0162], In some embodiment, the mini-PCR amplification methods described herein may be used for absolute quantification using spike calibrators. It may be used for mutation/minor allele quantification through very deep sequencing).

Regarding claim 99, Ryan discloses the method of claim 97. Ryan further discloses determining the presence or absence of a target locus in the sample (Para. [0174], the method disclosed herein is able to detect the presence or absence of a phenotype or genotype, for example, a chromosomal abnormality).

Regarding claim 100, Ryan discloses the method of claim 97. Ryan further discloses simultaneously amplifying at least 1,000 different target loci (Para. [0141], The number of genetic target sequences that may be run in parallel can range from one to ten, ten to one hundred, one hundred to one thousand, one thousand to ten thousand, ten thousand to one hundred thousand, one hundred thousand to one million, or one million to ten million. Para. [0158], In an embodiment, a 100-plex to- 500 plex, 500-plex to 1,000-plex, 1,000-plex to 2,000-plex, 2,000-plex to 5,000- plex, 5,000-plex to 10,000-plex, 10,000-plex to 20,000-plex, 20,000-plex to 50,000-plex, or 50,000-plex to 100,000-plex PCR assay pool is created).

Regarding claim 101, Ryan discloses the method of claim 97. Ryan further discloses wherein the target loci are present on the same chromosome of interest (Para. [0094], a combination of alleles at multiple
loci that are typically inherited together on the same chromosome).

Regarding claim 102, Ryan discloses the method of claim 97. Ryan further discloses wherein at least some of the target loci are present on different chromosomes of interest (Para. [0086], measured ratio of alleles at a heterozygous locus... different alleles).

Claims 15, 20, 21, 25, 55, 56, 88, and 91 lack an inventive step under PCT Article 33(3) as being obvious over Ryan et al. (hereafter Ryan).

Regarding claim 15, Ryan discloses the method of claim 1. Ryan further discloses wherein less than 1% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)), and elimination of primers to minimize formation of primer dimers (Para. [0164], choose primers for a library where the amount of non-mapping primer-dimer or other primer mischief products are minimized; Para. [0165], those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed; Para. [0166], create a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible with other the designs in the pool). Ryan fails to explicitly disclose wherein less than 0.1% of the amplified products are primer dimers.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to remove primers to achieve less than 0.1% primer dimers, since discovering the optimum value of a result effective variable involves only routine skill in the art. The motivation for doing so would be maximize the proportion of sequence reads that map to targeted loci, as taught by Ryan (Para. [0164], Removing these 'bad' primers can increase the percent of sequence reads that map to targeted loci).

Regarding claim 20, Ryan discloses the method of claim 19. Ryan further discloses reducing the number of candidate primers remaining in the library until the number of candidate primers remaining in the library is reduced to a desired number (Para. [0172], a kit maybe formulated that comprises a plurality of primers designed to achieve the methods described in this disclosure; Para. [0258], In one experiment, four maternal plasma samples were prepared and amplified using a hemi-nested 9,600-plex protocol; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol (the number of primers in a library is reduced to a desired number to achieve a desired result, as in a kit for a specific test)). Ryan fails to explicitly disclose reducing the number by decreasing the first minimum threshold used in step (ii) to a lower second minimum threshold and repeating steps (ii) and (iii) until the undesirability scores for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to select a reduced number of primers based on a second thermodynamic threshold, since discovering the optimum value of a result effective variable involves only routine skill in the art. The motivation for doing so would be to select the best (i.e. most specific, or most unlikely to form dimers) primers for the application, as taught by Ryan (Para. [0164], Removing these 'bad' primers can increase the percent of sequence reads that map to targeted loci).

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
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Regarding claim 21, Ryan discloses the method of claim 19. Ryan further discloses wherein the number of primer combinations are reduced until the number of candidate primers remaining in the library is reduced to a desired number (Para. [0172], a kit maybe formulated that comprises a plurality of primers designed to achieve the methods described in this disclosure; Para. [0258], In one experiment, four maternal plasma samples were prepared and amplified using a hemi-nested 9,600-plex protocol; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol (the number of primers in a library is reduced to a desired number to achieve a desired result, as in a kit for a specific test)), and wherein the primers are selected based on a thermodynamic model of dimer formation (i.e. an undesirability score) (Para. [0166], create a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible with other designs in the pool). Ryan fails to explicitly disclose reducing the primer numbers by increasing the first minimum threshold used in step (ii) to a higher second minimum threshold and repeating steps (ii) and (iii) until the undesirability scores for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to select a reduced number of primers based on a second thermodynamic threshold, since discovering the optimum value of a result effective variable involves only routine skill in the art. The motivation for doing so would be to select the best (i.e. most specific, or most unlikely to form dimers) primers for the application, as taught by Ryan (Para. [0164], Removing these 'bad' primers can increase the percent of sequence reads that map to targeted loci).

Regarding claim 25, Ryan discloses the method of claim 1. Ryan further discloses wherein the concentration of each test primer is in the low nM range (Para. [0263], forward primers and tagged reverse primers at 7.3 nM). Ryan fails to explicitly disclose wherein the primer concentration is less than 2 nM.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a primer concentration of less than 2 nM, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. The motivation for doing so would be to use a primer concentration effective for target amplification as taught by Ryan (Para. [0263], Relevant maternal and paternal genomic DNA samples amplified using a semi-nested 9600-plex protocol).

Regarding claim 55, Ryan discloses the method of claim 54. Ryan further discloses reducing the number of candidate primers remaining in the library until the number of candidate primers remaining in the library is reduced to a desired number (Para. [0172], a kit maybe formulated that comprises a plurality of primers designed to achieve the methods described in this disclosure; Para. [0258], In one experiment, four maternal plasma samples were prepared and amplified using a hemi-nested 9,600-plex protocol; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol (the number of primers in a library is reduced to a desired number to achieve a desired result, as in a kit for a specific test)). Ryan fails to explicitly disclose reducing the number by decreasing the first minimum threshold used in step (ii) to a lower second minimum threshold and repeating steps (ii) and (iii) until the undesirability scores for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to select a reduced number of primers based on a second thermodynamic threshold, since discovering the optimum value of a result effective variable involves only routine skill in the art. The motivation for doing so would be to select the best (i.e. most specific, or most unlikely to form dimers) primers for the application, as taught by Ryan (Para. [0164], Removing these 'bad' primers can increase the percent of sequence reads that map to targeted loci).

Regarding claim 56, Ryan discloses the method of claim 54. Ryan further discloses wherein the number of primer combinations are reduced until the number of candidate primers remaining in the library is reduced to a desired number (Para. [0172], a kit maybe formulated that comprises a plurality of primers designed to achieve the methods described in this disclosure; Para. [0258], In one experiment, four maternal plasma samples were prepared and amplified using a hemi-nested 9,600-plex protocol; Para. [0265], In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol (the number of primers in a library is reduced to a desired number to achieve a desired result, as in a kit for a specific test)), and wherein the primers are selected based on a thermodynamic model of dimer formation (i.e. an undesirability score) (Para. [0166], create a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible with other designs in the pool). Ryan fails to explicitly disclose reducing the primer numbers by increasing the first minimum threshold used in step (ii) to a higher second minimum threshold and repeating steps (ii) and (iii) until the undesirability scores for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to select a reduced number of primers based on a second thermodynamic threshold, since discovering the optimum value of a result effective variable involves only routine skill in the art. The motivation for doing so would be to select the best (i.e. most specific, or most unlikely to form dimers) primers for the application, as taught by Ryan (Para. [0164], Removing these 'bad' primers can increase the percent of sequence reads that map to targeted loci).

Regarding claim 88, Ryan discloses the library of claim 72. Ryan further discloses wherein less than 1% of the amplified products are primer dimers (Para. [0040], Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 99% DNA molecules that map to targeted loci (i.e. less than 1% primer dimers)), and elimination of primers to minimize formation of primer dimers (Para. [0164], choose primers for a library where the amount of non-mapping primer-dimer or other primer mischief products are minimized; Para. [0165], those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed; Para. [0166], create a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible with other the designs in the pool). Ryan fails to explicitly disclose wherein less than 0.1% of the amplified products are primer dimers.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to remove primers to achieve less than 0.1% primer dimers, since discovering the optimum value of a result effective variable involves only routine skill in the art. The motivation for doing so would be maximize the proportion of sequence reads that map to targeted loci, as taught by Ryan (Para. [0164], Removing these 'bad' primers can increase the percent of sequence reads that map to targeted loci).

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In case **the space in any of the preceding boxes is not sufficient.**
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Regarding claim 91, Ryan discloses a kit for amplifying target loci in a nucleic acid sample comprising (i) the library of claim 72 (Para. [0172], In some embodiments, a kit maybe formulated that comprises a plurality of primers designed to achieve the methods described in this disclosure). Ryan fails to explicitly disclose the kit comprising instructions for using the library to amplify the target loci.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to provide instructions for use with the kit, since it was known in the art that instructions were useful for the purpose of instructing proper use. The motivation for doing so would be to enable proper practice of the invention.

Claims 28-32, 34-36, and 39 lack an inventive step under PCT Article 33(3) as being obvious over Ryan et al. (hereafter Ryan) in view of Lietz.

Regarding claim 28, Ryan discloses the method of claim 1. Ryan fails to explicitly disclose wherein the GC content of the test primers is between 30% and 80%, inclusive.
Lietz discloses primers for target amplification (Para. [0023], performing at least one cycle of primer extension amplification on the sample) wherein the GC content of the test primers is between 30% and 80% (Para. [0131], chemically synthesizing the library by mixing different phosphoramidites at a substantially equal ratio (e.g. A:T:C:G=25%:25%:25%:25%) (the GC content would be about 50%)). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers of 50% GC content as taught by Lietz. The motivation would be generate a diverse primer library as taught by Lietz (Para. [0131], Complete randomization of the library maximizes the molecular diversity for an oligonucleotide at a certain length).

Regarding claim 29, Ryan discloses the method of claim 1. Ryan fails to explicitly disclose wherein the range of GC content of the test primers is less than 20%.
Lietz discloses primers for target amplification (Para. [0023], performing at least one cycle of primer extension amplification on the sample) wherein the range of GC content of the test primers is less than 20% (Para. [0131], chemically synthesizing the library by mixing different phosphoramidites at a substantially equal ratio (e.g. A:T:C:G=25%:25%:25%:25%) (the GC content of all the primers would be about 50%)).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers of 50% GC content as taught by Lietz. The motivation would be generate a diverse primer library as taught by Lietz (Para. [0131], Complete randomization of the library maximizes the molecular diversity for an oligonucleotide at a certain length).

Regarding claim 30, Ryan discloses the method of claim 1. Ryan fails to explicitly disclose wherein the melting temperature of the test primers is between 40 °C and 80 °C, inclusive.
Lietz discloses primers for target amplification (Para. [0023], performing at least one cycle of primer extension amplification on the sample) wherein the melting temperature of the test primers is between 40 °C and 80 °C (Para. [0055], the lengths of the first and second primers may optionally be between 10 and 80 nucleotides, preferably between 12 and 60 nucleotides and more preferably between 15 and 40 nucleotides; Para. [0131], chemically synthesizing the library by mixing different phosphoramidites at a substantially equal ratio (e.g. A:T:C:G=25%:25%:25%:25%) (the GC content of all the primers would be about 50%, yielding melting temperatures of 42oC to 78oC for primers of 14 to 26 nucleotides in length)).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers with melting temperatures according to the teaching of Lietz. The motivation would be to use melting temperatures appropriate for primer extension as taught by Lietz (Para. [0064], multiple cycles of primer extension polymerase amplification may be performed such that extension by the polymerase is performed by heating the amplification reaction mixture from a temperature between about 30° C. to 50° C. to a temperature between about 65° C. to 75° C. for at least 30 sec).

Regarding claim 31, Ryan discloses the method of claim 1. Ryan fails to explicitly disclose wherein the range of melting temperatures of the test primers is less than 5 °C.
Lietz discloses primers for target amplification (Para. [0023], performing at least one cycle of primer extension amplification on the sample) wherein the range of melting temperatures of the test primers is less than 5 °C (Para. [0131], chemically synthesizing the library by mixing different phosphoramidites at a substantially equal ratio (e.g. A:T:C:G=25%:25%:25%:25%). Complete randomization of the library maximizes the molecular diversity for an oligonucleotide at a certain length (the GC content of all the primers would be about 50%, yielding essentially identical melting temperatures for primers of a specified length); Para. [0139], In a library of oligonucleotides, oligonucleotides can have uniform lengths).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers with melting temperatures according to the teaching of Lietz. The motivation would be to use melting temperatures appropriate for primer extension as taught by Lietz (Para. [0064], multiple cycles of primer extension polymerase amplification may be performed such that extension by the polymerase is performed by heating the amplification reaction mixture from a temperature between about 30° C. to 50° C. to a temperature between about 65° C. to 75° C. for at least 30 sec).

Regarding claim 32, Ryan discloses the method of claim 1. Ryan fails to explicitly disclose wherein the length of the test primers is between 17 and 35 nucleotides, inclusive.
Lietz discloses primers for target amplification (Para. [0023], performing at least one cycle of primer extension amplification on the sample) wherein the length of the test primers is between 17 and 35 nucleotides, inclusive (Para. [0055], the lengths of the first and second primers may optionally be between 10 and 80 nucleotides, preferably between 12 and 60 nucleotides and more preferably between 15 and 40 nucleotides).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers with lengths according to the teaching of Lietz. The motivation would be to use primers with melting temperatures appropriate for primer extension as taught by Lietz (Para. [0064], multiple cycles of primer extension polymerase amplification may be performed such that extension by the polymerase is performed by heating the amplification reaction mixture from a temperature between about 30° C. to 50° C. to a temperature between about 65° C. to 75° C. for at least 30 sec).

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
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Regarding claim 34, Ryan discloses the method of claim 33. Ryan fails to explicitly disclose wherein the tag forms an internal loop structure.
Lietz teaches amplification of target sequences (Para. [0023], performing at least one cycle of primer extension amplification on the sample) using primers comprising a tag on an internal loop structure (Para. [0067], at least one cycle of primer extension amplification may include mismatches, bulges or loops; Para. [0096], As illustrated, the first and second oligonucleotides 24, 26 are not perfectly complementary to the target sequence and form imperfect double-stranded sequences including mismatches 30, bulges 32 and internal loops 34. (see figure 1, with bulge 32 representing an internal loop tag)).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers with an internal loop structure as taught by Lietz. The motivation would be to introduce heterologous sequences into amplicons as taught by Lietz (Para. [0003], methods for mutagenizing nucleic acids relative to an initial target nucleic acid sequence by the insertion; Para. [0053], primers preferably include at least one restriction site, which facilitates subcloning).

Regarding claim 35, Ryan as modified discloses the method of claim 34. Ryan fails to explicitly disclose wherein the tag is between two DNA binding regions.
Lietz teaches wherein the tag is between two DNA binding regions (Para. [0067], at least one cycle of primer extension amplification may include mismatches, bulges or loops; Para. [0096], As illustrated, the first and second oligonucleotides 24, 26 are not perfectly complementary to the target sequence and form imperfect double-stranded sequences including mismatches 30, bulges 32 and internal loops 34. (see figure 1, with bulge 32 representing an internal loop tag between two DNA binding regions)).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers with an internal loop structure as taught by Lietz. The motivation would be to introduce heterologous sequences into amplicons as taught by Lietz (Para. [0003], methods for mutagenizing nucleic acids relative to an initial target nucleic acid sequence by the insertion; Para. [0053], primers preferably include at least one restriction site, which facilitates subcloning).

Regarding claim 36, Ryan as modified discloses the method of claim 34. Ryan fails to explicitly disclose wherein the test primers comprise a 5' region that is specific for a target locus, an internal region that is not specific for the target locus and forms loop structure, and a 3' region that is specific for the target locus.
Lietz teaches wherein the test primers comprise a 5' region that is specific for a target locus, an internal region that is not specific for the target locus and forms loop structure, and a 3' region that is specific for the target locus (Para. [0067], at least one cycle of primer extension amplification may include mismatches, bulges or loops; Para. [0096], As illustrated, the first and second oligonucleotides 24, 26 are not perfectly complementary to the target sequence and form imperfect double-stranded sequences including mismatches 30, bulges 32 and internal loops 34. (see figure 1, with bulge 32 representing an internal loop tag between 5' and 3' target DNA binding regions)).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers with an internal loop structure as taught by Lietz. The motivation would be to introduce heterologous sequences into amplicons as taught by Lietz (Para. [0003], methods for mutagenizing nucleic acids relative to an initial target nucleic acid sequence by the insertion; Para. [0053], primers preferably include at least one restriction site, which facilitates subcloning).

Regarding claim 39, Ryan discloses the method of claim 33. Ryan further discloses wherein the test primers comprise a 5' region that is not specific for a target locus (Para. [0168], Primer tails may improve the detection of fragmented DNA from universally tagged libraries. If the library tag and the primer-tails contain a homologous sequence, hybridization can be improved (the tail is specific for the tag rather than the target)), followed by a region that is specific for a target locus, and a 3' region that is specific for the target locus (Para. [0168], Primer tails may improve the detection of fragmented DNA from universally tagged libraries. If the library tag and the primer-tails contain a homologous sequence, hybridization can be improved (for example, melting temperature (TM) is lowered) and primers can be extended if only a portion of the primer target sequence is in the sample DNA fragment...In some embodiments, 13 or more target specific base pairs may be used). Ryan fails to explicitly disclose an internal region that is not specific for the target locus and forms a loop structure.
Lietz teaches amplification of target sequences (Para. [0023], performing at least one cycle of primer extension amplification on the sample) using primers comprising a tag on an internal loop structure (Para. [0067], at least one cycle of primer extension amplification may include mismatches, bulges or loops; Para. [0096], As illustrated, the first and second oligonucleotides 24, 26 are not perfectly complementary to the target sequence and form imperfect double-stranded sequences including mismatches 30, bulges 32 and internal loops 34. (see figure 1, with bulge 32 representing an internal loop tag)).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise primers with an internal loop structure as taught by Lietz. The motivation would be to introduce heterologous sequences into amplicons as taught by Lietz (Para. [0003], methods for mutagenizing nucleic acids relative to an initial target nucleic acid sequence by the insertion; Para. [0053], primers preferably include at least one restriction site, which facilitates subcloning).

Claims 37 and 38 lack an inventive step under PCT Article 33(3) as being obvious over Ryan et al. (hereafter Ryan) in view of Lietz and Stanton.

Regarding claim 37, Ryan as modified discloses the method of claim 36. Ryan fails to explicitly disclose wherein the length of the 3' region is at least 7 nucleotides.
Stanton teaches primers for target amplification (Pg. 16, 4, the sample of DNA is obtained by amplification of a DNA molecule comprising two or more polymorphic sites of the selected gene) comprising an internal loop when annealed to the target (Figure 5, BsgI/PvuII loop), and wherein the length of the 3' region is at least 7 nucleotides (Figure 5, 3' region, CAAAAGGATTTA, is 12 nucleotides). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise the looped primers with a 3' region according to the teaching of Stanton. The motivation would be to introduce a restriction site into an amplicon as taught by Stanton (Pg. 43, 2-4, method of primer design involves the use of a primer with an internal loop... This loop will be incorporated into the amplicon during the amplification process, thereby introducing the Fok I and Fsp I restriction sites).

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In case **the space in any of the preceding boxes is not sufficient.**
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Regarding claim 38, Ryan as modified discloses the method of claim 37. Ryan fails to explicitly disclose wherein the length of the 3' region is between 7 and 20 nucleotides, inclusive.
Stanton teaches primers for target amplification (Pg. 16, 4, the sample of DNA is obtained by amplification of a DNA molecule comprising two or more polymorphic sites of the selected gene) comprising an internal loop when annealed to the target (Figure 5, BsgI/PvuII loop), and wherein the length of the 3' region is between 7 and 20 nucleotides (Figure 5, 3' region, CAAAAGGATTTA, is 12 nucleotides).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise the looped primers with a 3' region according to the teaching of Stanton. The motivation would be to introduce a restriction site into an amplicon as taught by Stanton (Pg. 43, 2-4, method of primer design involves the use of a primer with an internal loop... This loop will be incorporated into the amplicon during the amplification process, thereby introducing the Fok I and Fsp I restriction sites).

Claims 42 and 62 lack an inventive step under PCT Article 33(3) as being obvious over Ryan et al. (hereafter Ryan) in view of Green et al. (hereafter Green) and Meyerson et al. (hereafter Myerson).

Regarding claim 42, Ryan discloses the method of claim 41. Ryan fails to explicitly disclose wherein the length of the annealing step is greater than 10 minutes.
Green teaches PCR amplification of small amounts of DNA targets (Para. [0008], a method of amplifying target DNA on a microarray using PCR) wherein the yield may be increased by increasing the annealing time for hybridization of the primer to the target (Para. [0043], if the multiplex PCR product yields are lower than expected, it is envisioned that several options are available to improve yield. They are to perhaps decrease annealing temperature in small steps (2° C), or increase annealing time). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise an extended annealing time as taught by Green. The motivation would be to improve the yield of amplified products as taught by Green (Para. [0043], several options are available to improve yield...increase annealing time).
Meyerson teaches improved annealing (hybridization) of probes to a target (Para. [0006], a method for associating molecules with a surface, for example, hybridizing a first oligonucleotide bound to a surface with a second unbound oligonucleotide) by using hybridization times greater than 10 minutes (Para. [0141], The present method and composition perform well where conventional hybridization parameters fail, for example, at a hybridization time of about 6 hours and longer. The present method and composition perform particularly well for hybridizations taking at least 24 hours to complete, and more particularly, for hybridizations taking between about 12 hours to at least 24 hours).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise an annealing time of greater than 10 minutes as taught by Myerson. The motivation would be to improve the performance of the assay as taught by Myerson (Para. [0139], The method of the disclosure also provides superior hybridization assay performance; Para. [0141], The present method and composition perform well where conventional hybridization parameters fail).

Regarding claim 62, Ryan discloses the method of claim 58. Ryan further discloses wherein the undesirability scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus (Para. [0156], a partial nesting approach where one or both of the second round primers overlap the first binding sites extending internally some number of bases to achieve additional specificity (primers are selected to increase specificity)); size of the candidate primer, and size of the target amplicons (Para. [0156], Nested PCR typically entails designing primers completely internal to the previous primer binding sites, necessarily increasing the minimum DNA segment size required for amplification (larger amplicons allow selection of internal primers)); and wherein the test primers are used to simultaneously amplify at least 1,000 target loci (Para. [0040], Using one or a combination of these approaches allows multiplexing of at least 300, at least 800, at least 1,200, at least 4,000 or at least 10,000 primers in a single pool with the resulting amplified DNA comprising a majority of DNA molecules that, when sequenced, will map to targeted loci) in a forensic nucleic acid sample (Para. [0150], In an embodiment, the method may be applied for analysis of forensic samples). Ryan fails to explicitly disclose using an annealing time of at least 5 minutes.
Green teaches PCR amplification of small amounts of DNA targets (Para. [0008], a method of amplifying target DNA on a microarray using PCR) wherein the yield may be increased by increasing the annealing time for hybridization of the primer to the target (Para. [0043], if the multiplex PCR product yields are lower than expected, it is envisioned that several options are available to improve yield. They are to perhaps decrease annealing temperature in small steps (2° C), or increase annealing time).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise an extended annealing time as taught by Green. The motivation would be to improve the yield of amplified products as taught by Green (Para. [0043], several options are available to improve yield...increase annealing time).
Meyerson teaches improved annealing (hybridization) of probes to a target (Para. [0006], a method for associating molecules with a surface, for example, hybridizing a first oligonucleotide bound to a surface with a second unbound oligonucleotide) by using hybridization times greater than 5 minutes (Para. [0141], The present method and composition perform well where conventional hybridization parameters fail, for example, at a hybridization time of about 6 hours and longer. The present method and composition perform particularly well for hybridizations taking at least 24 hours to complete, and more particularly, for hybridizations taking between about 12 hours to at least 24 hours).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to comprise an annealing time of greater than 5 minutes as taught by Myerson. The motivation would be to improve the performance of the assay as taught by Myerson (Para. [0139], The method of the disclosure also provides superior hybridization assay performance; Para. [0141], The present method and composition perform well where conventional hybridization parameters fail).

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Claim 69 lacks an inventive step under PCT Article 33(3) as being obvious over Ryan et al. (hereafter Ryan) in view of Schumm et al. (hereafter Schumm).

Regarding claim 69, Ryan discloses the method of claim 53. Ryan fails to explicitly disclose prior to step (b), removing a primer pair from the library that produces a target amplicon that overlaps with a target amplicon produced by another primer pair.
Schumm teaches methods for multiplex amplification and analysis of polymorphic loci (Col. 4, Lns. 20-30, a method and materials for the simultaneous amplification of sets of loci, which include multiple distinct polymorphic short tandem repeat (STR) loci, in a single multiplex reaction, using PCR or other amplification systems), wherein primers are selected (or removed as candidates) to avoid producing a target amplicon that overlaps with a target amplicon produced by another primer pair (Col. 3, Lns. 20-35, Amplification protocols with STR loci can be designed to produce small products... by careful design of PCR primers such that all potential amplification products from an individual system do not overlap the range of alleles of other systems; Col. 11, lns. 20-30, Once a set of loci for co-amplification in a single multiplex reaction is identified, one can determine primers suitable for co-amplifying each locus in the set. Care should be used in selecting the sequence of primers used in the multiplex reaction. Inappropriate selection of primers can produce several undesirable effects such as production of alleles from one locus which overlap with alleles from another).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Ryan to eliminate overlapping amplicons as taught by Schumm. The motivation would be to eliminate spurious amplification products as taught by Schumm (Col. 11, Lns. 40-45, may generate amplification products which do not represent the alleles themselves. These extra fragments may result from any number of causes described above).

Claims 1-102 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.

INTERNATIONAL SEARCH REPORT

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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) - C07H 21/02, 21/04; C12N 1/08; C12P 19/34; C12Q 1/68; C40B 20/04, 20/08, 40/06, 50/02, 50/06, 50/14, 60/02, 60/14; G01N 33/483, 33/49, 33/50, 33/53 (2013.01)
USPC - 435/6.11, 91.1, 91.2; 436/813, 814; 506/2, 6, 16, 24, 26, 34; 536/2, 23.1, 24.33, 25.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC Class/Subclass(es): B01J 19/0046, 2219/00585, 2219/00707, 2219/00722; C07H 21/00; C12Q 1/686, 1/689, 1/6883

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit.com, Google Patents, Google Scholar, ProQuest

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2012/0122701 A1 (RYAN et al) 17 May 2012 (17.05.2012) entire document	1-27, 33, 40, 41, 43-61, 63-68, 70-102 --- 28-32, 34-39, 42, 62, 69
Y	US 2008/0234142 A1 (LIETZ) 25 September 2008 (25.09.2008) entire document	28-32, 34-39
Y	WO 2001/090419 A2 (STANTON) 29 November 2001 (29.11.2001) entire document	37, 38
Y	US 2005/0227263 A1 (GREEN et al) 13 October 2005 (13.10.2005) entire document	42, 62
Y	US 2006/0210997 A1 (MYERSON et al) 21 September 2006 (21.09.2006) entire document	42, 62
Y	US 6,479,235 B1 (SCHUMM et al) 12 November 2002 (12.11.2002) entire document	69

☐ 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 February 2013	05 MAR 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

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 W15	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2013/028378	International filing date *(day/month/year)* 28 February 2013 (28.02.2013)	Priority date *(day/month/year)* 29 February 2012 (29.02.2012)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 8 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 02 September 2014 (02.09.2014)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Philippe Bécamel e-mail: pt03.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 MAY 2013
Applicant's or agent's file reference 118103-012502/PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2013/028378	28 February 2013	29 February 2012

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11

Applicant NATERA, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	02 May 2013	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/028378

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/US2013/028378

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, 12, 13	YES
	Claims	1-6, 11, 14-32	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-32	NO
Industrial applicability (IA)	Claims	1-32	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-6, 11, and 14-32 lack novelty under PCT Article 33(2) as being anticipated by Rabinowitz et al. (hereafter Rabinowitz).

Regarding claim 1, Rabinowitz discloses, an ex vivo method for determining a number of copies of a chromosome or chromosome segment of interest in the genome of a fetus (the present disclosure provides ex vivo methods for determining the ploidy status of a chromosome in a gestating fetus, Para. [0038]), the method comprising: making genotypic measurements at a plurality of polymorphic loci on the chromosome or chromosome segment from a sample comprising cellular genetic material from the fetus and/or the mother of the fetus (from genotypic data measured from a mixed sample of DNA ,i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]); obtaining genotypic measurements at the plurality of polymorphic loci from genetic material from the mother (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]; the fraction of fetal DNA, or the percentage of fetal DNA in the mixture can be measured...the fraction can be calculated using only the genotyping measurements made on the maternal plasma sample itself, which is a mixture of fetal and maternal DNA, Para. [0322]); determining, on a computer, based on the genotypic measurements, whether the sample comprises (i) DNA from the fetus with essentially no DNA from the mother, (ii) maternal DNA with essentially no DNA from the fetus, or (iii) DNA from both the mother and the fetus (the fraction of fetal DNA, or the percentage of fetal DNA in the mixture can be measured...the fraction can be calculated using only the genotyping measurements made on the maternal plasma sample itself, which is a mixture of fetal and maternal DNA, Para. [0322]; any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]) and; determining, on a computer based on the genotypic measurements (analyzing the genotypic data, on a computer, and ex vivo, Para. [0047]), the number of copies of a chromosome or chromosome segment in the genome of the fetus when the sample comprises DNA from the fetus (determining a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0018]; non-invasive prenatal diagnosis involves a number of steps. Some of the steps may include: (1) obtaining the genetic material from the fetus; (2) enriching the genetic material of the fetus, ex vivo; (3) amplifying the genetic material, ex vivo; (4) preferentially enriching specific loci in the genetic material, ex vivo; (5) genotyping the genetic material, ex vivo; and (6) analyzing the genotypic data, on a computer, and ex vivo, Para. [0047]).

Regarding claim 2, Rabinowitz discloses the method of claim 1. Rabinowitz further discloses wherein the sample comprises DNA from the fetus, and wherein the determining the number of copies of the chromosome further comprises: creating, on a computer (any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]), a set of one or more hypotheses about the number of copies of the chromosome or chromosome segment in the genome of the fetus (one step involves creating a hypothesis. In some embodiments, the hypothesis is a copy number hypothesis, Para. [0136]; copy number hypothesis... refers to a hypothesis concerning the number of copies of a particular chromosome in an individual, Para. [0108]); determining, on a computer (any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]), the probability of each of the hypotheses given the genotypic measurements (once the set of hypotheses have been defined, when the algorithms operate on the input genetic data, they may output a determined statistical probability for each of the hypotheses under consideration, Para. [0141]); and using the probabilities associated with each hypothesis to determine the most likely number of copies of the chromosome or chromosome segment in the genome of the fetus (the probabilities of the various hypotheses may be determined by mathematically calculating, for each of the various hypotheses, the value that the probability equals, as stated by one or more of the expert techniques, algorithms, and/or methods described elsewhere in this disclosure, using the relevant genetic data as input, Para. [0141]; the method disclosed herein can be used to determine a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0353]).

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:

Regarding claim 3, Rabinowitz discloses an ex vivo method for determining a number of copies of a chromosome or chromosome segment of interest in the genome of a fetus (the present disclosure provides ex vivo methods for determining the ploidy status of a chromosome, Para. [0038]; methods are disclosed for determining a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0018]), the method comprising: making genotypic measurements at a plurality of polymorphic loci on the chromosome or chromosome segment from a mixed sample (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]) that comprises (i) cellular genetic material from the fetus and (ii) cellular genetic material from the mother of the fetus (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]); creating, on a computer any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]), a set of one or more hypotheses about the number of copies of the chromosome or chromosome segment in the genome of the fetus (one step involves creating a hypothesis...in some embodiments , the hypothesis is a copy number hypothesis, Para. [0136]; copy number hypothesis... refers to a hypothesis concerning the number of copies of a particular chromosome in an individual, Para. [0108]); determining, on a computer, the probability of each of the hypotheses given the genotypic measurements (once the set of hypotheses have been defined, when the algorithms operate on the input genetic data, they may output a determined statistical probability for each of the hypotheses under consideration, Para. [0141]); and using the probabilities associated with each hypothesis to determine the most likely number of copies of the chromosome or chromosome segment in the genome of the fetus (the probabilities of the various hypotheses may be determined by mathematically calculating, for each of the various hypotheses, the value that the probability equals, as stated by one or more of the expert techniques, algorithms, and/or methods described elsewhere in this disclosure, using the relevant genetic data as input, Para. [0141]).

Regarding claim 4, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the fetal cellular genetic material is from a gestating fetus (determining the ploidy status of a chromosome in a gestating fetus from genotypic data measured from a mixed sample of DNA, Para. [0038]).

Regarding claim 5, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein fetal cellular genetic material is from a products of conception sample (it is possible to phase the parental genotype from unphased genotypic information using grandparental genetic data, or genetic data from other born children, or a sample of a miscarriage, Para. [0419]).

Regarding claim 6, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the mixed sample is a maternal whole blood sample, cells isolated from a maternal blood sample, amniocentesis sample, placental tissue sample, cervical mucus sample, or sample from a fetus (the mixed sample is derived from maternal blood, Para. [0038]).

Regarding claim 11, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the mixed sample is enriched for fetal cells (obtaining a maternal blood sample, and enriching the fetal DNA. The method may involve amplifying and/or genotyping or sequencing the genetic material in the sample, Paras. [0054] and [0362]).

Regarding claim 14, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses obtaining genotypic measurements at the plurality of polymorphic loci from genetic material from the mother, wherein the probability of each hypotheses is determined using (i) the genotypic measurements made on the genetic material from the mother (obtaining genotypic data from one or both parents of the fetus... building on a computer, a joint distribution model for heterozygosity rates of each polymorphic allele on a chromosome for each ploidy hypothesis using genotypic data from the one or both parents, Para. [0009]) and (ii) the genotypic measurements made on the mixed sample (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus)... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Paras. [0009] and [0038]).

Regarding claim 15, Rabinowitz discloses the method of claim 14. Rabinowitz further discloses wherein the genotypic measurements made on the genetic material from the mother facilitate the detection or quantitation of maternal DNA in the mixed sample (the franction of fetal DNA, or the percentage of fetal DNA in the mixture can be measured...the fraction can be calculated using only the genotyping measurements made on the maternal plasma sample itself, which is a mixture of fetal and maternal DNA, Para. [0322]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Regarding claim 16, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses obtaining genotypic measurements at the plurality of polymorphic loci from genetic material from the mother and from genetic material from the father (genotypic data measured from a mixed sample of DNA, i.e., DNA from the mother of the fetus, and DNA from the fetus and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Para. [0038]), wherein the probability of each hypotheses is determined using (i) the genotypic measurements made on the genetic material from the mother (genotypic data measured from a mixed sample of DNA (i.e., DNA from the mother of the fetus, and DNA from the fetus) and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Para. [0038]), (ii) the genotypic measurements made on the genetic material from the father (genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Para. [0038]), and (iii) the genotypic measurements made on the mixed sample (genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus, and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Paras. [0038] and [0412]).

Regarding claim 17, Rabinowitz discloses the method of claim 16. Rabinowitz further discloses wherein DNA cross-linking inhibits the measuring of the genetic material from the mixed sample (determining the genetic state of a fetus where the fetal DNA is found in maternal blood, but this example is not meant to limit to possible contexts that this method may be applied to. In addition, the method may be applicable in cases where the amount of target DNA is in any proportion with the non-target DNA; for example, the target DNA could make up anywhere between 0.000001 and 99.999999 percent of the DNA present. In addition, the non-target DNA does not necessarily need to be from one individual, or even from a related individual, as long as genetic data from non-target individual(s) is known. In one embodiment of the present disclosure, the method can be used to determine genotypic data of a fetus from maternal blood that contains fetal DNA. In one embodiment, the method can be used in a case where there are multiple fetuses in the uterus of a pregnant woman, or where other contaminating DNA may be present in the sample, Paras. [0052] and [0364]); and wherein genotypic measurements made on the genetic material from the father facilitate the copy number determination (genotypic data measured from a mixed sample of DNA, i.e., DNA from the mother of the fetus, and DNA from the fetus and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Paras. [0038] and [0412]).

Regarding claim 18, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the plurality of loci comprise single nucleotide polymorphisms (SNPs) (the set of polymorphic alleles are SNPs, Para. [0011]; the targeting may focus entirely on SNPs, Para. [0158]).

Regarding claim 19, Rabinowitz discloses the method of claim 18. Rabinowitz further discloses wherein plurality of loci comprises at least 100 SNPs (it is possible to focus on a small number of SNP's, for example between 1 and 100 SNPs, or a larger number, for example between 100 and 1,000, between 1,000 and 10,000, between 10,000 and 100,000 or more than 100,000 SNPs, Para. [0158]).

Regarding claim 20, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the probability of each of the hypotheses is determined using data about the probability of chromosomes crossing over at different locations in the chromosome or chromosome segment of interest (building a joint distribution model is done by using data about the probability of chromosomes crossing over at different cross-over locations in a chromosome to model dependence between polymorphic alleles on the chromosome, Para. [0013]).

Regarding claim 21, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the method comprises calculating, on a computer, allele counts at the plurality of polymorphic loci from the genotypic measurements (calculating, on a computer, allele ratios at the set of polymorphic alleles from the DNA measurements made on the second sample, Para. [0009]), building, on a computer, a joint distribution model for the expected allele counts at the plurality of polymorphic loci on the chromosome or chromosome segment for each hypothesis (building, on a computer, a joint distribution model for heterozygosity rates of each polymorphic allele on the chromosome for each ploidy hypothesis using genotypic data from the one or both parents of the fetus, Para. [0009]); determining, on a computer, a relative probability of each of the copy number hypotheses using the joint distribution model and the allele counts (determining, on a computer, a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele ratios calculated for the second sample, Para. [0009]).

Regarding claim 22, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein a SNP genotyping array is used to make the genotypic measurements (parental context may refer to a set or subset of target SNPs, Para. [0147]; isolated DNA can then be measured by a number of means, such as by hybridizing to a genotyping array, Para. [0148]).

Regarding claim 23, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein high throughput DNA sequencing is used to make the genotypic measurements (This isolated DNA can then by measured by a number of means... sequencing on a high throughput sequencer, Para. [0148]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2013/028378

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 24, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein a confidence for the copy number determination is at least 95% (checking concordance of hit rates and given confidences, for a range of confidence >90%, Para. [0352]).

Regarding claim 25, Rabinowitz discloses the method of claim 3. Rabinowitz further comprising using the genotypic measurements to determine whether or not the fetus has inherited one or more disease linked haplotypes (the knowledge of the maternal genetic data may be used along with the genetic data measured from the free floating DNA found in the blood to determine which haplotypes from the mother and father are present in the fetus. This knowledge may be used to determine the presence or absence of specific disease related genes or other phenotypically correlated genes in the genotype of the fetus, Para. [0365]).

Regarding claim 26, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses comprising performing a clinical action based on the copy number determination (taking a clinical action based on the called ploidy state of the fetus, Para. [0016]).

Regarding claim 27, Rabinowitz discloses the method of claim 26. Rabinowitz further discloses wherein the clinical action is terminating the pregnancy or maintaining the pregnancy (taking a clinical action based on the called ploidy state of the fetus, wherein the clinical action is selected from one of terminating the pregnancy or maintaining the pregnancy, Para. [0016]).

Regarding claim 28, Rabinowitz discloses the method of claim 26. Rabinowitz further discloses wherein the clinical action is selected from the group consisting of performing amniocentesis on the fetus, performing amniocentesis on a subsequent fetus that that inherits genetic material from the mother and/or father, performing chorion villus biopsy on the fetus, performing chorion villus biopsy on a subsequent fetus that that inherits genetic material from the mother and/or father, performing in vitro fertilization, performing preimplantation genetic diagnosis on one or more embryos that inherited genetic material from the mother and/or father, karyotyping the mother, karyotyping the father, and combinations thereof (the method may be used in the context of in vitro fertilization, where it may be desirable to genotype a single cell blastomere biopsied from a cleavage state embryo for the purposes of determining the genetic state of the embyo, Para. [0189]).

Regarding claim 29, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the chromosome of interest is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, the X chromosome, the Y chromosome, and combinations thereof (it is possible to use this method to create a DNA sample that is enriched in SNPs located on one or a small number of chromosomes that are correlated with live trisomic births, for example chromosomes 13, 18, 21, X and Y, or some combination thereof, Para. [0157]).

Regarding claim 30, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the method comprises screening for a chromosomal abnormality selected from the group consisting of monosomy, uniparental disomy, trisomy, other aneuploidies, mosaicism, unbalanced translocations, insertions, deletions, and combinations thereof (it may also be used to infer the more complete genotypic information of the fetus, specifically, allele calls, the presence of insertions, deletions, transpositions, and other genetic modifications that may correlate with diseases, conditions, or other phenotypes, Para. [0365]).

Regarding claim 31, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the method comprises determining whether the individual has Down syndrome, Edwards syndrome, Patau syndrome, Klinefelters syndrome, 47,XXX, 47,XYY, Turner syndrome, triploidy, DiGeorge syndrome, Cri du Chat syndrome, Angelman syndrome, Praeder-Willi syndrome, Wolf-Hirschhorn syndrome, SmithMagenis syndrome, Williams-Beuren syndrome, Phelan-McDermid syndrome, Sotos Syndrome, Cystic Fibrosis, Muscular Dystrophy, Spinal Muscular Atrophy, Fragile X, Tay- Sachs disease, Gaucher disease, Torsion Dystonia, Niemann-Pick disease, Mucolipidosis, Fanconi Anemia, Canavan disease, Sickle Cell Anemia, or Bloom Syndrome (the congenital disorder is a malformation, neural tube defect, chromosome abnormality, Down syndrome, Para. [0393]).

Regarding claim 32, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses a report displaying a copy number determination for a chromosome or chromosome segment of interest in the genome of a fetus generated using the method of claim 3 (the knowledge of the determined ploidy state may be used to make a clinical decision. This knowledge, typically stored as a physical arrangement of matter in a memory device, may then be transformed into a report, Para. [0429];determining a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0018]).

Claim 8-10, 12, and 13 lack an inventive step under PCT Article 33(3) as being obvious over Rabinowitz et al (hereafter Rabinowitz)

Regarding claim 8, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein at least 10% of the DNA in the mixed sample are maternal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA... the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicilty disclose wherein the sample is cells.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/028378

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 9, Rabinowitz discloses the method of claim 8. Rabinowitz further discloses wherein at least 50% of the DNA in the mixed sample are maternal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA... the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, It would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Regarding claim 10, Rabinowitz discloses the method of claim 9. Rabinowitz further discloses wherein at least 90% of the DNA in the mixed sample are maternal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA... the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, It would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285];In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Regarding claim 12, Rabinowitz discloses the method of claim 11. Rabinowitz further discloses wherein at least 1% of the DNA in the mixed sample are fetal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA... the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, It would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Regarding claim 13, Rabinowitz discloses the method of claim 12. Rabinowitz further discloses wherein at least 4% of the DNA in the mixed sample are fetal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA... the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, It would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Claim 7 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz et al (hereafter Rabinowitz) in view of Hyland et al. (hereafter Hyland).

Regarding claim 7, Rabinowitz discloses the method of claim 6. Rabinowitz fails to explicitly disclose wherein the placental tissue sample is from chorionic villus, decidua, or placental membrane.
Hyland is in the field of the quantification of copy numbers (abstract) and teaches wherein the placental tissue sample is from chorionic villus, deciduas, or placental membrane (PCR typing of a fetus would be even be possible using any fetal cell containing DNA such as chorionic villi, Col. 7 Lns. 40-41).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Rabinowitz to include wherein the placental tissue sample is from chorionic villus as disclosed in Hyland because such a sample can be advantageously amplified in the absence of red blood cells or large quantities of tissue, leading to safer testing (Hyland Col. 7 Lns. 35-39).

Claims 1-32 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.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

RECEIVED
MAY 31 2013
GREENBERG TRAURIG
IP DEPT. BOSTON

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)*	28 MAY 2013
Applicant's or agent's file reference 118103-012502/PCT	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2013/028378	International filing date *(day/month/year)* 28 February 2013
Applicant NATERA, 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.

   Where? Directly to the International Bureau of WIPO, 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. 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. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   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.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 118103-012502/PCT	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2013/028378	International filing date *(day/month/year)* 28 February 2013	(Earliest) Priority Date *(day/month/year)* 29 February 2012
Applicant NATERA, 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. 27D

   ☒ 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.

NeoGenomics
Exhibit 1003 Page 1066

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/028378

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/US2013/028378

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) - C06F 19/18, 16/20; C12Q 1/68; G01N 33/50 (2013.01)
USPC - 435/6.11, 6.12, 287.2; 702/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- C12Q 1/6883 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0288780 A1 (RABINOWITZ et al) 24 November 2011 (24.11.2011) entire document	1-6, 8-32
Y		7
Y	US 5,972,602 A (HYLAND et al) 26 October 1999 (26.10.1999) entire document	7
A	US 2011/0105353 A1 (LO et al) 05 May 2011 (05.05.2011) entire document	1-32

☐ 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
02 May 2013	28 MAY 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DAVID J. DYKEMAN
GREENBERG TRAURIG, LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 MAY 2013
Applicant's or agent's file reference 118103-012502/PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2013/028378	28 February 2013	29 February 2012

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11

Applicant NATERA, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	02 May 2013	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/026378

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/US2013/028378

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, 12, 13	YES
	Claims	1-6, 11, 14-32	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-32	NO
Industrial applicability (IA)	Claims	1-32	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-6, 11, and 14-32 lack novelty under PCT Article 33(2) as being anticipated by Rabinowitz et al. (hereafter Rabinowitz).

Regarding claim 1, Rabinowitz discloses, an ex vivo method for determining a number of copies of a chromosome or chromosome segment of interest in the genome of a fetus (the present disclosure provides ex vivo methods for determining the ploidy status of a chromosome in a gestating fetus, Para. [0038]), the method comprising: making genotypic measurements at a plurality of polymorphic loci on the chromosome or chromosome segment from a sample comprising cellular genetic material from the fetus and/or the mother of the fetus (from genotypic data measured from a mixed sample of DNA ,i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]); obtaining genotypic measurements at the plurality of polymorphic loci from genetic material from the mother (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]; the fraction of fetal DNA, or the percentage of fetal DNA in the mixture can be measured...the fraction can be calculated using only the genotyping measurements made on the maternal plasma sample itself, which is a mixture of fetal and maternal DNA, Para. [0322]); determining, on a computer, based on the genotypic measurements, whether the sample comprises (i) DNA from the fetus with essentially no DNA from the mother, (ii) maternal DNA with essentially no DNA from the fetus, or (iii) DNA from both the mother and the fetus (the fraction of fetal DNA, or the percentage of fetal DNA in the mixture can be measured...the fraction can be calculated using only the genotyping measurements made on the maternal plasma sample itself, which is a mixture of fetal and maternal DNA, Para. [0322]; any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]) and; determining, on a computer based on the genotypic measurements (analyzing the genotypic data, on a computer, and ex vivo, Para. [0047]), the number of copies of a chromosome or chromosome segment in the genome of the fetus when the sample comprises DNA from the fetus (determining a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0018]; non-invasive prenatal diagnosis involves a number of steps. Some of the steps may include: (1) obtaining the genetic material from the fetus; (2) enriching the genetic material of the fetus, ex vivo; (3) amplifying the genetic material, ex vivo; (4) preferentially enriching specific loci in the genetic material, ex vivo; (5) genotyping the genetic material, ex vivo; and (6) analyzing the genotypic data, on a computer, and ex vivo, Para. [0047]).

Regarding claim 2, Rabinowitz discloses the method of claim 1. Rabinowitz further discloses wherein the sample comprises DNA from the fetus, and wherein the determining the number of copies of the chromosome further comprises: creating, on a computer (any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]), a set of one or more hypotheses about the number of copies of the chromosome or chromosome segment in the genome of the fetus (one step involves creating a hypothesis. In some embodiments, the hypothesis is a copy number hypothesis, Para. [0136]; copy number hypothesis... refers to a hypothesis concerning the number of copies of a particular chromosome in an individual, Para. [0108]); determining, on a computer (any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]), the probability of each of the hypotheses given the genotypic measurements (once the set of hypotheses have been defined, when the algorithms operate on the input genetic data, they may output a determined statistical probability for each of the hypotheses under consideration, Para. [0141]); and using the probabilities associated with each hypothesis to determine the most likely number of copies of the chromosome or chromosome segment in the genome of the fetus (the probabilities of the various hypotheses may be determined by mathematically calculating, for each of the various hypotheses, the value that the probability equals, as stated by one or more of the expert techniques, algorithms, and/or methods described elsewhere in this disclosure, using the relevant genetic data as input, Para. [0141]; the method disclosed herein can be used to determine a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0353]).

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 3, Rabinowitz discloses an ex vivo method for determining a number of copies of a chromosome or chromosome segment of interest in the genome of a fetus (the present disclosure provides ex vivo methods for determining the ploidy status of a chromosome, Para. [0038]; methods are disclosed for determining a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0018]), the method comprising: making genotypic measurements at a plurality of polymorphic loci on the chromosome or chromosome segment from a mixed sample (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]) that comprises (i) cellular genetic material from the fetus and (ii) cellular genetic material from the mother of the fetus (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Para. [0038]); creating, on a computer any of the embodiments disclosed herein may be implemented in... computer hardware, firmware, software, or in combinations thereof, Para. [0132]), a set of one or more hypotheses about the number of copies of the chromosome or chromosome segment in the genome of the fetus (one step involves creating a hypothesis...in some embodiments , the hypothesis is a copy number hypothesis. Para. [0136]; copy number hypothesis... refers to a hypothesis concerning the number of copies of a particular chromosome in an individual, Para. [0108]); determining, on a computer, the probability of each of the hypotheses given the genotypic measurements (once the set of hypotheses have been defined, when the algorithms operate on the input genetic data, they may output a determined statistical probability for each of the hypotheses under consideration, Para. [0141]); and using the probabilities associated with each hypothesis to determine the most likely number of copies of the chromosome or chromosome segment in the genome of the fetus (the probabilities of the various hypotheses may be determined by mathematically calculating, for each of the various hypotheses, the value that the probability equals, as stated by one or more of the expert techniques, algorithms, and/or methods described elsewhere in this disclosure, using the relevant genetic data as input, Para. [0141]).

Regarding claim 4, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the fetal cellular genetic material is from a gestating fetus (determining the ploidy status of a chromosome in a gestating fetus from genotypic data measured from a mixed sample of DNA, Para. [0038]).

Regarding claim 5, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein fetal cellular genetic material is from a products of conception sample (it is possible to phase the parental genotype from unphased genotypic information using grandparental genetic data, or genetic data from other born children, or a sample of a miscarriage, Para. [0419]).

Regarding claim 6, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the mixed sample is a maternal whole blood sample, cells isolated from a maternal blood sample, amniocentesis sample, placental tissue sample, cervical mucus sample, or sample from a fetus (the mixed sample is derived from maternal blood, Para. [0038]).

Regarding claim 11, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the mixed sample is enriched for fetal cells (obtaining a maternal blood sample, and enriching the fetal DNA. The method may involve amplifying and/or genotyping or sequencing the genetic material in the sample, Paras. [0054] and [0362]).

Regarding claim 14, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses obtaining genotypic measurements at the plurality of polymorphic loci from genetic material from the mother, wherein the probability of each hypotheses is determined using (i) the genotypic measurements made on the genetic material from the mother (obtaining genotypic data from one or both parents of the fetus... building on a computer, a joint distribution model for heterozygosity rates of each polymorphic allele on a chromosome for each ploidy hypothesis using genotypic data from the one or both parents, Para. [0009]) and (ii) the genotypic measurements made on the mixed sample (from genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus)... the mixed sample of DNA is preferentially enriched at a plurality of polymorphic loci, Paras. [0009] and [0038]).

Regarding claim 15, Rabinowitz discloses the method of claim 14. Rabinowitz further discloses wherein the genotypic measurements made on the genetic material from the mother facilitate the detection or quantitation of maternal DNA in the mixed sample (the franction of fetal DNA, or the percentage of fetal DNA in the mixture can be measured...the fraction can be calculated using only the genotyping measurements made on the maternal plasma sample itself, which is a mixture of fetal and maternal DNA, Para. [0322]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/028378

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 16, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses obtaining genotypic measurements at the plurality of polymorphic loci from genetic material from the mother and from genetic material from the father (genotypic data measured from a mixed sample of DNA, i.e., DNA from the mother of the fetus, and DNA from the fetus and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Para. [0038]), wherein the probability of each hypotheses is determined using (i) the genotypic measurements made on the genetic material from the mother (genotypic data measured from a mixed sample of DNA (i.e., DNA from the mother of the fetus, and DNA from the fetus) and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Para. [0038]), (ii) the genotypic measurements made on the genetic material from the father (genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Para. [0038]), and (iii) the genotypic measurements made on the mixed sample (genotypic data measured from a mixed sample of DNA i.e., DNA from the mother of the fetus, and DNA from the fetus, and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, Paras. [0038] and [0412]).

Regarding claim 17, Rabinowitz discloses the method of claim 16. Rabinowitz further discloses wherein DNA cross-linking inhibits the measuring of the genetic material from the mixed sample (determining the genetic state of a fetus where the fetal DNA is found in maternal blood, but this example is not meant to limit to possible contexts that this method may be applied to. In addition, the method may be applicable in cases where the amount of target DNA is in any proportion with the non-target DNA; for example, the target DNA could make up anywhere between 0.000001 and 99.999999 percent of the DNA present. In addition, the non-target DNA does not necessarily need to be from one individual, or even from a related individual, as long as genetic data from non-target individual(s) is known. In one embodiment of the present disclosure, the method can be used to determine genotypic data of a fetus from maternal blood that contains fetal DNA. In one embodiment, the method can be used in a case where there are multiple fetuses in the uterus of a pregnant woman, or where other contaminating DNA may be present in the sample, Paras. [0052] and [0364]); and wherein genotypic measurements made on the genetic material from the father facilitate the copy number determination (genotypic data measured from a mixed sample of DNA, i.e., DNA from the mother of the fetus, and DNA from the fetus and from genotypic data measured from a sample of genetic material from the mother and optionally also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data. Paras. [0038] and [0412]).

Regarding claim 18, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the plurality of loci comprise single nucleotide polymorphisms (SNPs) (the set of polymorphic alleles are SNPs, Para. [0011]; the targeting may focus entirely on SNPs, Para. [0158]).

Regarding claim 19, Rabinowitz discloses the method of claim 18. Rabinowitz further discloses wherein plurality of loci comprises at least 100 SNPs (it is possible to focus on a small number of SNP's, for example between 1 and 100 SNPs, or a larger number, for example between 100 and 1,000, between 1,000 and 10,000, between 10,000 and 100,000 or more than 100,000 SNPs, Para. [0158]).

Regarding claim 20, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the probability of each of the hypotheses is determined using data about the probability of chromosomes crossing over at different locations in the chromosome or chromosome segment of interest (building a joint distribution model is done by using data about the probability of chromosomes crossing over at different cross-over locations in a chromosome to model dependence between polymorphic alleles on the chromosome. Para. [0013]).

Regarding claim 21, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the method comprises calculating, on a computer, allele counts at the plurality of polymorphic loci from the genotypic measurements (calculating, on a computer, allele ratios at the set of polymorphic alleles from the DNA measurements made on the second sample, Para. [0009]), building, on a computer, a joint distribution model for the expected allele counts at the plurality of polymorphic loci on the chromosome or chromosome segment for each hypothesis (building, on a computer, a joint distribution model for heterozygosity rates of each polymorphic allele on the chromosome for each ploidy hypothesis using genotypic data from the one or both parents of the fetus, Para. [0009]); determining, on a computer, a relative probability of each of the copy number hypotheses using the joint distribution model and the allele counts (determining, on a computer, a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele ratios calculated for the second sample, Para. [0009]).

Regarding claim 22, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein a SNP genotyping array is used to make the genotypic measurements (parental context may refer to a set or subset of target SNPs, Para. [0147]; isolated DNA can then be measured by a number of means, such as by hybridizing to a genotyping array, Para. [0148]).

Regarding claim 23, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein high throughput DNA sequencing is used to make the genotypic measurements (This isolated DNA can then by measured by a number of means... sequencing on a high throughput sequencer, Para. [0148]).

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 24, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein a confidence for the copy number determination is at least 95% (checking concordance of hit rates and given confidences, for a range of confidence >90%, Para. [0352]).

Regarding claim 25, Rabinowitz discloses the method of claim 3. Rabinowitz further comprising using the genotypic measurements to determine whether or not the fetus has inherited one or more disease linked haplotypes (the knowledge of the maternal genetic data may be used along with the genetic data measured from the free floating DNA found in the blood to determine which haplotypes from the mother and father are present in the fetus. This knowledge may be used to determine the presence or absence of specific disease related genes or other phenotypically correlated genes in the genotype of the fetus, Para. [0365]).

Regarding claim 26, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses comprising performing a clinical action based on the copy number determination (taking a clinical action based on the called ploidy state of the fetus, Para. [0016]).

Regarding claim 27, Rabinowitz discloses the method of claim 26. Rabinowitz further discloses wherein the clinical action is terminating the pregnancy or maintaining the pregnancy (taking a clinical action based on the called ploidy state of the fetus, wherein the clinical action is selected from one of terminating the pregnancy or maintaining the pregnancy, Para. [0016]).

Regarding claim 28, Rabinowitz discloses the method of claim 26. Rabinowitz further discloses wherein the clinical action is selected from the group consisting of performing amniocentesis on the fetus, performing amniocentesis on a subsequent fetus that that inherits genetic material from the mother and/or father, performing chorion villus biopsy on the fetus, performing chorion villus biopsy on a subsequent fetus that that inherits genetic material from the mother and/or father, performing in vitro fertilization, performing preimplantation genetic diagnosis on one or more embryos that inherited genetic material from the mother and/or father, karyotyping the mother, karyotyping the father, and combinations thereof (the method may be used in the context of in vitro fertilization, where it may be desirable to genotype a single cell blastomere biopsied from a cleavage state embryo for the purposes of determining the genetic state of the embyo, Para. [0189]).

Regarding claim 29, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the chromosome of interest is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, the X chromosome, the Y chromosome, and combinations thereof (it is possible to use this method to create a DNA sample that is enriched in SNPs located on one or a small number of chromosomes that are correlated with live trisomic births, for example chromosomes 13, 18, 21, X and Y, or some combination thereof, Para. [0157]).

Regarding claim 30, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the method comprises screening for a chromosomal abnormality selected from the group consisting of monosomy, uniparental disomy, trisomy, other aneuploidies, mosaicism, unbalanced translocations, insertions, deletions, and combinations thereof (it may also be used to infer the more complete genotypic information of the fetus, specifically, allele calls, the presence of insertions, deletions, transpositions, and other genetic modifications that may correlate with diseases, conditions, or other phenotypes, Para. [0365]).

Regarding claim 31, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein the method comprises determining whether the individual has Down syndrome, Edwards syndrome, Patau syndrome, Klinefelters syndrome, 47,XXX, 47,XYY, Turner syndrome, triploidy, DiGeorge syndrome, Cri du Chat syndrome, Angelman syndrome, Praeder-Willi syndrome, Wolf-Hirschhorn syndrome, SmithMagenis syndrome, Williams-Beuren syndrome, Phelan-McDermid syndrome, Sotos Syndrome, Cystic Fibrosis, Muscular Dystrophy, Spinal Muscular Atrophy, Fragile X, Tay- Sachs disease, Gaucher disease, Torsion Dystonia, Niemann-Pick disease, Mucolipidosis, Fanconi Anemia, Canavan disease, Sickle Cell Anemia, or Bloom Syndrome (the congenital disorder is a malformation, neural tube defect, chromosome abnormality, Down syndrome, Para. [0393]).

Regarding claim 32, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses a report displaying a copy number determination for a chromosome or chromosome segment of interest in the genome of a fetus generated using the method of claim 3 (the knowledge of the determined ploidy state may be used to make a clinical decision. This knowledge, typically stored as a physical arrangement of matter in a memory device, may then be transformed into a report, Para. [0429];determining a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes, Para. [0018]).

Claim 8-10, 12, and 13 lack an inventive step under PCT Article 33(3) as being obvious over Rabinowitz et al (hereafter Rabinowitz)

Regarding claim 8, Rabinowitz discloses the method of claim 3. Rabinowitz further discloses wherein at least 10% of the DNA in the mixed sample are maternal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA... the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 9, Rabinowitz discloses the method of claim 8. Rabinowitz further discloses wherein at least 50% of the DNA in the mixed sample are maternal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA… the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Regarding claim 10, Rabinowitz discloses the method of claim 9. Rabinowitz further discloses wherein at least 90% of the DNA in the mixed sample are maternal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA… the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285];In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Regarding claim 12, Rabinowitz discloses the method of claim 11. Rabinowitz further discloses wherein at least 1% of the DNA in the mixed sample are fetal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA… the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Regarding claim 13, Rabinowitz discloses the method of claim 12. Rabinowitz further discloses wherein at least 4% of the DNA in the mixed sample are fetal DNA (the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA… the fetal DNA may be mixed with maternal DNA in ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%, Para. [0434]), but fails to explicitly disclose wherein the sample is cells.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize a percentage of cells in the sample instead of DNA, since selection of a known material on the basis of its suitability for an intended use involves only routine skill in the art. The motivation for doing so would be to determine the genotype of an individual from a mixed sample of cells (Rabinowitz, There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA...these methods target thousands of locations along each chromosome, Para. [0285]; In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood, Para. [0431]).

Claim 7 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz et al (hereafter Rabinowitz) in view of Hyland et al. (hereafter Hyland).

Regarding claim 7, Rabinowitz discloses the method of claim 6. Rabinowitz fails to explicitly disclose wherein the placental tissue sample is from chorionic villus, decidua, or placental membrane.
Hyland is in the field of the quantification of copy numbers (abstract) and teaches wherein the placental tissue sample is from chorionic villus, deciduas, or placental membrane (PCR typing of a fetus would be even be possible using any fetal cell containing DNA such as chorionic villi, Col. 7 Lns. 40-41).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Rabinowitz to include wherein the placental tissue sample is from chorionic villus as disclosed in Hyland because such a sample can be advantageously amplified in the absence of red blood cells or large quantities of tissue, leading to safer testing (Hyland Col. 7 Lns. 35-39).

Claims 1-32 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.

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 W40	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2013/055205	International filing date *(day/month/year)* 15 August 2013 (15.08.2013)	Priority date *(day/month/year)* 15 August 2012 (15.08.2012)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 13 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 17 February 2015 (17.02.2015)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Simin Baharlou e-mail: pt09.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Scott R. Bortner
Natera, Inc.
201 Industrial Road, Suite 410
San Carlos, CA 94070
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	23 DEC 2013

Applicant's or agent's file reference	FOR FURTHER ACTION
W40	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US13/55205	15 August 2013 (15.08.2013)	15 August 2012 (15.08.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 40/06, 40/08; C50B 50/06; C12P 19/34; C07H 21/04 (2013.01)
USPC - 435/6.1; 506/16, 17, 26

Applicant Natera, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	17 December 2013 (17.12.2013)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/55205

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/US13/55205

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	1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48, 49	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48, 49	NO
Industrial applicability (IA)	Claims	1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48, 49	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 44(B) and 45(B) lack an inventive step under PCT Article 33(3) as being obvious over the publication entitled 'Paired-End Sample Preparation Guide (1005063 Rev. E)' by Illumina, Inc. (hereinafter 'Illumina') in view of the article entitled 'Method For Improving Sequence Coverage Uniformity Of Targeted Genomic Intervals Amplified By LR-PCR Using Illumina GA Sequencing-By-Synthesis Technology' by Harismendy, et al. (hereinafter 'Harismendy').

As per claim 44(B), Illumina discloses a kit (Illumina kit or Paired-End Sample Prep Kit, page 3, paragraph 4; Table 1) for making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: adapters comprising a first universal priming site and a second universal priming site (two adapters contain sequences complimentary to two surface-bound amplification primers; figure 1; page 3, paragraph 3); a primer specific for the first universal priming site; and a primer specific for the second universal priming site (two primers anneal to the ends of the adapters (figure 1, page 3, paragraph 3; page 26 paragraph 2). Illumina does not disclose that the primers are non-ligatable. Harismendy discloses the use of non-ligatable primers (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked), abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the kit of Illumina, for including non-ligatable primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 45(B), Illumina and Harismendy, in combination, disclose the kit of claim 44(B), but Illumina does not disclose wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses the use of primers blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the primers of Illumina to be blocked at the 5' terminus, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

Claims 44(A)-47(A) lack an inventive step under PCT Article 33(3) as being obvious over Harismendy in view of the article entitled 'Nested Patch PCR Enables Highly Multiplexed Mutation Discovery In Candidate Genes' by Varley, et al. (hereinafter 'Varley').

As per claim 44(A), Harismendy discloses a genetic library (200 bp or 600 bp library; abstract; figures 1B, 2; page 230, columns 2-3) comprising a plurality of amplicons (6 amplicons were generated with one type of primer; page 229, column3, paragraph 1; figure 1A) having 2 non-ligatable termini (amplicons generated with 5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). Harismendy does not disclose wherein each amplicon comprises a polymorphic locus. Varley discloses a genetic library wherein each amplicon comprises a polymorphic locus (mutations and SNPs; page 1846, column 1, paragraph 5; Table 1; page 1847, column 1, paragraph 1-2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including a polymorphic locus comprising each amplicon, as previously disclosed by Varley, as utilizing amplicons comprising of polymorphic loci will to allow the genetic library to be used for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 45(A), Harismendy and Varley in combination disclose claim 44(A), and Harismendy further discloses wherein each amplicon comprises a universal priming site (adapter-ligated fragments; page 229, column 3, paragraph 1) suitable for use with a clonal amplification procedure (Cluster generation; page 229, column 3, paragraph 1).

As per claim 46(A), Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 100 genetic loci. Varley discloses wherein the genetic library comprises polymorphisms from at least 100 genetic loci (100-1000 candidate regions); page 1847, column 1, paragraph 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 disclosure of Harismendy, for providing for a genetic library comprising polymorphisms from at least 100 genetic loci, for maximizing the potential of discovery of a significant number of polymorphisms of clinical importance, without undue experimentation or testing.

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/55205

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Regarding Claim 1, in line 6 and 12 of Claim 1, the letter 's' after 'primers' likely should have been removed.

Regarding Claim 16, in line 2 of Claim 16, the word 'a' should likely have been added before the word 'pregnant.'

Claim 21 is missing a period after '21.'

Regarding Claim 22, in line 8 of Claim 22, the word 'a' should be added in front of the word 'primers' and the letter 's' after 'primers' should likely have bene removed.

Regarding Claim 33, in line 12 of Claim 33, the letter 's' after 'primers' should have likely been removed; in line 18 of Claim 33, the word 'a' likely should have been added in front of the word 'primers' and the letter 's' after 'primers' likely should have been removed.

Regarding Claim 45(B), in line 2, a period likely should have replaced the comma.

Regaridng Claim 47(B), a period is missing at the end of the claim.

Regarding Claim 45(B), in line 2, a period likely should have replaced the comma.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/55205

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:

Claim 44 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 44 of the instant application appears twice. For the purposes of this examination, an assumption has been made that the first Claim 44 will be considered as being Claim 44(A) and the second Claim 44 is Claim 44(B).

Claim 45 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 45 of the instant application appears twice. For the purposes of this examination, an assumption has been made that the first Claim 45 will be considered Claim 45(A), and the second Claim 45 is Claim 45(B).

Claim 46 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 46 of the instant application appears twice. For the purposes of this examination, an assumption has been made that the first Claim 46 will be considered Claim 46(A), and the second Claim 46 is Claim 46(B).

Claim 47 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 47 of the instant application appears three times. For the purposes of this examination, an assumption has been made that the first Claim 47 will be considered Claim 47(A), the second Claim 47 is Claim 47(B) and the third Claim 47 is Claim 47(C).

Claim 45(B) is objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claim is an incomplete claim. The claim does not disclose what type of primer is 'capable of binding to the first universal priming' site. Earlier claims describe the primer capable of binding to the first universal site 'comprises a barcode' or 'is blocked at the 5' terminus.' Based upon the Specification of the instant PCT application, 'the kit' comprises 'ligation blocked universal primers.' For the purposes of this examination, Claim 45(B) has been interpreted to have instead been 'the primer capable of binding to the first universal priming site is blocked at the 5' terminus.'

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As per claim 47(A), Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 1000 genetic loci. Varley discloses a genetic library which comprises polymorphisms from at least 1000 genetic loci (100-1000 candidate regions); page 1847, column 1, paragraph 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 previous disclosure of Harismendy, for providing for a genetic library comprising polymorphisms from at least 1000 genetic loci, for maximizing the potential of discovery of a significant number of polymorphisms of clinical importance, without undue experimentation or testing.

Claims 1-4, 6-8, 10, 11/1-4, 11/6-8, 11/10, 12-15, 17-20, 21/12-15, 21/17-20, 22-25, 27-29, 31, 32/22-25, 32/27-29, 32/31, 33-36, 38-40, 42, 43/33-36, 43/38-40 and 43/42 lack an inventive step under PCT Article 33(3) as being obvious over Illumina and further in view of Varley and Harismendy.

As per claim 1, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: ligating a set of universal adapters to nucleic acid fragments in a sample preparation (ligate adapters/adaptors' figures 1, 2; page 21, paragraph 2), the universal adapters having a first universal primer binding site and a second universal primer binding site (page 3, paragraph 3; page 26, paragraph 2); amplifying a subset of the adapter modified nucleic acid fragments (PCR to selectively enrich DNA fragments that have adapter molecules on both ends; page 26, paragraph 2), wherein the amplification step comprises adding a primer capable of binding to the first universal binding site (PCR Primer PE 1.0; page 26, paragraph 11) and adding a primer capable of binding to the second universal binding site (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose the use of a plurality of different target-specific primers, wherein the primers capable of binding to the first universal priming site are non-ligatable primers whereby a set of partially selected amplicons are formed; and amplifying the set of partially selected genetic amplicons using a plurality of different target-specific primers, wherein the primers capable of binding to the second universal priming site are non-ligatable primers, whereby a set of non-ligatable amplification products are formed. Varley discloses the use of a plurality of different target-specific primers (amplified 90 targeted human exons, abstract; page 1845, column 2, paragraph; by PCR containing 2 primers for each target, page 1845, column 1, paragraph 4; figure 1A). Harismendy discloses that the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a plurality of different target-specific primers, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including non-ligatable universal primers, as disclosed by Harismendy, for reducing overrepresentation of amplicon ends (abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (page 231, column 1, paragraph 2).

As per claim 2, Illumina, Varley and Harismendy, in combination, disclose the method of claim 1, but Illumina does not disclose wherein the set of non-ligatable amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses wherein the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column 1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine, page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, to have modified the method of Illumina, for including next generation sequencing, as disclosed Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 3, Illumina, Varley and Harismendy, in combination, disclose the method of claim 1, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

As per claim 4, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy;page 231, column 1, paragraph 2).

As per claim 6, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherein the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5; mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (abstract).

As per claim 7, Illumina, Varley and Harismendy, in combination, disclose claim 6, but Illumina does not disclose wherein the polymorphic region is a SNP. Varley discloses wherein the polymorphic region is a SNP (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as the target-specific amplification of SNPs would have allowed for the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

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As per claim 8, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein at least 1000 target specific primers are added. Varley discloses wherein at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 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 method of Illumina, for including at least 1000 target specific primers to be added, as previously disclosed by Varley, as multiplexing would have allowed for medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 10, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprise a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (Varley; page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 11/1-4, 11/6-8 and 11/10, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 1-4, 6-8 and 10, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 12, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: providing a genetic library comprising DNA fragments having a first end and a second end (figure 1), wherein a first universal priming site is joined to the first end and a second universal priming site is joined to the second end (two adapter join DNA fragment ends; figure 2); and amplifying the genetic library with a primer specific for the first universal priming site (PCR Primer PE 1.0; page 26, paragraph 11) and a primer specific for the second universal priming site (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose a plurality of amplified target regions or non-ligatable universal primers. Varley discloses a plurality of amplified target regions (amplified 90 targeted human exons; abstract; page 1845, column 2, paragraph 3). Harismendy discloses that the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a plurality of amplified target regions, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the method of Illumina, for including non-ligatable universal primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 13, Illumina, Varley and Harismendy, in combination disclose the method of claim 12, but Illumina does not disclose that the amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column 1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine; page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including next generation sequencing, as previously disclosed by Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 14, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

As per claim 15, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses that the capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 17, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherein the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5; mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs, abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as previously disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

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As per claim 18, Illumina, Varley and Harismendy, in combination, disclose claim 17, but Illumina does not disclose that the polymorphic region is a SNP. Varley discloses the polymorphic region is a SNP (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as target-specific amplification of SNPs would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 19, Illumina, Varley and Harismendy, in combination, disclose claim 12, but Illumina does not disclose wherein at least 1000 target specific primers are added. Varley discloses wherein at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 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 method of Illumina, for including at least 1000 target specific primers are added, as previously disclosed by Varley, as multiplexing would have allowed medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 20, Illumina, Varley and Harismendy, in combination, disclose claim 12, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses the use of primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 21/12-15 and 21/17-20, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 12-15 and 17-20, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 22, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: ligating a first universal adapter and a second universal adapter to a set of nucleic acid fragments from a nucleic sample preparation (ligate adapters/adaptors figures 1, 2; page 21, paragraph 2), the first universal adapter and the second universal adapter having a first universal primer binding site and a second universal primer binding site (page 3, paragraph 3; page 26, paragraph 2); amplifying a subset of the adapter modified nucleic acid fragments (PCR to selectively enrich DNA fragments that have adapter molecules on both ends; page 26, paragraph 2) wherein the amplification step comprises adding primers capable of binding to the first universal binding region (PCR Primer PE 1.0; page 26, paragraph 11) and adding a primer capable of binding to the second universal binding region (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose that disclose amplifying a subset of pre-amplified adapter modified nucleic acid fragments using a plurality of different target-specific primers, wherein the primers capable of binding to the first universal priming site are non-ligatable, whereby a set of partially selected amplicons are formed or amplifying the set of partially selected genetic amplicons using a plurality of different target-specific primers, wherein the primers capable of binding to the second universal priming site are non-ligatable, whereby a set of non-ligatable amplification products are formed. Varley discloses a subset of pre-amplified adapter modified nucleic acid fragments (targets amplified by PCR, then ligated to universal primers by Nested Patch oligonucleotides; pages 1845, column 1, paragraph 4; figure 1A, 1C) and the use of a plurality of different target-specific primers (amplified 90 targeted human exons; abstract; page 1845, column 2, paragraph 3) by PCR containing 2 primers for each target (page 1845, column 1, paragraph 4; figure 1A). Harismendy discloses the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a subset of pre-amplified adapter modified nucleic acid fragments and the use of a plurality of different target-specific primers, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including non-ligatable universal primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 23, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine, page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including next generation sequencing, as previously disclosed by Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 24, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

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As per claim 25, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1) thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 27, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherein the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5); mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs, abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as previously disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Harismendy; abstract).

As per claim 28, Illumina, Varley and Harismendy, in combination, disclose claim 27, but Illumina does not disclose wherein the polymorphic region is a SNP. Varley discloses wherein the polymorphic region is a SNP (Varley; abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as target-specific amplification of SNPs would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 29, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein at least 1000 target specific primers are added. Varley teaches wherein at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 1). It would have been obvious to one of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including at least 1000 target specific primers are added, as previously disclosed by Varley, as multiplexing would have allowed medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 31, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (Varley; page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 32/22-25, 32/27-29 and 32/31, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 22-25, 27-29 and 31, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 33, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: ligating a first universal adapter and a second universal adapter to a set of nucleic acid fragments from a nucleic sample preparation (ligate adapters/adaptors; figures 1, 2; page 21, paragraph 2), the first universal adapter and the second universal adapter have a first universal primer binding site and a second universal primer binding site site (page 3, paragraph 3; page 26, paragraph 2); amplifying a subset of the adapter modified nucleic acid fragments (PCR to selectively enrich DNA fragments that have adapter molecules on both ends; page 26, paragraph 2), wherein the amplification step comprises adding [a primer] capable of binding to the first universal binding region (PCR Primer PE 1.0; page 26, paragraph 11) and adding a primer capable of binding to the second universal binding site (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose amplifying a subset of pre-amplified adapter modified nucleic acid fragments using a plurality of different target-specific primers, whereby a set of partially selected amplicons are formed or amplifying the set of partially selected genetic amplicons using a plurality of different target-specific primers, whereby a set of selected amplicons is formed; and amplifying the set of selected amplicons with primers specific for universal binding sites, wherein the primers are non-ligatable primers, whereby a set of non-ligatable amplicons are produced. Varley discloses a subset of pre-amplified adapter modified nucleic acid fragments (targets amplified by PCR then ligated to universal primers by Nested Patch oligonucleotides; pages 1845, column 1, paragraph 4; figures 1A, 1C) and the use of a plurality of different target-specific primers (amplified 90 targeted human exons, abstract; page 1845, column 2, paragraph 3; by PCR containing 2 primers for each target, page 1845, column 1, paragraph 4; figure 1A). Harismendy discloses the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a subset of pre-amplified adapter modified nucleic acid fragments and the use of a plurality of different target-specific primers, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modiifed the method of Illumina, for including non-ligatable universal primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

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As per claim 34, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses wherein the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column 1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine, page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including next generation sequencing, as previously disclosed by Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 35, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

As per claim 36, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 38, Illumina, Varley and Harismendy, in combination disclose, claim 33, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherien the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5; mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs, abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as previously disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 39, Illumina, Varley and Harismendy, in combination, disclose claim 38, but Illumina does not disclose wherein the polymorphic region is a SNP. Varley discloses wherein the polymorphic region is a SNP (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as target-specific amplification of SNPs would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 40, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose that at least 1000 target specific primers are added. Varley teaches that at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 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 method of Illumina, for including at least 1000 target specific primers are added, as previously disclosed by Varley, as multiplexing would have allowed medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 42, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (Varley; page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 43/33-36, 43/38-40 amd 43/42, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 33-36, 38-40 and 42, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

Claim 46(B) lacks an inventive step under PCT Article 33(3) as being obvious over Illumina in view of Harismendy in view of the article entitled 'Accurate Whole Human Genome Sequencing Using Reversible Terminator Chemistry' by Bentley, et al. (hereinafter 'Bentley').

As per claim 46(B), Illumina and Harismendy, in combination, disclose the kit of claim 44(B), but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses wherein the universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the kit of Illumina to include universal adapters which are Y-shaped adapters, as previously disclosed by Bentley, as this would have allowed DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/55205

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:

Claim 47(B) lacks an inventive step under PCT Article 33(3) as being obvious over Harismendy in view of Varley in view of the article entitled 'Non-Invasive Prenatal Diagnosis By Massively Parallel Sequencing Of Maternal Plasma DNA' by Lo (hereinafter 'Lo').

As per claim 47(B), Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the amplicons are derived from a genetic composition comprising a mixture of maternal and fetal DNA. Lo discloses amplicons which are derived from a genetic composition comprising a mixture of maternal and fetal DNA (fetal DNA represents a minor fraction of maternal plasma DNA, summary; page 3, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including amplicons which are derived from a genetic composition comprising a mixture of maternal and fetal DNA, as previously disclosed by Lo, as improvement would have been expected to have allowed the the genetic library to be used for non-invasive prenatal diagnosis (Lo; Summary; page 3, column 2, paragraph 6).

Claim 47(C) lacks an inventive step under PCT Article 33(3) as being obvious over Illumina in view of Harismendy in view of Varley.

As per claim 47(C), Illumina and Harismendy, in combination, disclose the kit of claim 44(B), but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine, page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (page 1845, column 1, paragraph 4), the primer being incomplete and non-ligatable.

Claims 48 and 49 lack an inventive step under PCT Article 33(3) as being obvious over Harismendy and Varley and further in view of Bentley.

As per claim 48, Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 5000 genetic loci. Bentley discloses a genetic library comprising polymorphisms which from at least 5000 genetic loci (about 4 million SNPs, page 55, column 2, paragraph 4; in whole human genome (about 20,000 genes), page 55, column 2, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including polymorphisms from at least 5000 genetic loci, as previously disclosed by Bentley, as large genetic libraries (i.e. whole-genome) would have been expected to maximize the opportunity to discover polymorphisms of important biological or medical benefit (Bentley; abstract).

As per claim 49, Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 10000 genetic loci. Bentley discloses wherein the genetic library comprises polymorphisms from at least 10000 genetic loci (about 4 million SNPs, page 55, column 2, paragraph 4; in whole human genome (about 20,000 genes), page 55, column 2, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including polymorphisms from at least 10000 genetic loci, as previously disclosed by Bentley, as large genetic libraries (i.e. whole-genome) would have been expected to enable discoveries of important biological or medical benefit (Bentley; abstract).

Claims 5, 11/5, 16, 21/16, 26, 32/26, 37 and 43/37 lack an inventive step under PCT Article 33(3) as being obvious over Illumina, Varley and Harismendy and further in view of Lo.

As per claim 5, Illumina, Varley and Harismendy in combination, disclose the method of claim 1, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses wherein the nucleic acids are obtained from the blood of pregnant female (foetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Varley, for including nucleic acids obtained from the blood of pregnant female, as previously disclosed by Lo, as this additional feature would have allowed the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 11/5, Illumina, Varley and Harismendy, in combination, disclose the method of claim 5, and Illumina further discloses a genetic library (libraries of genomic DNA (page 3, paragraph 2)).

As per claim 16, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses that the nucleic acids are obtained from the blood of pregnant female (fetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modiifed the genetic library of Varley, for including that the nucleic acids are obtained from the blood of pregnant female, as previously disclosed by Lo, for allowing the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 21/16, Illumina, Varley, Harismendy and Lo, in combination, disclose the method of claim 16, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/55205

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 26, Illumina, Varley and Harismendy, in combination, disclose the method of claim 22, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses wherein the nucleic acids are obtained from the blood of pregnant female (fetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the genetic library of Varley, for including the nucleic acids are obtained from the blood of pregnant female, as previously disclosed by Lo, as this feature disclosed by Lo would have allowed the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 32/26, Illumina, Varley, Harismendy and Lo in combination disclose the method of claim 26, and Illumina discloses a genetic library (libraries of genomic DNA (page 3, paragraph 2)).

As per claim 37, Illumina, Varley and Harismendy, in combination, disclose the method of claim 33, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses wherein the nucleic acids are obtained from the blood of pregnant female (fetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have implemented the genetic library of Varley, for including the nucleic acids obtained from the blood of pregnant female, as previously by Lo, as this feature disclosed by Lo would have allowed the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 43/37, Illumina, Varley, Harismendy and Lo, in combination, disclose the method of claim 33, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

Claims 9; 11/9, 30, 32/30, 41 and 43/41 lack an inventive step under PCT Article 33(3) as being obvious over Illunima, Varley and Harismendy and further in view of Bentley.

As per claim 9, Illumina, Varley and Harismendy, in combination, disclose the method of claim 1, but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the kit of Illumina, for including wherein the universal adapters are Y-shaped adapters, as previously disclosed by Bentley, as this adaption would have allowed DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

As per claim 11/9, Illumina, Varley, Harismendy and Bentley, in combination disclose the method of claim 9, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 30, Illumina, Varley and Harismendy, in combination, disclose the method of claim 22, but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the kit of Illumina, for including wherein the universal adapters are Y-shaped adapters, as previously disclosed by Bentley, as this adaption would have allowed DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

As per claim 32/30, Illumina, Varley, Harismendy and Bentley in combination disclose the method of claim 30, and Illumina discloses a genetic library (libraries of genomic DNA (page 3, paragraph 2)).

As per claim 41, Illumina, Varley and Harismendy, in combination, disclose the method of claim 33, but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses wherein the universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the kit of Illumina, for including wherein the universal adapters are Y-shaped adapters, as previously disclosed by Bentley, as this adaption would have allowed for DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

As per claim 43/41, Illumina, Varley, Harismendy and Bentley, in combination, disclose the method of claim 41, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

Claims 1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48 and 49 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 W40	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US13/55205	International filing date *(day/month/year)* 15 August 2013 (15.08.2013)	(Earliest) Priority Date *(day/month/year)* 15 August 2012 (15.08.2012)
Applicant Natera, 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. 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 figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

PCT/US2013/055205 23.12.2013

International application No.
PCT/US13/55205

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C40B 40/06, 40/08; C50B 50/06; C12P 19/34; C07H 21/04 (2013.01)

USPC - 435/6.1; 506/16, 17, 26

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): C40B 30/00,02, 40/00, 40/04, 40/06, 40/08, 40/10, 50/06; C12Q 1/68; C12P 19/3; C07H 21/04 (2013.01)
USPC: 435/4, 6.1, 442, 912, 915; 506/7, 8, 9, 13, 15, 16, 17, 18, 26

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; Google Scholar; ProQuest; 'genetic library,' 'universal adapter,' 'universal primer,' 'massively parallel DNA sequencer,' '5' blocked primer,' 'Y-shaped adaptor'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ILLUMINA, INC. Paired-End Sample Preparation Guide (1005063 Rev. E). February 2011; page 3, paragraphs 2-4; figure 1; Table 1; figure 2; page 21, paragraph 2; pg 26, paragraphs 2, 11, 12.	1-43, 44(B)-46(B), 47(C)
Y	VARLEY, KE et al. 'Nested Patch PCR Enables Highly Multiplexed Mutation Discovery In Candidate Genes. Epub. 10 October 2008; abstract; page 1845, column 1, paragraphs 1, 4; page 1845, column 2, paragraphs 1, 3; figures 1A-C, 2; page 1846, column 1, paragraph 5; Table 1; page 1847, column 1, paragraphs 1-2; page 1847, column 2, paragraphs 3-4; page 1848, column 1, paragraphs 1, 5; page 1849, columnn 1, paragraphs 3.	1-43, 44(A)-47(A), 47(B), 47(C), 48, 49
Y	HARISMENDY, O et al. 'Method For Improving Sequence Coverage Uniformity Of Targeted Genomic Intervals Amplified By LR-PCR Using Illumina GA Sequencing-By-Synthesis Technology. March 2009, Vol. 46: pages 229-31; abstract; page 229, column 2, paragraph 2; page 229, column 3, paragraph 1; figures 1, 2; page 230, columns 2-3; page 231, column 1, paragraph 2.	1-43, 44(A)-47(A), 44 (B)-47(B), 47(C), 48, 49
Y	LO, YM. Non-Invasive Prenatal Diagnosis By Massively Parallel Sequencing Of Maternal Plasma DNA. June 2012, Vol. 2: 120086; pages 1-5; Summary; page 3, column 1, paragraph 3; page 3 column 2, paragraph 6.	5, 11/5, 16, 21/16, 26, 32/26, 37, 43/37, 47(B)
Y	BENTLEY, DR et al. Accurate Whole Human Genome Sequencing Using Reversible Terminator Chemistry. Nature. 06 November 2008, Vol. 456, No. 6; pages 53-59; abstract; page 53, column 1, paragraph 4; figures 1A-C; page 55, column 2, paragraphs 3-4.	9, 11/9, 30, 32/30, 41, 43/41, 46(B), 48, 49

☐ 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
17 December 2013 (17.12.2013)	23 DEC 2013

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Scott R. Bortner
Natera, Inc.
201 Industrial Road, Suite 410
San Carlos, CA 94070
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 23 DEC 2013

Applicant's or agent's file reference
W40

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US13/55205	15 August 2013 (15.08.2013)	15 August 2012 (15.08.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 40/06, 40/08; C50B 50/06; C12P 19/34; C07H 21/04 (2013.01)
USPC - 435/6.1; 506/16, 17, 26

Applicant Natera, 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	17 December 2013 (17.12.2013)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/55205

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/US13/55205

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	1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48, 49	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48, 49	NO
Industrial applicability (IA)	Claims	1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48, 49	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 44(B) and 45(B) lack an inventive step under PCT Article 33(3) as being obvious over the publication entitled 'Paired-End Sample Preparation Guide (1005063 Rev. E)' by Illumina, Inc. (hereinafter 'Illumina') in view of the article entitled 'Method For Improving Sequence Coverage Uniformity Of Targeted Genomic Intervals Amplified By LR-PCR Using Illumina GA Sequencing-By-Synthesis Technology' by Harismendy, et al. (hereinafter 'Harismendy').

As per claim 44(B), Illumina discloses a kit (Illumina kit or Paired-End Sample Prep Kit, page 3, paragraph 4; Table 1) for making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: adapters comprising a first universal priming site and a second universal priming site (two adapters contain sequences complimentary to two surface-bound amplification primers; figure 1; page 3, paragraph 3); a primer specific for the first universal priming site; and a primer specific for the second universal priming site (two primers anneal to the ends of the adapters (figure 1, page 3, paragraph 3; page 26 paragraph 2). Illumina does not disclose that the primers are non-ligatable. Harismendy discloses the use of non-ligatable primers (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked), abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the kit of Illumina, for including non-ligatable primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 45(B), Illumina and Harismendy, in combination, disclose the kit of claim 44(B), but Illumina does not disclose wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses the use of primers blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the primers of Illumina to be blocked at the 5' terminus, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

Claims 44(A)-47(A) lack an inventive step under PCT Article 33(3) as being obvious over Harismendy in view of the article entitled 'Nested Patch PCR Enables Highly Multiplexed Mutation Discovery In Candidate Genes' by Varley, et al. (hereinafter 'Varley').

As per claim 44(A), Harismendy discloses a genetic library (200 bp or 600 bp library; abstract; figures 1B, 2; page 230, columns 2-3) comprising a plurality of amplicons (6 amplicons were generated with one type of primer; page 229, column3, paragraph 1; figure 1A) having 2 non-ligatable termini (amplicons generated with 5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). Harismendy does not disclose wherein each amplicon comprises a polymorphic locus. Varley discloses a genetic library wherein each amplicon comprises a polymorphic locus (mutations and SNPs; page 1846, column 1, paragraph 5; Table 1; page 1847, column 1, paragraph 1-2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including a polymorphic locus comprising each amplicon, as previously disclosed by Varley, as utilizing amplicons comprising of polymorphic loci will to allow the genetic library to be used for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 45(A), Harismendy and Varley in combination disclose claim 44(A), and Harismendy further discloses wherein each amplicon comprises a universal priming site (adapter-ligated fragments; page 229, column 3, paragraph 1) suitable for use with a clonal amplification procedure (Cluster generation; page 229, column 3, paragraph 1).

As per claim 46(A), Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 100 genetic loci. Varley discloses wherein the genetic library comprises polymorphisms from at least 100 genetic loci (100-1000 candidate regions); page 1847, column 1, paragraph 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 disclosure of Harismendy, for providing for a genetic library comprising polymorphisms from at least 100 genetic loci, for maximizing the potential of discovery of a significant number of polymorphisms of clinical importance, without undue experimentation or testing.

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Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Regarding Claim 1, in line 6 and 12 of Claim 1, the letter 's' after 'primers' likely should have been removed.

Regarding Claim 16, in line 2 of Claim 16, the word 'a' should likely have been added before the word 'pregnant.'

Claim 21 is missing a period after '21.'

Regarding Claim 22, in line 8 of Claim 22, the word 'a' should be added in front of the word 'primers' and the letter 's' after 'primers' should likely have bene removed.

Regarding Claim 33, in line 12 of Claim 33, the letter 's' after 'primers' should have likely been removed; in line 18 of Claim 33, the word 'a' likely should have been added in front of the word 'primers' and the letter 's' after 'primers' likely should have been removed.

Regarding Claim 45(B), in line 2, a period likely should have replaced the comma.

Regaridng Claim 47(B), a period is missing at the end of the claim.

Regarding Claim 45(B), in line 2, a period likely should have replaced the comma.

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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:

Claim 44 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 44 of the instant application appears twice. For the purposes of this examination, an assumption has been made that the first Claim 44 will be considered as being Claim 44(A) and the second Claim 44 is Claim 44(B).

Claim 45 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 45 of the instant application appears twice. For the purposes of this examination, an assumption has been made that the first Claim 45 will be considered Claim 45(A), and the second Claim 45 is Claim 45(B).

Claim 46 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 46 of the instant application appears twice. For the purposes of this examination, an assumption has been made that the first Claim 46 will be considered Claim 46(A), and the second Claim 46 is Claim 46(B).

Claim 47 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect in the form or contents thereof: Claim 47 of the instant application appears three times. For the purposes of this examination, an assumption has been made that the first Claim 47 will be considered Claim 47(A), the second Claim 47 is Claim 47(B) and the third Claim 47 is Claim 47(C).

Claim 45(B) is objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claim is an incomplete claim. The claim does not disclose what type of primer is 'capable of binding to the first universal priming' site. Earlier claims describe the primer capable of binding to the first universal site 'comprises a barcode' or 'is blocked at the 5' terminus.' Based upon the Specification of the instant PCT application, 'the kit' comprises 'ligation blocked universal primers.' For the purposes of this examination, Claim 45(B) has been interpreted to have instead been 'the primer capable of binding to the first universal priming site is blocked at the 5' terminus.'

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As per claim 47(A), Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 1000 genetic loci. Varley discloses a genetic library which comprises polymorphisms from at least 1000 genetic loci (100-1000 candidate regions); page 1847, column 1, paragraph 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 previous disclosure of Harismendy, for providing for a genetic library comprising polymorphisms from at least 1000 genetic loci, for maximizing the potential of discovery of a significant number of polymorphisms of clinical importance, without undue experimentation or testing.

Claims 1-4, 6-8, 10, 11/1-4, 11/6-8, 11/10, 12-15, 17-20, 21/12-15, 21/17-20, 22-25, 27-29, 31, 32/22-25, 32/27-29, 32/31, 33-36, 38-40, 42, 43/33-36, 43/38-40 and 43/42 lack an inventive step under PCT Article 33(3) as being obvious over Illumina and further in view of Varley and Harismendy.

As per claim 1, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: ligating a set of universal adapters to nucleic acid fragments in a sample preparation (ligate adapters/adaptors' figures 1, 2; page 21, paragraph 2), the universal adapters having a first universal primer binding site and a second universal primer binding site (page 3, paragraph 3; page 26, paragraph 2); amplifying a subset of the adapter modified nucleic acid fragments (PCR to selectively enrich DNA fragments that have adapter molecules on both ends; page 26, paragraph 2), wherein the amplification step comprises adding a primer capable of binding to the first universal binding site (PCR Primer PE 1.0; page 26, paragraph 11) and adding a primer capable of binding to the second universal binding site (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose the use of a plurality of different target-specific primers, wherein the primers capable of binding to the first universal priming site are non-ligatable primers whereby a set of partially selected amplicons are formed; and amplifying the set of partially selected genetic amplicons using a plurality of different target-specific primers, wherein the primers capable of binding to the second universal priming site are non-ligatable primers, whereby a set of non-ligatable amplification products are formed. Varley discloses the use of a plurality of different target-specific primers (amplified 90 targeted human exons, abstract; page 1845, column 2, paragraph; by PCR containing 2 primers for each target, page 1845, column 1, paragraph 4; figure 1A). Harismendy discloses that the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a plurality of different target-specific primers, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including non-ligatable universal primers, as disclosed by Harismendy, for reducing overrepresentation of amplicon ends (abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (page 231, column 1, paragraph 2).

As per claim 2, Illumina, Varley and Harismendy, in combination, disclose the method of claim 1, but Illumina does not disclose wherein the set of non-ligatable amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses wherein the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column 1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine, page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, to have modified the method of Illumina, for including next generation sequencing, as disclosed Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 3, Illumina, Varley and Harismendy, in combination, disclose the method of claim 1, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

As per claim 4, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy;page 231, column 1, paragraph 2).

As per claim 6, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherein the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5; mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (abstract).

As per claim 7, Illumina, Varley and Harismendy, in combination, disclose claim 6, but Illumina does not disclose wherein the polymorphic region is a SNP. Varley discloses wherein the polymorphic region is a SNP (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as the target-specific amplification of SNPs would have allowed for the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

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As per claim 8, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein at least 1000 target specific primers are added. Varley discloses wherein at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 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 method of Illumina, for including at least 1000 target specific primers to be added, as previously disclosed by Varley, as multiplexing would have allowed for medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 10, Illumina, Varley and Harismendy, in combination, disclose claim 1, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprise a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (Varley; page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 11/1-4, 11/6-8 and 11/10, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 1-4, 6-8 and 10, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 12, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: providing a genetic library comprising DNA fragments having a first end and a second end (figure 1), wherein a first universal priming site is joined to the first end and a second universal priming site is joined to the second end (two adapter join DNA fragment ends; figure 2); and amplifying the genetic library with a primer specific for the first universal priming site (PCR Primer PE 1.0; page 26, paragraph 11) and a primer specific for the second universal priming site (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose a plurality of amplified target regions or non-ligatable universal primers. Varley discloses a plurality of amplified target regions (amplified 90 targeted human exons; abstract; page 1845, column 2, paragraph 3). Harismendy discloses that the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a plurality of amplified target regions, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the method of Illumina, for including non-ligatable universal primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 13, Illumina, Varley and Harismendy, in combination disclose the method of claim 12, but Illumina does not disclose that the amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column 1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine; page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including next generation sequencing, as previously disclosed by Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 14, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer is capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

As per claim 15, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the primer is capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses that the capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 17, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherein the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5; mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs, abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as previously disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

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As per claim 18, Illumina, Varley and Harismendy, in combination, disclose claim 17, but Illumina does not disclose that the polymorphic region is a SNP. Varley discloses the polymorphic region is a SNP (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as target-specific amplification of SNPs would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 19, Illumina, Varley and Harismendy, in combination, disclose claim 12, but Illumina does not disclose wherein at least 1000 target specific primers are added. Varley discloses wherein at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 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 method of Illumina, for including at least 1000 target specific primers are added, as previously disclosed by Varley, as multiplexing would have allowed medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 20, Illumina, Varley and Harismendy, in combination, disclose claim 12, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses the use of primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 21/12-15 and 21/17-20, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 12-15 and 17-20, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 22, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: ligating a first universal adapter and a second universal adapter to a set of nucleic acid fragments from a nucleic sample preparation (ligate adapters/adaptors figures 1, 2; page 21, paragraph 2), the first universal adapter and the second universal adapter having a first universal primer binding site and a second universal primer binding site (page 3, paragraph 3; page 26, paragraph 2); amplifying a subset of the adapter modified nucleic acid fragments (PCR to selectively enrich DNA fragments that have adapter molecules on both ends; page 26, paragraph 2) wherein the amplification step comprises adding primers capable of binding to the first universal binding region (PCR Primer PE 1.0; page 26, paragraph 11) and adding a primer capable of binding to the second universal binding region (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose that disclose amplifying a subset of pre-amplified adapter modified nucleic acid fragments using a plurality of different target-specific primers, wherein the primers capable of binding to the first universal priming site are non-ligatable, whereby a set of partially selected amplicons are formed or amplifying the set of partially selected genetic amplicons using a plurality of different target-specific primers, wherein the primers capable of binding to the second universal priming site are non-ligatable, whereby a set of non-ligatable amplification products are formed. Varley discloses a subset of pre-amplified adapter modified nucleic acid fragments (targets amplified by PCR, then ligated to universal primers by Nested Patch oligonucleotides; pages 1845, column 1, paragraph 4; figure 1A, 1C) and the use of a plurality of different target-specific primers (amplified 90 targeted human exons; abstract; page 1845, column 2, paragraph 3) by PCR containing 2 primers for each target (page 1845, column 1, paragraph 4; figure 1A). Harismendy discloses the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a subset of pre-amplified adapter modified nucleic acid fragments and the use of a plurality of different target-specific primers, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including non-ligatable universal primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 23, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine, page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including next generation sequencing, as previously disclosed by Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 24, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

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As per claim 25, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1) thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 27, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherein the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5); mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs, abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as previously disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Harismendy; abstract).

As per claim 28, Illumina, Varley and Harismendy, in combination, disclose claim 27, but Illumina does not disclose wherein the polymorphic region is a SNP. Varley discloses wherein the polymorphic region is a SNP (Varley; abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as target-specific amplification of SNPs would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 29, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein at least 1000 target specific primers are added. Varley teaches wherein at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 1). It would have been obvious to one of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including at least 1000 target specific primers are added, as previously disclosed by Varley, as multiplexing would have allowed medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 31, Illumina, Varley and Harismendy, in combination, disclose claim 22, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (Varley; page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 32/22-25, 32/27-29 and 32/31, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 22-25, 27-29 and 31, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 33, Illumina discloses a method (protocol; page 3, paragraph 2) of making a genetic library (libraries of genomic DNA; page 3, paragraph 2) comprising: ligating a first universal adapter and a second universal adapter to a set of nucleic acid fragments from a nucleic sample preparation (ligate adapters/adaptors; figures 1, 2; page 21, paragraph 2), the first universal adapter and the second universal adapter have a first universal primer binding site and a second universal primer binding site site (page 3, paragraph 3; page 26, paragraph 2); amplifying a subset of the adapter modified nucleic acid fragments (PCR to selectively enrich DNA fragments that have adapter molecules on both ends; page 26, paragraph 2), wherein the amplification step comprises adding [a primer] capable of binding to the first universal binding region (PCR Primer PE 1.0; page 26, paragraph 11) and adding a primer capable of binding to the second universal binding site (PCR Primer PE 2.0; page 26, paragraph 12). Illumina does not disclose amplifying a subset of pre-amplified adapter modified nucleic acid fragments using a plurality of different target-specific primers, whereby a set of partially selected amplicons are formed or amplifying the set of partially selected genetic amplicons using a plurality of different target-specific primers, whereby a set of selected amplicons is formed; and amplifying the set of selected amplicons with primers specific for universal binding sites, wherein the primers are non-ligatable primers, whereby a set of non-ligatable amplicons are produced. Varley discloses a subset of pre-amplified adapter modified nucleic acid fragments (targets amplified by PCR then ligated to universal primers by Nested Patch oligonucleotides; pages 1845, column 1, paragraph 4; figures 1A, 1C) and the use of a plurality of different target-specific primers (amplified 90 targeted human exons, abstract; page 1845, column 2, paragraph 3; by PCR containing 2 primers for each target, page 1845, column 1, paragraph 4; figure 1A). Harismendy discloses the universal primers are non-ligatable (5'-blocked primers - Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including a subset of pre-amplified adapter modified nucleic acid fragments and the use of a plurality of different target-specific primers, as previously disclosed by Varley, as target-specific amplification would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract). Additionally, it would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modiifed the method of Illumina, for including non-ligatable universal primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

-***-Continued Within the Next Supplemental Box-***-

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.**
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Box No. V:
-Continued from Citations and Explanations:

As per claim 34, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the amplification products are sequenced in a massively parallel DNA sequencer. Varley discloses wherein the amplification products are sequenced in a massively parallel DNA sequencer (next-generation or second-generation sequencing, abstract; page 1845, column 1, paragraph 1; page 1848, column 1, paragraph 5; using 454 Life Sciences (Roche) FLX machine, page 1847, column 1, paragraph 1; page 1849, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including next generation sequencing, as previously disclosed by Varley, as next generation sequencing would have allowed for medical resequencing of many candidate genes across multiple individual patient samples (Varley; abstract).

As per claim 35, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site comprises a barcode sequence. Varley discloses wherein the primer capable of binding to the first universal priming site comprises a barcode sequence (sample-specific barcodes are incorporated into universal primers; page 1845, column 2, paragraph 1; figure 2). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the method of Illumina, for including barcodes in the universal primer, as previously disclosed by Varley, as DNA barcodes would have allowed for multiple samples to be sequenced as a pool (Varley; page 1847, column 2, paragraph 3).

As per claim 36, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus. Harismendy discloses wherein the primer capable of binding to the first universal priming site is blocked at the 5' terminus (5'-blocked primers - 5' modification of either Amino Modifier C6 (NH2-blocked) or C3 spacer (C3-blocked); abstract; page 229, column 3, paragraph 1; figures 1, 2). It would have been obvious to a person of ordinary skill in the art, at the time of the invention to have modified the method of Illumina, for including 5'-blocked primers, as previously disclosed by Harismendy, for reducing overrepresentation of amplicon ends (Harismendy; abstract; page 229, column 2, paragraph 2; figure 1), thereby maximizing coverage yield and limiting coverage variability (Harismendy; page 231, column 1, paragraph 2).

As per claim 38, Illumina, Varley and Harismendy, in combination disclose, claim 33, but Illumina does not disclose wherein the target regions comprise a polymorphic region. Varley discloses wherien the target regions comprise a polymorphic region (variants, page 1846, column 1, paragraph 5; mutations, abstract; Table 1; page 1847, column 1, paragraph 2; or SNPs, abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including target regions comprise a polymorphic region, as previously disclosed by Varley, as target-specific amplification of polymorphic regions would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 39, Illumina, Varley and Harismendy, in combination, disclose claim 38, but Illumina does not disclose wherein the polymorphic region is a SNP. Varley discloses wherein the polymorphic region is a SNP (abstract; Table 1; page 1847, column 1, paragraph 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 method of Illumina, for including the polymorphic region is a SNP, as previously disclosed by Varley, as target-specific amplification of SNPs would have allowed the genetic library to be useful for medical resequencing of candidate gene mutations (Varley; abstract).

As per claim 40, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose that at least 1000 target specific primers are added. Varley teaches that at least 1000 target specific primers can be added (amplification of 100-1000 candidate regions (200-2000 primers); page 1847; column 2, paragraph 4 to page 1848, column 1, paragraph 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 method of Illumina, for including at least 1000 target specific primers are added, as previously disclosed by Varley, as multiplexing would have allowed medical resequencing of many candidate genes across multiple samples (Varley; abstract).

As per claim 42, Illumina, Varley and Harismendy, in combination, disclose claim 33, but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine; page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (Varley; page 1845, column 1, paragraph 4), thereby rendering the primer incomplete and non-ligatable.

As per claims 43/33-36, 43/38-40 amd 43/42, Illumina, Varley and Harismendy, in combination, disclose the methods of claims 33-36, 38-40 and 42, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

Claim 46(B) lacks an inventive step under PCT Article 33(3) as being obvious over Illumina in view of Harismendy in view of the article entitled 'Accurate Whole Human Genome Sequencing Using Reversible Terminator Chemistry' by Bentley, et al. (hereinafter 'Bentley').

As per claim 46(B), Illumina and Harismendy, in combination, disclose the kit of claim 44(B), but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses wherein the universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the kit of Illumina to include universal adapters which are Y-shaped adapters, as previously disclosed by Bentley, as this would have allowed DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

-***-Continued Within the Next 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.**
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Claim 47(B) lacks an inventive step under PCT Article 33(3) as being obvious over Harismendy in view of Varley in view of the article entitled 'Non-Invasive Prenatal Diagnosis By Massively Parallel Sequencing Of Maternal Plasma DNA' by Lo (hereinafter 'Lo').

As per claim 47(B), Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the amplicons are derived from a genetic composition comprising a mixture of maternal and fetal DNA. Lo discloses amplicons which are derived from a genetic composition comprising a mixture of maternal and fetal DNA (fetal DNA represents a minor fraction of maternal plasma DNA, summary; page 3, column 1, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including amplicons which are derived from a genetic composition comprising a mixture of maternal and fetal DNA, as previously disclosed by Lo, as improvement would have been expected to have allowed the the genetic library to be used for non-invasive prenatal diagnosis (Lo; Summary; page 3, column 2, paragraph 6).

Claim 47(C) lacks an inventive step under PCT Article 33(3) as being obvious over Illumina in view of Harismendy in view of Varley.

As per claim 47(C), Illumina and Harismendy, in combination, disclose the kit of claim 44(B), but Illumina does not disclose wherein the non-ligatable primer binding to the first universal primer site or the second universal primer site comprises a uracil. Varley discloses primers that comprise a uracil (target-specific primers that contain uracil substituted for thymine, page 1845, column 1, paragraph 4; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Illumina, for including primers that comprise a uracil, as previously disclosed by Varley, allowing the primer to be degraded by uracil DNA glycosylase (page 1845, column 1, paragraph 4), the primer being incomplete and non-ligatable.

Claims 48 and 49 lack an inventive step under PCT Article 33(3) as being obvious over Harismendy and Varley and further in view of Bentley.

As per claim 48, Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 5000 genetic loci. Bentley discloses a genetic library comprising polymorphisms which from at least 5000 genetic loci (about 4 million SNPs, page 55, column 2, paragraph 4; in whole human genome (about 20,000 genes), page 55, column 2, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including polymorphisms from at least 5000 genetic loci, as previously disclosed by Bentley, as large genetic libraries (i.e. whole-genome) would have been expected to maximize the opportunity to discover polymorphisms of important biological or medical benefit (Bentley; abstract).

As per claim 49, Harismendy and Varley, in combination, disclose the genetic library of claim 44(A), but Harismendy does not disclose wherein the genetic library comprises polymorphisms from at least 10000 genetic loci. Bentley discloses wherein the genetic library comprises polymorphisms from at least 10000 genetic loci (about 4 million SNPs, page 55, column 2, paragraph 4; in whole human genome (about 20,000 genes), page 55, column 2, paragraph 3). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Harismendy, for including polymorphisms from at least 10000 genetic loci, as previously disclosed by Bentley, as large genetic libraries (i.e. whole-genome) would have been expected to enable discoveries of important biological or medical benefit (Bentley; abstract).

Claims 5, 11/5, 16, 21/16, 26, 32/26, 37 and 43/37 lack an inventive step under PCT Article 33(3) as being obvious over Illumina, Varley and Harismendy and further in view of Lo.

As per claim 5, Illumina, Varley and Harismendy in combination, disclose the method of claim 1, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses wherein the nucleic acids are obtained from the blood of pregnant female (foetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the genetic library of Varley, for including nucleic acids obtained from the blood of pregnant female, as previously disclosed by Lo, as this additional feature would have allowed the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 11/5, Illumina, Varley and Harismendy, in combination, disclose the method of claim 5, and Illumina further discloses a genetic library (libraries of genomic DNA (page 3, paragraph 2)).

As per claim 16, Illumina, Varley and Harismendy, in combination, disclose the method of claim 12, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses that the nucleic acids are obtained from the blood of pregnant female (fetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modiifed the genetic library of Varley, for including that the nucleic acids are obtained from the blood of pregnant female, as previously disclosed by Lo, for allowing the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 21/16, Illumina, Varley, Harismendy and Lo, in combination, disclose the method of claim 16, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Supplemental Box

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Box No. V:
-Continued from Citations and Explanations:

As per claim 26, Illumina, Varley and Harismendy, in combination, disclose the method of claim 22, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses wherein the nucleic acids are obtained from the blood of pregnant female (fetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the genetic library of Varley, for including the nucleic acids are obtained from the blood of pregnant female, as previously disclosed by Lo, as this feature disclosed by Lo would have allowed the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 32/26, Illumina, Varley, Harismendy and Lo in combination disclose the method of claim 26, and Illumina discloses a genetic library (libraries of genomic DNA (page 3, paragraph 2)).

As per claim 37, Illumina, Varley and Harismendy, in combination, disclose the method of claim 33, but Illumina does not disclose wherein the nucleic acids are obtained from the blood of pregnant female. Lo discloses wherein the nucleic acids are obtained from the blood of pregnant female (fetal DNA in the plasma of pregnant women; Summary section). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have implemented the genetic library of Varley, for including the nucleic acids obtained from the blood of pregnant female, as previously by Lo, as this feature disclosed by Lo would have allowed the genetic library to be used for non-invasive prenatal diagnosis (Lo; summary; page 3, column 2, paragraph 6).

As per claim 43/37, Illumina, Varley, Harismendy and Lo, in combination, disclose the method of claim 33, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

Claims 9; 11/9, 30, 32/30, 41 and 43/41 lack an inventive step under PCT Article 33(3) as being obvious over Illunima, Varley and Harismendy and further in view of Bentley.

As per claim 9, Illumina, Varley and Harismendy, in combination, disclose the method of claim 1, but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the kit of Illumina, for including wherein the universal adapters are Y-shaped adapters, as previously disclosed by Bentley, as this adaption would have allowed DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

As per claim 11/9, Illumina, Varley, Harismendy and Bentley, in combination disclose the method of claim 9, and Illumina further discloses a genetic library (libraries of genomic DNA; page 3, paragraph 2).

As per claim 30, Illumina, Varley and Harismendy, in combination, disclose the method of claim 22, but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the kit of Illumina, for including wherein the universal adapters are Y-shaped adapters, as previously disclosed by Bentley, as this adaption would have allowed DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

As per claim 32/30, Illumina, Varley, Harismendy and Bentley in combination disclose the method of claim 30, and Illumina discloses a genetic library (libraries of genomic DNA (page 3, paragraph 2)).

As per claim 41, Illumina, Varley and Harismendy, in combination, disclose the method of claim 33, but Illumina does not disclose wherein the universal adapters are Y-shaped adapters. Bentley discloses wherein the universal adapters which are Y-shaped adapters (a pair of oligonucleotides in a forked adaptor configuration; figure 1A). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the kit of Illumina, for including wherein the universal adapters are Y-shaped adapters, as previously disclosed by Bentley, as this adaption would have allowed for DNA fragments to attach to the surface of the reaction chamber and bridging amplification to form DNA clusters (Bentley; page 53, column 1, paragraph 4; figures 1A-C).

As per claim 43/41, Illumina, Varley, Harismendy and Bentley, in combination, disclose the method of claim 41, and Illumina further discloses the use of a genetic library (libraries of genomic DNA; page 3, paragraph 2).

Claims 1-43, 44(A)-47(A), 44(B)-47(B), 47(C), 48 and 49 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 PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference W16	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2013/057924	International filing date *(day/month/year)* 04 September 2013 (04.09.2013)	Priority date *(day/month/year)* 04 September 2012 (04.09.2012)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 5 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 10 March 2015 (10.03.2015)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Nora Lindner e-mail: pt05.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: VICKI L. HEALY
NATERA, INC.
201 INDUSTRIAL ROAD
SUITE 410
SAN CARLOS, CA 94070

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	18 FEB 2014

Applicant's or agent's file reference	FOR FURTHER ACTION
W16	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 13/57924	04 September 2013 (04.09.2013)	04 September 2012 (04.09.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 20/00 (2013.01)
USPC - 506/2,12; 702/1,19; 435/6.1,7.2

Applicant NATERA, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [x] 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	23 January 2014 (23.01.2014)	Lee W. Young PCT Helpdesk: 571-272-4300 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 13/57924

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 13/57924

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. 5-29

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. 5-29 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 5-29 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ 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. 5-29

☐ 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 13/57924

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	1-4, 30, 32-34	YES
	Claims	31	NO
Inventive step (IS)	Claims	1-4, 30, 32-34	YES
	Claims	31	NO
Industrial applicability (IA)	Claims	1-4, 30-34	YES
	Claims	None	NO

2. Citations and explanations:

Claim 31 lacks novelty under PCT Article 33(2) as being anticipated by US 5,716,776 A (Bogart).

As to claim 31, Bogart discloses the use of (ii) a stimulant (col 4, ln 34-38) for increasing the fetal fraction in the blood, plasma, or serum of a pregnant woman (col 3, ln 64 to pg 4, ln 4).

Claims 1-4, 30 and 32-34 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the claimed subject matter, specifically increasing the fetal fraction in the blood, plasma, serum of a pregnant woman by administering a nutritious composition or stimulant prior to obtaining the sample.

The best prior art on record that discloses increased fetal fractions in prenatal testing are below:

US 5,716,776 A (Bogart) discloses the use of a stimulant (pg 4, ln 34-38) to increase the fetal fraction in the blood of a pregnant woman (col 3, ln 64 to pg 4, ln 4), but does not disclose wherein the administration of the stimulant occurs before the blood is taken from the pregnant woman.

US 2011/0251149 A1 to Perrine, et al. (hereinafter 'Perrine') discloses increasing the percentage of fetal hemoglobin in the blood of subject by administering a substance to the subject (para [0008]), but does not disclose wherein the substance is a stimulant or a nutritious composition or wherein the increase is used in prenatal testing.

US 2008/0138809 A1 to Kapur, et al. (hereinafter 'Kapur') discloses the enrichment of fetal cells from blood samples that are used in prenatal testing (para [0005] and [0009]), but does not disclose wherein the increase occurs before the blood is taken from the pregnant woman.

The article titled "Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: Effect of maternal and fetal factors" by Ashoor, et al. (hereinafter 'Ashoor') discloses wherein the fetal fraction in maternal plasma cfDNA increases with serum PAPP-A and free B-hCG and decreases with maternal weight and wherein (pg 1, 1st paragraph) and wherein the fetal fraction is used in prenatal testing (pg 1, 2nd paragraph), but does not disclose administering any substance to a pregnant woman to increase the fetal fraction before the sample is taken. There is no prior art on record that discloses the claimed subject matter, specifically increasing the fetal fraction in the blood, plasma, serum of a pregnant woman by administering a nutritious composition or stimulant prior to obtaining the sample. Thus, claims 1-4, 30 and 32-34 meet the criteria set out in PCT Article 33(2)-(3).

Claims 1-4 and 30-34 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in the industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference W19	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US14/51926	International filing date *(day/month/year)* 20 August 2014 (20.08.2014)	(Earliest) Priority Date *(day/month/year)* 20 August 2013 (20.08.2013)
Applicant NATERA, 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. _____

☐ 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/US14/51926

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.: 5-14
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:

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.:

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/US14/51926

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2014.01)
CPC - C12Q 1/6883, 1/6827, 1/6816
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 (2014.01)
CPC: C12Q 1/6883, 1/6827, 1/6816; USPC: 435/6.1, 4

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; Google Scholar; Dialog ProQuest; Entrez Pubmed; 'blood samples,' 'fetal DNA fraction,' percentiles, 'chromosomal abnormalities,' 'ploidy,' 'placental detachment,' amniocentesis, subpopulations

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0196862 A1 (RABINOWITZ, M et al.) August 1, 2013; abstract; paragraphs [0008], [0011], [0029], [0034], [0037], [0105], [0106], [0218], [0232], [0384], [0510]	1, 2 ----- 3, 4
Y	US 2012/0270212 A1 (RABINOWITZ, M et al.) October 25, 2012; paragraphs [0062], [0088]-[0090], [0200], [0508]	3, 4
Y	US 2013/0190653 A1 (ALVAREZ-RAMOS, AG) July 25, 2013; abstract; paragraphs [0033], [0036]	4
A	US 2012/0264121 A1 (RAVA, RP et al.) October 18, 2012; abstract	1-4

☐ 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
17 November 2014 (17.11.2014)	09 DEC 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Scott Bortner
201 Industrial Road
Suite 410
San Carlos, California 94070
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: W19	FOR FURTHER ACTION: See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US14/51926	20 August 2014 (20.08.2014)	20 August 2013 (20.08.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2014.01)
CPC - C12Q 1/6883, 1/6827, 1/6816

Applicant: NATERA, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [x] 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 Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	17 November 2014 (17.11.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/51926

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/US14/51926

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. 5-14

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. 5-14 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 5-14 are dependent claims which are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ 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. 5-14

☐ 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/US14/51926

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	YES
	Claims	1	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-4	NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by US 2013/0196862 A1 to Rabinowitz, et al. (hereinafter 'Rabinowitz'862').

Regarding claim 1, Rabinowitz'862 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), determining if the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is within the lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]), and generating a report (generating a ploidy status report; paragraphs [0232], [0510]) indicating that an invasive genetic analysis procedure should be performed (report with results for determining need for amniocentesis; paragraph [0034]).

Claim 2 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz'862.

Regarding claim 2, Rabinowitz'862 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), determining if the ratio (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample to maternal cell free DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is in lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]), and performing an invasive genetic test (performing amniocentesis; paragraph [0034]). Rabinowitz'862 does not disclose, in a single, concise embodiment, wherein the invasive genetic test is performed if the fetal fraction is in lowest 1 percentile of a reference population. However, since Rabinowitz'862 discloses determining a fetal fraction in lowest 1 percentile of a reference and performing confirmative, invasive studies, such as amniocentesis, both as disclosed above, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have implemented the use of the separate disclosures of Rabinowitz'862, for including the invasive test step if warranted by results indicative of a fetal DNA fraction, which is in lowest 1 percentile of a reference population, through routine experimentation and testing.

-***-Continued on Next Supplemental Page-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/51926

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:

Claim 3 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz'682 in view of US 2012/0270212 A1 to Rabinowitz et al. (hereinafter 'Rabinowitz'212').

Regarding claim 3, Rabinowitz'862 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), determining if the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is within the lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]), and performing an invasive genetic test (performing amniocentesis; paragraph [0034]) if the fetal fraction is in lowest 1 percentile of a reference population (report with results for determining need for amniocentesis; paragraph [0034]; if fetal DNA is low in a mixed sample; paragraph [0105]). Rabinowitz'682 does not disclose the steps of: obtaining a plurality blood samples from a test population of pregnant human subjects, measuring the fetal fraction of the cell free fetal DNA in the blood samples, determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing an invasive genetic test on at least one member of the population subset. Rabinowitz'212 discloses obtaining a plurality blood samples from a test population of pregnant human subjects (blood samples derived from four euploid and two trisomy-indicated pregnancies; paragraph [0508]), and measuring the fetal fraction of the cell free fetal DNA in the blood samples (estimating fetal DNA fractions; paragraph [0062]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Rabinowitz'682, for implementing the step of determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing an invasive genetic test on at least one member of the population subset, since the data on the cell-free fetal fraction of DNA obtained from analysis of a plurality of blood samples from a test population of pregnant human subjects, as previously disclosed by Rabinowitz'212, would have allowed individuals exhibiting fetal fractions in the lowest 1 percentile to be selected for performing an invasive genetic test, as previously disclose by Rabinowitz'682 (paragraph [0034]).

Claim 4 lacks an inventive step under PCT Article 33(3) as being obvious over Rabinowitz'682 and further in view of Rabinowitz'212 and US 2013/0190653 A1 (ALVAREZ-RAMOS).

Regarding claim 4, Rabinowitz'682 discloses a method of evaluating a fetus (determining ploidy status of a fetus; abstract; paragraph [0008]) for genetic abnormalities (screening for chromosomal abnormalities; paragraph [0029]), comprising, obtaining a blood sample from a pregnant human subject (blood sample from pregnant mother; paragraphs [0011], [0114]), measuring (paragraph [0106]) the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) in the blood sample (paragraphs [0011], [0114]), and determining if the fetal fraction (fraction or ratio of fetal DNA to maternal DNA; paragraphs [0037], [0218], [0384]) of cell free fetal DNA in the blood sample (free-floating DNA in mixed sample of maternal and fetal DNA; paragraph [0106]) is within the lowest 1 percentile of a reference population (fetal fraction is 1% or less of compared to fraction of fetal DNA in individuals with disomic reference chromosomes; paragraph [0218]). Rabinowitz'682 does not disclose obtaining a plurality blood samples from a test population of pregnant human subjects, measuring the fetal fraction of the cell free fetal DNA in the blood sample to maternal cell free DNA in the blood samples, determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing a test for a detached placenta on at least one member of the population subset. Rabinowitz'212 discloses obtaining a plurality blood samples from a test population of pregnant human subjects (blood samples derived from four euploid and two trisomy-indicated pregnancies; paragraph [0508]), and measuring the fetal fraction of the cell free fetal DNA in the blood samples (estimating fetal DNA fractions; paragraph [0062]). Alvarez-Ramos discloses blood sample collections appropriate for pregnancies susceptible to placental detachment (blood sampling to prevent placental detachment; abstract; paragraphs [0033], [0036]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Rabinowitz'682, for implementing the steps of determining a population subset consisting of members of the test population that have fetal fraction that is in the lowest 1 percentile of the test population, and performing a test for a detached placenta on at least one member of the population subset, since the data on the cell-free fetal fraction of DNA obtained from analysis of a plurality of blood samples from a test population of pregnant human subjects, as disclosed by Rabinowitz'212, would have allowed individuals exhibiting fetal fractions in the lowest 1 percentile to be selected for testing for a detached placenta, as disclosed by Alvarez-Ramos, on at least one member of the population subset, as determined by the analysis for low fetal DNA fractions, as previously disclosed by Rabinowitz'682.

Claims 1-4 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 PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference W20	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2014/057843	International filing date *(day/month/year)* 26 September 2014 (26.09.2014)	Priority date *(day/month/year)* 27 September 2013 (27.09.2013)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 10 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 29 March 2016 (29.03.2016)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pt04.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2014/057843	26.09.2014	27.09.2013

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68

Applicant
NATERA, 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 and 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 usually 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 notifed 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:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Telephone No. +31 70 340-0

Form PCT/ISA/237 (Cover Sheet) (July 2009)

Box No. I Basis of the 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 43bis.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:

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)	Yes:	Claims	13, 18, 19
	No:	Claims	1-12, 14-17, 20
Inventive step (IS)	Yes:	Claims	13, 18, 19
	No:	Claims	1-12, 14-17, 20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

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:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 CITATIONS

Reference is made to the following documents:

D1 WO 2011/057094 A1 (UNIV HONG KONG CHINESE [CN]; SEQUENOM I [CN) 12 May 2011 (2011-05-12)

D2 WO 2013/030577 A1 (SINGAPORE VOLITION PTE LTD [SG]; MICALLEF 2013 (2013-03-07)

D3 FORSHEW T ET AL: "Noninvasive identification and monitoring of cancer sequencing of plasma DNA",
SCIENCE TRANSLATION MEDICINE, AMERICAN ASSOCIATION FOR T SCIENCE, US
vol. 4, no. 136 30 May 2012 (2012-05-30), pages 1-12, XP003030868,
ISSN: 1946-6234, DOI: 10.1126/SCITRANSLMED.3003726
Retrieved from the Internet:
URL:http://www.bonuobio.com/khzc/pdf/NoninvasiveIdentificationandMonit
TargetedDeepSequencingofPlasmaDNA.pdf

D4 WO 2011/087760 A2 (SEQUENOM INC [US]; EHRICH MATHIAS [US]; MI COS) 21 July 2011 (2011-07-21)

D5 ANUPAMA SRINIVASAN ET AL: "Noninvasive Detection of Fetal Subchro Sequencing of Maternal Plasma",
THE AMERICAN JOURNAL OF HUMAN GENETICS,
vol. 92, no. 2, 1 February 2013 (2013-02-01), pages 167-176, XP0551258
ISSN: 0002-9297, DOI: 10.1016/j.ajhg.2012.12.006
cited in the application

D6 GB 2 488 358 A (UNIV PLYMOUTH [GB]) 29 August 2012 (2012-08-29)

D7 WO 02/076377 A2 (KANAI YOSHIYUKI [JP]; CT FOR ADVANCED SCIEN (2002-10-03)

D8 Megan P Hall: "Panorama (TM) non-Invasive Prenatal screening for mIcroc , 1 April 2014 (2014-04-01), XP055157224,
Retrieved from the Internet:
URL:http://www.panoramatest.com/sites/default/files/files/Panorama Microc
[retrieved on 2014-12-08]

2 NOVELTY (Art. 33(2) PCT)

2.1 The purpose of the composition of claim 1 is to mimic a plasma DNA extract from comprising two different DNA populations, such as the one deriving from a pregnant female or of a cancer patients. The composition of claim 1 is therefore indistinguishable from any such plasma DNA extract and any prior art document disclosing this matter is detrimental fro the novelty of the claim. D1, for instance, discloses extraction and analysis of maternal plasma DNA containing fetal DNA (par. [0226]) and explains that plasma DNA consists of nucleosomal DNA fragments corresponding to the size of a nucleosome that are derived from an apoptotic enzymatic process (par. [0240]-[0246]). D1 further discloses that fetal DNA represents 5-10% of the total maternal plasma DNA (par. [0067]).

Therefore, claims 1, 2, 4, 5, 7 and 10 are not novel in view of D1.

2.2 Similarly, D2 discloses plasma DNA extracts containing nucleosomal DNA from healthy and cancer patients (ex. 4-7). It goes without saying that plasma DNA from cancer patients comprises a mixture of normal and cancer cell DNA. D2 explains that during the process of apoptosis, chromatin is broken down into mononucleosomes and oligonucleosomes which are released from the cells (p. 2, par. 2). Even though it is not specified, it is very plausible that oligonucleosomes include a di-nucleosomal fraction. In fact, since most of the plasma DNA is as short as mono-nucleosomes, the oligonucleosomal fraction is more likely to comprise di-nucleosomes than longer oligonucleosomes.

Therefore, claims 1, 2, 4-6, 8 and 10 are not novel in view of D2.

2.3 D3 discloses a method to simulate circulating DNA to test the accuracy of a sequencing method by mixing plasma DNA from two healthy individuals. The two DNA samples were mixed so that one type (identified by specific SNPs) ranged from 0.16% to 40% of the total (p. 3, right-hand col., last par.). It is implicit that plasma DNA generally consists of free nucleosomal DNA derived from apoptotic events as it is commonly known.

Hence, in view of D3, claims 1, 2, 5, 10-12, 15-17 and 20 are not novel.

2.4 None of the cited documents discloses that the nucleic acid preparations derive from cell lines. However, it is not clear how a nucleic acid preparation obtained from a general cell line and treated so as to produce nucleosomal DNA would differ from a plasma sample preparation. In absence of particular distinguishing features, the compositions of claims 3 and 9 even though derived from cultured cell lines cannot be considered novel with respect to the compositions disclosed in D1-D3.

2.5 In view of the vague meaning of the term "genetically related", also claim 14 is not in new in view of D3. Two individuals of the same species can be considered "genetically related" in a broad interpretation of the feature.

2.6 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-12, 14-17 and 20 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

3 INVENTIVE STEP (Art. 33(3) PCT)

3.1 Document D3 is considered to represent the most relevant state of the art.

3.1.1 The subject-matter of claim 13 differs in that the nucleosomal DNA preparation is derived from a cell line. The technical effect is an increased availability of nucleic acid source.

The problem to be solved may be therefore considered as how to increase the availability of the nucleic acid source. The proposed solution is by deriving the nucleic acid from a cell line.

This solution can be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D4 discloses the preparation of model systems to test maternal-fetal free DNA by mixing DNA samples extracted from cell lines (ex. 3). However, the preparations of D4 are not treated so as to obtain nucleosomal DNA. The skilled person would not therefore consider preparing nucleosomal DNA from a cell line nucleic acid extract. D7 teaches how to obtain nucleosomal nucleic acid by micrococcal endonuclease treatment of cell line nucleic acid extract but it does not relate to cell-free plasma DNA and it cannot be therefore combined with either D3 or D4.

Therefore, claim 13 is inventive.

3.1.2 For the same reason, claim 19 is also inventive.

3.2 If claim 14 referred to mother and child or father and child cell sources, the purpose would be simulating a mother-fetus DNA commixtion of pregnant female plasma DNA. In this case, D5 would be a better closest prior art because it deals with this type of analysis. D5 discloses the preparation of artificial mixtures to be used as a standard control for maternal plasma DNA. The nucleic acid preparations of D5 are however treated by sonication, which generates short fragments (200 bp) but it does not exactly mimic the size of plasma nucleosomal DNA.

This solution proposed by the claim could be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D6 attempts to solve the problem by mixing plasma DNA (nucleosomal) with artificial fetal DNA obtained by PCR amplification creating fragments of the size of nucleosomal DNA (ex. 1 and 2). The method of D6 is however only useful for general quantification of fetal DNA in maternal plasma. It does not allow to test specific genetic analysis assays for copy number alterations because the whole fetal genome is not represented. The method would not be suitable for the purpose of D5. There is no hint in the prior art to the use of nucleosomal maternal and child DNA.

Therefore, the claim would be inventive.

3.3 D3 is the closest prior art for claim 18. The claim differs in that the second source is a cancerous cell. The technical effect is the possibility of testing detection assays for specific cancer-related mutations.

This solution proposed by the claim can be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

No hint is present in the prior art suggesting the use of nucleosomal DNA from cancer cells to mimic the cancerous DNA fraction found in plasma DNA from cancer patients.

The claim is therefore inventive.

3.4 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1, 2, 5, 10-12, 15-17 and 20 does not involve an inventive step (Rule 65(1)(2) PCT).

Re Item VI

Certain documents cited

D8 was published on 01.04.2014 in between the priority and filing dates of the present application. The document discloses the plasmART method corresponding to the claimed invention. Although D9 does not constitute prior art within the meaning of Rule 64.1(b), it might be taken into consideration in the regional phase before the EPO. No check has been made as to wether the priority of this application has been validly claimed, because the priority documents pertaining to the present application were not available at the time of establishing this first written opinion.

Re Item VIII

Certain observations on the international application

The term "genetically related" used in claims 4 and 14 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear, Article 6 PCT. Other acceptable definitions are provided in the application, such as mother and child or father and child.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
W20		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2014/057843	26 September 2014 (26-09-2014)	27 September 2013 (27-09-2013)
Applicant		
NATERA, 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.

[X] 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:
      [X] 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,**
   [X] 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,**
   [X] 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. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/057843

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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)
C12Q

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)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/057094 A1 (UNIV HONG KONG CHINESE [CN]; SEQUENOM INC [US]; LO YUK MING DENNIS [CN) 12 May 2011 (2011-05-12) paragraph [0240] - paragraph [0246] paragraph [0226] paragraph [0067]	1-7,9,10
X	WO 2013/030577 A1 (SINGAPORE VOLITION PTE LTD [SG]; MICALLEF JACOB VINCENT [GB]) 7 March 2013 (2013-03-07) page 2, paragraph 2; examples 4-7	1,3-6, 8-10

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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 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
9 December 2014	22/12/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Santagati, Fabio

1

Form PCT/ISA/210 (second sheet) (April 2005)

NeoGenomics
Exhibit 1003 Page 1127

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FORSHEW T ET AL: "Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA", SCIENCE TRANSLATION MEDICINE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, US , vol. 4, no. 136 30 May 2012 (2012-05-30), pages 1-12, XP003030868, ISSN: 1946-6234, DOI: 10.1126/SCITRANSLMED.3003726 Retrieved from the Internet: URL:http://www.bonuobio.com/khzc/pdf/NoninvasiveIdentificationandMonitoringofCancerMutationsbyTargetedDeepSequencingofPlasmaDNA.pdf page 3, last paragraph	1-3,5, 9-12, 14-17,20
A	WO 2011/087760 A2 (SEQUENOM INC [US]; EHRICH MATHIAS [US]; MISTRO GUY DEL [US]; DECIU COS) 21 July 2011 (2011-07-21) page 413, line 24 - page 415, line 34; example 3	1-20
A	ANUPAMA SRINIVASAN ET AL: "Noninvasive Detection of Fetal Subchromosome Abnormalities via Deep Sequencing of Maternal Plasma", THE AMERICAN JOURNAL OF HUMAN GENETICS, vol. 92, no. 2, 1 February 2013 (2013-02-01), pages 167-176, XP055125834, ISSN: 0002-9297, DOI: 10.1016/j.ajhg.2012.12.006 cited in the application page 168, left-hand column, paragraph 2	1-20
A	GB 2 488 358 A (UNIV PLYMOUTH [GB]) 29 August 2012 (2012-08-29) examples 1,2	1-20
A	WO 02/076377 A2 (KANAI YOSHIYUKI [JP]; CT FOR ADVANCED SCIENCE & TECH [JP]) 3 October 2002 (2002-10-03) the whole document	1-20

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Megan P Hall: "Panorama (TM) non-Invasive Prenatal screening for mIcrodeletion syndromes", , 1 April 2014 (2014-04-01), XP055157224, Retrieved from the Internet: URL:http://www.panoramatest.com/sites/default/files/files/Panorama Microdeletions White Paper-2.pdf [retrieved on 2014-12-08] page 3, paragraph 2 -----	1-20
A	A. O. H. NYGREN ET AL: "Quantification of Fetal DNA by Use of Methylation-Based DNA Discrimination", CLINICAL CHEMISTRY, vol. 56, no. 10, 20 August 2010 (2010-08-20), pages 1627-1635, XP055089811, ISSN: 0009-9147, DOI: 10.1373/clinchem.2010.146290 page 1628, right-hand column, paragraph 2 page 1631 -----	1-20

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/057843

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011057094 A1	12-05-2011	AU 2010315037 A1	07-06-2012
		CA 2779695 A1	12-05-2011
		CN 102770558 A	07-11-2012
		EA 201200690 A1	30-05-2013
		EP 2496717 A1	12-09-2012
		JP 5540105 B2	02-07-2014
		JP 2013509884 A	21-03-2013
		JP 2014193165 A	09-10-2014
		TW 201122470 A	01-07-2011
		US 2011105353 A1	05-05-2011
		US 2013253844 A1	26-09-2013
		US 2013323731 A1	05-12-2013
		WO 2011057094 A1	12-05-2011
WO 2013030577 A1	07-03-2013	AU 2012300641 A1	06-03-2014
		CA 2845993 A1	07-03-2013
		CN 104067124 A	24-09-2014
		EP 2751568 A1	09-07-2014
		KR 20140078637 A	25-06-2014
		WO 2013030577 A1	07-03-2013
WO 2011087760 A2	21-07-2011	CA 2785020 A1	21-07-2011
		EP 2516680 A2	31-10-2012
		US 2013130923 A1	23-05-2013
		WO 2011087760 A2	21-07-2011
GB 2488358 A	29-08-2012	GB 2488358 A	29-08-2012
		WO 2012114075 A1	30-08-2012
WO 02076377 A2	03-10-2002	AU 2002238994 A1	08-10-2002
		JP 4276843 B2	10-06-2009
		WO 02076377 A2	03-10-2002

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2014/057843	26.09.2014	27.09.2013

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68

Applicant
NATERA, 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 and 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 usually 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 notifed 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:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Telephone No. +31 70 340-0

Form PCT/ISA/237 (Cover Sheet) (July 2009)

Box No. I Basis of the 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 43bis.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:

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)	Yes:	Claims	13, 18, 19
	No:	Claims	1-12, 14-17, 20
Inventive step (IS)	Yes:	Claims	13, 18, 19
	No:	Claims	1-12, 14-17, 20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

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:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 CITATIONS

Reference is made to the following documents:

D1 WO 2011/057094 A1 (UNIV HONG KONG CHINESE [CN]; SEQUENOM I
[CN) 12 May 2011 (2011-05-12)

D2 WO 2013/030577 A1 (SINGAPORE VOLITION PTE LTD [SG]; MICALLEF
2013 (2013-03-07)

D3 FORSHEW T ET AL: "Noninvasive identification and monitoring of cancer
sequencing of plasma DNA",
SCIENCE TRANSLATION MEDICINE, AMERICAN ASSOCIATION FOR T
SCIENCE, US
vol. 4, no. 136 30 May 2012 (2012-05-30), pages 1-12, XP003030868,
ISSN: 1946-6234, DOI: 10.1126/SCITRANSLMED.3003726
Retrieved from the Internet:
URL:http://www.bonuobio.com/khzc/pdf/NoninvasiveIdentificationandMonit
TargetedDeepSequencingofPlasmaDNA.pdf

D4 WO 2011/087760 A2 (SEQUENOM INC [US]; EHRICH MATHIAS [US]; MI
COS) 21 July 2011 (2011-07-21)

D5 ANUPAMA SRINIVASAN ET AL: "Noninvasive Detection of Fetal Subchro
Sequencing of Maternal Plasma",
THE AMERICAN JOURNAL OF HUMAN GENETICS,
vol. 92, no. 2, 1 February 2013 (2013-02-01), pages 167-176, XP0551258
ISSN: 0002-9297, DOI: 10.1016/j.ajhg.2012.12.006
cited in the application

D6 GB 2 488 358 A (UNIV PLYMOUTH [GB]) 29 August 2012 (2012-08-29)

D7 WO 02/076377 A2 (KANAI YOSHIYUKI [JP]; CT FOR ADVANCED SCIEN
(2002-10-03)

D8 Megan P Hall: "Panorama (TM) non-Invasive Prenatal screening for mIcroc , 1 April 2014 (2014-04-01), XP055157224,
Retrieved from the Internet:
URL:http://www.panoramatest.com/sites/default/files/files/Panorama Microc
[retrieved on 2014-12-08]

2 NOVELTY (Art. 33(2) PCT)

2.1 The purpose of the composition of claim 1 is to mimic a plasma DNA extract from comprising two different DNA populations, such as the one deriving from a pregnant female or of a cancer patients. The composition of claim 1 is therefore indistinguishable from any such plasma DNA extract and any prior art document disclosing this matter is detrimental fro the novelty of the claim. D1, for instance, discloses extraction and analysis of maternal plasma DNA containing fetal DNA (par. [0226]) and explains that plasma DNA consists of nucleosomal DNA fragments corresponding to the size of a nucleosome that are derived from an apoptotic enzymatic process (par. [0240]-[0246]). D1 further discloses that fetal DNA represents 5-10% of the total maternal plasma DNA (par. [0067]).

Therefore, claims 1, 2, 4, 5, 7 and 10 are not novel in view of D1.

2.2 Similarly, D2 discloses plasma DNA extracts containing nucleosomal DNA from healthy and cancer patients (ex. 4-7). It goes without saying that plasma DNA from cancer patients comprises a mixture of normal and cancer cell DNA. D2 explains that during the process of apoptosis, chromatin is broken down into mononucleosomes and oligonucleosomes which are released from the cells (p. 2, par. 2). Even though it is not specified, it is very plausible that oligonucleosomes include a di-nucleosomal fraction. In fact, since most of the plasma DNA is as short as mono-nucleosomes, the oligonucleosomal fraction is more likely to comprise di-nucleosomes than longer oligonucleosomes.

Therefore, claims 1, 2, 4-6, 8 and 10 are not novel in view of D2.

2.3 D3 discloses a method to simulate circulating DNA to test the accuracy of a sequencing method by mixing plasma DNA from two healthy individuals. The two DNA samples were mixed so that one type (identified by specific SNPs) ranged from 0.16% to 40% of the total (p. 3, right-hand col., last par.). It is implicit that plasma DNA generally consists of free nucleosomal DNA derived from apoptotic events as it is commonly known.

Hence, in view of D3, claims 1, 2, 5, 10-12, 15-17 and 20 are not novel.

2.4 None of the cited documents discloses that the nucleic acid preparations derive from cell lines. However, it is not clear how a nucleic acid preparation obtained from a general cell line and treated so as to produce nucleosomal DNA would differ from a plasma sample preparation. In absence of particular distinguishing features, the compositions of claims 3 and 9 even though derived from cultured cell lines cannot be considered novel with respect to the compositions disclosed in D1-D3.

2.5 In view of the vague meaning of the term "genetically related", also claim 14 is not in new in view of D3. Two individuals of the same species can be considered "genetically related" in a broad interpretation of the feature.

2.6 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-12, 14-17 and 20 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

3 INVENTIVE STEP (Art. 33(3) PCT)

3.1 Document D3 is considered to represent the most relevant state of the art.

3.1.1 The subject-matter of claim 13 differs in that the nucleosomal DNA preparation is derived from a cell line. The technical effect is an increased availability of nucleic acid source.

The problem to be solved may be therefore considered as how to increase the availability of the nucleic acid source. The proposed solution is by deriving the nucleic acid from a cell line.

This solution can be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D4 discloses the preparation of model systems to test maternal-fetal free DNA by mixing DNA samples extracted from cell lines (ex. 3). However, the preparations of D4 are not treated so as to obtain nucleosomal DNA. The skilled person would not therefore consider preparing nucleosomal DNA from a cell line nucleic acid extract. D7 teaches how to obtain nucleosomal nucleic acid by micrococcal endonuclease treatment of cell line nucleic acid extract but it does not relate to cell-free plasma DNA and it cannot be therefore combined with either D3 or D4.

Therefore, claim 13 is inventive.

3.1.2 For the same reason, claim 19 is also inventive.

3.2 If claim 14 referred to mother and child or father and child cell sources, the purpose would be simulating a mother-fetus DNA commixtion of pregnant female plasma DNA. In this case, D5 would be a better closest prior art because it deals with this type of analysis. D5 discloses the preparation of artificial mixtures to be used as a standard control for maternal plasma DNA. The nucleic acid preparations of D5 are however treated by sonication, which generates short fragments (200 bp) but it does not exactly mimic the size of plasma nucleosomal DNA.

This solution proposed by the claim could be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D6 attempts to solve the problem by mixing plasma DNA (nucleosomal) with artificial fetal DNA obtained by PCR amplification creating fragments of the size of nucleosomal DNA (ex. 1 and 2). The method of D6 is however only useful for general quantification of fetal DNA in maternal plasma. It does not allow to test specific genetic analysis assays for copy number alterations because the whole fetal genome is not represented. The method would not be suitable for the purpose of D5. There is no hint in the prior art to the use of nucleosomal maternal and child DNA.

Therefore, the claim would be inventive.

3.3 D3 is the closest prior art for claim 18. The claim differs in that the second source is a cancerous cell. The technical effect is the possibility of testing detection assays for specific cancer-related mutations.

This solution proposed by the claim can be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

No hint is present in the prior art suggesting the use of nucleosomal DNA from cancer cells to mimic the cancerous DNA fraction found in plasma DNA from cancer patients.

The claim is therefore inventive.

3.4 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1, 2, 5, 10-12, 15-17 and 20 does not involve an inventive step (Rule 65(1)(2) PCT).

Re Item VI

Certain documents cited

D8 was published on 01.04.2014 in between the priority and filing dates of the present application. The document discloses the plasmART method corresponding to the claimed invention. Although D9 does not constitute prior art within the meaning of Rule 64.1(b), it might be taken into consideration in the regional phase before the EPO. No check has been made as to wether the priority of this application has been validly claimed, because the priority documents pertaining to the present application were not available at the time of establishing this first written opinion.

Re Item VIII

Certain observations on the international application

The term "genetically related" used in claims 4 and 14 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear, Article 6 PCT. Other acceptable definitions are provided in the application, such as mother and child or father and child.

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 N.012.WO.01	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2015/026957	International filing date *(day/month/year)* 21 April 2015 (21.04.2015)	Priority date *(day/month/year)* 21 April 2014 (21.04.2014)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NATERA, 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 11 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 25 October 2016 (25.10.2016)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Mineko Mohri e-mail: pt08.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2015/026957	21.04.2015	21.04.2014

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68 G06F19/16

Applicant
NATERA, 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 and 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 usually 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 notifed 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:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Knudsen, Henrik Telephone No. +49 89 2399-0

Form PCT/ISA/237 (Cover Sheet) (January 2015)

Box No. I Basis of the 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:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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. 44-61

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. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ 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 the whole application or for said claims Nos. 44-61

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file 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 the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ 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

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:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-43

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)	Yes:	Claims	13, 18-22, 24-43
	No:	Claims	1-12, 14-17, 23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-43
Industrial applicability (IA)	Yes:	Claims	1-43
	No:	Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

D1 S. Y. Su ET AL: "Inferring combined CNV/SNP haplotypes from genotype data", Bioinformatics, vol. 26, no. 11, 1 June 2010 (2010-06-01), pages 1437-1445, XP55199108, ISSN: 1367-4803, DOI: 10.1093/bioinformatics/btq157

D2 WO 2013/130848 A1 (NATERA INC [US]; RABINOWITZ MATTHEW [US]; BANER JOHAN [US]; GEMELOS GE) 6 September 2013 (2013-09-06)

D3 WO 2014/018080 A1 (NATERA INC [US]; ZIMMERMANN BERNHARD [US]; HILL MATTHEW M [US]; LACROU) 30 January 2014 (2014-01-30)

D4 WO 2012/071621 A1 (UNIV HONG KONG CHINESE [CN]; LO YUK MING DENNIS [CN]; CHAN KWAN CHEE [) 7 June 2012 (2012-06-07)

Re Item IV

Lack of unity of invention

Invention no.	Claims and Subject-Matter
1.	claims: 1-43 Method of determining ploidy of a chromosomal segment in a sample from an individual based on allele frequency data, phasing of the alleles, generating individual and joint probabilities and selecting a best fit for use in pregnant women or with patients suspected of suffering from cancer. Computer systems and media for use with the method.
2.	claims: 44-49 Method for determining circulating tumour nucleic acids by determining a ploidy and a level of allelic imbalance higher than 0.45%
3.	claims: 50-55 Method for determining whether a single nucleotide variant is present or the percent of single nucleotide variants at a genomic position by estimating the efficiency and error rate per cycle for an amplicon spanning the single nucleotide variant and receiving observed nucleotide identity information

4. claims: 56-61

Method for detecting one or more single nucleotide variants by determining a median variant allele frequency for a plurality of control samples from each of a plurality of control individuals; identifying selected single nucleotide variants having variant median allele frequencies below a threshold value and to determine background error; and determining an observed depth of read weighted mean and variance.

The claimed subject-matter contains the four separate and independent inventions identified above. The inventions do not share a common inventive concept for the following reasons:

Independent claim 1 and independent claim 44 both relate to the determination of a ploidy. However, claim 1 employs the means of phasing the alleles and providing individual and joint probabilities and a best fit model indicative of chromosomal ploidy, whereas claim 44 is based on the determination of an allelic imbalance higher than 0.45%. In view of the fact that methods for determining chromosomal ploidy are plentiful in the field (see e.g. D1 and D2), claims 1 and 44 are not linked by any special technical features and are considered to represent separate and independent inventions.

Independent claims 50, 54 and 56 are directed to methods of determining single nucleotide variants and employs methods which do not share any common features with the method of claim 1. Thus, each of these claims represents inventions which are not linked by a common inventive concept to the method of claim 1.

Equally, claims 50, 54 and 56 do not share any common features with the method of claim 44 and each of claims 50, 54 and 56 is therefore considered a separate and independent invention over the method of claim 44.

Claims 50 and 54 (i.e. Invention 3) concerns a method for determining a single nucleotide variant and therefore shares the same purpose as the method of claim 56. However, whereas claims 50 and 54 determine the single nucleotide variant by estimating the efficiency and error rate per cycle for an amplicon, claim 56 determines the single nucleotide variant by comparison with a median variant allele frequency for a plurality of control samples from a plurality of control individuals. Considering that methods of determining single nucleotide variants based on controls are well-known (see D1 and D2). Claim 50 and claim 56 do not share any special technical features and are therefore considered separate and independent inventions.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

NOVELTY & INVENTIVE STEP:

1 D1 discloses a polyHap based method of determining CNV and SNP haplotypes based on genotype data (see abstract). The SNP/CNV genotypes were combined into pairs to create diploid genomes (see page 1441, left column, 2nd paragraph). The probabilities of the CNV haplotypes are inferred by treating the CNV as a region of variable ploidy, polyHap, which is based on a comparison with ancestral haplotypes can be used in phasing SNPs and CNVs. The method is based on the determination of individual and joint probabilities and the calculation of a maximum likelihood (see pages 1439 - 1440). Thus, claim 1 lacks novelty over D1.

1.1 The emission and transition probabilities used in D1 take the LD into consideration and therefore claim 6 is also not considered novel over D1.

2 D2 (see abstract and page 162) discloses a method for determining the ploidy status of a chromosome in a fetus from genotypic data. The ploidy status is determined by using a joint distribution model to create a plurality of expected allele distributions for different possible fetal ploidy states given the parental genotypic data and comparing the measured allelic distributions to and choosing the ploidy status whose expected allelic distribution pattern most closely matches (fits best) the observed allelic distribution pattern.

2.1 In addition, D2 (see page 161) discloses that the method may be used in an embodiment in which the entire haplotype of a fetus is determined (i.e. the phase is determined). Alleles at the polymorphic loci may be counted on a computer (see page 162, lines 4-5). Thus, D2 is novelty destroying for claims 1-2 and 23.

2.2 The possibility of correcting for amplification bias is disclosed on pages 133-134 of D2 and in the context of the system PARENTAL SUPPORT on page 40 of D2. Thus, claims 3-4 lack novelty over D2.

2.3 D2 (see page 138, first paragraph) further discloses that the likelihood of each hypothesis is derived based on the response model, the estimated fetal fraction, the mother genotypes, allele population frequencies, and the plasma allele counts and therefore considers an allelic imbalance. D2 therefore is considered novelty destroying for claim 5 as well.

2.4 D2 (see page 143, lines 12-13) also discloses that genetic linkage is used in the genetic model and (page 148, lines 17-20) discloses that "the method disclosed herein is able to make accurate ploidy calls in such a situation, and the reduction to practice is disclosed elsewhere in this document, using a joint probability maximum likelihood method and optionally utilizes SNP noise and bias models, as well as a linkage model." Thus, D2 is novelty destroying for claim 6 as well.

2.5 It follows from the above that D2 discloses all features of claim 7.

2.6 The determination of homologs and their inheritance from parents using phased genotypes and PARENTAL SUPPORT is disclosed in the last paragraph on page 159 of D2. Thus, claim 8 does not appear to be novel.

2.7 The use of the system PARENTAL SUPPORT to determine maternal cell contamination is disclosed on page 40 of D2. D2 (page 180) further discloses the use of homozygous genotypes for the calculation and that contamination with foreign tissue may be detected. Thus, claims 9-10 do not appear to be novel over D2.

2.8 The use of expected alleles and actual allele count in phased genotypes in creating individual probabilities which are then combined into joint probabilities using a binomial model is disclosed in the section "Platform modelling" on pages 135-142 of D2. Claims 11-12 are therefore not considered novel over D2.

2.9 The use of a Bayesian classifier appears to be routine in the field and claim 13 therefore does not appear to add anything inventive to the claims to which it refers.

2.10 The use of sequencing of amplicons resulting from multiplexed PCR for amplifying polymorphic loci of the target DNA is disclosed on pages 133-134 of D2. Claim 14 therefore lacks novelty over D2.

2.11 D2 (page 103, lines 7-9) also discloses that a limiting primer concentration improves the accuracy of the amplification of the polymorphic loci and claim 15 therefore lacks novelty.

2.12 When the entire fetal haplotype is determined (see page 161-162 of D2), it is clear that the ploidy status of a number of segments is determined with the same method and also claim 16 therefore lacks novelty.

2.13 D2 (see page 91) further discloses that more than 100,000 loci may be amplified in a multiplex PCR and also claim 17 therefore lacks novelty.

2.14 D2 does not disclose an average allelic imbalance of between 0.4 and 5% but provides means and methods for dealing with different fetal fractions in a sample and it would therefore appear to be obvious to the skilled person that also samples with such allelic imbalance may be dealt with in the method of D2. Claim 18 is therefore not considered inventive.

2.15 D2 also discloses that the methods disclosed therein may be used in the determination of ploidy of cancer cells from mixed samples (see page 68, last paragraph). Thus, it would appear to be obvious to the skilled person that the methods disclosed in D2 may be employed in the detection of cancer ploidy (see also abstract of D4) and claims 19-22 are not therefore not considered inventive over D2.

2.16 The use of a control with a known ratio of alleles is routinely used in the field as well as the removal of outlier results before modelling. Thus, claims 24-25 do not add anything inventive to the claims to which they refer.

2.17 Claims 26-43 are considered either not novel or not inventive over D2 since D2 discloses computer systems for carrying out the methods disclosed therein.

3 D3 (see Figure 29) appears to be novelty destroying at least for claim 1 for the same reasons as D2.

INDUSTRIAL APPLICABILITY:

4 Present claims 1-43 are considered industrially applicable.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
3409053WO1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2015/026957	21 April 2015 (21-04-2015)	21 April 2014 (21-04-2014)
Applicant		
NATERA, 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 7 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. 20
      ☒ 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

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The invention provides methods, systems, and computer readable medium for detecting ploidy of chromosome segments or entire chromosomes based on phasing of the alleles and determination of individual and joint probabilities and a best-fit model. In some aspects, the invention provides methods, systems, and computer readable medium for detecting cancer or a chromosomal abnormality in a gestating fetus. The invention also provides methods for detecting circulating tumor nucleic acids based on the level of allelic imbalance present at the polymorphic loci found by ploidy determination. The invention also provides a method for detecting single nucleotide variants based on an estimation of amplification efficiency and error rate and a method for detecting single nucleotide variants based on a median variant allele frequency for a plurality of control samples from individuals.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/026957

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:

see additional 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 an 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.:

1-43

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/US2015/026957

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G06F19/16
ADD.

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)
C12Q G06F

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)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. Y. SU ET AL: "Inferring combined CNV/SNP haplotypes from genotype data", BIOINFORMATICS, vol. 26, no. 11, 1 June 2010 (2010-06-01), pages 1437-1445, XP055199108, ISSN: 1367-4803, DOI: 10.1093/bioinformatics/btq157 page 1441, left-hand column, paragraph 2 page 1439 - page 1440 ----- -/--	1,6

[X] Further documents are listed in the continuation of Box C.

[X] 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 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
8 July 2015	28/09/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Knudsen, Henrik

1

Form PCT/ISA/210 (second sheet) (April 2005)

NeoGenomics
Exhibit 1003 Page 1154

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/026957

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/130848 A1 (NATERA INC [US]; RABINOWITZ MATTHEW [US]; BANER JOHAN [US]; GEMELOS GE) 6 September 2013 (2013-09-06) pages 161-162 pages 133-134 page 40 page 138, paragraph 1 page 143, lines 12-13 page 148, lines 17-20 page 159 page 180 pages 135-142 page 103, lines 17-19 page 91 page 68, last paragraph	1-43

X	WO 2014/018080 A1 (NATERA INC [US]; ZIMMERMANN BERNHARD [US]; HILL MATTHEW M [US]; LACROU) 30 January 2014 (2014-01-30) figure 29	1

A	WO 2012/071621 A1 (UNIV HONG KONG CHINESE [CN]; LO YUK MING DENNIS [CN]; CHAN KWAN CHEE [) 7 June 2012 (2012-06-07) abstract	18-22

A	H CHRISTINA FAN ET AL: "Whole-genome molecular haplotyping of single cells", NATURE BIOTECHNOLOGY, vol. 29, no. 1, 1 January 2011 (2011-01-01), pages 51-57, XP055026438, ISSN: 1087-0156, DOI: 10.1038/nbt.1739 abstract	1-43

A	RYAN TEWHEY ET AL: "The importance of phase information for human genomics", NATURE REVIEWS GENETICS, vol. 12, no. 3, 1 March 2011 (2011-03-01), pages 215-223, XP055199104, ISSN: 1471-0056, DOI: 10.1038/nrg2950 tables 1-2	1-43

A	BRYAN HOWIE ET AL: "Fast and accurate genotype imputation in genome-wide association studies through pre-phasing", NATURE GENETICS, vol. 44, no. 8, 22 July 2012 (2012-07-22), pages 955-959, XP055151106, ISSN: 1061-4036, DOI: 10.1038/ng.2354 abstract	1-43

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/026957

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013130848 A1	06-09-2013	NONE	
WO 2014018080 A1	30-01-2014	AU 2012385961 A1	12-02-2015
		CA 2877493 A1	30-01-2014
		CN 104685064 A	03-06-2015
		IL 236435 A	26-02-2015
		KR 20150038216 A	08-04-2015
		WO 2014018080 A1	30-01-2014
WO 2012071621 A1	07-06-2012	AU 2011335891 A1	02-05-2013
		CA 2817370 A1	07-06-2012
		CN 103403182 A	20-11-2013
		EA 201300649 A1	30-10-2013
		EP 2646579 A1	09-10-2013
		JP 2014502155 A	30-01-2014
		KR 20140091650 A	22-07-2014
		NZ 611599 A	29-05-2015
		SG 190344 A1	28-06-2013
		TW 201307570 A	16-02-2013
		US 2013040824 A1	14-02-2013
		US 2014227699 A1	14-08-2014
		WO 2012071621 A1	07-06-2012

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-43

   Method of determining ploidy of a chromosomal segment in a sample from an individual based on allele frequency data, phasing of the alleles, generating individual and joint probabilities and selecting a best fit for use in pregnant women or with patients suspected of suffering from cancer. Computer systems and media for use with the method.

   ---

2. claims: 44-49

   Method for determining circulating tumour nucleic acids by determining a ploidy and a level of allelic imbalance higher than 0.45%

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3. claims: 50-55

   Method for determining whether a single nucleotide variant is present or the percent of single nucleotide variants at a genomic position by estimating the efficiency and error rate per cycle for an amplicon spanning the single nucleotide variant and receiving observed nucleotide identity information

   ---

4. claims: 56-61

   Method for detecting one or more single nucleotide variants by determining a median variant allele frequency for a plurality of control samples from each of a plurality of control individuals; identifying selected single nucleotide variants having variant median allele frequencies below a threshold value and to determine background error; and determining an observed depth of read weighted mean and variance.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2015/026957	21.04.2015	21.04.2014

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68 G06F19/16

Applicant
NATERA, 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 and 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 usually 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 notifed 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:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Knudsen, Henrik Telephone No. +49 89 2399-0

Form PCT/ISA/237 (Cover Sheet) (January 2015)

Box No. I Basis of the 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:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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. 44-61

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. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ 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 the whole application or for said claims Nos. 44-61

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file 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 the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ 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

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:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-43

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)	Yes:	Claims	13, 18-22, 24-43
	No:	Claims	1-12, 14-17, 23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-43
Industrial applicability (IA)	Yes:	Claims	1-43
	No:	Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

D1 S. Y. Su ET AL: "Inferring combined CNV/SNP haplotypes from genotype data", Bioinformatics, vol. 26, no. 11, 1 June 2010 (2010-06-01), pages 1437-1445, XP55199108, ISSN: 1367-4803, DOI: 10.1093/bioinformatics/btq157

D2 WO 2013/130848 A1 (NATERA INC [US]; RABINOWITZ MATTHEW [US]; BANER JOHAN [US]; GEMELOS GE) 6 September 2013 (2013-09-06)

D3 WO 2014/018080 A1 (NATERA INC [US]; ZIMMERMANN BERNHARD [US]; HILL MATTHEW M [US]; LACROU) 30 January 2014 (2014-01-30)

D4 WO 2012/071621 A1 (UNIV HONG KONG CHINESE [CN]; LO YUK MING DENNIS [CN]; CHAN KWAN CHEE [) 7 June 2012 (2012-06-07)

Re Item IV

Lack of unity of invention

Invention no.	Claims and Subject-Matter
1.	claims: 1-43 Method of determining ploidy of a chromosomal segment in a sample from an individual based on allele frequency data, phasing of the alleles, generating individual and joint probabilities and selecting a best fit for use in pregnant women or with patients suspected of suffering from cancer. Computer systems and media for use with the method.
2.	claims: 44-49 Method for determining circulating tumour nucleic acids by determining a ploidy and a level of allelic imbalance higher than 0.45%
3.	claims: 50-55 Method for determining whether a single nucleotide variant is present or the percent of single nucleotide variants at a genomic position by estimating the efficiency and error rate per cycle for an amplicon spanning the single nucleotide variant and receiving observed nucleotide identity information

4. claims: 56-61

Method for detecting one or more single nucleotide variants by determining a median variant allele frequency for a plurality of control samples from each of a plurality of control individuals; identifying selected single nucleotide variants having variant median allele frequencies below a threshold value and to determine background error; and determining an observed depth of read weighted mean and variance.

The claimed subject-matter contains the four separate and independent inventions identified above. The inventions do not share a common inventive concept for the following reasons:

Independent claim 1 and independent claim 44 both relate to the determination of a ploidy. However, claim 1 employs the means of phasing the alleles and providing individual and joint probabilities and a best fit model indicative of chromosomal ploidy, whereas claim 44 is based on the determination of an allelic imbalance higher than 0.45%. In view of the fact that methods for determining chromosomal ploidy are plentiful in the field (see e.g. D1 and D2), claims 1 and 44 are not linked by any special technical features and are considered to represent separate and independent inventions.

Independent claims 50, 54 and 56 are directed to methods of determining single nucleotide variants and employs methods which do not share any common features with the method of claim 1. Thus, each of these claims represents inventions which are not linked by a common inventive concept to the method of claim 1.

Equally, claims 50, 54 and 56 do not share any common features with the method of claim 44 and each of claims 50, 54 and 56 is therefore considered a separate and independent invention over the method of claim 44.

Claims 50 and 54 (i.e. Invention 3) concerns a method for determining a single nucleotide variant and therefore shares the same purpose as the method of claim 56. However, whereas claims 50 and 54 determine the single nucleotide variant by estimating the efficiency and error rate per cycle for an amplicon, claim 56 determines the single nucleotide variant by comparison with a median variant allele frequency for a plurality of control samples from a plurality of control individuals. Considering that methods of determining single nucleotide variants based on controls are well-known (see D1 and D2). Claim 50 and claim 56 do not share any special technical features and are therefore considered separate and independent inventions.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

NOVELTY & INVENTIVE STEP:

1 D1 discloses a polyHap based method of determining CNV and SNP haplotypes based on genotype data (see abstract). The SNP/CNV genotypes were combined into pairs to create diploid genomes (see page 1441, left column, 2nd paragraph). The probabilities of the CNV haplotypes are inferred by treating the CNV as a region of variable ploidy, polyHap, which is based on a comparison with ancestral haplotypes can be used in phasing SNPs and CNVs. The method is based on the determination of individual and joint probabilities and the calculation of a maximum likelihood (see pages 1439 - 1440). Thus, claim 1 lacks novelty over D1.

1.1 The emission and transition probabilities used in D1 take the LD into consideration and therefore claim 6 is also not considered novel over D1.

2 D2 (see abstract and page 162) discloses a method for determining the ploidy status of a chromosome in a fetus from genotypic data. The ploidy status is determined by using a joint distribution model to create a plurality of expected allele distributions for different possible fetal ploidy states given the parental genotypic data and comparing the measured allelic distributions to and choosing the ploidy status whose expected allelic distribution pattern most closely matches (fits best) the observed allelic distribution pattern.

2.1 In addition, D2 (see page 161) discloses that the method may be used in an embodiment in which the entire haplotype of a fetus is determined (i.e. the phase is determined). Alleles at the polymorphic loci may be counted on a computer (see page 162, lines 4-5). Thus, D2 is novelty destroying for claims 1-2 and 23.

2.2 The possibility of correcting for amplification bias is disclosed on pages 133-134 of D2 and in the context of the system PARENTAL SUPPORT on page 40 of D2. Thus, claims 3-4 lack novelty over D2.

2.3 D2 (see page 138, first paragraph) further discloses that the likelihood of each hypothesis is derived based on the response model, the estimated fetal fraction, the mother genotypes, allele population frequencies, and the plasma allele counts and therefore considers an allelic imbalance. D2 therefore is considered novelty destroying for claim 5 as well.

2.4 D2 (see page 143, lines 12-13) also discloses that genetic linkage is used in the genetic model and (page 148, lines 17-20) discloses that "the method disclosed herein is able to make accurate ploidy calls in such a situation, and the reduction to practice is disclosed elsewhere in this document, using a joint probability maximum likelihood method and optionally utilizes SNP noise and bias models, as well as a linkage model." Thus, D2 is novelty destroying for claim 6 as well.

2.5 It follows from the above that D2 discloses all features of claim 7.

2.6 The determination of homologs and their inheritance from parents using phased genotypes and PARENTAL SUPPORT is disclosed in the last paragraph on page 159 of D2. Thus, claim 8 does not appear to be novel.

2.7 The use of the system PARENTAL SUPPORT to determine maternal cell contamination is disclosed on page 40 of D2. D2 (page 180) further discloses the use of homozygous genotypes for the calculation and that contamination with foreign tissue may be detected. Thus, claims 9-10 do not appear to be novel over D2.

2.8 The use of expected alleles and actual allele count in phased genotypes in creating individual probabilities which are then combined into joint probabilities using a binomial model is disclosed in the section "Platform modelling" on pages 135-142 of D2. Claims 11-12 are therefore not considered novel over D2.

2.9 The use of a Bayesian classifier appears to be routine in the field and claim 13 therefore does not appear to add anything inventive to the claims to which it refers.

2.10 The use of sequencing of amplicons resulting from multiplexed PCR for amplifying polymorphic loci of the target DNA is disclosed on pages 133-134 of D2. Claim 14 therefore lacks novelty over D2.

2.11 D2 (page 103, lines 7-9) also discloses that a limiting primer concentration improves the accuracy of the amplification of the polymorphic loci and claim 15 therefore lacks novelty.

2.12 When the entire fetal haplotype is determined (see page 161-162 of D2), it is clear that the ploidy status of a number of segments is determined with the same method and also claim 16 therefore lacks novelty.

2.13 D2 (see page 91) further discloses that more than 100,000 loci may be amplified in a multiplex PCR and also claim 17 therefore lacks novelty.

2.14 D2 does not disclose an average allelic imbalance of between 0.4 and 5% but provides means and methods for dealing with different fetal fractions in a sample and it would therefore appear to be obvious to the skilled person that also samples with such allelic imbalance may be dealt with in the method of D2. Claim 18 is therefore not considered inventive.

2.15 D2 also discloses that the methods disclosed therein may be used in the determination of ploidy of cancer cells from mixed samples (see page 68, last paragraph). Thus, it would appear to be obvious to the skilled person that the methods disclosed in D2 may be employed in the detection of cancer ploidy (see also abstract of D4) and claims 19-22 are not therefore not considered inventive over D2.

2.16 The use of a control with a known ratio of alleles is routinely used in the field as well as the removal of outlier results before modelling. Thus, claims 24-25 do not add anything inventive to the claims to which they refer.

2.17 Claims 26-43 are considered either not novel or not inventive over D2 since D2 discloses computer systems for carrying out the methods disclosed therein.

3 D3 (see Figure 29) appears to be novelty destroying at least for claim 1 for the same reasons as D2.

INDUSTRIAL APPLICABILITY:

4 Present claims 1-43 are considered industrially applicable.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
N015WO01		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2016/031686	10 May 2016 (10-05-2016)	11 May 2015 (11-05-2015)
Applicant		
NATERA, 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.

[X] 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:
      [X] 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. [X] 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,**
   [X] 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
   [X] 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
      [X] 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/US2016/031686

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:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The invention provides improved methods, compositions, and kits for detecting ploidy of chromosome regions, e.g. for detecting cancer or a chromosomal abnormality in a gestating fetus. The methods can utilize a set of more than 200 SNPs that are found within haploblocks and can include analyzing a series of target chromosomal regions related to cancer or a chromosomal abnormality in a gestating fetus. Finally the method may use knowledge about chromosome crossover locations or a best fit algorithm for the analysis. The compositions may comprise more than 200 primers located within haplotype blocks known to show CNV.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/031686

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G06F19/16
ADD.

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)
C12Q G06F

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)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2011/301854 A1 (CURRY BO U [US] ET AL) 8 December 2011 (2011-12-08) paragraph [0097]; claims 1,15-16	1-3,5,16 4,6-15, 17-44
X	US 2014/287934 A1 (SZELINGER SZABOLCS [US] ET AL) 25 September 2014 (2014-09-25) paragraphs [0011] - [0012], [0023] - [0025], [0159]	1,5
X	WO 2012/142531 A2 (COMPLETE GENOMICS INC [US]; DRMANAC RADOJE [US]; PETERS BROCK A [US];) 18 October 2012 (2012-10-18) paragraphs [0078], [0094], [0275]; figure 21	1,5

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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 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
12 September 2016	19/09/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Knudsen, Henrik

4

Form PCT/ISA/210 (second sheet) (April 2005)

NeoGenomics
Exhibit 1003 Page 1171

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/031686

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/108920 A1 (NATERA INC [US]; RABINOWITZ MATTHEW [US]; GEMELOS GEORGE [US]; BANJEVI) 16 August 2012 (2012-08-16)	45-51
Y	page 82	1-44, 52-54

Y	WO 2013/130848 A1 (NATERA INC [US]; RABINOWITZ MATTHEW [US]; BANER JOHAN [US]; GEMELOS GE) 6 September 2013 (2013-09-06) the whole document	1-44, 51-54

Y	WO 2014/018080 A1 (NATERA INC [US]; ZIMMERMANN BERNHARD [US]; HILL MATTHEW M [US]; LACROU) 30 January 2014 (2014-01-30) the whole document	1-44, 51-54

A	S. Y. SU ET AL: "Inferring combined CNV/SNP haplotypes from genotype data", BIOINFORMATICS, vol. 26, no. 11, 1 June 2010 (2010-06-01), pages 1437-1445, XP055199108, ISSN: 1367-4803, DOI: 10.1093/bioinformatics/btq157 abstract	1-54

A	DANIEL TALIUN ET AL: "Efficient haplotype block recognition of very long and dense genetic sequences", BMC BIOINFORMATICS, BIOMED CENTRAL, LONDON, GB, vol. 15, no. 1, 14 January 2014 (2014-01-14), page 10, XP021171925, ISSN: 1471-2105, DOI: 10.1186/1471-2105-15-10 abstract	1-54

A	JIANNIS RAGOUSSIS: "Genotyping Technologies for Genetic Research", ANNUAL REVIEW OF GENOMICS AND HUMAN GENETICS, vol. 10, no. 1, 1 September 2009 (2009-09-01), pages 117-133, XP055116403, ISSN: 1527-8204, DOI: 10.1146/annurev-genom-082908-150116 abstract	1-54

X,P	WO 2015/164432 A1 (NATERA INC [US]) 29 October 2015 (2015-10-29) paragraphs [0056], [0195], [0440]	1

4

NeoGenomics
Exhibit 1003 Page 1172

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/031686

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011301854 A1	08-12-2011	NONE	
US 2014287934 A1	25-09-2014	NONE	
WO 2012142531 A2	18-10-2012	AU 2012242525 A1	02-05-2013
		CA 2833165 A1	18-10-2012
		CN 103843001 A	04-06-2014
		EP 2754078 A2	16-07-2014
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		US 2013059740 A1	07-03-2013
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		CA 2824387 A1	16-08-2012
		CN 103608818 A	26-02-2014
		JP 2014507141 A	27-03-2014
		RU 2013141237 A	20-03-2015
		US 2012270212 A1	25-10-2012
		US 2013178373 A1	11-07-2013
		US 2015051087 A1	19-02-2015
		WO 2012108920 A1	16-08-2012
WO 2013130848 A1	06-09-2013	NONE	
WO 2014018080 A1	30-01-2014	AU 2012385961 A1	12-02-2015
		CA 2877493 A1	30-01-2014
		CN 104685064 A	03-06-2015
		HK 1211058 A1	13-05-2016
		JP 2015526073 A	10-09-2015
		KR 20150038216 A	08-04-2015
		SG 11201408813V A	27-02-2015
		WO 2014018080 A1	30-01-2014
WO 2015164432 A1	29-10-2015	NONE	

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2016/031686	10.05.2016	11.05.2015

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68 G06F19/16

Applicant
NATERA, 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 and 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 usually 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 notifed 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:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Knudsen, Henrik Telephone No. +49 89 2399-0

Form PCT/ISA/237 (Cover Sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2016/031686

Box No. I Basis of the 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:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

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)	Yes:	Claims	4, 6-15, 17-44, 52-54
	No:	Claims	1-3, 5, 16, 45-51
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-54
Industrial applicability (IA)	Yes:	Claims	1-54
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

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:

see separate sheet

Reference is made to the following documents:

D1 US 2011/301854 A1 (CURRY BO U [US] ET AL) 8 December 2011 (2011-12-08)

D2 US 2014/287934 A1 (SZELINGER SZABOLCS [US] ET AL) 25 September 2014 (2014-09-25)

D3 WO 2012/142531 A2 (COMPLETE GENOMICS INC [US]; DRMANAC RADOJE [US]; PETERS BROCK A [US];) 18 October 2012 (2012-10-18)

D4 WO 2012/108920 A1 (NATERA INC [US]; RABINOWITZ MATTHEW [US]; GEMELOS GEORGE [US]; BANJEVI) 16 August 2012 (2012-08-16)

D5 WO 2013/130848 A1 (NATERA INC [US]; RABINOWITZ MATTHEW [US]; BANER JOHAN [US]; GEMELOS GE) 6 September 2013 (2013-09-06)

D5 WO 2014/018080 A1 (NATERA INC [US]; ZIMMERMANN BERNHARD [US]; HILL MATTHEW M [US]; LACROU) 30 January 2014 (2014-01-30)

D7 S. Y. Su ET AL: "Inferring combined CNV/SNP haplotypes from genotype data", Bioinformatics,
vol. 26, no. 11, 1 June 2010 (2010-06-01), pages 1437-1445,
XP055199108, ISSN: 1367-4803, DOI: 10.1093/bioinformatics/btq157
WO 2014/018080 A1 (NATERA INC [US]; ZIMMERMANN BERNHARD [US]; HILL MATTHEW M [US]; LACROU) 30 January 2014 (2014-01-30)

D8 WO 2015/164432 A1 (NATERA INC [US]) 29 October 2015 (2015-10-29)

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

NOVELTY & INVENTIVE STEP:

1 D1 (see claims 1 and 15-16) discloses a method of genotyping whereby a chromosomal aberration may be identified by aligning a reference and test genome. The method applies a probability distribution function based on the ratios of SNPs. D1 does not explicitly mention that the phase of the test genome is determined, however, the purpose of the comparison of the test and reference genomes appears to be the determination of one phase for the aligned SNPs. D1 ([0097]) also mentions that the determination of a large number, e.g. 1000, of SNPs enable the use of a Gaussian distribution. Thus, claims 1-3, 5 and 16 do not appear to be novel over D1.

2 D2 (see [0011-0012] and [0023-0025]) discloses that the size of an X-chromosome heterozygous deletion can be determined by computer simulation of phased chromosome-wide X-linked heterozygous SNPs. D2 also mentions that only whole-genome sequencing procedures allows the exact determination of the size of the deletion (see [0159]). D2 does not disclose that more than 200 SNPs in LD with each other is determined. However, it is clear from D2 that NGS is used in determining the size of the deletion and since whole-genome sequencing will detect every SNP, it may be assumed that the 200 SNPs in LD are also determined in D2. Thus, at least claims 1 and 5 do not appear to be novel over D2.

3 D3 (see Figure 21) which discloses how whole-chromosome sequencing is used in phasing the reads [0078] and creating haplotypes which take into account chromosome cross-over locations, per definition, [0094] and mentions that the models may be used in determining copy number variants of chromosomes and chromosome arms (see [0275]). D3 does not disclose that more than 200 SNPs in LD with each other is determined. However, it is clear from D3 that whole genome sequencing is used and since whole-genome sequencing will detect every SNP, it may be assumed that the 200 SNPs in LD are also determined in D3. Thus, at least claims 1 and 5 appear to lack novelty over D3.

4 According to claim 23, the use of 500 different primers which bind to primer binding sequence within one or more of a plurality of haploblocks would only appear to solve a technical problem in case the 500 primers amplify different SNPs in a haploblock. However, present dependent claim 24 mentions that each of the primers may be used in amplifying the same SNP of a set of SNPs. Moreover, dependent claim 30 mentions that the haploblock need only contain 2 SNPs. Thus, in view of the dependent claims, it appears that the method of claim 23 does not solve any technical problem over the prior art which uses a single primer for amplifying SNPs in a haploblock (see e.g. D7)

5 None of the prior art documents appear to disclose the use of at least 500 primers or primer pairs which specifically bind to a primer binding sequence within one or more of a plurality of haploblocks in a chromosome region known to exhibit CNV. However, D1 mentions that the analysis of 1000 SNPs assist in the determination of the chromosomal aberration Thus, it would appear to be obvious to analyse 1000 SNPs to determine the CNV. Moreover, D4 discloses the use of more than 10000 PCR for SNP loci and the use of more than 500 primers would therefore be obvious to the skilled person. Thus, claims 23-30 are not considered inventive.

6 Each of D4 (page 82) and D5-D6 discloses that in an embodiment, a multiplex pool of about 10,000 PCR assays (10,000-plex) are designed to amplify potentially heterozygous SNP loci on chromosomes X, Y, 13, 18, and 21 and 1 or 2 and these assays are used in a single reaction to amplify cfDNA obtained from a material plasma sample, chorion villus samples, amniocentesis samples, single or a small number of cells, other bodily fluids or tissues, cancers, or other genetic matter. Thus, it is clear that the primers used in D4 comprise at least 200 primers which bind to a chromosome or chromosome region which is known to exhibit CNV and since the entire genome can be distributed in haploblocks also more than 90% of the primers will bind within a haploblock region. Claims 45-51 therefore appear to lack novelty in view of D4.

7 In addition, the features of claims 4 and 6-22 are routinely used in the field and therefore do not appear to add anything inventive to the claims to which they refer (see e.g. D5-D6).

8 In view of each of D4-D6, claims 31-44 do not add anything inventive to the claims to which they refer.

9 The subject-matter of claim 52 appears to be routinely used in the field and is therefore not considered inventive.

10 D4 (see page 55) and D5-D6 also discloses that the therapy of a cancer may be carried out based on the ploidy status of the cancer and also claims 53 and 54 therefore do not appear to be inventive.

INDUSTRIAL APPLICABILITY:

11 Present claims 1-54 are considered industrially applicable.

Re Item VI

Certain documents cited

12 D8 which is published after the present application's priority date, but before its filing date, is relevant only for those parts of the present application, if any, which do not have a valid claim to priority. Moreover, the applicant is reminded that D8 may become relevant prior art in the European regional phase of the application.

Re Item VIII

Certain observations on the international application

13 The invention appears to be differently defined in independent claims 1 and 5 since claim 1 requires that 90% of the 200 SNPs have strong LD with at least one other SNP of the 200 SNPs. This means that at least 180 SNPs must be in one haploblock according to claim 1, whereas claim 5 only requires that 200 SNPs on a chromosome or in region of interest are analysed. Thus, it is unclear what are the essential technical features of the invention.

13.1 Moreover, claim 5 appears to be inconsistent with the description (see [0148]) which teaches that the invention is based on the finding that by choosing SNPs that are found within haploblocks.

13.2 Also claim 23 does not appear to contain the same essential features as claim 1 since it requires only that at least 500 primers which specifically bind within one or more haploblocks are used. Thus, it is not excluded that the 500 primers may bind specifically to 500 different haploblocks within the chromosome. Thus, also claim 23 does not require that many SNPs within one haploblock are analysed.

13.3 This is also clear from claims 45 and 49 which requires only two SNPs to be analysed within one haploblock.

Electronic Acknowledgement Receipt

EFS ID:	37484079
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
Filing Date:	28-FEB-2019
Time Stamp:	13:06:09
Application Type:	Utility under 35 USC 111(a)

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	OROZCO.pdf	1878876 439427637b506d813f415126037752e270d8d62b	no	7

Warnings:

Information:					
2	Non Patent Literature	OZAWA.pdf	985105 9765355f53433df18062acf01de45e8fd79c9d90	no	8
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Information:					
3	Non Patent Literature	PAEZ.pdf	896564 621134854d200d1dbc22d39d8ab2af694374b5fc	no	11
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4	Non Patent Literature	PAGE.pdf	1386755 4439f3cafb08b26ae573091344c64989d71ccec8	no	5
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Information:					
5	Non Patent Literature	PALOMAKI_2011.pdf	1898827 d460ad5af39630e795f61641a58e61c9552cf4b4	no	8
Warnings:					
Information:					
6	Non Patent Literature	PALOMAKI_2011_913_NPL.pdf	1176430 a348486d76b205a7ba4c70c6feeb7e7cd454b6b9	no	8
Warnings:					
Information:					
7	Non Patent Literature	PALOMAKI_2012.pdf	792022 8ace4dd6ffc77ef9c28a940e41066877c90d3649	no	10
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Information:					
8	Non Patent Literature	PAPADOPOULOU_2004_439_NPL.pdf	605153 fa9232e17823b9c04a3778000527ae464dc03dd1	no	7
Warnings:					
Information:					

9	Non Patent Literature	PAPAGEORGIOU.pdf	1048761 3a3fa7b0d377fb755ab34350905af650b4122053	no	5
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Information:					
10	Non Patent Literature	PASTINEN_1997_606_NPL.pdf	1208270 74eec0c2355032452d67e1dec1679163a98d3325	no	10
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Information:					
11	Non Patent Literature	Pathak_2006.pdf	633853 ab24220306743301c76ee9cc1f990e7941b4fb3e	no	10
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12	Non Patent Literature	PCTUS1451926_writtenopinion.pdf	270572 3d0039f8109ce5588709be6a59b30fb3c3c783e4	no	5
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13	Non Patent Literature	PCTUS201357924_2014-02-18.pdf	1128659 bdd43b6633476e84dade7f949b5a68f14c46af06	no	8
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14	Non Patent Literature	PCTUS2006045281_2007-09-28_ISRWO.pdf	1174989 aae33cbc9e0aed9cf417816e6ebce0aacbd0f97b	no	7
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15	Non Patent Literature	PCTUS2006045281_2008-05-27_IPRP.pdf	110359 b74247b554fa8b03ab48f5737613351225d373a2	no	1
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16	Non Patent Literature	PCTUS2008003547_IPRP_22Sep2009.pdf	413098 cca6794ca54ff07be8a95b937b4a6ab92296da6b	no	9
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17	Non Patent Literature	PCTUS2008003547_ISR_14Apr2009.pdf	575307 343aa4a57594342 6f31d0403ccf59fe1c708a2f6	no	5
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18	Non Patent Literature	PCTUS2009034506_IPRP_24Aug2010.pdf	280565 50c40e214ee6ec9d7b941e719f8a4d100c263d68	no	6
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19	Non Patent Literature	PCTUS2009034506_ISR_08Jul2009.pdf	344071 aa2cb62f391b48d77b7f77f4b78819aef739f695	no	2
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20	Non Patent Literature	PCTUS2009045335_IPRP_30Nov2010.pdf	220246 6e01dcf386c2cbd24439333490183701ffff18bd	no	5
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Information:					
21	Non Patent Literature	PCTUS2009045335_ISR_27Jul2009.pdf	156183 77d38c38d773850be8b5baa0557b89937c49d6fa	no	1
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22	Non Patent Literature	PCTUS2009052730_IPRP_08Feb2011.pdf	329752 6dd1f7be0dbc9b7c8e9d1be582c0e6be56aef2ab	no	6
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Information:					

23	Non Patent Literature	PCTUS2009052730_ISR_28Sept2009.pdf	153839 8f05e995a8c5d4a88f57d9e237a8e5dbe0dc0820	no	1
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Information:					
24	Non Patent Literature	PCTUS2010050824_IPRP_03Apr2012.pdf	242250 f00b52f354d9d6a9c2716c91e0751636a7631ef6	no	5
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Information:					
25	Non Patent Literature	PCTUS2010050824_ISR_15Nov2010.pdf	327512 07afd2f7cc10e87b48985057562082e9e0cb2c52	no	2
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Information:					
26	Non Patent Literature	PCTUS2011037018_IPRP_20Nov2012.pdf	562748 de54b8243526c8325f193a001bfefe5d3b3bd7a2	no	9
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27	Non Patent Literature	PCTUS2011037018_ISR_27Sept2011.pdf	272302 933b21a9da026ae1cb68b0d8cf1980c8ba04befe	no	2
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28	Non Patent Literature	PCTUS2011061506_IPRP_13Aug2013.pdf	600365 95f08e40c206298a840b73c439d43af697a3fedb	no	9
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Information:					
29	Non Patent Literature	PCTUS2011061506_ISR_16Mar2012.pdf	177334 9cf7871b162a91f83481473a64c520c144c37cae	no	1
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Information:					

30	Non Patent Literature	PCTUS2011066938_IPRP_10Sep2013.pdf	573610 f97e9065e860d6f38dbdd7805a6b2b61b594baf8	no	10
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31	Non Patent Literature	PCTUS2011066938_ISR_20Jun2012.pdf	161208 3885284bc81c1e58f81c168ad6c4bee03e43640d	no	1
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32	Non Patent Literature	PCTUS2012058578_IPRP_8Apr2014.pdf	395940 489ad142c57bf27dbc7ce4c2bcc2990bb503284f	no	6
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33	Non Patent Literature	PCTUS2012058578_ISR_WO_25Mar2013.pdf	474605 7863fa4fc9971d4678923eec3bf2ce5f2e7e83fa	no	7
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Information:					
34	Non Patent Literature	PCTUS2012066339_IPRP_27Jan2015.pdf	1683869 f19c149d2b7f4b503f7252ea61d2e6363a137779	no	20
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Information:					
35	Non Patent Literature	PCTUS2012066339_ISR_05Mar2013.pdf	170948 51d2bf2d8c3261bf68ae4a7d5ce3bebda0643f72	no	1
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36	Non Patent Literature	PCTUS2013028378_IPRP__2Sep2014.pdf	591131 89ac4624c1f4c486ea8afdf1af8f9b317a619026	no	8
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Information:					

37	Non Patent Literature	PCTUS2013028378_ISR_20May2013.pdf	2068861 7aa225e5fc5072ee19793347a70c0e638d0aaf6a	no	11
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38	Non Patent Literature	PCTUS2013055205_IPRP_17Feb2015.pdf	1088787 a4ad0b4cbca3accc6e3d7dcc39fb16b1f47d9c4f	no	13
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Information:					
39	Non Patent Literature	PCTUS2013055205_ISR_WO_23Dec2013.pdf	1183672 ac7f8d4b45ad74177cf7e983f7ccdb35f8c3aaa7	no	14
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Information:					
40	Non Patent Literature	PCTUS2013057924_IPRP_10Mar2015.pdf	194296 76f80542f29980daf890ef0757097f13f83d4ee9	no	5
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Information:					
41	Non Patent Literature	PCTUS2014051926_12_09_2014_ISRWO.pdf	1478382 3c5398352c275e83b7e5ee6b91e28f28c4c1df64	no	8
Warnings:					
Information:					
42	Non Patent Literature	PCTUS2014057843_IPRP_29Mar2016.pdf	349324 c3eae10fc48e781a6f44caa2e4a95b85db282695	no	10
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43	Non Patent Literature	PCTUS2014057843_ISR_WO_22Dec2014.pdf	486811 42829e389140d271ae30d9dc16319ce25d55911d	no	14
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44	Non Patent Literature	PCTUS2015026957_IPRP_25Oct2016.pdf	427635 9328233fec14c4302957f989afc4c4b869dc2670	no	11
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45	Non Patent Literature	PCTUS2015026957_ISR_WO_28Sep2015.pdf	626701 2a382251e3fc7ba9bba20e605edf00abb6ce7e77	no	17
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46	Non Patent Literature	PCTUS2016031686_ISR_WO_19Sep2016.pdf	501383 7f23b55ae024ade34c82e68f98491c262eb5f67d	no	14
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47	Non Patent Literature	PEARSON_1900.pdf	2165807 283bdede01b20ed9ecbfde9724c72360f8b427bf	no	19
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48	Non Patent Literature	PENA.pdf	1948259 10039b3595d68ef3b220a52f0f0dd87a9642 7eb5	no	6
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49	Non Patent Literature	PERGAMENT_2014_210_NPL.pdf	1374298 c1dd6eb1059476f2181feff6c69241716589c6ac	no	26
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50	Non Patent Literature	Perkel_OvercomingChallenges_2012.pdf	281244 9f4ba79104cafca37a7b1da69f1142fc42b3d708	no	5
Warnings:					
Information:					

51	Non Patent Literature	Perkel-Biocompare.pdf	201071 dbf1fb55527bbf03c5024bd8c2b07894fcb9a2ed	no	5
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Information:					
52	Non Patent Literature	PERRY.pdf	2253670 4e0398a4ea3f14b2852cb1bed1af2030804c4aa5	no	10
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Information:					
53	Non Patent Literature	PERTL2000.pdf	1406693 4b563e9210ff5ee7a44c65aafa7db04337fbc01d	no	5
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Information:					
54	Non Patent Literature	PETERS.pdf	381392 d12f8d1777bee84fd411fb68877e03488e4d86ca	no	2
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55	Non Patent Literature	PETERS_List_2pgs_NPL.pdf	132551 363a7103566b26c6ad7d1a6ce58c6da2ddd86e06	no	2
Warnings:					
Information:					
56	Non Patent Literature	PFAFFL.pdf	2021399 37b43e8aafbb85b1e6156ef1ec133e395187477d	no	10
Warnings:					
Information:					
57	Non Patent Literature	PHILLIPS.pdf	1356186 401e0104e70e8c74e614c6785d6470dda4c6299a	no	7
Warnings:					
Information:					

58	Non Patent Literature	PODDER2008.pdf	3840122 d789dfff1083df9ca9766e9216a93158e7ffa308	no	15
Warnings:					
Information:					
59	Non Patent Literature	POIRIER_2005_45_NPL.pdf	412763 ee2987c927a8e7e3698c0607c7c84747f0c43b02	no	4
Warnings:					
Information:					
60	Non Patent Literature	POON_2002_35_NPL.pdf	795233 156bcd3f5e2921e7268884d51c6fd10280b908a4	no	7
Warnings:					
Information:					
		Total Files Size (in bytes):	50878618		

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.

Electronic Acknowledgement Receipt

EFS ID:	37484373
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
Filing Date:	28-FEB-2019
Time Stamp:	13:05:25
Application Type:	Utility under 35 USC 111(a)

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	POPOVA_2009_NPL.pdf	798494 d30c5efbdb33e23d903177c0689f895f9b6114ff	no	14

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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

EFS ID:	37484671
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
Filing Date:	28-FEB-2019
Time Stamp:	13:04:39
Application Type:	Utility under 35 USC 111(a)

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Submitted with Payment	no

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<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

EFS ID:	37485374
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
Filing Date:	28-FEB-2019
Time Stamp:	13:02:55
Application Type:	Utility under 35 USC 111(a)

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	EP13829596_EESR_15Mar2016.pdf	159196 b308f7d64218df37b8194acfb1a88c48a97e0326	no	5

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2	Non Patent Literature	EP13835916_EESR_23Jun2016.pdf	202611 669a2dd2753b05d974b825029ee313e242d57910	no	7
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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

EFS ID:	37485023
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
Filing Date:	28-FEB-2019
Time Stamp:	13:03:41
Application Type:	Utility under 35 USC 111(a)

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.)
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59	Non Patent Literature	EP11858061_PSESR_07May2015.pdf	249096 6efaf7c78c124f60504c17fcc514adfab7a6408d	no	7
Warnings:					
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60	Non Patent Literature	EP12881774_EESR_24Nov2015.pdf	200960 d3f344a52ec6e6c3e7a036555739577d308098bdc	no	7
Warnings:					
Information:					
		Total Files Size (in bytes):	62294453		

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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

EFS ID:	37485939
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
Filing Date:	28-FEB-2019
Time Stamp:	13:01:22
Application Type:	Utility under 35 USC 111(a)

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	Foreign Reference	WO2012019200A2.pdf	1328559 1a90c68e4a4473ffd483be4e97c65b693f50a18c	no	147

Warnings:

Information:					
2	Foreign Reference	WO2012028746A1.pdf	5599952 1d9e65e81a3faa4639819716 2c0a0d421378b093	no	128
Warnings:					
Information:					
3	Foreign Reference	WO2012083250.pdf	3866594 9823d19a0dc29b777a48d5edd522efeb659c37a8	no	71
Warnings:					
Information:					
4	Foreign Reference	WO2012108920A1.pdf	14997756 97b8eaea868a7636125993d772620e9c26eaf8d7	no	197
Warnings:					
Information:					
5	Foreign Reference	WO2012142531A2.pdf	9127920 11c96889205be27f590bc9a39787b3bb1a4298e9	no	94
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Information:					
6	Foreign Reference	WO2013030577.pdf	3023115 c2f042f51a6b2f9f93d269c0401680bc21f96ffd	no	71
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7	Foreign Reference	WO2013045432.pdf	6809883 8aedcd671c806175e227df9123aaca832758f981	no	90
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8	Foreign Reference	WO2013049892A1.pdf	1416080 614bd284316a07eb2c87134e14e302dfbca8034c	no	68
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Information:					

9	Foreign Reference	WO2013078470A2.pdf	1368103 ff21e093a79a6b10e9607b0ac772ccc9a25626d9	no	29
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10	Foreign Reference	WO2013086464A1.pdf	10998281 41c06ee29924564914df1f04778924cf5e5d8352	no	218
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11	Foreign Reference	WO2013130848.pdf	13360184 ae028b1cd691fc4983294b11b3634fc3e92768d0	no	221
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12	Foreign Reference	WO2013159035A2.pdf	1685426 feeb46017c69d429dfe54e694122f48afecc75c8	no	95
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13	Foreign Reference	WO2013181651A1.pdf	774267 749644e1711c357be05b56d486abc9ce4207efeb	no	31
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14	Foreign Reference	WO2014004726A1.pdf	9818421 1bc5d72db6ad2fd18ab65ea0218121f77fe48946	no	93
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15	Foreign Reference	WO2014014497.pdf	25891655 8602578c6a286f68b06d942d168f5c4fabe63358	no	473
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16	Foreign Reference	WO2013052557A2.pdf	13996375 400a1c7313d4e464fb2d80114ac04a277800e599	no	91
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17	Foreign Reference	WO2012088456.pdf	13985368 168bd2593b004d1f0bb39ec44fdab6a00647f3d0	no	98
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18	Foreign Reference	WO2011087760.pdf	24130761 cf52e7988d96424d42229c152380271a02f3eb50	no	529
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19	Foreign Reference	WO2009036525.pdf	1718959 9021ef10f95185cec68deaf6c6cec1a44eede3c5	no	32
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21	Non Patent Literature	GARCIA_MURILLAS_2015_1_NPL.PDF	133220 8017419e1fba1b565d0b9f7b4326d72438525739	no	2
Warnings:					
Information:					
22	Non Patent Literature	HORNAK_2012_6pgs_NPL.PDF	270888 7e2687fd7ba04b52677787c17b5c5ffed7fe3349	no	6
Warnings:					
Information:					

23	Non Patent Literature	HULTIN_2005.PDF	1204410 189bf117e28d2679cb50bac5dc0fcb131eaf4570	no	10
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24	Non Patent Literature	JAMAL_HANJANI_2017_2109_NPL.PDF	1391478 cef98e141135802ad842adf45f2b82cfd21551ab	no	13
Warnings:					
Information:					
		Total Files Size (in bytes):	167791863		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16/288,351
	Filing Date	Feb 28, 2019
	First Named Inventor	Joshua BABIARZ
	Art Unit	1637
	Examiner Name	Teresa E. Strzelecka
	Attorney Docket Number	N.012.US.10

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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
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	Attorney Docket Number	N.012.US.10

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	Attorney Docket Number	N.012.US.10

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	Examiner Name	Teresa E. Strzelecka
	Attorney Docket Number	N.012.US.10

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	Examiner Name	Teresa E. Strzelecka
	Attorney Docket Number	N.012.US.10

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	Art Unit	1637
	Examiner Name	Teresa E. Strzelecka
	Attorney Docket Number	N.012.US.10

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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
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	Examiner Name	Teresa E. Strzelecka
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	Attorney Docket Number	N.012.US.10

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	Examiner Name	Teresa E. Strzelecka
	Attorney Docket Number	N.012.US.10

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	Art Unit	1637
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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

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Electronic Acknowledgement Receipt

EFS ID:	37549779
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Anton John Bokal/Angela McGowan
Filer Authorized By:	Anton John Bokal
Attorney Docket Number:	N.012.US.10
Receipt Date:	24-OCT-2019
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Time Stamp:	12:59:22
Application Type:	Utility under 35 USC 111(a)

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1	Transmittal Letter	N012US10_Transmittal_Ltr_SIGNED.pdf	80137 7dca8d2062b1b54a6f8c70d9dcd3299c91dbcafe	no	8

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8	Non Patent Literature	TYNAN_2011_382_NPL.pdf	824208 a672591951d06dca98145c9120f8bc37f1914571	no	8
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New Applications Under 35 U.S.C. 111

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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.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Joshua BABIARZ *et al.*	Confirmation No.: 1764
Applicant: Natera, Inc.	Art Unit: 1637
Application No.: 16/288,351	Examiner: STRZELECKA, TERESA E
Filing Date: Feb. 28, 2019	Atty. Docket: N.012.US.10
Title: DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS	

INFORMATION DISCLOSURE STATEMENT

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

Dear Commissioner:

Applicant submits information listed on the attached Form PTO/SB/08.

This Information Disclosure Statement:

☐ (a) accompanies a new patent application submitted herewith.

☐ (b) is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 C.F.R. § 1.491.

☒ (c) as far as is known to the undersigned, is filed before the mailing date of a first Office Action on the merits.

☐ (d) as far as is known to the undersigned, is filed before the mailing date of a first Office Action on the merits after the filing of a Request for Continued Examination (RCE).

☐ (e) is filed after the first Office Action and more than three months after the application's filing date or PCT National Stage entry date, but as far as is known to the undersigned, prior to the mailing date of either a Final Office Action or a Notice of Allowance, whichever occurs first, and is accompanied by either the fee ($240)

set forth in 37 C.F.R. § 1.17(p) **or** a certification as specified in 37 C.F.R. § 1.97(e), as checked below. Should any fee be due, Applicant authorizes the U.S. Patent and Trademark Office to charge Deposit Account No. 50-6075 in the amount of $240.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account.

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☐ Those references appearing on Form PTO/SB/08 and indicated by the double asterisk (**) are not supplied because they were previously cited by or submitted to the Office in prior Application Serial No. **********, filed on ******* and relied upon in this application for an earlier effective filing date under 35 U.S.C. § 120.

The submission of this Information Disclosure Statement does not constitute an admission that a search was made, does not constitute an admission that any information cited is prior art, and does not constitute an admission that no other or better art exists.

Applicant wishes to inform the Examiner of the issued, pending and abandoned U.S. patent applications, which are at least partially owned by Applicant, listed on the attached **Exhibit A**.

Applicant submits that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and MPEP § 609 and respectfully requests that the Examiner consider the cited information and acknowledge such consideration with appropriate markings on Form PTO/SB/08.

Respectfully submitted,

Date: 10-22-2019

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EXHIBIT A

Docket No.	Application No. Publication No. Patent No.	Filing Date Publication Date Grant Date	Title	Art Unit	Assigned Examiner
N.001.US.01	11/004,274 2006/0052945 8,024,128	December 3, 2004 March 9, 2006 September 2, 2011	System and Method for Improving Clinical Decisions By Aggregating, Validating and Analysing Genetic and Phenotypic Data	1631	Skowronek, Karlheinz R.
N.002.US.01	11/496,982 2007/0027636	July 31, 2006 February 1, 2007	System and method for using genetic, phentoypic and clinical data to make predictions for clinical or lifestyle decisions	1631	Sims, Jason M.
N.002.US.02	11/603,406 2007/0184467 8,532,930	November 22, 2006 August 9, 2007 September 10, 2013	Method for Determining the Number of Copies of a Chromosome in the Genome of a Target Individual Using Genetic Data From Genetically Related Individuals	1631	Riggs II, Larry D.
N.002.US.03	11/634,550 2007/0178501	December 6, 2006 August 2, 2007	System and method for integrating and validating genotypic, phenotypic and medical information into a database according to a	1631	Sims, Jason M.
N.002.US.04	12/076,348 2008/0243398 8,515,679	March 17, 2008 October 2, 2008 August 20, 2013	System and Method for Cleaning Noisy Genetic Data and Determining Chromosome Copy Number	1631	Riggs II, Larry D.
N.002.US.05	13/793,133 2013/0253369 9,424,392	March 11, 2013 Sept. 26, 2013 August 23, 2016	System And Method For Cleaning Noisy Genetic Data From Target Individuals Using Genetic Data From Genetically Related Individuals	1634	Riggs II, Larry D.
N.002.US.06	13/793,186 2013/0252824 8,682,592	March 11, 2013 Sept. 26, 2013 March 25, 2014	System And Method For Cleaning Noisy Genetic Data From Target Individuals Using Genetic Data From Genetically Related Individuals	1634	Lin, Jerry
N.002.US.07	13/949,212 2014/0032128	July 23, 2013 January 30, 2014	System and Method for Cleaning Noisy Genetic Data and Determining Chromosome Copy Number	1637	Bertagna, Angela M.
N.002.US.08	14/092,457 2014/0087385 9,430,611	November 27, 2013 March 27, 2014 August 30, 2016	System And Method For Cleaning Noisy Genetic Data From Target Individuals Using Genetic Data From Genetically Related Individuals	1631	Riggs II, Larry D.
N.002.US.09	14/156,433 2014/0193816	January 14, 2014 July 10, 2014	System And Method For Cleaning Noisy Genetic Data From Target Individuals Using Genetic Data From Genetically Related Individuals	1631	Riggs II, Larry D.
N.002.US.10	14/286,895 2014/0256569	May 23, 2014 Sept. 11, 2014	System And Method For Cleaning Noisy Genetic Data From Target Individuals Using Genetic Data From Genetically Related Individuals	1634	Riggs II, Larry D
N.002.US.11	15/187,555 2016-0298188	Jun 20, 2016 Oct. 13, 2016	System And Method For Cleaning Noisy Genetic Data From Target Individuals Using Genetic Data From Genetically Related Individuals	1631	Riggs II, Larry D

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Serial Number: 16/288,351
Filing Date: Feb. 28, 2019
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Docket No.	Application No. Publication No. Patent No.	Filing Date Publication Date Grant Date	Title	Art Unit	Assigned Examiner
N.002.US.12	15/191,197 2016-0369345	Jun 23, 2016 Dec. 22, 2016	System And Method For Cleaning Noisy Genetic Data From Target Individuals Using Genetic Data From Genetically Related Individuals	1631	Riggs II, Larry D
N.002.US.13	15/293,257 2017-0029893 9,695,477	Oct 13, 2016 Feb. 2, 2017 Jul. 4, 2017	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA FROM TARGET INDIVIDUALS USING GENETIC DATA FROM GENETICALLY RELATED INDIVIDUALS	1631	Riggs II, Larry D
N.002.US.14	15/413,200 2017-0175187	Jan. 23, 2017 June 22, 2017	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER	1637	Bertagna, Angela M.
N.002.US.15	15/446,778 2017-0166971	Mar. 1, 2017 June 15, 2017	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER	1637	Bertagna, Angela M.
N.002.US.16	15/676,233	Aug 14, 2017	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER	1637	Bertagna, Angela M.
N.002.US.17	15/676,759	Aug 14, 2017	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER	1637	Bertagna, Angela M.
N.002.US.18	15/688,604	Aug 28, 2017	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER	1639	
N.002.US.19	15/881,263	Jan 26, 2018	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER	1634	
N.002.US.20	15/887,746	Feb 2, 2018	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER		
N.002.US.21	15/881,384	Jan 26, 2018	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER	1634	
N.002.US.22	15/881,488	Jan 26, 2018	SYSTEM AND METHOD FOR CLEANING NOISY GENETIC DATA AND DETERMINING CHROMOSOME COPY NUMBER		

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Filing Date: Feb. 28, 2019
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Docket No.	Application No. Publication No. Patent No.	Filing Date Publication Date Grant Date	Title	Art Unit	Assigned Examiner
N.003.US.01	12/918,445 2011-0033862	October 7, 2010 February 10, 2011	Methods for Cell Genotyping	1637	Horlick, Kenneth R
N.003.US.02	12/994,260 2011/0092763	December 20, 2010 April 21, 2011	Methods for Embryo Characterization and Comparison	3735	Canty, Shannon Cantina
N.003.US.03	13/057,350 2011-0178719	March 29, 2011 July 21, 2011	Methods for Allele Calling and Ploidy Calling	1631	Sims, Jason M.
N.003.US.04	13/846,111 2013/0225422 9,639,657	March 18, 2013 August 29, 2013 May 2, 2017	Methods For Allele Calling And Ploidy Calling	1639	Sims, Jason M.
N.004.US.01	13/110,685 2011/0288780 8,825,412	May 18, 2011 November 24, 2011 Sept. 2, 2014	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.02	13/300,235 2012/0270212	November 18, 2011 October 25, 2012	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.03	13/335,043 2012/0122701	December 22, 2011 May 17, 2012	Methods for Non-Invasive Prenatal Paternity Testing	1631	Vanni, George S.
N.004.US.04	13/499,086 2012-0185176	March 29, 2012 July 19, 2012	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.05	13/683,604 2013/0123120	November 21, 2012 May 16, 2013	Highly Multiplex PCR Methods and Compositions	1634	Pohnert, Steven C.
N.004.US.06	13/780,022 2013/0196862	February 28, 2013 August 1, 2013	Informatics Enhanced Analysis of Fetal Samples Subject to Maternal Contamination	1714	Bratland Jr., Kenneth A
N.004.US.07	13/791,397 2013/0178373 9,163,282	March 8, 2013 July 11, 2013 October 20, 2015	Methods For Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.08	13/846,160 2013/0261004	March 18, 2013 October 3, 2013	Methods For Non-Invasive Prenatal Paternity Testing	1634	Vanni, George S.
N.004.US.09	13/896,293 2013/0274116	May 16, 2013 Oct. 17, 2013	Methods For Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.10	13/968,302 2014/0051585	August 15, 2013 February 20, 2014	Methods And Compositions For Reducing Genetic Library Contamination	1637	Wessendorf, Teresa D.
N.004.US.11	14/044,434 2014/0094373	October 2, 2013 April 3, 2014	Highly Multiplex PCR Methods And Compositions	1634	Pohnert, Steven C.
N.004.US.12	14/080,656 2014/0154682 9,228,234	November 14, 2013 June 5, 2014 January 5, 2016	Methods For Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.13	14/100,928 2014/0100134 8,949,036	December 9, 2013 April 1, 2014 February 3, 2015	Methods For Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.14	14/171,587 2014/0141981	February 3, 2014 May 22, 2014	Highly Multiplex PCR Methods And Compositions	1636	Pohnert, Steven C.
N.004.US.15	14/179,399 2014/0162269	February 12, 2014 June 12, 2014	Methods For Non-Invasive Prenatal Ploidy Calling	1637	Bertagna, Angela M.
N.004.US.16	14/225,356 2014/0206552	March 25, 2014 July 24, 2014	Methods For Preimplantation Genetic Diagnosis By Sequencing	1639	Riggs II, Larry D.
N.004.US.17	14/446,232 2014/0336060 9334541	July 29, 2014 November 13, 2014 May 10, 2016	Methods For Non-Invasive Prenatal Ploidy Calling	1634	Riggs II, Larry D.

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Docket No.	Application No. Publication No. Patent No.	Filing Date Publication Date Grant Date	Title	Art Unit	Assigned Examiner
N.004.US.18	14/532,666 2015/0051087	November 4, 2014 February 19, 2015	Methods For Non-Invasive Prenatal Ploidy Calling	1637	Bertagna, Angela M.
N.004.US.19	14/546,321 2015/0072872	November 18, 2014 March 12, 2015	Methods For Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.20	14/866,223 2016-0024564	September 25, 2015 Jan. 28, 2016	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.21	14/877,925 2017/0051355	October 7, 2015 February 23, 2017	Highly Multiplex Pcr Methods and Compositions	1634	Pohnert, Steven C.
N.004.US.22	14/983,128 2016-0171152	December 29, 2015 June 16, 2016	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.23	15/243,915 2016-0357904	August 22, 2016 Dec. 8, 2016	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D.
N.004.US.24	15/252,795 2016-0369346	August 31, 2016 December 22, 2016	Methods for Non-Invasive Prenatal Ploidy Calling	1637	Bertagna, Angela M.
N.004.US.25	15/273,332 2017-0011166	September 22, 2016 January 13, 2017	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D
N.004.US.26	15/343,003 2017-0076038	November 3, 2016 March 16, 2017	Methods for Non-Invasive Prenatal Ploidy Calling	1631	Riggs II, Larry D
N.004.US.27	15/586,013 2017-0242960-A1	May 3, 2017 August 24, 2017	Methods For Non-Invasive Prenatal Ploidy Calling	1637	Bertagna, Angela M.
N.004.US.28	15/724,020	Oct 3, 2017	METHODS FOR NON-INVASIVE PRENATAL PLOIDY CALLING	1637	Bertagna, Angela M.
N.004.US.29	15/727,428	Oct 6, 2017	METHODS FOR NON-INVASIVE PRENATAL PLOIDY CALLING	1637	Bertagna, Angela M.
N.004.US.30	15/805,871	Nov 7, 2017	METHODS FOR NON-INVASIVE PRENATAL PLOIDY CALLING	1637	Bertagna, Angela M.
N.004.US.31	15/806,047	Nov 7, 2017	METHODS FOR NON-INVASIVE PRENATAL PLOIDY CALLING	1637	Bertagna, Angela M.
N.005.US.01	13/970,436 2014/0100126	August 19, 2013 April 10, 2014	Method for Non-Invasive Prenatal Testing Using Parental Mosaicism Data	1634	Weiler, Karen S.
N.005.US.02	15/433,950 2017-0177786	February 15, 2017 June 22, 2017	Method for Non-Invasive Prenatal Testing Using Parental Mosaicism Data	1631	Brusca, John S.
N.006.US.01	13/793,316 2014/0065621	March 11, 2013 March 6, 2014	Methods For Increasing Fetal Fraction In Maternal Blood	1637	Marschel, Ardin H.
N.009.US.01	14/498,629 2015/0147815 9,499,870	September 26, 2014 May 28, 2015 Nov 22, 2016	Cell Free Dna Diagnostic Testing Standards	1797	Maureen Wallenhorst
N.009.US.02	14/996,097 2016/0244838	January 14, 2016 August 25, 2016	Cell Free Dna Diagnostic Testing Standards	1797	Maureen Wallenhorst
N.010.US.01	14/732,632	November 12, 2014	Systems and Methods for Detection of Aneuploidy	1631	Negin, Russell S.
N.010.US.02	15/887,864	Feb 2, 2018	Systems and Methods for Detection of Aneuploidy	1634	
N.010.US.03	15/887,914	Feb 2, 2018	Systems and Methods for Detection of Aneuploidy		
N.012.US.01	14/538,998	November 24, 2014	Methods for Simultaneous Amplification of Target Loci	1631	Woitach, Joseph T

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Serial Number: 16/288,351
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Dkt: N.012.US.10

Docket No.	Application No. Publication No. Patent No.	Filing Date Publication Date Grant Date	Title	Art Unit	Assigned Examiner
N.012.US.02	14/538,982 2015/0322507 9,677,118	November 24, 2014 November 12, 2015 June 13, 2017	Methods for Simultaneous Amplification of Target Loci	1637	Mummert, Stephanie
N.012.US.03	14/692,703 2017-0107576	April 21, 2015 April 20, 2017	Detecting Mutations and Ploidy in Chromosomal Segments	1631	Riggs II, Larry D
N.012.US.04	14/882,763 2016-0333416	October 14, 2015 Nov. 17, 2016	Detecting Cancer Mutations And Aneuploidy In Chromosomal Segments	1637	Mummert, Stephanie
N.012.US.05	14/918,544 2016-0369333	October 20, 2015 December 22, 2016	Methods For Simultaneous Amplification Of Target Loci	1637	Mummert, Stephanie
N.012.US.06	15/336,630 2017-0145474	October 27, 2016 May 25, 2017	Methods for Simultaneous Amplification of Target Loci	1637	Mummert, Stephanie
N.015.US.01	15/573,800	May 10, 2016	METHODS AND COMPOSITIONS FOR DETERMINING PLOIDY		
N.016.US.01	15/186,774 2016-0371428	Jun 20, 2016 December 22, 2016	Systems and Methods for Determining Aneuploidy Risk Using Sample Fetal Fraction	1631	Harward, Soren T.
N.018.US.01	15/372,279	Dec. 7, 2016	COMPOSITIONS AND METHODS FOR IDENTIFYING NUCLEIC ACID MOLECULES	1637	Woolwine, Samuel C.
N.018.US.02	15/716,058	Sept. 26, 2017	COMPOSITIONS AND METHODS FOR IDENTIFYING NUCLEIC ACID MOLECULES	1637	Woolwine, Samuel C.
N.018.US.03	15/716,331	Sept. 26, 2017	COMPOSITIONS AND METHODS FOR IDENTIFYING NUCLEIC ACID MOLECULES	1637	Woolwine, Samuel C.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Joshua BABIARZ
Title:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
Application No.:	16/288,351
Filing Date:	2/28/2019
Examiner:	Unassigned
Art Unit:	1631
Conf. No.:	1764

PRELIMINARY AMENDMENT UNDER 37 CFR 1.115

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

Commissioner:

Prior to examination of the present Continuing Application, Applicant respectfully requests that the application be amended as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this document.

Remarks begin on page 7 of this document.

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Amendments to the Claims:

This listing of claims will replace all prior versions and listing of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for monitoring the progression of cancer in a subject comprising:

(a) performing ~~whole exome~~ sequencing on a ~~tumor biopsy~~ cancer sample of the subject to identify a plurality of cancer-specific ~~tumor specific~~ mutations; and

(b) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points for the presence or absence of the cancer-specific ~~tumor-specific~~ mutations, thereby monitoring the progression of cancer.

2. (Original) The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.

3. (Currently Amended) The method of claim 1, wherein the cancer-specific ~~tumor specific~~ mutations comprise one or more single nucleotide variant (SNV) mutations.

4. (Currently Amended) The method of claim 1, wherein the cancer-specific ~~tumor specific~~ mutations comprise one or more copy number variation (CNV) mutations.

5. (Currently Amended) The method of claim 1, wherein the cancer-specific ~~tumor specific~~ mutations comprise one or more SNV mutations in a gene selected from the group consisting of TP53, PTEN, PIK3CA, APC, EGFR, NRAS, NF2, FBXW7, ERBBs, ATAD5, KRAS, BRAF, VEGF, EGFR, HER2, ALK, p53, BRCA, BRCA1, BRCA2, SETD2, LRP1B, PBRM, SPTA1, DNMT3A, ARID1A, GRIN2A, TRRAP, STAG2, EPHA3/5/7, POLE, SYNE1, C20orf80, CSMD1, CTNNB1, ERBB2. FBXW7, KIT, MUC4, ATM, CDH1, DDX11, DDX12, DSPP, EPPK1, FAM186A, GNAS, HRNR, KRTAP4-11, MAP2K4, MLL3, NRAS, RB1, SMAD4, TTN, ABCC9, ACVR1B, ADAM29, ADAMTS19, AGAP10, AKT1, AMBN, AMPD2, ANKRD30A, ANKRD40, APOBR, AR, BIRC6, BMP2, BRAT1, BTNL8, C12orf4, C1QTNF7,

C20orf186, CAPRIN2, CBWD1, CCDC30, CCDC93, CD5L, CDC27, CDC42BPA, CDH9, CDKN2A, CHD8, CHEK2, CHRNA9, CIZ1, CLSPN, CNTN6, COL14A1, CREBBP, CROCC, CTSF, CYP1A2, DCLK1, DHDDS, DHX32, DKK2, DLEC1, DNAH14, DNAH5, DNAH9, DNASE1L3, DUSP16, DYNC2H1, ECT2, EFHB, RRN3P2, TRIM49B, TUBB8P5, EPHA7, ERBB3, ERCC6, FAM21A, FAM21C, FCGBP, FGFR2, FLG2, FLT1, FOLR2, FRYL, FSCB, GAB1, GABRA4, GABRP, GH2, GOLGA6L1, GPHB5, GPR32, GPX5, GTF3C3, HECW1, HIST1H3B, HLA-A, HRAS, HS3ST1, HS6ST1, HSPD1, IDH1, JAK2, KDM5B, KIAA0528, KRT15, KRT38, KRTAP21-1, KRTAP4-5, KRTAP4-7, KRTAP5-4, KRTAP5-5, LAMA4, LATS1, LMF1, LPAR4, LPPR4, LRRFIP1, LUM, LYST, MAP2K1, MARCH1, MARCO, MB21D2, MEGF10, MMP16, MORC1, MRE11A, MTMR3, MUC12, MUC17, MUC2, MUC20, NBPF10, NBPF20, NEK1, NFE2L2, NLRP4, NOTCH2, NRK, NUP93, OBSCN, OR11H1, OR2B11, OR2M4, OR4Q3, OR5D13, OR8I2, OXSM, PIK3R1, PPP2R5C, PRAME, PRF1, PRG4, PRPF19, PTH2, PTPRC, PTPRJ, RAC1, RAD50, RBM12, RGPD3, RGS22, ROR1, RP11-671M22.1, RP13-996F3.4, RP1L1, RSBN1L, RYR3, SAMD3, SCN3A, SEC31A, SF1, SF3B1, SLC25A2, SLC44A1, SLC4A1, SMAD2, SPTA1, ST6GAL2, STK11, SZT2, TAF1L, TAX1BP1, TBP, TGFBI, TIF1, TMEM14B, TMEM74, TPTE, TRAPPC8, TRPS1, TXNDC6, USP32, UTP20, VASN, VPS72, WASH3P, WWTR1, XPO1, ZFHX4, ZMIZ1, ZNF167, ZNF436, ZNF492, ZNF598, ZRSR2, ABL1, AKT2, AKT3, ARAF, ARFRP1, ARID2, ASXL1, ATR, ATRX, AURKA, AURKB, AXL, BAP1, BARD1, BCL2, BCL2L2, BCL6, BCOR, BCORL1, BLM, BRIP1, BTK, CARD11, CBFB, CBL, CCND1, CCND2, CCND3, CCNE1, CD79A, CD79B, CDC73, CDK12, CDK4, CDK6, CDK8, CDKN1B, CDKN2B, CDKN2C, CEBPA, CHEK1, CIC, CRKL, CRLF2, CSF1R, CTCF, CTNNA1, DAXX, DDR2, DOT1L, EMSY (C11orf30), EP300, EPHA3, EPHA5, EPHB1, ERBB4, ERG, ESR1, EZH2, FAM123B (WTX), FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, FGF10, FGF14, FGF19, FGF23, FGF3, FGF4, FGF6, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FLT4, FOXL2, GATA1, GATA2, GATA3, GID4 (C17orf39), GNA11, GNA13, GNAQ, GNAS, GPR124, GSK3B, HGF, IDH1, IDH2, IGF1R, IKBKE, IKZF1, IL7R, INHBA, IRF4, IRS2, JAK1, JAK3, JUN, KAT6A (MYST3), KDM5A, KDM5C, KDM6A, KDR, KEAP1, KLHL6, MAP2K2, MAP2K4, MAP3K1, MCL1, MDM2, MDM4, MED12, MEF2B,

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MEN1, MET, MITF, MLH1, MLL, MLL2, MPL, MSH2, MSH6, MTOR, MUTYH, MYC, MYCL1, MYCN, MYD88, NF1, NFKBIA, NKX2-1, NOTCH1, NPM1, NRAS, NTRK1, NTRK2, NTRK3, PAK3, PALB2, PAX5, PBRM1, PDGFRA, PDGFRB, PDK1, PIK3CG, PIK3R2, PPP2R1A, PRDM1, PRKAR1A, PRKDC, PTCH1, PTPN11, RAD51, RAF1, RARA, RET, RICTOR, RNF43, RPTOR, RUNX1, SMARCA4, SMARCB1, SMO, SOCS1, SOX10, SOX2, SPEN, SPOP, SRC, STAT4, SUFU, TET2, TGFBR2, TNFAIP3, TNFRSF14, TOP1, TP53, TSC1, TSC2, TSHR, VHL, WISP3, WT1, ZNF217, and ZNF703.

6. (Currently Amended) The method of claim 1, wherein the cancer-specific ~~tumor-specific~~ mutations comprise one or more SNV mutations in a gene selected from the group consisting of CYFIP1, FAT1, MLLT4, RASA1, HERC4, JAK2, MSH2, MTOR, PLCG2, GABRG1, and TRIM67.

7. (Currently Amended) The method of claim 1, wherein the cancer-specific ~~tumor-specific~~ mutations comprise one or more clonal SNV mutations.

8. (Original) The method of claim 7, wherein at least one clonal SNV mutation is in a gene selected from the group consisting of BRIP1, CARS, FAT1, MLLT4, NFE2L2, TP53, TP53, EGFR, EGFR, TP53, KDM6A, and ROS1.

9. (Currently Amended) The method of claim 1, wherein the cancer-specific ~~tumor-specific~~ mutations comprise one or more subclonal SNV mutations.

10. (Original) The method of claim 9, wherein at least one subclonal SNV mutation is in a gene selected from the group consisting of CIC, KDM6A, NF1, and TRIM67.

11. (Currently Amended) The method of claim 1, wherein the cancer-specific ~~tumor-specific~~ mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.

12. (Currently Amended) The method of claim 1, wherein the method further comprises determining clonal heterogeneity of the ~~tumor biopsy~~ cancer sample.

13. (Currently Amended) The method of claim 1, wherein the method further comprises designing targeted PCR assays for the cancer-specific ~~tumor-specific~~ mutations identified in the ~~tumor~~ cancer sample.

14. (Currently Amended) The method of claim 1, wherein step (b) comprises targeted multiplex PCR amplification of a plurality of target loci comprising the cancer-specific ~~tumor-specific~~ mutations from the cell-free DNA.

15. (Original) The method of claim 14, wherein step (b) comprises targeted multiplex PCR amplification of 10 to 50 of the target loci.

16. (Original) The method of claim 14, wherein step (b) comprises determining the sequences of the amplified target loci by high-throughput sequencing.

17. (Original) The method of claim 14, wherein step (b) comprises determining the sequences of the amplified target loci by microarray.

18. (Currently Amended) The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the cancer-specific ~~tumor-specific~~ mutations detected in the cell-free DNA.

19. (Original) The method of claim 1, wherein the cancer is colorectal cancer.

20. (Original) The method of claim 1, wherein the cancer is lung cancer.

21. (Original) The method of claim 1, wherein the cancer is bladder cancer.

22. (Original) The method of claim 1, wherein the cancer is breast cancer.

23. (Original) The method of claim 1, wherein the biological sample is a blood, serum, plasma, or urine sample.

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24. (New) The method of claim 1, wherein step (a) comprises performing whole exome sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations.

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REMARKS

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application.

Status of Claims

Claims 1, 3-7, 9, 11-14 and 18 are amended to more clearly and particularly describe the claimed invention. Claim 24 is newly added. No new matter has been introduced. These amendments are made without prejudice or disclaimer. After amending the claims as set forth above, claims 1-24 will be pending in this application.

Concluding Remarks

Applicant believes that the application is in condition for allowance. Favorable consideration is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance prosecution.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-6075. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extension fees to Deposit Account No. 50-6075.

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Respectfully submitted,

Date July 1, 2019

By /Linda X. Wu/

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NeoGenomics
Exhibit 1003 Page 1347

Electronic Acknowledgement Receipt

EFS ID:	36466442
Application Number:	16288351
International Application Number:	
Confirmation Number:	1764
Title of Invention:	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
First Named Inventor/Applicant Name:	Joshua BABIARZ
Customer Number:	110711
Filer:	Linda X. Wu/Angela McGowan
Filer Authorized By:	Linda X. Wu
Attorney Docket Number:	N.012.US.10
Receipt Date:	01-JUL-2019
Filing Date:	28-FEB-2019
Time Stamp:	16:21:29
Application Type:	Utility under 35 USC 111(a)

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		01_N012US10_Preliminary_Amendment.pdf	41880 f5675d6c06900f55d1ac6301eb453cb62b94a00f	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Preliminary Amendment	1	1
	Claims	2	6
	Applicant Arguments/Remarks Made in an Amendment	7	8
Warnings:			
Information:			
	Total Files Size (in bytes):	41880	

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New International Application Filed with the USPTO as a Receiving Office

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/288,351	02/28/2019	Joshua BABIARZ	N.012.US.10

CONFIRMATION NO. 1764

110711
Natera, Inc.
201 Industrial Road
Suite 410
San Carlos, CA 94070

PUBLICATION NOTICE

OC000000109230037

Title:DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS

Publication No.US-2019-0194758-A1
Publication Date:06/27/2019

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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In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

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Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/288,351

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	660
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	760
TOTAL CLAIMS (37 CFR 1.16(i))	23 minus 20 =	* 3			OR	x 100 =	300
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 460 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 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).						1600
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	3620

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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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
16/288,351	02/28/2019	1634	3620	N.012.US.10	23	1

CONFIRMATION NO. 1764

FILING RECEIPT

110711
Natera, Inc.
201 Industrial Road
Suite 410
San Carlos, CA 94070

OC000000106823433

Date Mailed: 03/19/2019

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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Assignment For Published Patent Application

Natera, Inc., San Carlos, CA

Power of Attorney: The patent practitioners associated with Customer Number 110711

Domestic Priority data as claimed by applicant

This application is a CON of 16/014,961 06/21/2018
which is a CON of 14/692,703 04/21/2015 PAT 10179937
which claims benefit of 61/982,245 04/21/2014
and claims benefit of 61/987,407 05/01/2014
and claims benefit of 61/994,791 05/16/2014
and claims benefit of 62/066,514 10/21/2014
and claims benefit of 62/146,188 04/10/2015
and claims benefit of 62/147,377 04/14/2015

and claims benefit of 62/148,173 04/15/2015

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 03/15/2019
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/288,351**
Projected Publication Date: 06/27/2019
Non-Publication Request: No
Early Publication Request: No
Title

DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS

Preliminary Class

435

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Doc Code: PA..
Document Description: Power of Attorney

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Application Number	To be assigned
Filing Date	Feb. 28, 2019
First Named Inventor	Joshua BABIARZ
Title	DETECTING MUTATIONS AND PLOIDY IN CHROMOSOMAL SEGMENTS
Art Unit	To be assigned
Examiner Name	To be assigned
Attorney Docket Number	N.012.US.10

SIGNATURE of Applicant or Patent Practitioner

Signature	/Anton Bokal/	Date (Optional)	
Name	Anton Bokal	Registration Number	51,243
Title (if Applicant is a juristic entity)	Chief IP Counsel		
Applicant Name (if Applicant is a juristic entity)	Natera, Inc.		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ *Total of 2 forms are submitted.

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Doc Code: PA..
Document Description: Power of Attorney

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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 110711

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Natera, Inc.

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Anton Bokal/	Date (Optional)	
Name	Anton Bokal		
Title	Chief IP Counsel		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 2 forms are submitted.

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