TECAN

ORAL ARGUMENT FOR IPR2025-00026

U.S. Patent No. 11,098,357

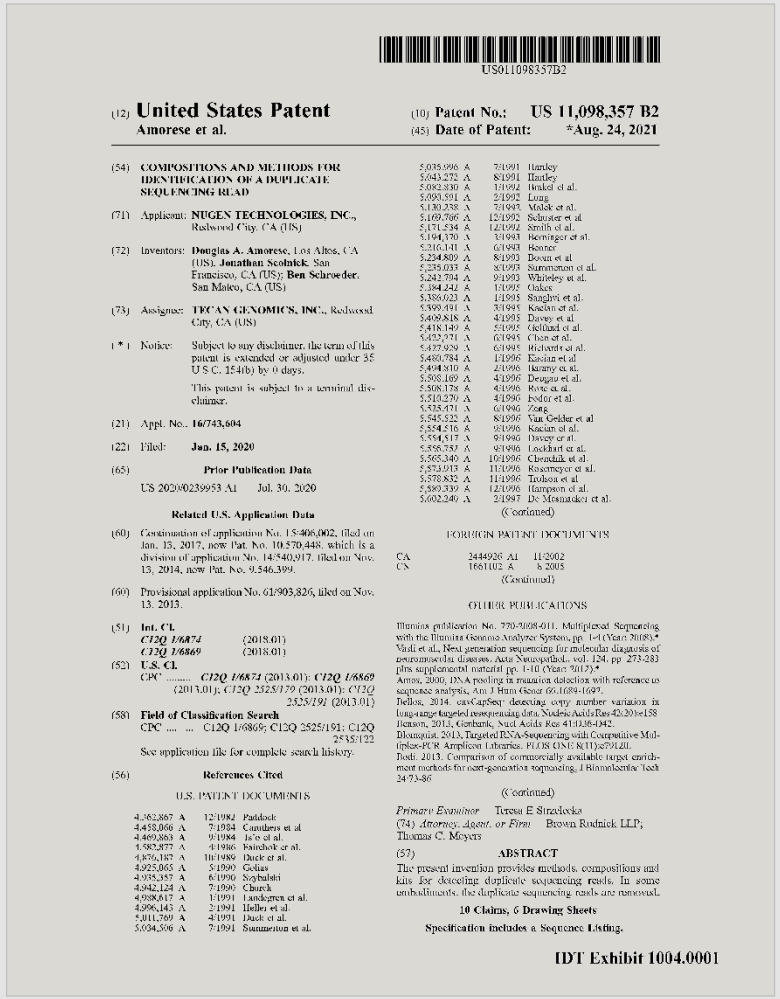
Patent Owner's Hearing Demonstratives

February 11, 2026

Petition Fails to Establish Invalidity

- The challenged claims require use of the identifier and target sequence in combination
- Petitioner's Ground 1 fails
  - **Porreca** fails to disclose an embodiment with both limitations 1.a and 1.c
  - **Porreca** does not disclose the claimed method of identifying duplicates
- Petitioner's Ground 2 fails
  - The combination of **Schmitt** and **McCloskey** fails to disclose limitation 1.g
  - Petitioner's motivation to combine **Schmitt** and **McCloskey** is deficient
- Petitioner's Grounds 3-6 fail for the same reasons as Ground 1 and 2

Claim Construction – "identifying sequence reads with identical identifier and target sequences as duplicates"



US011098357B2

(12) **United States Patent**
Amorese et al.

(10) **Patent No.: US 11,098,357 B2**
(45) **Date of Patent: *Aug. 24, 2021**

(54) **COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ**

(71) Applicant: **NUGEN TECHNOLOGIES, INC.**, Redwood City, CA (US)

(72) Inventors: **Douglas A. Amorese**, Los Altos, CA (US); **Jonathan Scolnick**, San Francisco, CA (US); **Ben Schroeder**, San Mateo, CA (US)

(73) Assignee: **TECAN GENOMICS, INC.**, Redwood City, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **16/743,604**

(22) Filed: **Jan. 15, 2020**

(65) **Prior Publication Data**

US 2020/0239953 A1 Jul. 30, 2020

Related U.S. Application Data

(63) Continuation of application No. 15/406,002, filed on Jan. 13, 2017, now Pat. No. 10,570,448, which is a division of application No. 14/540,917, filed on Nov. 13, 2014, now Pat. No. 9,546,399.

(60) Provisional application No. 61/903,826, filed on Nov. 13, 2013.

(51) **Int. Cl.**
C12Q 1/6874 (2018.01)
C12Q 1/6869 (2018.01)

(52) **U.S. Cl.**
CPC ***C12Q 1/6874*** (2013.01); ***C12Q 1/6869*** (2013.01); *C12Q 2525/179* (2013.01); *C12Q 2525/191* (2013.01)

(58) **Field of Classification Search**
CPC C12Q 1/6869; C12Q 2525/191; C12Q 2535/122
See application file for complete search history.

(56) **References Cited**

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(Continued)

Primary Examiner Teresa E Strzelecka
(74) *Attorney, Agent, or Firm* Brown Rudnick LLP; Thomas C. Meyers

(57) **ABSTRACT**

The present invention provides methods, compositions and kits for detecting duplicate sequencing reads. In some embodiments, the duplicate sequencing reads are removed.

10 Claims, 6 Drawing Sheets

Specification includes a Sequence Listing.

IDT Exhibit 1004.0001

The invention claimed is:

1. A method for detecting duplicate sequencing reads, the method comprising:

ligating an adaptor to each of a plurality of nucleic acid fragments, wherein each adaptor comprises a unique identifier having from about 1 to about 8 nucleotides, an indexing site unique to a subset of the adaptors, and a primer binding site;

amplifying the adaptor-ligated fragments into amplicons;

sequencing the amplicons to produce sequence reads that include identifier and target sequences; and

identifying sequence reads with identical identifier and target sequences as duplicates.

Tecan's Construction	Petitioner's Construction
"identifying sequence reads as duplicates through a step that uses both the identifier and at least a portion of the target sequence"	**"Plain and ordinary meaning, which is identifying sequence reads with identical identifier and at least a portion of the target sequences as duplicates"**

Paper 19, 14-20; Paper 28, 1-7; EX1002.0024

Prosecution History Is Critical Intrinsic Evidence

"The prosecution history is often of critical significance in determining the meaning of the claims because it contains the complete record of all the proceedings before the Patent and Trademark Office, including any express representations made by the applicant regarding the scope of the claims."

Genentech, Inc. v. Iancu, 809 F. App'x 781, 783 (Fed. Cir. 2020)

"Unambiguous intrinsic evidence in turn provides sufficient input to the rules of claim construction, in particular in this case, the rule that explicit statements made by a patent applicant during prosecution to distinguish a claimed invention over prior art may serve to narrow the scope of a claim."

Spectrum Int'l, Inc. v. Sterilite Corp., 164 F.3d 1372, 1378 (Fed. Cir. 1998); *see also Phillips v. AWH Corp.*, 415 F.3d 1303, 1317 (Fed. Cir. 2005)

Paper 19, 14-20; Paper 28, 1-7

The Prosecution History of the ’357 Patent

Attorney Docket No. NUGN-001/01US 301735-2003 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	AMORESE, Douglas, et al.	Confirmation No.:	7076
Application No.:	14/540,917	Group Art Unit:	1637
Filed:	November 13, 2014	Examiner:	Teresa E. Strzelecka

FOR: **COMPOSITIONS AND METHODS FOR IDENTIFICATION OF A DUPLICATE SEQUENCING READ**

Filed *via* EFS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT/RESPONSE TO OFFICE ACTION

In response to the Final Office Action dated September 28, 2015, this paper is timely filed within the 3-month period for reply ending December 28, 2015. Please amend the above-identified patent application in the following manner:

Amendments to the Claims are reflected in the listing of the claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

1

125135876 v1

Dec. 28, 2015, Amendment/Response to Office Action

1. (Currently Amended) A method for detecting a duplicate sequencing read from a population of sample sequencing reads comprising:

   a) ligating an adaptor to a 5’ end of each nucleic acid fragment of a plurality of nucleic acid fragments from one or more samples, wherein the adaptor comprises:

      (i) an indexing primer binding site;

      (ii) an indexing site;

      (iii) an identifier site consisting of~~comprising at least~~ between 1 and 8 nucleotides; and

      (iv) a target sequence primer binding site;

   b) amplifying the adapter-nucleic acid fragment ligated products;

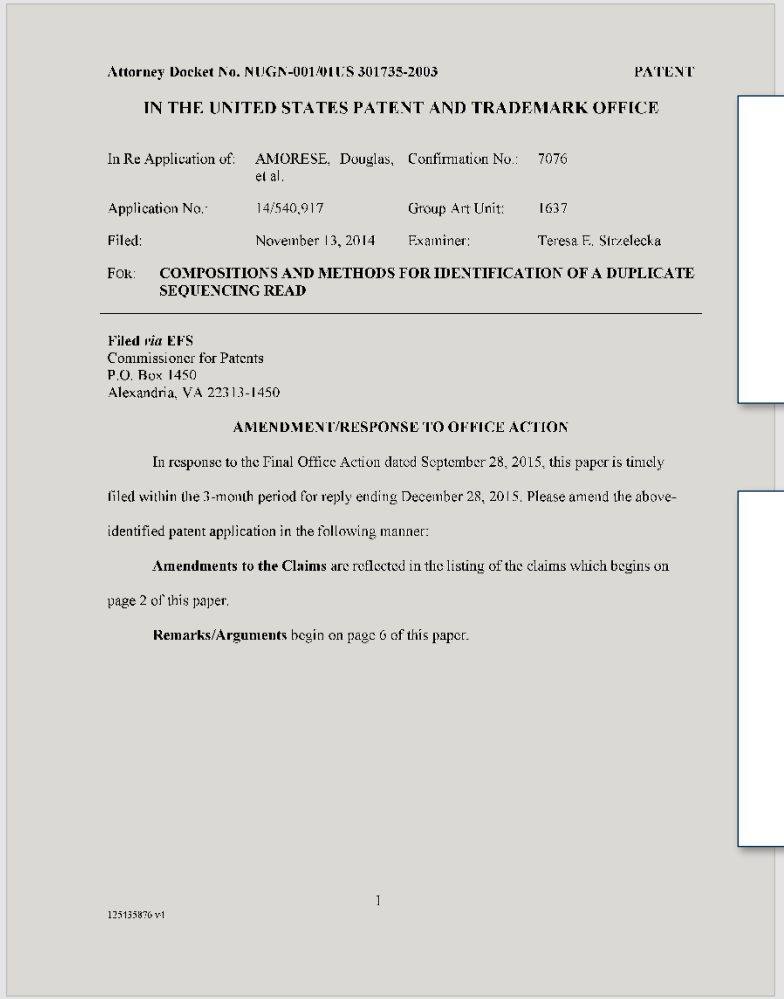
   c) generating a population of sequencing reads from amplified adapter-nucleic acid fragment ligated products; and

   d) ~~detecting~~ identifying a duplicate sequencing read as the ~~population of~~ sequencing read~~s~~ comprising the same identifier site and nucleic acid fragment as another sequencing read in the population of sequencing reads~~a sequencing read with a duplicate identifier site and target sequence~~;

Paper 19, 18-20; Paper 28, 1-5

EX2021.0794, 807

The Prosecution History of the ’357 Patent



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1

125135876 v1

Dec. 28, 2015, Amendment/Response to Office Action

Samuels *et al.* do not teach (1) the active step of “identifying a duplicate sequencing read as the sequencing read comprising the same identifier site and nucleic acid fragment as another sequencing read in the population of sequencing reads;” (emphasis added); or (2) adaptors

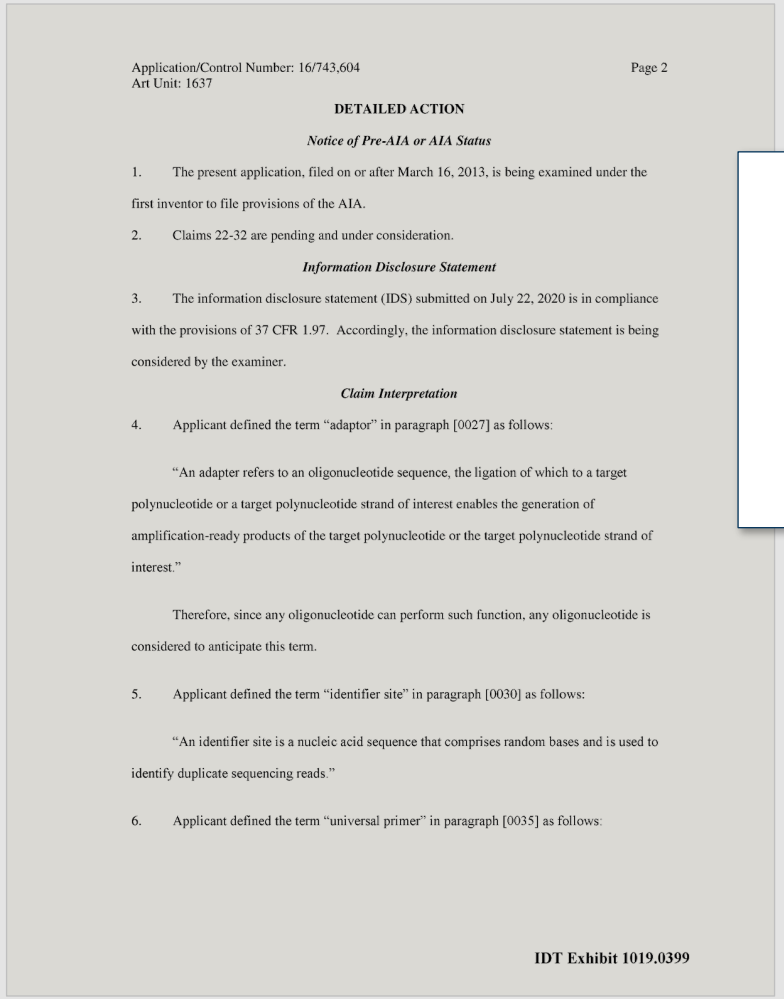
EX2021.0797-798

purposes of comparing the instant claims with the disclosure of McCloskey *et al.* However, the instant invention relies on the indexing site for batch identification of samples, and the sequence of the identifier site and the sequence of the fragment that is amplified are used in combination to discern duplication. This is very different than the method disclosed in McCloskey *et al.*

EX2021.0799

Paper 19, 18-20; Paper 28, 1-5

The Prosecution History of the ’357 Patent



Application/Control Number: 16/743,604 Page 2
Art Unit: 1637

DETAILED ACTION

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. Claims 22-32 are pending and under consideration.

Information Disclosure Statement

3. The information disclosure statement (IDS) submitted on July 22, 2020 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Interpretation

4. Applicant defined the term “adaptor” in paragraph [0027] as follows:

“An adapter refers to an oligonucleotide sequence, the ligation of which to a target polynucleotide or a target polynucleotide strand of interest enables the generation of amplification-ready products of the target polynucleotide or the target polynucleotide strand of interest.”

Therefore, since any oligonucleotide can perform such function, any oligonucleotide is considered to anticipate this term.

5. Applicant defined the term “identifier site” in paragraph [0030] as follows:

“An identifier site is a nucleic acid sequence that comprises random bases and is used to identify duplicate sequencing reads.”

6. Applicant defined the term “universal primer” in paragraph [0035] as follows:

IDT Exhibit 1019.0399

Mar. 5, 2021, Amendment/Response to Office Action

This is obviated in the claimed methods by using the identifier and fragment sequence to identify duplicate reads. Moreover, because the fragment sequence is used in conjunction with the identifier to find duplicates, the identifier can be less complex than the “barcode” in McCloskey. The lower complexity of adaptors comprising fewer nucleotides proves more cost-

EX1013.0070

Paper 19, 18-20; Paper 28, 1-5

Example 5 of the ’357 Patent

Identifier Site Index Site

Sequencing Primer Binding Site

Coralville, Iowa). The reverse adaptors each contain a unique identifier (underlined) that enables libraries made with these adapters to be distinguished. In this case N represents an equimolar mixture of A, C, G, and T. A mixture of 5 uM forward, 5 uM reverse, and 10 uM common in 10

* * *

GAIIx, MiSeq, or Hi Seq sequencing instruments (Illumina Inc., San Diego, Calif.). The following series is run: 50 base first read, 6 base second read. A third read is not required for counting purposes or duplication analysis.

Data Analysis: The sequencer output was processed in accordance with manufacturer’s recommendation. The 6 bases of the index read is used for standard library parsing, separating the data files from the two sample types. The first 50 bases of the target sequence read are compared to each other. Any read that has identical sequence to any other read is identified as a duplicate and removed from the population, thus, only a single copy is retained within the file. Once the

EX1002.0021, 20:19-23; 20:51-62

Paper 19, 16-18; Paper 28, 5-7

Expert Testimony Supports Tecan's Construction

Carlos Bustamante, Ph.D.
Tecan's Expert

93. It is my opinion that based on the claim language, specification, and prosecution history, a POSA would understand the term "identifying sequence reads with identical identifier and target sequences as duplicates" to mean "identifying sequence reads as duplicates through a step that uses both the identifier and at least a portion of the target sequence." In my opinion, this construction is consistent with how "identifying sequence reads with identical identifier and target sequences as duplicates" is used by the '357 patent.

94. For example, Example 5 of the '357 patent describes a process of identifying duplicate sequencing reads. Example 5 is an experiment wherein "[t]otal

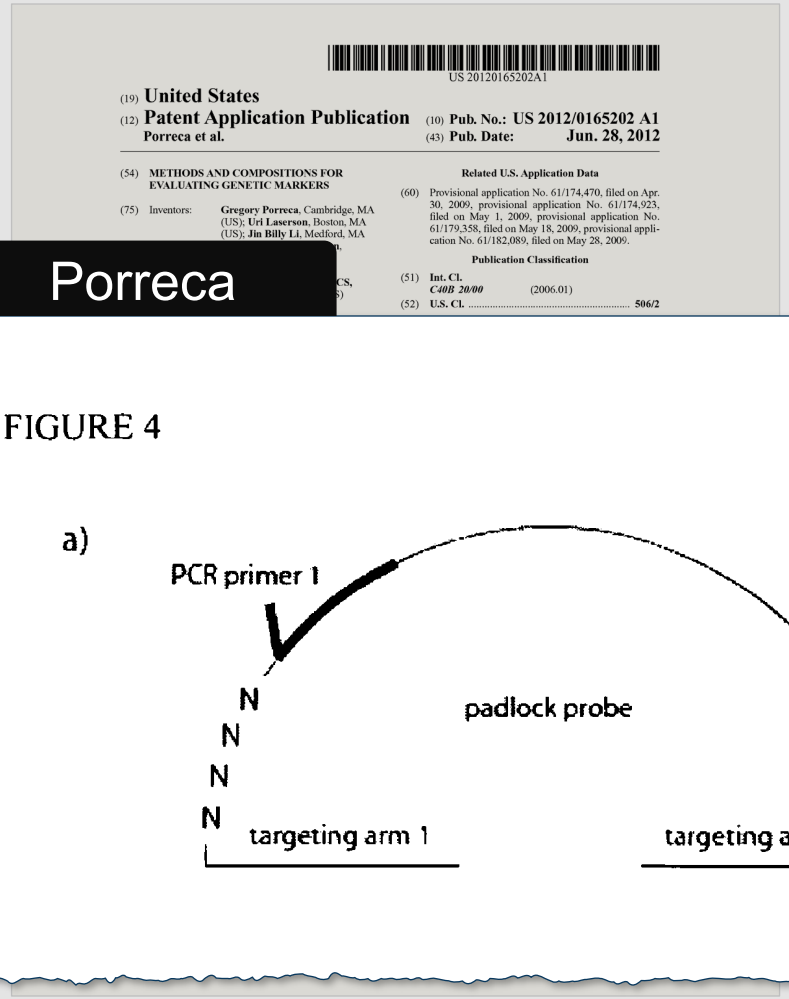
EX2107.¶¶93-94

Paper 28, 6-7

Petition Fails to Establish Invalidity

- The challenged claims require use of the identifier and target sequence in combination
- Petitioner's Ground 1 fails
  - **Porreca** fails to disclose an embodiment with both limitations 1.a and 1.c
  - **Porreca** does not disclose the claimed method of identifying duplicates
- Petitioner's Ground 2 fails
  - The combination of **Schmitt** and **McCloskey** fails to disclose limitation 1.g
  - Petitioner's motivation to combine **Schmitt** and **McCloskey** is deficient
- Petitioner's Grounds 3-6 fail for the same reasons as Ground 1 and 2

Porreca Discloses Multiple Different Embodiments



US 20120165202A1

(19) United States

(12) Patent Application Publication

Porreca et al.

(10) Pub. No.: US 2012/0165202 A1

(43) Pub. Date: Jun. 28, 2012

(54) METHODS AND COMPOSITIONS FOR EVALUATING GENETIC MARKERS

(75) Inventors: Gregory Porreca, Cambridge, MA (US); Uri Laserson, Boston, MA (US); Jin Billy Li, Medford, MA

Related U.S. Application Data

(60) Provisional application No. 61/174,470, filed on Apr. 30, 2009, provisional application No. 61/174,923, filed on May 1, 2009, provisional application No. 61/179,358, filed on May 18, 2009, provisional application No. 61/182,089, filed on May 28, 2009.

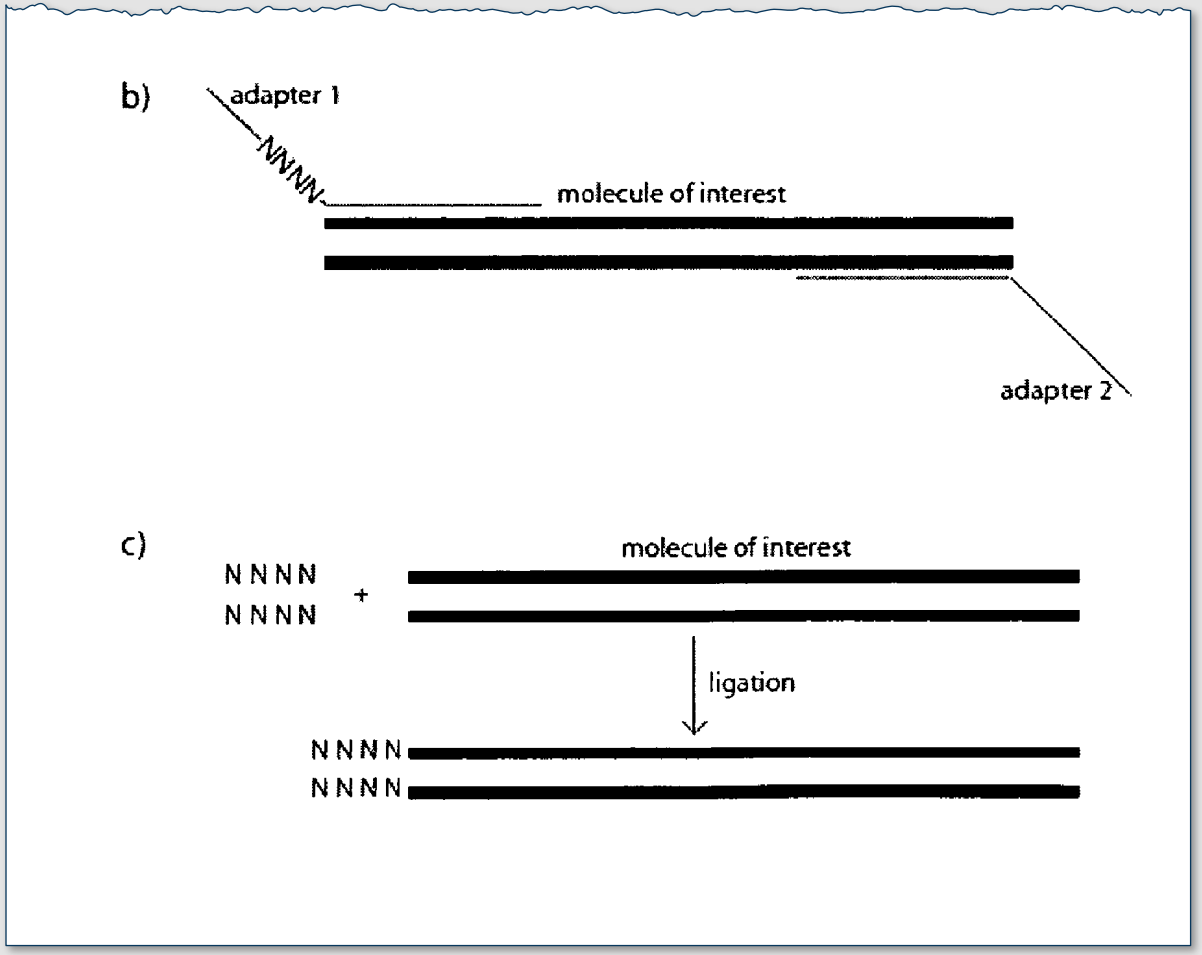
Publication Classification

(51) Int. Cl.
C40B 20/00 (2006.01)

(52) U.S. Cl. 506/2

Porreca

FIGURE 4



EX1006.0005

Paper 19, 21-27; Paper 28, 7-15

Anticipation Requires Disclosure of the Claimed Elements as Arranged in the Claims

"[I]t is not enough that the prior art reference discloses part of the claimed invention, which an ordinary artisan might supplement to make the whole, or that it includes multiple, distinct teachings that the artisan might somehow combine to achieve the claimed invention."

Net MoneyIN, Inc. v. VeriSign, Inc., 545 F.3d 1359, 1369, 1371 (Fed. Cir. 2008)

"[A]nticipation requires that the reference provide specific guidance that would lead a person of ordinary skill to an embodiment within the claim."

Otonomy, Inc. v. Auris Med., AG, 743 F. App'x 430, 439 (Fed. Cir. 2018)

Paper 19, 21-22; Paper 28, 7-9

Anticipation Requires the POSA to "At Once Envisage" the Challenged Claims

"[A] reference can anticipate a claim even if it does not expressly spell out all the limitations arranged or combined as in the claim, if a person of skill in the art, reading the reference, would at once envisage the claimed arrangement or combination."

Kennametal, Inc. v. Ingersoll Cutting Tool Co., 780 F.3d 1376, 1381 (Fed. Cir. 2015)

Paper 19, 22-23; Paper 28, 8-10

Unlike Porreca, Disclosure in *Kennametal* Was Narrow

> **5**. The coated cutting insert of claim **1** wherein the binder alloy further includes one or more of tungsten, iron, nickel, ruthenium, and rhenium.

"Claim 5 of Grab recites using a binder consisting of one of five metals, one of which is ruthenium, together with a coating. Grab only discloses three coating methods, one of which is PVD. While CVD and MTCVD coatings are the coatings on which Grab focuses, it "also contemplate[d] that one or more layers of a coating scheme may be applied by physical vapor deposition." Grab col.4 ll.59–61. Because all the limitations of Kennametal's claim are specifically disclosed in Grab, the question for the purposes of anticipation is 'whether the number of categories and components' disclosed in Grab is so large that the combination of ruthenium and PVD coatings 'would not be immediately apparent to one of ordinary skill in the art.'"

Kennametal, Inc. v. Ingersoll Cutting Tool Co., 780 F.3d 1376, 1382 (Fed. Cir. 2015) (quoting *Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1361 (Fed. Cir. 2012))

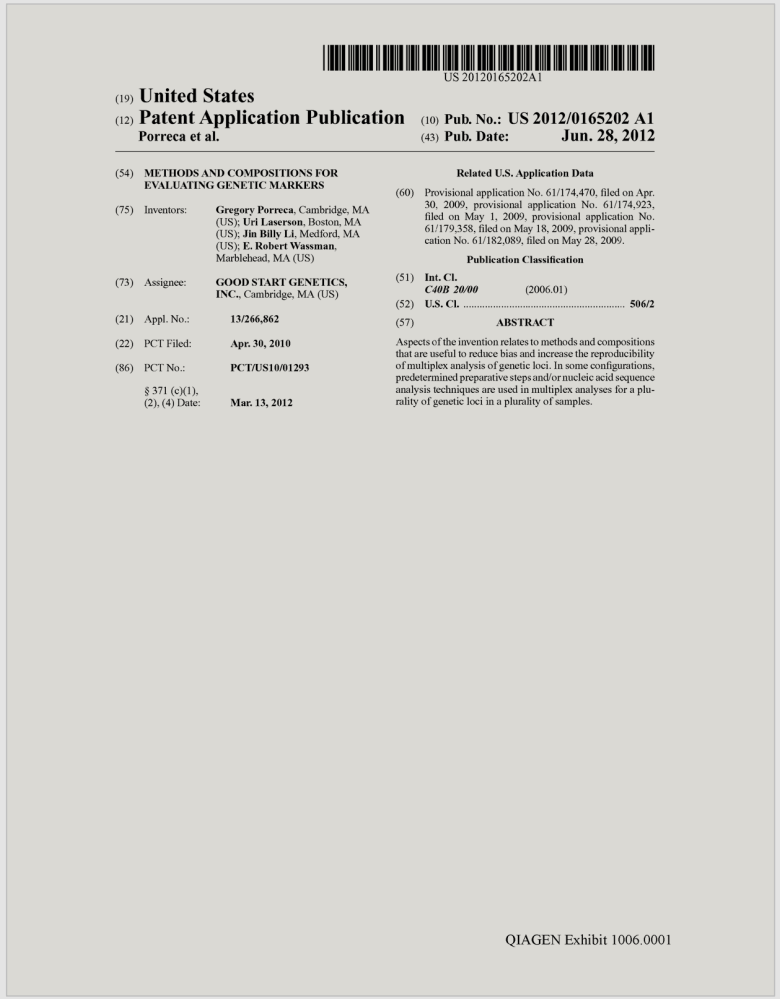
Paper 19, 22-23; Paper 28, 8-10

Claim 1 of the ’357 Patent

1.p	A method for detecting duplicate sequencing reads, the method comprising:
1.a	ligating an adaptor to each of a plurality of nucleic acid fragments
1.b	wherein each adaptor comprises a unique identifier having from about 1 to about 8 nucleotides,
1.c	an indexing site unique to a subset of the adaptors,
1.d	and a primer binding site;
1.e	amplifying the adaptor-ligated fragments into amplicons;
1.f	sequencing the amplicons to produce sequence reads that include identifier and target sequences; and
1.g	identifying sequence reads with identical identifier and target sequences as duplicates.

EX1002.0024

Petition Relies on Ligation Embodiment of Figure 4.C



US 20120165202A1

(19) **United States**

(12) **Patent Application Publication**

Porreca et al.

(10) **Pub. No.: US 2012/0165202 A1**

(43) **Pub. Date: Jun. 28, 2012**

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

(75) Inventors: **Gregory Porreca**, Cambridge, MA (US); **Uri Laserson**, Boston, MA (US); **Jin Billy Li**, Medford, MA (US); **E. Robert Wassman**, Marblehead, MA (US)

(73) Assignee: **GOOD START GENETICS, INC.**, Cambridge, MA (US)

(21) Appl. No.: **13/266,862**

(22) PCT Filed: **Apr. 30, 2010**

(86) PCT No.: **PCT/US10/01293**

§ 371 (c)(1), (2), (4) Date: **Mar. 13, 2012**

Related U.S. Application Data

(60) Provisional application No. 61/174,470, filed on Apr. 30, 2009, provisional application No. 61/174,923, filed on May 1, 2009, provisional application No. 61/179,358, filed on May 18, 2009, provisional application No. 61/182,089, filed on May 28, 2009.

Publication Classification

(51) **Int. Cl.**
C40B 20/00 (2006.01)

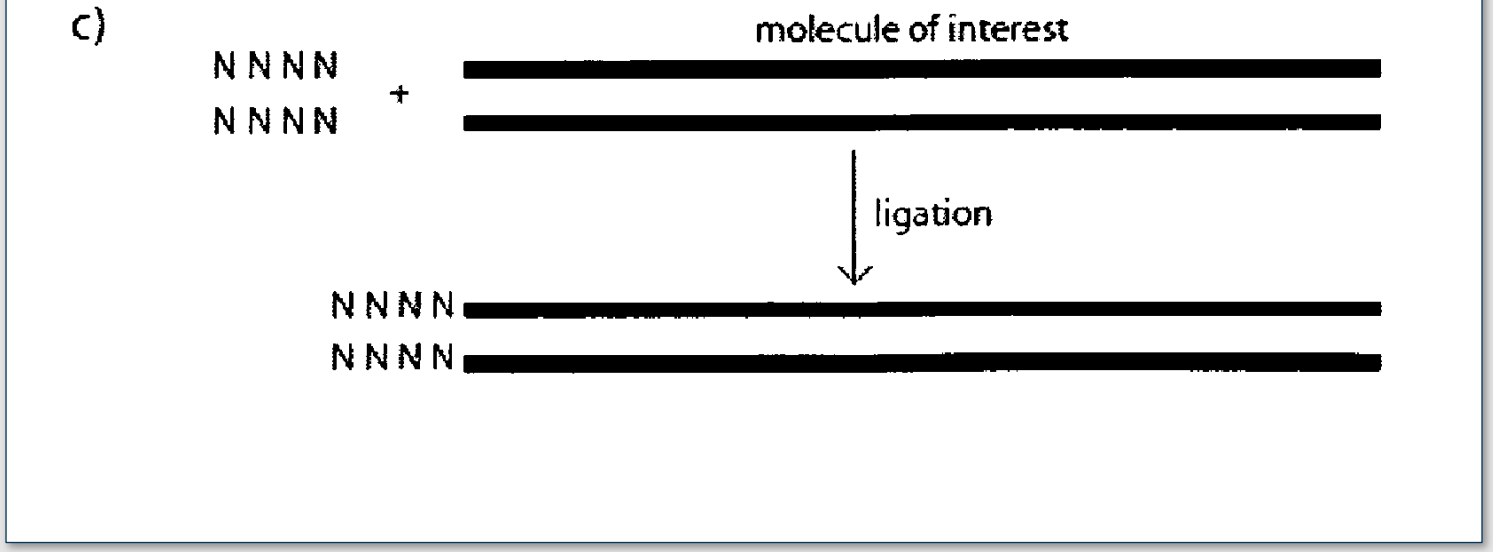
(52) **U.S. Cl.** **506/2**

(57) **ABSTRACT**

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

QIAGEN Exhibit 1006.0001

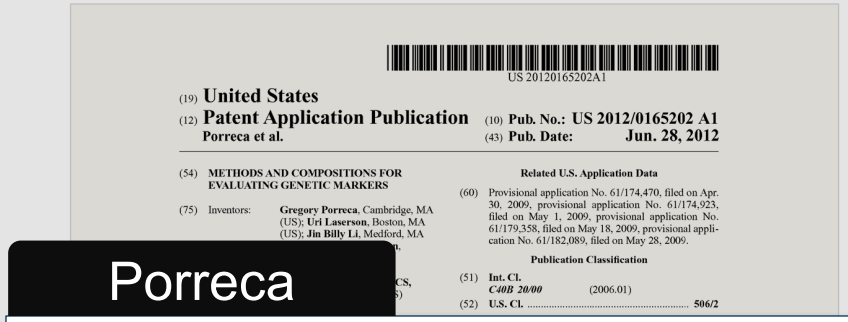
Porreca



EX1006.0005

Paper 2, 17-27; Paper 19, 22-27; Paper 28, 7-10

Porreca Focused on MIP Capture Technology



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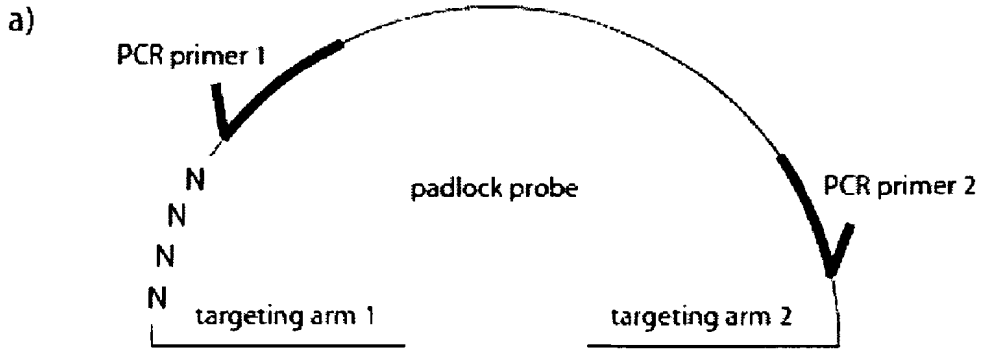
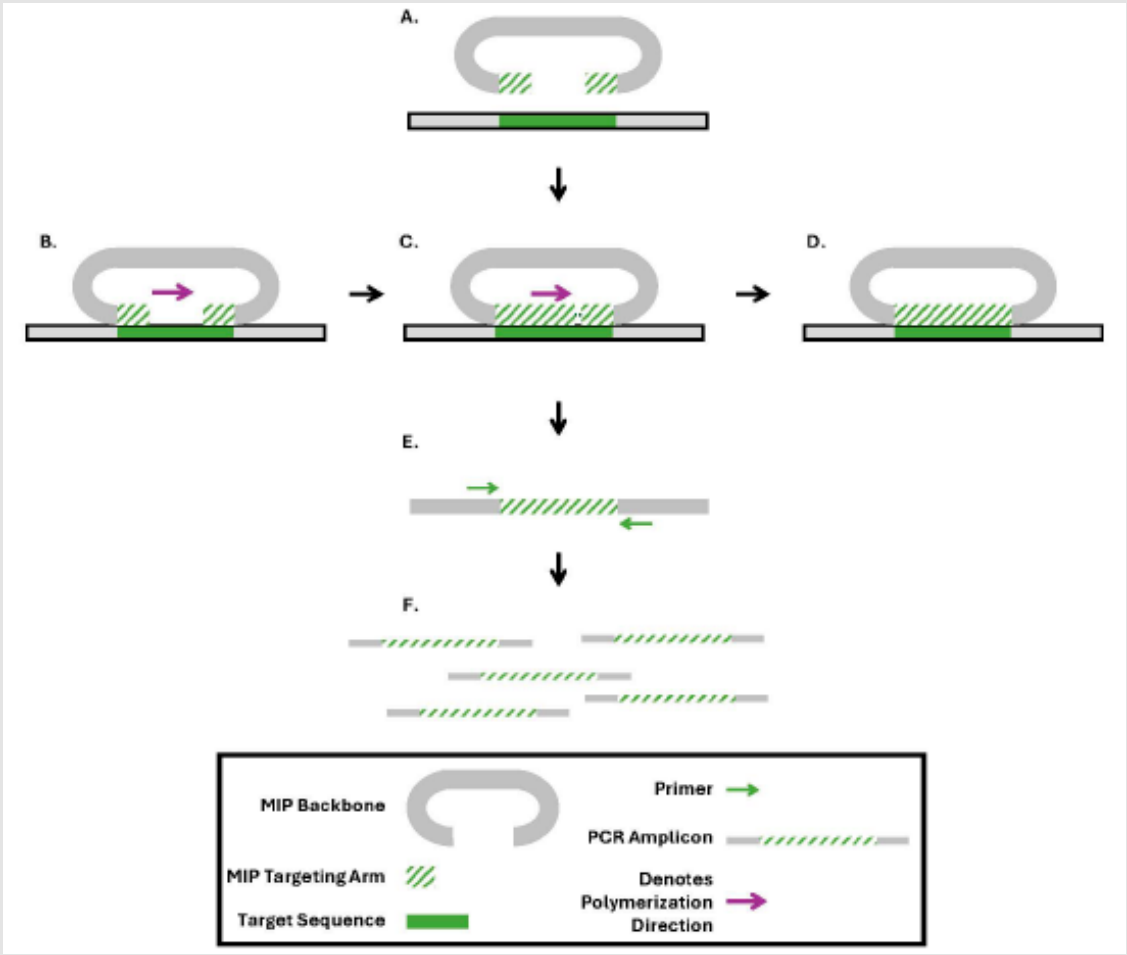
Porreca

FIGURE 4



EX1006.0005



Paper 19, 9-12, 22-27; Paper 28, 9-15

Porreca Only Teaches Using an Indexing Site With the MIP Preparative Method

Example 1

Design a Set of Capture Probes for a Human Target Exon

* * *

Hybridize Capture Probes to Human Genomic Sample

[0213] Assemble hybridization reaction:

* * *

[0234] Amplify Circular Material by PCR Using Primers Specific to the 'Constant Region' of the Probes

* * *

[0248] Prepare a Shotgun Next-Generation Sequencing Library for Analysis

[0249] Purify desired amplicon population from non-specific amplification products by gel extraction.

[0250] Concatemerize amplicons into high-molecular weight products suitable for shearing

[0251] Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.

[0252] Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

Example 2

Use of Differentiator Tag Sequences to Detect and Correct Bias in a MIP-Capture Reaction of a Set of Exon Targets

* * *

[0254] Hybridize Capture Probes to Human Genomic Sample

* * *

[0276] Amplify Circular Material by PCR Using Primers Specific to the 'Constant Region' of the Probes

* * *

[0290] Prepare a shotgun next-generation sequencing library for analysis

[0291] Purify desired amplicon population from non-specific amplification products by gel extraction.

[0292] Concatemerize amplicons into high-molecular weight products suitable for shearing

[0293] Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in lenth.

[0294] Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

Example 6

MIP-Capture Reaction of a Set of Exon Target Nucleic Acids

* * *

[0340] Hybridize Capture Probes to Human Genomic Sample

* * *

[0362] Amplify Circular Material by PCR Using Primers Specific to the 'Constant Region' of the Probes

* * *

[0376] Prepare a Shotgun Next-Generation Sequencing Library for Analysis

[0377] Purify desired amplicon population from non-specific amplification products by gel extraction.

[0378] Concatemerize amplicons into high-molecular weight products suitable for shearing

[0379] Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.

[0380] Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

EX1006.0051-53, 55

Paper 19, 22-27; Paper 28, 9-15

Porreca Does Not Teach the Claimed Arrangement

1. A method for detecting duplicate sequencing reads, the method comprising:

ligating an adaptor to each of a plurality of nucleic acid fragments, wherein each adaptor comprises a unique identifier having from about 1 to about 8 nucleotides, an indexing site unique to a subset of the adaptors, and a primer binding site;

amplifying the adaptor-ligated fragments into amplicons;

sequencing the amplicons to produce sequence reads that include identifier and target sequences; and

identifying sequence reads with identical identifier and target sequences as duplicates.

EX1002.0024

Example 1

Design a Set of Capture Probes for a Human Target Exon

* * *

Hybridize Capture Probes to Human Genomic Sample

[0213] Assemble hybridization reaction:

* * *

[0234] Amplify Circular Material by PCR Using Primers Specific to the 'Constant Region' of the Probes

* * *

[0248] Prepare a Shotgun Next-Generation Sequencing Library for Analysis

[0249] Purify desired amplicon population from non-specific amplification products by gel extraction.

[0250] Concatemerize amplicons into high-molecular weight products suitable for shearing

[0251] Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length.

[0252] Ligate adapters required for amplification by the sequencing platform used. If necessary, purify ligated product from unligated product and adapters.

EX1006.0051-52

Paper 19, 22-27; Paper 28, 9-15

Petitioner Relies on Disclosures That Relate to MIPs

Porreca

[0149] The skilled artisan will appreciate that as part of a MIP library preparation process, adapters may be ligated onto the ends of the molecules of interest. Adapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites. In addition, barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007/0020640 A1 (McCloskey et al.)

EX1006.0028, -0033

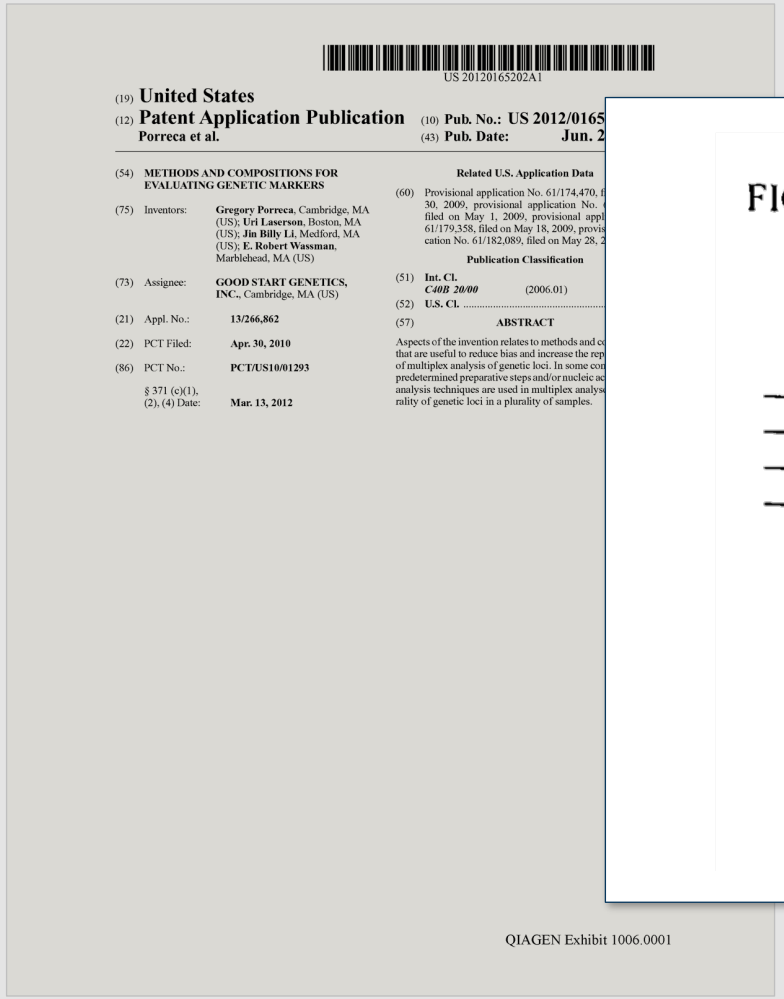
[0102] It should be appreciated that aspects of the invention may be useful for high throughput, cost-effective, yet reliable, genotyping of multiple patient samples (e.g., in parallel, for example in multiplex reactions). In some embodiments,

* * *

more or intermediate numbers of patient samples). It should be appreciated that different embodiments of the invention may involve conducting two or more target capture reactions and/or two or more patient sample analyses in parallel in a single multiplex reaction. For example, in some embodiments a plurality of capture reactions (e.g., using different capture probes for different target loci) may be performed in a single multiplex reaction on a single patient sample. In some embodiments, a plurality of captured nucleic acids from each one of a plurality of patient samples may be combined in a single multiplex analysis reaction. In some embodiments, samples from different subjects are tagged with subject-specific (e.g., patient-specific) tags (e.g., unique sequence tags) so that the information from each product can be assigned to an identified subject. In some embodiments, each of the different capture probes used for each patient sample have a common patient-specific tag. In some embodiments, the capture probes do not have patient-specific tags, but the captured products from each subject may be amplified using one or a pair of amplification primers that are labeled with a patient-specific tag. Other techniques for associating a patient-spe-

Paper 2, 20-21; Paper 19, 23-27; Paper 28, 10-15

Porreca Does Not Disclose the Claimed Method of Identifying Duplicates



US 20120165202A1

(19) **United States**

(12) **Patent Application Publication**

Porreca et al.

(10) **Pub. No.: US 2012/0165**

(43) **Pub. Date: Jun. 2**

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

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(73) Assignee: **GOOD START GENETICS, INC.**, Cambridge, MA (US)

(21) Appl. No.: **13/266,862**

(22) PCT Filed: **Apr. 30, 2010**

(86) PCT No.: **PCT/US10/01293**

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(51) **Int. Cl.**
C40B 20/00 (2006.01)

(52) **U.S. Cl.** .....

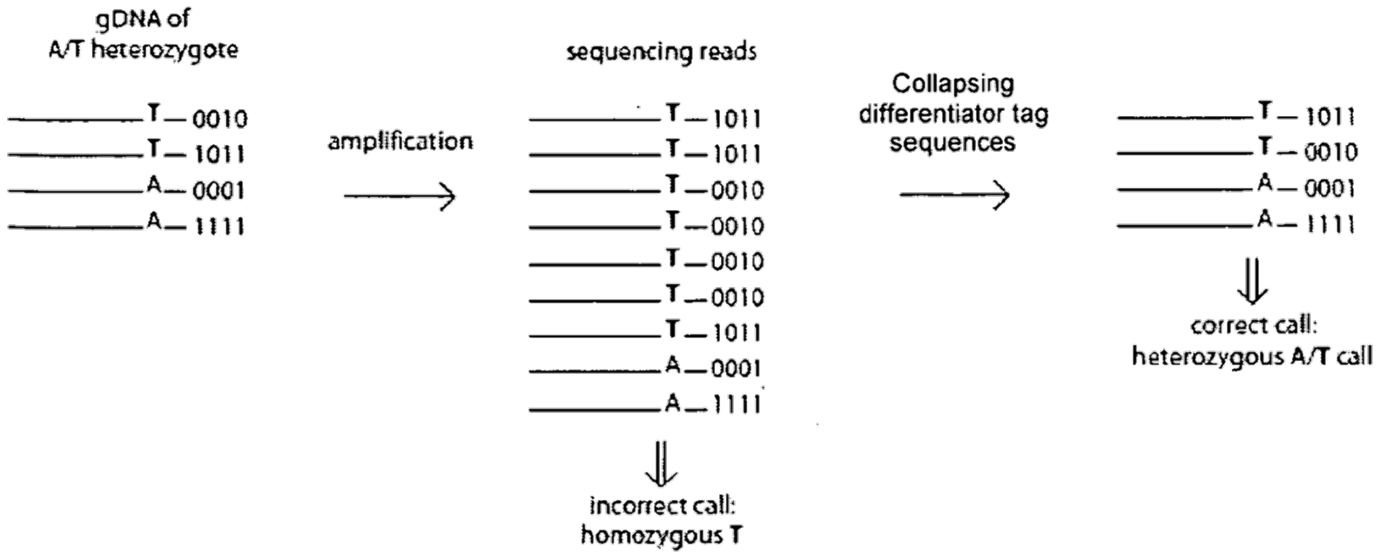
(57) **ABSTRACT**

Aspects of the invention relates to methods and c that are useful to reduce bias and increase the rep of multiplex analysis of genetic loci. In some con predetermined preparative steps and/or nucleic ac analysis techniques are used in multiplex analys rality of genetic loci in a plurality of samples.

QIAGEN Exhibit 1006.0001

Porreca

FIGURE 5



EX1006.0006

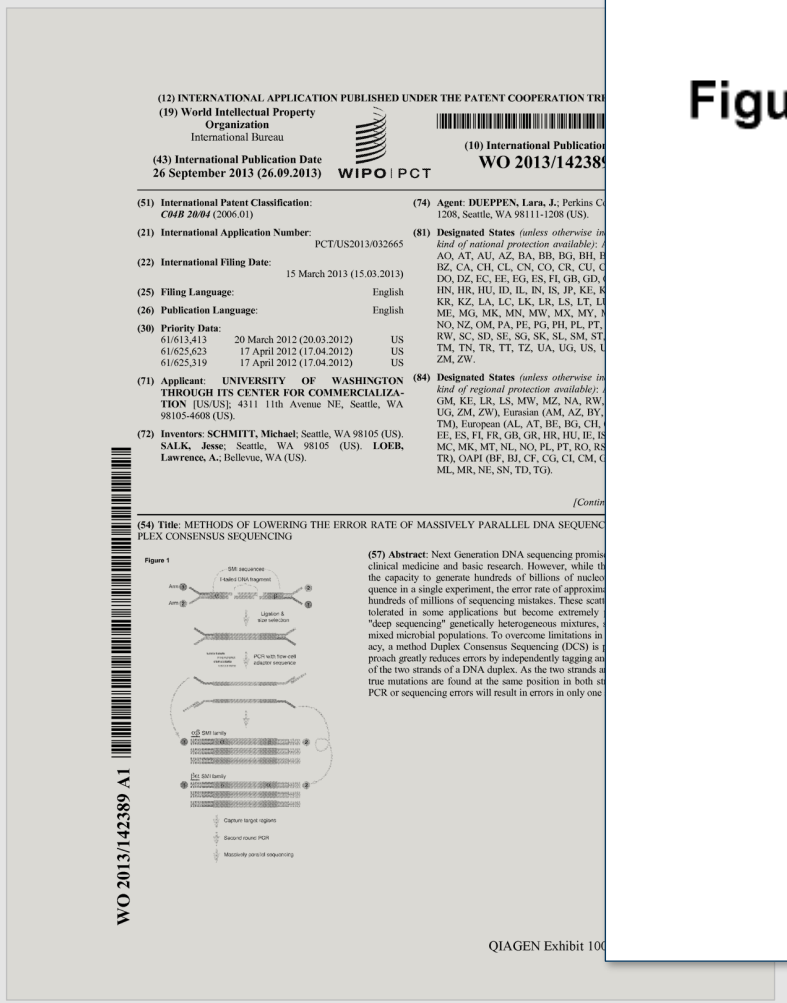
Paper 19, 28-29; Paper 28, 15-16

Petition Fails to Establish Invalidity

- The challenged claims require use of the identifier and target sequence in combination
- Petitioner's Ground 1 fails
  - **Porreca** fails to disclose an embodiment with both limitations 1.a and 1.c
  - **Porreca** does not disclose the claimed method of identifying duplicates
- Petitioner's Ground 2 fails
  - The combination of **Schmitt** and **McCloskey** fails to disclose limitation 1.g
  - Petitioner's motivation to combine **Schmitt** and **McCloskey** is deficient
- Petitioner's Grounds 3-6 fail for the same reasons as Ground 1 and 2

Schmitt Uses Only SMI Tags to Group Reads

Schmitt



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(81) Designated States (unless otherwise in
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KR, KZ, LA, LC, LK, LR, LS, LT, L
ME, MG, MK, MN, MW, MX, MY,
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MC, MK, MT, NL, NO, PL, PT, RO, RS
TR), OAPI (BF, BJ, CF, CG, CI, CM,
ML, MR, NE, SN, TD, TG).

[Contin

(54) Title: METHODS OF LOWERING THE ERROR RATE OF MASSIVELY PARALLEL DNA SEQUENC
PLEX CONSENSUS SEQUENCING

Figure 1

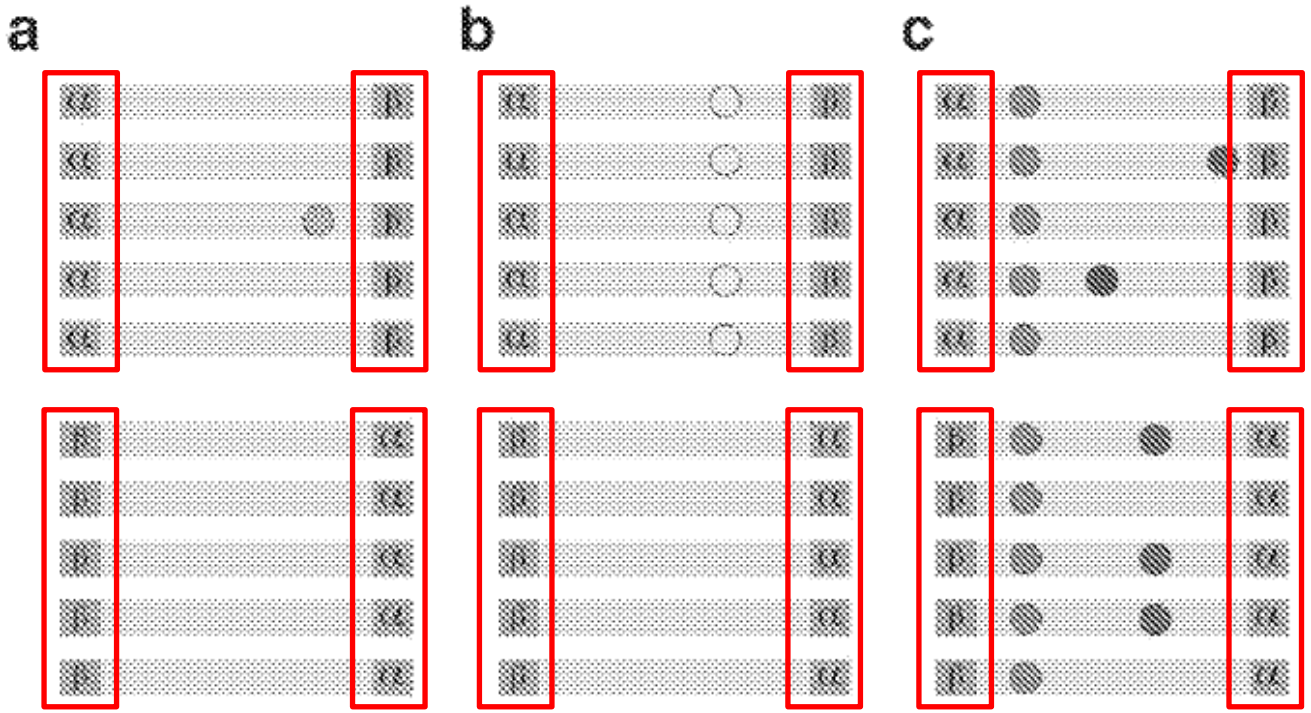
(57) Abstract: Next Generation DNA sequencing promis
clinical medicine and basic research. However, while th
the capacity to generate hundreds of billions of nucleo
quence in a single experiment, the error rate of approxim
hundreds of millions of sequencing mistakes. These scatt
tolerated in some applications but become extremely
"deep sequencing" genetically heterogeneous mixtures,
mixed microbial populations. To overcome limitations in
acy, a method Duplex Consensus Sequencing (DCS) is
proach greatly reduces errors by independently tagging an
of the two strands of a DNA duplex. As the two strands a
true mutations are found at the same position in both st
PCR or sequencing errors will result in errors in only one

WO 2013/142389 A1

QIAGEN Exhibit 100

3/12

Figure 3



EX1007.0060

Paper 19, 30-32; Paper 28, 17-20

Schmitt Identifies Duplicates Using Only SMI Tags

Schmitt

[0085] In another embodiment, the SMI adaptor molecules may be used in methods for unambiguous identification of PCR duplicates. In order to restrict sequencing analysis to uniquely sequenced DNA fragments, many sequencing studies include a step to filter out PCR duplicates by using the shear points at the ends of DNA

* * *

increasingly likely to be seen [48]. Because the use of SMI tags (or "double-stranded SMI sequences") allows every molecule to be uniquely labeled prior to PCR duplication, true PCR duplicates may be unambiguously identified by virtue of having a common (i.e., the same or identical) SMI sequence. This approach would thereby minimize the

EX1007.0029-30

Overview

[0098] To overcome limitations in the sensitivity of variant detection by single-

* * *

Every duplicate that arises from a single strand of DNA will have the same SMI, and thus each strand in a DNA duplex pair generates a distinct, yet related population of PCR duplicates after amplification owing to the complementary nature of the SMIs on the two strands of the duplex. Comparing the sequence obtained from each of the two

[0099] Following tagging with a double-stranded SMI and PCR amplification, a family of molecules is obtained that arose from a single DNA molecule; members of the same PCR "family" are then grouped together by virtue of having a common (i.e., the same) SMI tag sequence. The sequences of uniquely tagged PCR duplicates are

EX1007.0034

Paper 19, 30-32; Paper 28, 17-20

Dr. Metzker's Testimony Is at Odds With Schmitt

Michael Metzker, Ph.D.
Petitioner's Expert

(h) Limitation 1.g

127. Limitation 1.g recites "identifying sequence reads with identical identifier and target sequences as duplicates." In my opinion, the '389 PCT Appl. (Schmitt) discloses this limitation. For example, the '389 PCT Appl. (Schmitt) teaches that identical target sequences that also bear the same SMI sequence are duplicates of one another. For example, the '389 PCT Appl. (Schmitt) teaches that "SMI adaptor molecules may be used in methods ***for unambiguous identification of PCR duplicates***."[194] The '389 PCT Appl. (Schmitt) also explains that duplicate sequence read will have identical identifier and target sequences.

* * *

The '389 PCT Appl. (Schmitt) further explains that duplicate sequence read will have identical identifier and target sequences (*i.e.*, identical shear points).

Paper 28, 19-20

Schmitt

[0014] Figure 3 illustrates error correction through Duplex Consensus Sequencing (DCS) analysis according to one embodiment. (a-c) shows sequence reads (brown) sharing a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the αβ or βα orientation. Each family pair

EX1007.0007

EX1003.¶127
(citing [0085] & [0098])

The Board Should Not Rely on Dr. Metzker's Contradictory Testimony

"[C]onclusory expert testimony is 'particularly problematic in cases where, like here, expert testimony is offered not simply to provide a motivation to combine prior-art teachings, but rather to supply a limitation missing from the prior art'"

Xerox Corp., et al. v. Bytemark, Inc., IPR2022-00624, Paper 9, at 16 (PTAB Aug. 24, 2022)

"[W]e must disregard the testimony of an expert that is plainly inconsistent with the record, or based on an incorrect understanding of the claim[s]."

Tinnus Enters., LLC v. Telebrands, Corp., 733 F. App'x 1011, 1022 (Fed. Cir. 2018)

Paper 28, 19-20

Petitioner's Motivation to Combine Arguments Also Fail

"It is impermissible, however, simply to engage in a hindsight reconstruction of the claimed invention, using the applicant's structure as a template and selecting elements from references to fill the gaps."

In re Gorman, 933 F.2d 982, 987 (Fed. Cir. 1991);
see also InTouch Techs., Inc. v. VGO Commc'ns, Inc., 751 F.3d 1327, 1351 (Fed. Cir. 2014)

Paper 19, 32-34; Paper 28, 20-23

No Motivation When a Reference Criticizes the Claimed Combination

"The district court's reliance on hindsight is further underscored by the prior art's criticism of the one-compartment solution. . . . Because PCT '015 criticizes the use of one compartment to deliver both compounds, the person of ordinary skill would not be motivated to modify PCT '015 to make a one-compartment ring."

Merck Sharp & Dohme B.V. v. Warner Chilcott Co., LLC, 711 F. App'x 633, 637 (Fed. Cir. 2017)

Paper 19, 32-34; Paper 28, 20-23

Schmitt's Inventive Method Improves Upon "Fundamentally Limited" Techniques

Schmitt

[0080] The accuracy of current approaches to next-generation sequencing is limited due to their dependence on interrogating single-stranded DNA. This dependence makes potential sources of error such as PCR amplification errors and DNA damage fundamentally limiting. However, the complementary strands of a double-

* * *

[0081] As described above, one approach that has previously been reported for DNA sequencing involves incorporation of a random tag sequence into a PCR primer [36]. This approach results in an improvement in accuracy relative to standard Illumina sequencing, but is fundamentally limited in that it is based upon amplification and sequencing of single-stranded DNA and thus cannot overcome limitations in sensitivity owing to single-stranded DNA damage events. In the methods described herein, PCR

EX1007.0027-28

Paper 19, 32-34; Paper 28, 20-23

McCloskey Uses a “Fundamentally Limited” Technique

Schmitt

[0006]

* * *

The ability to resolve subpopulations below 0.1%, however, has remained elusive. Although several groups have attempted to increase sensitivity of sequencing, several limitations remain. For example techniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41] have been reported.

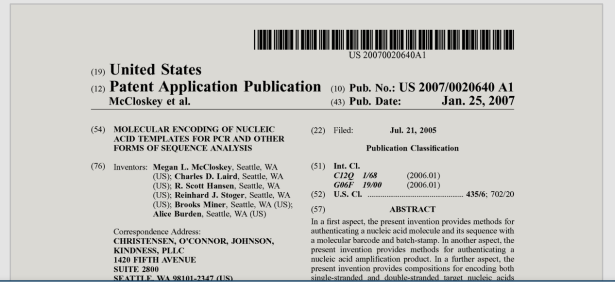
* * *

[34] Miner BE, Stöger RJ, Burden AF, Laird CD, Hansen RS. Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR. Nucleic Acids Research. 2004;32:e135.

[35] McCloskey ML, Stöger R, Hansen RS, Laird CD. Encoding PCR products with batch-stamps and barcodes. Biochem Genet. 2007;45:761-7.

EX1007.0004

McCloskey



(19) United States
(12) Patent Application Publication
McCloskey et al.

(10) Pub. No.: US 2007/0020640 A1
(43) Pub. Date: Jan. 25, 2007

(54) MOLECULAR ENCODING OF NUCLEIC ACID TEMPLATES FOR PCR AND OTHER FORMS OF SEQUENCE ANALYSIS

(22) Filed: Jul. 21, 2005

Publication Classification

(51) Int. Cl.
C12Q 1/68 (2006.01)
G06F 19/00 (2006.01)
(52) U.S. Cl. 435/6; 702/20

(57) ABSTRACT

In a first aspect, the present invention provides methods for authenticating a nucleic acid molecule and its sequence with a molecular barcode and batch-stamp. In another aspect, the present invention provides methods for authenticating a nucleic acid amplification product. In a further aspect, the present invention provides compositions for encoding both

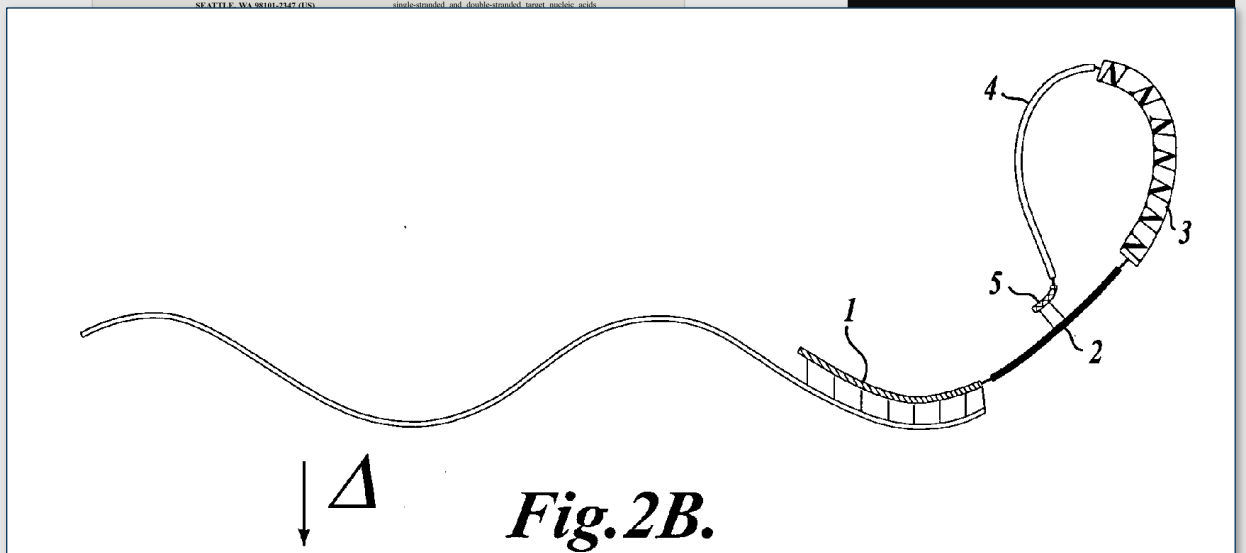


Fig. 2B.

EX1005.0003

Paper 19, 32-34; Paper 28, 20-23

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing **PATENT OWNER'S HEARING DEMONSTRATIVES** was served on February 6, 2026 and February 9, 2026 by email to Petitioner by serving the below counsel of record:

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Dated: February 9, 2026

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