UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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GUARDANT HEALTH, INC.
Petitioner,

v.

COLD SPRING HARBOR LABORATORY
Patent Owner

_____

Case No. IPR2025-____
U.S. Patent No. 10,947,589

_____

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 10,947,589

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Exhibit #	Description
EX1001	U.S. Patent No. 10,947,589 ("the '589 patent")
EX1002	Declaration of Gregory Cooper, Ph.D.
EX1003	Plaintiff's First Amended Complaint for Patent Infringement, *Cold Spring Harbor Laboratory v. Guardant Health, Inc.,* C.A. No. 25-263-JCG (May 1, 2025 D. Del.) (No. 15)
EX1004	Dan Levy et al., *Facilitated sequence counting and assembly by template mutagenesis,* PNAS (October 13, 2014) ("Levy-Wigler")
EX1005	Final Written Decision, *Parse Biosciences, Inc., v. 10X Genomics, Inc.,* IPR2023-00958 (February 3, 2025) (Paper 48)
EX1006	Petition for *Inter Partes* Review of U.S. Patent No. 10,697,013, *Parse Biosciences, Inc., v. 10X Genomics, Inc.,* IPR2023-00958 (May 25, 2023) (Paper 1)
EX1007	Brooks E. Miner et al., *Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR,* 32 Nucleic Acids Research 1-4 (2004) ("Miner")
EX1008	Megan L McCloskey et al., *Encoding PCR Products with Batch-Stamps and Barcodes,* 45 Biochemical Genetics, 761-767 (2007) ("McCloskey")
EX1009	Prosecution File History of U.S. Patent No. 10,947,589
EX1010	U.S. Patent Application 2009/0029377 ("Lo")
EX1011	Rossa W. K. Chiu et al., *Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma,* PNAS (December 23, 2008) ("Chiu")
EX1012	Tian-Li Wang et al*., Digital karyotyping,* PNAS (December 10, 2002) ("Wang")
EX1013	Derek Y. Chiang et al., *High-resolution mapping of copy-number alterations with massively parallel sequencing,* Nature Methods, Vol 6. No. 1 (January 2009) ("Chiang")
EX1014	U.S. Patent No. 7,537,897 ("Brenner")
EX1015	U.S. Patent No. 9,085,798 ("Chee")
EX1016	WO 2010/127186 (filed on April 29, 2010) ("Chee PCT")
EX1017	U.S. Patent No. 11,840,730 ("Porreca")
EX1018	WO 2010/126614 (filed on April 30, 2010) ("Porreca PCT")
EX1019	U.S. Patent No. 8,195,415 ("Quake")

EX1020	Plaintiff's Disclosure of Asserted Claims and Infringement Contentions for U.S. Patent Nos. 10,947,589 and 12,234,510, *Cold Spring Harbor Laboratory v. Guardant Health, Inc.,* C.A. No. 25-263-JCG (July 11, 2025 D. Del.)
EX1021	U.S. Patent No. 8,741,606
EX1022	DE 10 2008 025 656 B4 Translation
EX1023	Curriculum Vitae of Gregory Cooper
EX1024	U.S. Patent No. 10,697,013
EX1025	Decision Granting Petitioner's request on Rehearing of Decision Denying Institution of *Inter Partes* Review, *Parse Biosciences, Inc., v. 10X Genomics, Inc.,* IPR2023-00958 (February 5, 2024) (Paper 9)
EX1026	PCT Patent Publication No. WO 2010/117620 to Linnarsson et al. ("Linnarsson")
EX1027	U.S. Patent Application 2007/0020640 ("McCloskey II")
EX1028	Declaration of Gregory Cooper, Ph.D. In Support of Petitioner for *Inter Partes* Review, *Parse Biosciences, Inc., v. 10X Genomics, Inc.,* IPR2023-00958 (May 24, 2023) (Exhibit 1002)
EX1029	National Human Genome Research Institute, Nucleotide (March 21, 2023) at https://www.genome.gov/genetics-glossary/Nucleotide
EX1030	Gerald Schochetman et al., Polymerase Chain Reaction, 158 J. of Infectious Diseases 1154-1157 (1988) ("Schochetman")
EX1031	National Human Genome Research Institute, Polymerase Chain Reaction (March 21, 2023) at https://www.genome.gov/geneticsglossary/Polymerase-Chain-Reaction
EX1032	Severine A Degrelle et al., Amplification biases: possible difference among deviating gene expressions, 9 BMC Genomics 1-15 (2008) ("Degrelle")
EX1033	H.C. Yang et al., A genome-wide study of preferential amplification/hybridization in microarray-based pooled DNA experiments, 34 Nucleic Acids Research 1-11 (2006) ("Yang")
EX1034	Elaine R. Mardis, The impact of next-generation sequencing technology on genetics, 24(3) Trends in Genetics 133-141 (2008) ("Mardis")
EX1035	Andy Itsara et al., *Population Analysis of Large Copy Number Variants and Hotspots of Human Genetic Disease,* The American Journal of Human Genetics 84, 148-161 (February 13, 2009)

EX1036	Heather C. Mefford et al., *A method for rapid, targeted CNV genotyping identifies rare variants associated with neurocognitive disease,* Genome Research (June 8, 2009)
EX1037	Intentionally left blank
EX1038	Intentionally left blank.
EX1039	Roche Technical Bulletin for Genome Sequencer FLX System, TCB No. 004-2009 (April 2009)
EX1040	DE 10 2008 025 656 B4
EX1041	DE 10 2008 025 656 B4 Translator Certification
EX1042	Juliane C. Dohm et al., Substantial biases in ultra-short read data sets from high-throughput DNA sequencing, Nucleic Acids Research, 2008, Vol. 36, No. 16 e105 (December 21, 2007)

I. INTRODUCTION

Guardant Health, Inc. ("Petitioner") requests *inter partes* review and cancellation of claims 1-18 of U.S. Patent No. 10,947,589 ("the '589 patent"), which is assigned to Cold Spring Harbor Laboratory ("Patent Owner" or "PO").

The '589 patent is entitled "Varietal counting of nucleic acids for obtaining genomic copy number information," and purportedly pertains to determining "copy number information" in a DNA sample. According to PO, copy number information includes things such as "variations in the numbers of copies of particular genes" in a person's genome, which supposedly can be a "key genetic indicator for cancer as well as other disorders." EX1003 ¶ 26.

The claimed method for determining such copy number information is straightforward and obvious, involving little more than sequencing the DNA in a sample and then counting the number of DNA molecules from a location of interest, "thereby obtaining copy number information." *See* EX1001 at claim 1. Nothing could be more obvious than determining how many copies of something are present by counting the number of copies. And, in fact, multiple prior art references disclose precisely this method for genetic copy number, including the Lo reference, which is the primary reference relied upon herein. The '589 patent never suggests that determining copy number information is new or novel.

What, then, is supposedly new in the '589 patent? According to the patent, it is overcoming so-called "amplification distortion," which is an unnatural distortion in the numbers of copies of sample molecules that stems from commonly used procedures, such as PCR, to copy sample molecules for analysis. As the '589 patent states, the "invention described herein may be used to determine the original genome copy number (or the gene expression profile) of a sample without amplification distortion." *Id.* at 28:62-64. The way this is supposedly done, the patent explains, is by modifying the sample molecules with "tags," which then allow one to account for amplification distortion and accurately count the molecules originally present in a sample. *See id.* at 19:19:33-55.

At first blush, this might sound like a nice invention to a non-POSA. The fact is, however, that using "tags" to overcome amplification distortion was standard and had long been used by POSAs. If anything, by characterizing tagging to overcome amplification distortion as the alleged invention, the '589 patent admits unpatentability. In fact, Dr. Michael Wigler, the primary named inventor, admitted in a scientific publication that "[m]odifying molecules to facilitate counting is not a new idea." EX1004 at 1. To support his assertion that the use of tagging to facilitate counting was not new, he cited 10 different references, some of which pre-dated the '589 patent by as much as six years. *Id.*

At bottom, and as Dr. Wigler confirmed, the '589 patent claims nothing more than the obvious application of a well-known technique (*i.e.*, tagging) to address a known issue (*i.e.*, amplification bias when counting molecules to determine copy number information). In fact, ***four*** different prior art references expressly suggested this combination long before the '589 patent. The case for obviousness here is thus unusually compelling.

In this regard, this tribunal has already considered whether tagging for molecular counting to overcome amplification bias was obvious. In IPR2023-00958, the Board considered U.S. Patent No. 10,697,013 ("the '013 patent"), which has claims analogous to those at issue here, reciting the use of "tagged polynucleotides" to "count sample polynucleotides." EX1005 at 4-5; EX1002 ¶¶ 37-41. Critically, the '013 patent has a substantially ***earlier*** priority date than the '589 patent at issue here. After a full review of the merits, the Board found all claims of the '013 patent unpatentable as obvious following the petitioner's arguments that the desire to avoid amplification bias would motivate tagging. EX1005 at 36-37, 51; EX1006 at 35-37. The IPR against the '013 patent relied upon one of the very references relied upon here as disclosing tagging for counting—McCloskey. Because the '589 patent came after the '013 patent, it is even more unpatentable in view of the prior art. The Board should now cancel the challenged claims.

II. MANDATORY NOTICES

A. Real Party-in-Interest

The real party-in-interest is Guardant Health, Inc.

B. Related Matters

As of the filing date of this Petition, the '589 patent is involved in litigation in *Cold Spring Harbor Laboratory v. Guardant Health, Inc.*, 1:25-cv-00263 (D. Del. filed Mar. 6, 2025). Additionally, in parallel with the instant Petition, Petitioner is seeking review of U.S. Patent No. 12,234,510 in a co-pending IPR.

C. Grounds for Standing

Petitioner certifies that the '589 patent is available for IPR and that Petitioner is not barred or estopped from requesting IPR challenging the patent claims on the grounds identified in this Petition.

D. Lead and Back-Up Counsel and Service Information

Petitioner provides the following designation of Lead and Back-Up Counsel.

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III. IDENTIFICATION OF CHALLENGES

Petitioner presents the following challenges:

- **Ground 1**: Claims 1-3, 6-8, 11, and 17-18 are unpatentable as obvious over Lo in view of the knowledge of a POSA, as reflected in McCloskey, Brenner, Chee, and Porreca.
- **Ground 2**: Claim 9 is unpatentable as obvious over Lo in view of the knowledge of a POSA in further view of specific disclosure in Brenner or McCloskey.
- **Ground 3**: Claims 12 and 14-16 are unpatentable as obvious over Lo in view of the knowledge of a POSA in further view of specific disclosure in Brenner, McCloskey, or Porreca.
- **Ground 4**: Claim 13 is unpatentable as obvious over Lo in view of the knowledge of a POSA in further view of specific disclosure in Chee or Porreca.
- **Ground 5**: Claims 4 and 5 are unpatentable as obvious over Lo in view of the knowledge of a POSA in further view of Quake.
- **Ground 6**: Claim 10 is unpatentable as obvious over Lo in view of the knowledge of a POSA in further view of specific disclosure in Chee, Porreca, or Brenner.

IV. TECHNICAL BACKGROUND AND STATE OF THE ART

A. DNA Sequencing, Reference Genomes, And Alignment

Deoxyribonucleic acid ("DNA") is a double-stranded nucleic acid molecule composed of a string of four different monomeric units called nucleotides. EX1002

¶¶ 47-50. The four nucleotides are adenine ("A"), thymine ("T"), guanine, ("G"), and cytosine ("C"). *Id.* ¶ 49. An oligonucleotide, or "oligo," is a short polynucleotide. Oligonucleotides are commonly used as primers with various biological techniques such as DNA sequencing, polymerase chain reaction ("PCR"), and molecular cloning. *Id.* ¶ 48. The two strands of DNA are connected through complementary base pairing via chemical bonds, also known as "hybridization" in which A pairs with T, and C pairs with G. In certain circumstances, *e.g.*, in the presence of certain forms of cancer or fetal congenital disorders, copy number variations ("CNV") can occur, wherein genes are duplicated or deleted, resulting in a deviation from the standard two-copy state.

DNA sequencing is the process of determining the order of the four nucleotides and has been practiced since at least 1977. *Id.* ¶¶ 70-76. In the 2000s, so-called next-generation sequencing ("NGS") methods became commercially available. *Id.* ¶ 71. These methods were distinguished from prior sequencing methods, because they allow scientists and researchers to sequence millions of DNA molecules simultaneously. *Id.* ¶ 72.

Once the sequences of such molecules have been obtained, they can be further analyzed using a so-called "reference genome." *See id.* ¶ 73. A reference genome is a genome sequence from the species that is being analyzed for which the sequence is already known. When sequence information from an unknown sample is

determined, one can compare that unknown sequence information to the reference genome to determine where the unknown sequence came from in the species of the genome. The process of comparing an unknown sequence to a reference genome is known as "mapping" or "alignment."

B. Amplification Bias

DNA sequencing using NGS generally requires a minimum amount of DNA. *Id.* ¶ 51, 72. Accordingly, before sequencing, DNA samples must often first be "amplified," particularly in situations where the samples include limited amounts of DNA. *Id.* ¶ 51. Amplification is simply the process of creating multiple copies of the DNA molecules in a sample so there is more DNA. *Id.* Various amplification methods were well-known in the art before 2011, including, for instance, the polymerase chain reaction ("PCR") method. *Id.* ¶¶ 51-57.

The amplification of DNA samples presented a significant problem for NGS sequencing analysis. *Id.* ¶¶ 65-69. Specifically, not all DNA sequences amplify equally. *Id.* ¶ 65. As a result, after amplification, the relative quantity of DNA molecules may not reflect the distribution of molecules in the original sample pre-amplification. *Id.* ¶¶ 65-66. This clouds one's ability to understand, for example, how much of a particular gene is being expressed in a particular cell of interest or the copy number of a particular gene in the DNA of an input sample. *Id.* Thus, for

studies that require determining the ***quantity*** of molecules that are present in addition the identity, amplification bias can be a particular problem. *Id.*

C. Molecular Tags

As discussed above, researchers faced a problem in NGS workflows: how to obtain accurate molecule counts despite amplification bias. *See supra*. Long before the priority date of the '589 patent, this problem was solved using so called "molecular tags," which are short, synthetic DNA sequences that can be added to individual DNA molecules to act as identifiers. *Id.* ¶¶ 58-64, 67-69.

Molecular tagging to address amplification bias works as follows. Before the DNA molecules in a sample are amplified, they are all randomly given a tag. *Id.* ¶ 67. In the simplest case, each molecule in a sample gets its own tag that is completely different from the tag applied to every other molecule. *Id.* If, for instance, there are two DNA molecules in the original sample that are identical, they will each get their own tag and be rendered distinguishable by virtue of the tag. *Id.* After amplification of the DNA molecules in the sample, there may be multiple copies of a particular tagged molecule, but one will nonetheless be able to tell that all such copies descended from the same single parent molecule in the original sample because they will all share the same tag. *Id.* ¶¶ 67-69. This will allow one to determine precisely how many copies of a particular molecule were in the original sample by counting the number of different tags. *Id.*

The use of tags before amplification was a well-known solution to avoid amplification bias, and this solution is admitted prior art in the '589 patent. *See* EX1001 at 2:12-28. As one of the many prior art examples, consider the 2004 Miner reference, which teaches unique tagging to avoid amplification bias: "Each genomic fragment is marked prior to amplification, allowing us to identify contaminant and redundant sequences and to quantify accurately the proportion of cells carrying a particular sequence variant by counting only distinctly tagged sequences." EX1007 at 1. The patent also cites McCloskey, which describes the advantages in tagging polynucleotides with "nonredundant" barcodes before amplification. As McCloskey states, an "additional benefit of our method for mosaic samples is that counting sequences with nonredundant barcodes provides a quantitative measure of template diversity." EX1008 at 766.

It is hardly surprising that these tagging concepts were so well-known. They amount to little more than the use of labels to keep track of and distinguish different objects—in this case, molecules. In fact, Dr. Michael Wigler, named inventor of the '589 patent admitted in a scientific publication that "[m]odifying molecules to facilitate counting is not a new idea." EX1004 at 1. Dr. Wigler noted that the idea had been applied under a variety of names to identify PCR duplicates and improve counting of DNA templates. *Id.*

This Petition relies upon four references that teach the use of tagging, amplification, and sequencing for molecular counting: McCloskey, Chee, Brenner, and Porreca. As documented herein, each of these references disclose the concept discussed in this section wherein tags are used to confer uniqueness prior to amplification so that any effects of distortion can be overcome.

D. Copy Number

The concept of "copy number" is straightforward. In most cases, individuals have two copies of each gene in their genome, one copy on each chromosome. Yet, in certain situations, a gene, or some other region of a person's genome, can deviate from this norm, and can instead be abnormally duplicated or deleted. The concept of copy number simply pertains to the frequency with which a gene, or some other genetic region, is present in a person's genome. *See* EX1002 ¶¶ 77-79.

V. OVERVIEW OF THE '589 PATENT

The '589 patent, which was filed on March 7, 2016, identifies two provisional applications, the earlier of which was filed on October 22, 2010. EX1001 at (60). Thus, the earliest possible priority date of the '589 patent is October 22, 2010. During prosecution, the examiner made no rejections based on prior art, instead focusing solely on §§ 101 and 112 rejections. *See* EX1009 at 305-316, 591-602. Thus, during prosecution, the claims received no meaningful scrutiny as to whether they are patentable over prior art.

Claim 1 of the ’589 patent, which is reproduced in full in the appendix, can be summarized as reciting the following general steps:

a) Obtaining a sample having “segments” of nucleic acid.

b) “Tagging” the nucleic acid segments to generate “unique tagged nucleic acid molecules.”

c) Subjecting the tagged molecules to PCR amplification.

d) Sequencing the tagged and amplified PCR molecules.

e) Assigning each of the molecules to a location on the genome by mapping the sequence to a reference genome.

f) Counting the number of molecules at the locations to “obtain copy number information.”

EX1001 at claim 1. Thus, claim 1 generally recites a process for determining “copy number information” based on using tagging to aid in counting molecules at locations in a genome. This approach, as well as the features recited in the dependent claims, are obvious in view of the prior art relied upon herein, as proven below.

VI. THE PRIOR ART RELIED UPON HEREIN

A. Lo

1. Lo Is § 102 Prior Art

U.S. Patent Application Publication No. 2009/0029377 (“Lo”) entitled “Diagnosing Fetal Chromosomal Aneuploidy Using Massively Parallel Genomic Sequencing,” was published on January 29, 2009. EX1010. Lo is prior art under

pre-AIA 35 U.S.C. § 102(a) and (b) because it was published at least one year before the earliest claimed priority date of the '589 patent. Lo is analogous art to the '589 patent because it is in the same field of genetic analysis and is directed to the problem of using molecular counting to determine copy number information.

2. Lo Overview

Dr. Cooper provides a detailed summary of Lo, the key aspects of which are enumerated here. EX1002 ¶¶ 80-93. Briefly, Lo teaches a method for determining copy number information based on sequencing DNA from a sample, mapping the sequence reads to a reference genome, and counting the number reads that correspond to a location in the genome. In other words, Lo teaches a method that corresponds to the great bulk of the elements in claim 1 of the '589 patent. *Id.*

Lo's method begins with a biological sample from, for instance, a pregnant female. EX1010 ¶ 54. The sample contains nucleic acid molecules constituting fragments of chromosomes from the fetus and the mother. *Id.* Next, the nucleic acid molecules from the biological sample are sequenced. *Id.* ¶ 55. The sequences of the molecules are frequently referred to in Lo as "sequenced tags" or just "tags." To avoid confusion, it is important to note that Lo's use of the term "tags" in this manner does not connote the "tags" that are discussed elsewhere herein, which are the short DNA molecules that are appended to DNA molecules for identification purposes.

Rather, in Lo, the terms "sequenced tags" or "tags" merely refers to a DNA sequence in the sample that comes from a particular chromosome.

Nevertheless, Lo goes on to explain that the "resultant sequenced reads form a representative profile of the mix of nucleic acid species in the original specimen." *Id.* ¶ 57. Lo teaches that an imbalance in the number of sequence reads coming from a chromosome or chromosome region is indicative of copy number variation and can be inferred from the percentage representation of the sequences from the region of interest relative to the other "sequenced tags" of the specimen. *Id.* ¶ 67. If the percentage is too high, it indicates a copy number alteration.

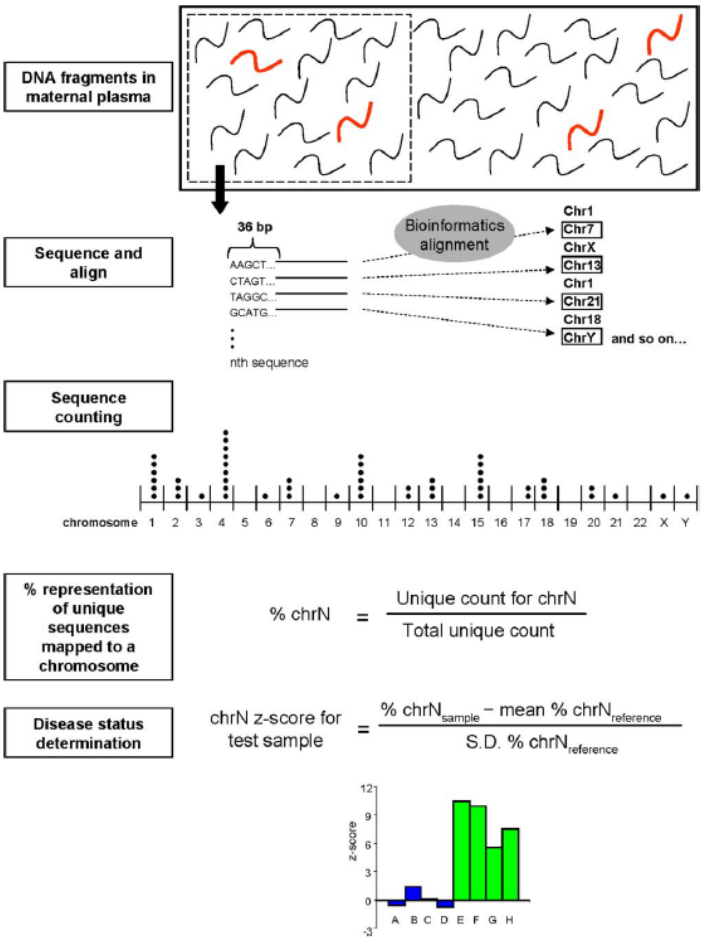
Lo discloses several ways to assess this percentage. *Id.* ¶ 60. These can include "counting the number of sequenced tags, the number of sequenced nucleotides (basepairs) or the accumulated lengths of sequenced nucleotides (basepairs) originating from particular chromosome(s) or chromosomal regions." *Id.* In one embodiment, Lo teaches aligning the sequenced tags to a human reference genome sequence to determine chromosomal origin. *Id.* ¶ 70. This process of "aligning" is nothing more than the process of determining which particular chromosome a DNA sequence came from, and long before the priority date of the '589 patent. EX1002 ¶ 86.

Following alignment, the total number of "sequenced tags" aligned to each chromosome can be tabulated. EX1010 ¶ 70. Lo then teaches determining a ratio

of the number of sequence reads from the chromosome or chromosome region of interest to some other "reference" chromosome or group of chromosomes within the same sample that one expects will not have a copy number change. *Id.* ¶ 62. This ratio may be a percentage of the total number of reads coming from the chromosome or chromosome region of interest relative to all of the other reads. *Id.* ¶ 63.

Finally, this ratio or percentage is compared to one or more cutoff values, which in turn, allows for a determination of whether a copy number change exists for the first chromosome or chromosomal region. *Id.* ¶¶ 64, 66. Lo discloses that the cutoff values are determined using an external standard based on normal genomes. As Lo sates, the "parameter (*e.g.*, the fractional representation of the clinically relevant chromosome) is then compared to a reference range established in pregnancies involving normal (i.e. euploid) fetuses." *Id.* ¶ 65; *see also id.* ¶¶ 70, 95.

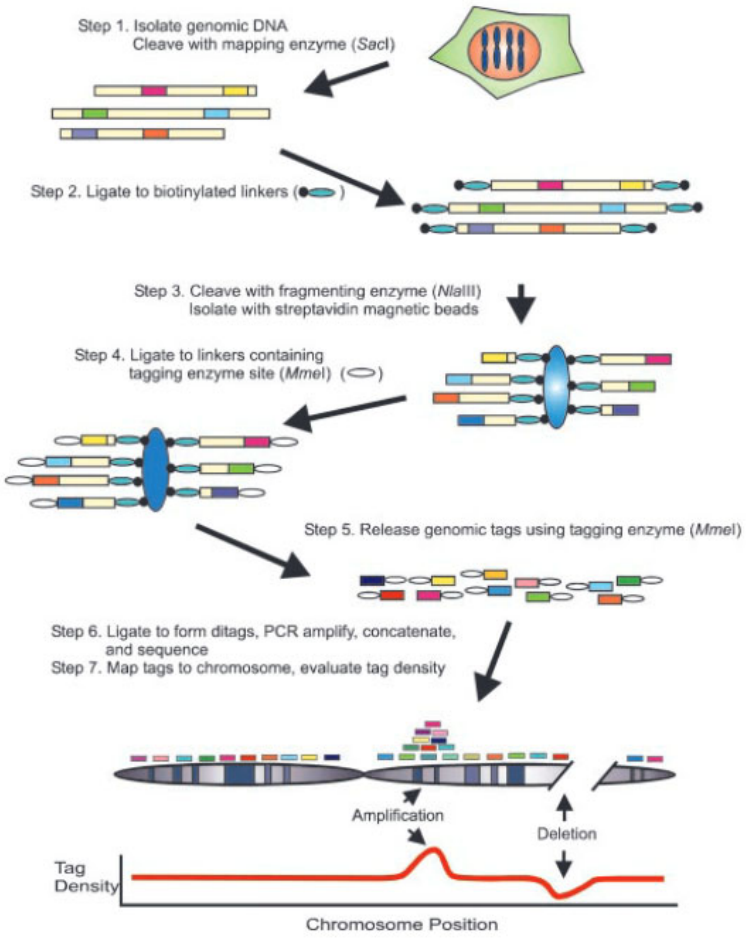
FIG. 1 of a related 2008 publication by Lo (EX1011) neatly explains the Lo method as applied to a sample of DNA from a pregnant woman, which has DNA from both the mother and fetus:



Id. at 20459. Fragments of DNA from the fetus are highlighted in red. From each fragment, 36 basepairs are sequenced. That short sequence is used to identify which chromosome or chromosomal region the fragment comes from. The count of the number of fragments from each chromosome or chromosomal region is compared with the total count of fragments to compute a percentage of sequences mapped to a particular chromosome or chromosomal region. That calculated percentage is compared against the percentage for that chromosome or chromosomal region in a reference standard genome. Samples containing several standard deviations more

chromosomal content than the reference genome are likely aneuploidy and are highlighted in green.

While the examples and embodiment in Lo focuses on the use of so-called "cell free DNA" that is found in the blood of pregnant women, the basic counting method described in Lo was standard and had been previously applied straightforwardly to other sources of DNA to detect copy number information. EX1002 ¶¶ 89-93. For example, Wang et al. (EX1012) describes a tagging and sequencing method to detect copy number changes called "digital karyotyping." Wang and co-workers described characterizing "210,245 genomic tags from the lymphoblastoid cells of a normal individual (NLB) and 171,795 genomic tags from the colorectal cancer cell line (DiFi)." *Id.* at 16158. In step 7 of FIG. 1, reproduced below, an abnormal number of sequences mapped to a chromosomal region indicates amplification while an absence indicates deletion:



Id. at 16157. The bottom of the figure with the chart labeled "Tag Density" clearly illustrates how Wang's method identifies copy number information (either an amplification or a deletion) by looking for imbalances in the number of sequence reads mapping to a region of a chromosome.

As another example, a 2009 paper by Chiang et al. (EX1013) titled "High-resolution mapping of copy-number alterations with massively parallel sequencing" describes a similar segmentation algorithm to identify copy number variations using a variable window size. *Id.* at 100-101. This method was applied to sequencing data

"from three tumor cell lines…and their matched normal cell lines." *Id.* at 101. Sequencing counts in a given genomic window were compared between the tumor and reference samples to identify "194 copy-number alterations in the HCC1954 cell line, 126 alterations in the HCC1143 cell line and 15 alterations in the NCI-H2347 cell line." *Id.* Notably, Chiang, via citation to the Dohm publication, flagged PCR bias as a potential problem for copy number determination by read counting. *See* EX1042 at 8.

Thus, while Lo focuses primarily on applications where the sample was cell-free DNA from pregnant women, there is nothing about Lo's method that would limit it to such applications. EX1002 ¶¶ 89-93. In fact, a POSA would immediately recognize and understand that Lo's method is applicable to other sources of DNA and had, in fact, been applied to other traditional DNA sample types that were not cell-free DNA well before Lo's patent publication. *Id.*

B. McCloskey, Brenner, Chee, and Porreca

As Dr. Cooper explains, McCloskey, Brenner, Chee, and Porreca each teach molecular counting based on tagging, amplification, and sequencing. *See* EX1002 ¶¶ 94-125. The disclosure in these references about tagging to distinguish molecules and enable more accurate counting reflects the common knowledge of the POSA before the priority date, as lead inventor Michael Wigler confirmed. *See id.* ¶¶ 64, 94-125; EX1004 at 1. Each of these references are analogous to the '589 patent

because, as documented herein, they are all in the field of genetic analysis using sequencing and address the problem of molecular counting using sequencing.

Each of McCloskey, Brenner, Chee, and Porreca are prior art:

- U.S. Patent No. 7,537,897 ("Brenner") entitled "Molecular Counting" was published on May 26, 2009. EX1014. Brenner is thus prior art under pre-AIA 35 U.S.C. § 102(a) and (b).
- McCloskey et. al., "Encoding PCR Products with Batch-Stamps and Barcodes," ("McCloskey") is an article that was published in Biochemical Genetics on October 23, 2007. EX1008. McCloskey is thus prior art under pre-AIA 35 U.S.C. § 102(a) and (b).
- U.S. Patent No. 9,085,798 ("Chee") entitled "Nucleic Acid Constructs and Methods of Use" was published on July 21, 2015. EX1015. Chee issued from U.S. Patent Application No. 13/266,568, which is the national phase entry of International PCT Application No. US2010/033064, filed on April 29, 2010. Chee and this PCT share a specification, and the PCT designated the United States and was published in English. EX1016. As such, Chee is prior art under pre-AIA 35 U.S.C. § 102(e).
- U.S. Patent No. 11,840,730 ("Porreca") entitled "Methods and Compositions for Evaluating Genetic Markers" was published on December 12, 2023. EX1017. Porreca claims the benefit of PCT Application No. US2010/001293, filed on April 30, 2010. Porreca and this PCT share a specification, and the PCT designated the United States and was published in English.

EX1018. Porreca is therefore prior art under pre-AIA 35 U.S.C. § 102(e).

C. Quake

U.S. Patent No. 8,195,415 ("Quake") entitled "Noninvasive Diagnosis of Fetal Aneuploidy by Sequencing" issued on June 5, 2012 from U.S. Patent Application No. 12,696,509, filed on January 29, 2010 and published on June 3, 2010. EX1019. Quake is thus prior art under pre-AIA 35 U.S.C. § 102(e).

Quake discloses a method similar to Lo based on determining an imbalance in the number of sequence reads in a genomic region . EX1002 ¶¶ 126-133. The difference between Quake and Lo has to do with the standard that is used to assess whether a sample has a copy number variation. Lo's method relies upon an external standard derived from samples that are normal in that they do not have a copy number variation. Quake's method, in contrast, does not require the use of an external standard and instead uses an internal standard. *Id.* ¶¶ 128-132. Quake is analogous prior art because it pertains to genetic analysis using sequencing and addresses the problem of using molecular counting to determine copy number information.

VII. CLAIM CONSTRUCTION

For purposes of this Petition, Petitioner interprets the claims consistent with PO's arguments and positions in the parallel district court proceeding. EX1020. Specifically, for purposes of this Petition, Petitioner requests that the Board

understand the claims consistent with the apparent interpretations that PO is pursuing in parallel district court litigation, particularly in view of its contentions regarding how the '589 patent is allegedly infringed. *See Hospira, Inc. v. Amgen Inc.*, IPR2021-00528, Paper 7 at 7 (PTAB Aug. 17, 2021) ("all that rule [42.104(b)(3)] requires is for the Petition to identify '[h]ow the challenged claim is to be construed'"). The portions of PO's infringement contentions illustrating their claim interpretations are cited herein where appropriate.

VIII. LEVEL OF ORDINARY SKILL IN THE ART

As explained by Dr. Cooper, a person of ordinary skill in the art ("POSA") relating to the '589 patent would have had (1) a master's degree and/or Ph.D. in molecular biology, genetics, chemistry, engineering, or a related discipline and at least two years of post-master's, postdoctoral, or industry experience or (2) a Bachelor of Science in such disciplines and at least five years of industry or academic experience (including any experimental work toward a graduate degree) relating to DNA labeling techniques, DNA amplification methods, and DNA sequencing technologies. EX1002 ¶¶ 43-44. Further, a POSA would have been familiar with associated tools, methods, and techniques including: (1) DNA tagging, (2) amplification, (3) use of tags to overcome amplification bias, (4) sequencing, (5) aligning and counting of sequence reads. *Id.* ¶ 45.

IX. IDENTIFICATION OF HOW THE CHALLENGED CLAIMS ARE UNPATENTABLE

Lo teaches the same general approach set forth in claim 1 of the '589 patent, wherein one determines copy number information by sequencing genomic segments, mapping the sequences to a reference genome, and then determining copy number information by counting sequences for the genomic region of interest.

To the extent any aspect of the independent claims is missing in Lo, it is tagging the DNA sequences to generate unique tagged nucleic acid molecules followed by amplification of these tagged nucleic acid molecules. This, however, was well-known to the skilled artisan and was a basic technique in his or her toolkit to improve molecular counting. Using tagging and amplification with Lo to enhance the accuracy of the counting process would have been the simple application of a known method to improve an existing process based on molecular counting. As such, claim 1 of the '589 patent is obvious. And, as to the dependent claims, they recite features that are already part of Lo's method or that are otherwise similarly obvious techniques that a POSA would have been motivated to use with a reasonable expectation of success. *See* EX1002 ¶¶ 134-137.

A. Ground 1: Claims 1-3, 6-8, 11, And 17-18 Are Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA, As Reflected In McCloskey, Brenner, Chee, And Porreca

1. Claim 1

a. [1A] A method comprising: a) obtaining segments of genomic nucleic acids from a sample;

Lo teaches a "biological sample" that "may be plasma, urine, serum, or any other suitable sample" will include "fragments from chromosomes." EX1010 ¶ 54. According to Lo "the term 'biological sample' as used herein refers to any sample that is taken from a subject which contains one or more nucleic acid molecule(s) of interest." *Id*. ¶ 33; *see also id.* ¶ 83("In one embodiment, random sequencing is performed on DNA fragments that are present in the plasma of a pregnant woman, and one obtains genomic sequences which would originally have come from either the fetus or the mother."); EX1002 ¶¶ 138-141.

b. [1B] tagging the segments with nucleic acid tags, thereby generating a plurality of unique tagged nucleic acid molecules, such that each of the unique tagged nucleic acid molecules comprises a segment of the segments of the genomic nucleic acids from step (a) and a nucleic acid tag of the nucleic acid tags;

To the extent Lo does not teach tagging to generate "unique tagged nucleic acid molecules," it would have nonetheless been obvious to perform this step in combination with Lo in view of the knowledge of the skilled artisan, as reflected in McCloskey, Porreca, Chee, or Brenner. *See* EX1002 ¶¶ 142-165. *See Koninklijke Philips N.V. v. Google LLC*, 948 F.3d 1330, 1338 (Fed. Cir. 2020) (The "Board did

not violate § 311(b) or the inter partes review statute in determining that the claims would have been obvious over SMIL 1.0 in light of the general knowledge of a skilled artisan.").

As Dr. Cooper explains, as of the earliest priority date of the '589 patent, it was well-known and routine in the art to attach tag sequences to nucleic acid fragments prior to amplification to obtain accurate copy number information and minimize bias and distortion. EX1002 ¶¶ 29-32, 65-69, 143. Indeed, the '589 patent admits as much in its discussion of Miner, McCloskey, and Brenner. *See* EX1001 at 2:1-28. Consistent with this, each of Brenner, Chee, McCloskey, and Porreca describe tagging for molecular counting in conjunction with amplification, reflecting the general knowledge of the skilled artisan as to tagging for molecular counting. *See* EX1002 ¶¶ 144-153.

- McCloskey teaches a tag with five distinct informational regions, including a "random barcode" that "distinguishes among sequences arising from different cell or allele copies." EX1008 at 762. As Dr. Cooper explains, the "random barcode" is the same type of tag in the '589 patent that distinguishes among molecules and allows one to count molecules accurately after amplification, which is what McCloskey is referring to when it mentions using the barcodes to "detect template redundancy" and being able to measure "sequence diversity"/"template diversity." *Id*. at Abstract, 766; *see also* EX1002 ¶¶ 146-147. Allowing one

to distinguish sequences from different "cell or allele copies," McCloskey's "random barcode" creates the claimed "unique tagged nucleic acid molecules."

- Porreca discloses "differentiator tag sequences to ***uniquely tag individual nucleic acid molecules***."[1] EX1017 at 5:4-5. This creates the claimed "unique tagged nucleic acid molecules" and, as Porreca explains, permits "the detection of bias based on the occurrence of combinations of differentiator tag and target sequences observed in a sequencing reaction." *Id.* at 5:3-8, 23:63-67. As Porreca explains, to count molecules, the "number of each differentiator tag sequence is counted, for example, by next-generation sequencing." *Id.* at 60:65-67; *see also id*. at 61:8-10; EX1002 ¶¶ 148-149.

- Chee teaches tagging "regions of interest coupled with ***unique identifiers***," and hence teaches "unique tagged nucleic caid molecules." EX1015 at Claim 1, 23:2-3, 24:59-63. These unique identifiers, Chee explains, can be used to identify "technical bias" in the count of regions of interest because the "unique identifier will be amplified with the ROI of each construct and thus the number of starting molecules can be inferred by association of each initial ROI present in a sample with its unique identifier." *Id.* at 10:42-46; EX1002 ¶¶ 150-151.

1 Emphasis supplied unless otherwise noted.

- Finally, Brenner teaches methods "for counting molecules in a sample, wherein each molecule is labeled with a ***unique oligonucleotide tag***," and hence also teaches "unique tagged nucleic acid molecules." EX1014 at Abstract. The tags, Brenner teaches, can be used for molecular counting, because each of the DNA molecules "of a particular genetic locus in a sample of genomic DNA, is associated with a probe having a different tag, so that the process of counting multiple copies of the same molecule is transformed into a process of counting the number of different kinds of associated tags." *Id.* at 5:16-23; *see also id.* at 9:23-24 (disclosing a process for using binary tag counting for estimating DNA fragment copy numbers); EX1002 ¶ 152.

As Dr. Cooper explains, McCloskey, Porreca, Chee, and Brenner demonstrate that it was well-known and routine to tag nucleic acid fragments to generate unique tagged nucleic acid molecules. *Id.* ¶¶ 143, 153. As Dr. Cooper further explains, for several reasons a POSA would have been motivated to use this technique with Lo.

First, the method of tagging for counting molecules was part of the standard tool kit of a POSA as of the priority date of the '589 patent. It offered a known solution to a known problem: the need to improve the accuracy of counting molecules, particularly in situations where few sample DNA molecules are provided. A POSA would have been motivated to use the tagging in McCloskey, Porreca, Chee, and Brenner, since this technique was one of only a small number of options available for improving the accuracy of counting and was well within the technical

grasp of a skilled artisan. *Id.* ¶¶ 154-155. As the Supreme Court has explained, a person of ordinary skill "has good reason to pursue the known options within his or her technical grasp" such that § 103 bars the patentability of obvious variations. *KSR Intl. Co. v. Teleflex Inc.*, 550 U.S. 398, 421 (2007); *see also Recor Med., Inc. v. Medtronic Ireland Mfg. Unlimited Co.,* No. 2023-2251, 2025 WL 944511, at *4 (Fed. Cir. Mar. 27, 2025).

Second, adding tagging to Lo would be a natural and obvious choice for a POSA. Lo is based on counting, and therefore a POSA would naturally think to include tagging as an extension of Lo's method because tagging was specifically intended for improving the accuracy of molecular counting. EX1002 ¶ 156. *Google LLC v. Sonos, Inc.*, No. 2023-1259, 2024 WL 2350509, at *3 (Fed. Cir. May 23, 2024) (affirming finding of obviousness where "[n]ot only was there knowledge of the problem and motivation to solve it, but…Baker and McMillin have similar network structures,…both networks…would suffer from the same problem, and that McMillin disclosed solutions to the collision problems").

Third, and consistent with the foregoing, multiple references mentioned above expressly contemplate using tagging to determine copy number information. Brenner discusses using tagging and subsequent amplification to for the "measurement of genetic copy number variation." EX1014 at 4:6-7. Porreca likewise discloses "detecting nucleic acid deletions or insertions in regions

containing nucleic acid sequence repeats" for cancer detection "which has been associated with micro satellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci." EX1017 at 55:48-50, 56:54-58. Yet additional prior art references expressly contemplate using tagging and amplification with counting for determining copy number information. *See* EX1021 at Abstract, 1:55-2:6, 21:62-65, 5:56-6:46; *see also id.* at 18:30-49 (explaining that the disclosed method can be used to "increase the confidence in calling copy number variations"); *id.* at 21:62-65; EX10222 ¶ 2 ("determining the frequency of copies of gene segments for karyotyping is an important diagnostic option for diseases such as cancer); *id.* ¶ 13 ("The task is solved by labeling all nucleic acids of a nucleic acid mixture, regardless of whether they are double-stranded or single-stranded, with a 'random tag' (RAT) derived from a mixture of synthetic oligonucleotides, wherein the mixture contains sufficient RATs with different sequences to ensure that the resulting random tag nucleic acid (RAT-NS) combinations are practically unique."). Thus, the prior art includes at least four references with an express teaching to include tagging with an approach for determining copy number variation based on molecular counting, as is taught in Lo. EX1002 ¶ 157.

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2 Certified translation of German original EX1040. *See* EX1041.

Fourth, adding tagging would help solve the signal-to-noise challenge described in Lo. Lo explains that "fetal DNA is present in low absolute concentration" in maternal plasma, creating a low signal problem. *See* EX1010 ¶ 13. A POSA would have been motivated to combine the tagging techniques known in the art with the amplification techniques described below in step [1C] to increase the signal-to-noise ratio and thereby improve the sensitivity and accuracy of detecting aneuploidy described in Lo. EX1002 ¶ 158.

A POSA would have had a reasonable expectation of successfully using tagging with Lo because the machinery for preparing and attaching tags was well-known and straightforward. *See id.* ¶¶ 159-165. A POSA would know, for instance, that it is straightforward to design tag sequences to achieve the claimed uniqueness. Brenner, for instance, describes using oligonucleotide tagging sets that are "preferably at least ten times the number of target polynucleotides to be counted." EX1014 at 25:36-46. Brenner similarly contemplates that the tags should be "maximally different," *id.* at 21:14-18, and teaches well-known ways of achieving this, including through "distance measures" known in the art for designing such sequences. Brenner cites several references to this effect. *Id.* at 25:58-66. Chee and Porreca likewise disclose creating a "large library of unique differentiator tag sequences…using degenerate, random-sequence polynucleotides of defined length."

EX1017 at 29:37-39; EX1015 at 11:26-38. Thus, methods were available for preparing a large number of oligonucleotide tags.

So too were methods for attaching tags. *See* EX1002 ¶¶ 162-163. For example, Porreca describes combining tags with target sequences. *See, e.g.*, EX1017 at FIG. 4A-4C. Brenner likewise details a procedure for attaching an oligonucleotide tag to one end of a polynucleotide, including methods for attachment chemically or enzymatically. EX1014 at FIG. 2A-2B. Moreover, since the '589 patent admits that various methods of tagging existed in the art, there would be a reasonable expectation of success here for a POSA. *See* EX1001 at 2:20-22 (describing labeling by ligation); *id.* at 2:23-26 (describing labeling through specific primers); *id.* at 21:38-40 ("Tags may be associated with nucleic acid molecules by using, singly or in combination, ligation, nick-ligation, terminal transferase, hybridization, priming, and the like.").

Further, as noted above, the '589 patent characterizes the Miner and McCloskey methods based on tagging and amplification as successfully implemented prior art methods, a point that confirms reasonable expectation of success. Likewise, inventor Michael Wigler admitted in a scientific publication that "[m]odifying molecules to facilitate counting is not a new idea." EX1004 at 1. He noted that this had been previously done under a variety of names to identify PCR duplicates and improve counting of DNA templates. *Id.* This confirms reasonable

expectation of success with respect to not just tagging, but also amplification of tagged molecules, as discussed in the claim element immediately below.

c. [1C] subjecting the plurality of the unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating amplified tagged nucleic acid molecules;

As explained by Dr. Cooper, a POSA would have found it obvious to include amplification of tagged molecules with the molecular counting method of Lo for determining copy number information. *See* EX1002 ¶¶ 166-176.

As an initial matter, tagging and amplification for molecular counting was part of the standard toolkit of a POSA, as reflected in McCloskey, Porreca, Chee, and Brenner:

- McCloskey tags the relevant DNA molecules with a random barcode after which "standard PCR amplification" is used. EX1008 at 766.
- Porreca teaches tagging DNA fragments, followed by amplification and then counting of the tagged reads, all for the purpose of reducing amplification bias, noting that PCR- based techniques are common for amplification. *See* EX1017 at FIG. 5; *id.* at 15:64-16:1-2, 58:43-49, 58:50-53.
- Brenner teaches amplification after sequence tagging, stating for instance that "oligonucleotide tags may be amplified using emulsion PCR, e.g. as also disclosed in the cited references, so that clonal populations of each oligonucleotide tag of a sample

are formed on beads, which are then sequenced." EX1014 at 17:53-55; *see also id.* at 17:14-20 (explaining that it is "desirable to be able to PCR the tags"); *id.* at Example 1, 29:27-54.

- Finally, Chee further teaches amplification after tagging, explaining that the tagging enables one to overcome any bias that may arise from the amplification. As Chee states, his methods "can identify technical bias in, e.g., amplification, as the unique identifier will be amplified with the ROI of each construct and thus the number of starting molecules can be inferred by association of each initial ROI present in a sample with its unique identifier." EX1015 at 10:41-46; *see also id.* at 15:64-66.

A POSA would have been motivated to use amplification of tagged molecules for several reasons. Most important, amplification is part and parcel of the methods in Brenner, McCloskey, Chee, and Porreca, which is all reflective of the knowledge of the skilled artisan. EX1002 ¶¶ 171. As Dr. Cooper explains, there is no reason to utilize tagging without subsequent amplification because the whole point of the tagging is to permit one to subsequently perform amplification and hence ensure that one captures all the molecules of interest while maintaining an avenue to avoid amplification bias. *Id.* Thus, all the reasons set forth above in connection with element [1B] as to why a POSA would be motivated to apply tagging to Lo's method apply equally to the use of amplification.

Further, exemplary samples in Lo's method were bodily fluids from a subject, such as blood or urine samples. EX1010 ¶ 14. As Dr. Cooper explains, and as Lo confirms, with such samples there is a limited amount of DNA overall and even less DNA from the fetus that one is interested in testing. EX1002 ¶ 172; s*ee also id.* ¶¶ 13, 16, 18. Therefore, a POSA would have naturally been motivated to perform amplification to increase the overall amount of DNA for testing. *See* EX1002 ¶ 172.

Yet additional motivation to utilize amplification is present within Lo itself. Specifically, Lo teaches enriching nucleic acid sequences prior to sequencing and the motivation for doing so, namely for cost-saving and efficiency:

- Lo teaches that in "some embodiments of the present invention, the random sequencing may be preceded by procedures to enrich a biological sample with particular populations of nucleic acid molecules sharing certain common features." EX1010 ¶ 47.
- Lo explains that it would be "possible and cost-effective to enrich the pool of nucleic acids to be sequenced prior to random sequencing of a fraction of the enriched pool." *Id.* ¶ 77.
- Lo teaches that since the fetal DNA found in the maternal plasma is generally of shorter length than the maternal chromosomal fragments, one method to accomplish enrichment would be to "fractionate the nucleic acid sequences in the sample according to molecule size, e.g. by gel electrophoresis or size exclusion columns or by microfluidics-based approach." *Id.* ¶ 72.

- Lo also teaches the use of hybridization techniques to accomplish enrichment of potentially aneuploid chromosomes and one or more normal chromosomes "for example onto oligonucelotide microarrays," followed by random sequencing. *Id.* ¶ 79. This is a known cost-saving technique to those skilled in the art. EX1002 ¶ 173.
- Lo describes that pre-sequencing enrichment in fetal cell free DNA could be accomplished through suppression of the maternal DNA relative to fetal DNA with the use of chemical treatments such as formaldehyde. EX1010 ¶ 72.
- Lo teaches the concept of sub-selecting some desired sequences from the pool of nucleic acids prior to sequencing. "For example, hybridization based techniques such as oligonucleotide array could be used to first sub-select for nucleic acid sequences from certain chromosomes, e.g. a potentially aneuploid chromosome and other chromosome(s) not involved in the aneuploidy tested. Another example is that a certain sub-population of nucleic acid sequences from the sample pool is sub-selected or enriched prior to sequencing." *Id.*

The enrichment taught by Lo is an improvement over previous whole-genome sequencing methods and is well-known to those of skill in the art, providing enhanced efficiency and cost-savings, as described above. The amplification as taught by McCloskey, Brenner, Porreca or Chee is another form of enrichment, and

therefore provides the same benefit but with the additional increase in accuracy by virtue of the tagging. *See* EX1002 ¶¶ 173-175.

Further, a POSA would have had a reasonable expectation of success in amplifying tagged nucleic acids because amplification was so routine and widely used. EX1002 ¶ 176. The '589 patent acknowledges PCR, for instance, as a well-known prior art method. Specifically, the specification explains that "[g]enerally…the [amplification] steps are performed multiple times (e.g., polymerase chain reaction)." EX1001 at 13:50-54; *see also id.* at 1:37-2:28 (describing prior art methods employing PCR). The Abstract also references "subjecting the tagged nucleic acid molecules to amplification by polymerase chain reaction." In other words, the '589 patent characterizes PCR as a well-known prior method that people of skill in the art had no trouble utilizing. EX1002 ¶ 176.

d. [1D] generating tag associated sequence reads by sequencing the amplified tagged nucleic acid molecules of step (c);

As discussed above in connection with claim elements [1C] and [1D], a POSA would have been motivated to utilize tagging and amplification of sequences in connection with Lo's method for the detection of copy number variation. Lo further discloses sequencing the nucleic acid molecules for the purpose of determining copy number information, and a POSA would have been motivated to perform such

sequencing of tagged and amplified molecules with Lo's method. *See* EX1002 ¶¶ 177-182.

As Dr. Cooper explains, Lo's method is a sequencing-based method for molecular counting. *Id.* ¶¶ 178-180. For example, in Experiments I and II, and as represented in FIG. 6, Lo teaches that sequencing of DNA fragments was used to demonstrate the presence of copy number information from chromosome 21. EX1010 at FIG. 6, ¶ 112. The only difference between this claim step and the method in Lo is that this claim step involves sequencing tagged and amplified nucleic acid molecules whereas Lo directly sequences the original sample molecules.

A POSA would be motivated to combine the well-known tagging and amplification method (as reflected in McCloskey, Brenner, Chee, and Porreca) as described above in claim elements [1B] and [1C] with Lo's sequencing methods for all the reasons stated above in connection with these two claim elements. Further, a POSA would have had a reasonable expectation of successfully sequencing such tagged and amplified molecules because, as Dr. Cooper explains, the relevant sequencing techniques were well-known methods at the priority date. *See* EX1002 ¶¶ 181-182. Indeed, Lo itself shows that the sequencing method for determining copy number information was a technique well-known to a POSA.

e. [1E] assigning each of the amplified tagged nucleic acid molecules to a location on a reference genome from the same species from which the sample has been obtained by mapping each of the tag associated sequence read of step (d) to a location of the reference genome; and

As Dr. Cooper explains, Lo teaches this claim element, which generally pertains to aligning the sequence reads to a reference genome. *See* EX1002 ¶¶ 183-188. Lo teaches, for instance, that "based on the sequencing (e.g. data from the sequencing), a first amount of a first chromosome (e.g. the clinically relevant chromosome) is determined. The first amount is determined from sequences identified as originating from the first chromosome. For example, a bioinformatics procedure may then be used to locate each of these DNA sequences to the human genome." EX1010 ¶ 58; *see also id.* ¶ 70. The Lo reference further provides, in Example I, experiments wherein maternal plasma DNA was collected from women with aneuploid and euploid pregnancies. After DNA from each maternal plasma specimen was sequenced, "[s]equenced reads were compiled using Solexa Analysis Pipeline. All reads were then aligned to the repeat-masked reference human genomic sequence, NCBI 36 assembly." *Id.* ¶ 93. *See also id*. at Figure 4A (showing percentage alignment of maternal plasma DNA for euploid v. aneuploid pregnancies).

As Dr. Cooper explains, the only difference between this claim step and the method set forth in Lo is that this claim step involves mapping tagged and amplified

nucleic acid molecules, whereas Lo aligns the sequences of the original sample molecules. Yet, as set forth above in connection with elements [1B] and [1C], a skilled artisan would have found it obvious to include tagging and amplification with Lo's method. Having been motivated to modify Lo's method to use tagging and amplification, a POSA would have necessarily sequenced and then aligned the sequences of the tagged and amplified molecules, according to the exact methods and techniques set forth in Lo as summarized above. *See supra* §§ VI.A.2, IX.A.1.b-c; EX1002 ¶ 188. A POSA also would have had a reasonable expectation of successfully doing this given that alignment methods were well-known and routine. Lo even reports actual data showing that it was easily achievable to align sequence reads and use the aligned sequence reads to determine copy number information. EX1010 ¶¶ 98, 102, FIG. 4A.

f. [1F] at a plurality of locations on the reference genome, counting the number of unique tagged nucleic acid molecules that have been assigned to the same location on the reference genome based on the mapping step to obtain a count for each location of the plurality of locations on the reference genome, thereby obtaining copy number information from genomic nucleic acids in the sample.

As Dr. Cooper explains, Lo teaches this claim element. *See* EX1002 ¶¶ 189-197. Lo discloses "counting the number of sequenced tags, the number of sequenced nucleotides (basepairs) or the accumulated lengths of sequenced nucleotides (basepairs) originating from particular chromosome(s) or chromosomal regions."

EX1010 ¶ 60. Referring to counting the number of mapped reads in "chromosomal regions," Lo teaches counting nucleic acid molecules that have been assigned the same location at a plurality of locations in the reference genome. Lo's mapping to a "chromosomal region" is consistent with PO's broad reading of "location" in the parallel district court litigation. *See* EX1020 (interpreting locations as any of "genes," "genetic locus," and "start/stop positions"). Nevertheless, this count of molecules in a region represents "copy number information."

Lo, however, further discloses how to utilize the counted sequence tags to derive yet additional copy number information, specifically an estimation of copy number. EX1002 ¶¶ 190-193. Lo teaches, for instance, considering a "random representative fraction of DNA molecules from the specimen pool" and then comparing the "number of different sequenced tags aligned to various chromosomal regions…between specimens containing or not containing the DNA species of interest. Chromosomal aberrations would be revealed by differences in the number (or percentage) of sequences aligned to any given chromosomal region in the specimens." EX1010 ¶ 108. Lo explains that one type of clinically relevant sequence that one may identify is "copy number variations." *Id.* ¶ 37. Thus, Lo's method is based on counting nucleic acid molecules to estimate copy number.

Moreover, Lo teaches that the counting for this purpose can be done at a "plurality of locations" in the reference genome. Specifically, Lo teaches that the

analysis can be done at a plurality of chromosomes or a plurality of regions in the genome. *See id.* ¶ 109 ("Changes (amplifications and deletions) in multiple chromosomal regions may be used. Thus, there would be an increased proportion of sequences aligned to the amplified regions and a decreased proportion of sequences aligned to decreased regions."); *see also id.* at claim 5, ¶¶ 14, 67, 71, 108 (all teaching analysis of multiple chromosomes or regions).

As Dr. Cooper explains, a skilled artisan would understand that the only difference between this claim step and the method set forth in Lo is that this claim step involves counting "unique tagged nucleic acid molecules," whereas Lo counts original sample molecules. EX1002 ¶¶ 195-196. Yet, as set forth above for elements [1B] and [1C], a skilled artisan would have found it obvious to include tagging and amplification with Lo's method. Having been motivated to modify Lo's method to use tagging and amplification, a POSA would have naturally counted the "unique" molecules. Indeed, as explained above in connection with steps [1B] and [1C], the reason for using tagging and amplification is to increase the accuracy of the counting, which is accomplished by employing the tags to identify and count only the unique molecules, as is done in each of Chee, Brenner, McCloskey, and Porreca. To count anything other than the unique molecules would defeat the purpose of using the tagging and amplification that the skilled artisan would have been motivated to use.

In this regard, even in Lo's method, he proposed to only count the molecules that could be uniquely mapped to any single, specific location. *See, e.g.*, EX1010 ¶¶ 85, 112. In this way, Lo avoided including sequence reads that might erroneously overinflate the count of sequence reads mapped to a particular region. A skilled artisan using tagging and amplification in Lo's method would have naturally followed this guidance and used only the unique sequence reads rather than counting duplicates and hence overinflating read counts.

2. Claim 2: The method of claim 1, further comprising estimating a copy number at multiple locations within a region of the reference genome.

Lo teaches the additional features of claim 2, or, at a minimum, renders them obvious.

As explained above in connection with claim 1 and by Dr. Cooper, Lo teaches that counting for determination of copy number can be done at a plurality of chromosomal regions, and hence teaches estimating copy number of at multiple locations (*e.g.*, regions of a chromosome) within a region of the reference genome (*e.g.*, a chromosome). *See, e.g.*, EX1010 ¶ 14 ("One or more cutoff values are chosen for determining whether a change compared to a reference quantity exists (i.e. an imbalance), for example, with regards to the ratio of amounts of two chromosomal regions (or sets of regions"); *id.* ¶¶ 67, 71, 108; EX1002 ¶¶ 198-203. Using a chromosome as a region is consistent with PO's interpretation of "region" in the

parallel district court litigation as a section of the genome comprising multiple genes. *See* EX1020.050 (discussing "the copy number of different genes, which are located at different locations in a given region of the reference genome.").

A POSA would have at a minimum found this claim element obvious in view of Lo. As noted above, Lo explicitly contemplates analyzing ***plural*** "chromosomal regions." Lo's method is based on determining an imbalance in the amount of DNA arising from a region of the genome, and this approach is not limited to a particular chromosome, as is clear from Lo. EX1002 ¶ 200. A POSA would have found it obvious to analyze multiple discrete regions of a particular chromosome and would have been motivated to do so because, as Dr. Cooper explains, there were several known copy number variations that existed on a single chromosome that were of clinical relevance. *Id.* ¶¶ 200-202. There would be a reasonable expectation of success because the method would simply involve counting at multiple locations instead of a single location. *See id.* ¶ 203.

3. Claim 3: The method of claim 1, further comprising comparing a count from counts obtained in step (f) for a location on the reference genome to a count for the same location on the reference genome obtained from a reference sample, thereby estimating a relative copy number at the location.

As Dr. Cooper explains, Lo in combination with the knowledge of the skilled artisan teaches and renders obvious claim 3. EX1002 ¶¶ 204-209.

Lo discloses determining a parameter from "first amounts" and "second amounts" of sequenced chromosomes, *e.g.* as a ratio or sum of the amounts, and then comparing the parameter to a pre-determined "cutoff value." Lo defines "cutoff value" as a "numerical value whose value is used to arbitrate between two or more states (e.g. diseased and non-diseased) of classification for a biological sample." EX1010 ¶ 44. As explained above in connection with claim element [1F] and by Dr. Cooper, these cutoff values are based on comparison to counts obtained from the "same location" on a "reference sample." *See supra* § IX.A.1.f; EX1002 ¶¶ 191-192, 205-209. These are used to determine, for instance, relative copy number at the location.

4. Claim 6: The method of claim 1, further comprising (l) summing the counts from the counts obtained in step (f) for locations at a region of the reference genome; (m) summing the counts obtained from a reference sample for the same region of the reference genome; and comparing the sums of the counts obtained in step (l) and step (m), thereby estimating a relative copy number at the region of the reference genome.

As described above for claims 1-3, Lo teaches an approach for determining the existence of chromosomal imbalance in a biological sample obtained from, for instance, a pregnant female, using a "cutoff value[]" to determine whether a "change compared to a reference quantity exists… for example, with regards to the ratio of amounts of two chromosomal regions (or sets of regions)." EX1010 ¶ 14.

Specifically, Lo describes determining the percentage of sequences reads in a region of interest from the sample and comparing this percentage to that observed in a normal sample. *See id.* ¶ 99. This approach entails the "summing" of claim 6. *See* EX1002 ¶¶ 210-213. This is because the percentage involves summing counts from regions of interest. Indeed, the percentage is nothing more than an expression of the count of sequences at a region normalized to a total. As set forth in ¶ 99 of Lo, this percentage from the sample is compared to a normal by virtue of taking the difference between the sample and normal percentage and assessing how wide of a gap there is in terms the number of standard deviations in the distribution of percentages observed in normal samples.

5. Claim 7: The method of claim 1, wherein the unique tagged nucleic acid molecules are subject to a hybrid capture method prior to PCR in step (c) or the amplified tagged nucleic acid molecules produced in step (c) are subject to a hybrid capture method prior to step (d).

As Dr. Cooper explains, Lo further teaches the features of claim 7. *See* EX1002 ¶¶ 214-217. Specifically, Lo teaches subselection of nucleic acid molecules prior to sequencing, and states that "hybridization based techniques such as oligonucleotide array could be used to first subselect for nucleic acid sequences from certain chromosomes, e.g. a potentially aneuploid chromosome and other chromosome(s) not involved in the aneuploidy tested." EX1010 ¶ 72.

Further, Lo establishes why a POSA would be motivated to carry out the hybrid capture of claim 7, and that there would be a reasonable expectation of success. As Lo explains, "only a portion of the human genome needs to be sequenced to differentiate trisomy 21 from euploid cases. Thus, it would be possible and cost-effective to enrich the pool of nucleic acids to be sequenced prior to random sequencing of a fraction of the enriched pool." *Id.* ¶ 77; *see also id.* ¶ 79. Thus, as Lo makes clear, a POSA would be motivated to perform advance enrichment because this would reduce the cost of the sequencing and, as Lo teaches, there would be a reasonable expectation of success because this would have simply involved the use of existing oligonucleotide array technologies.

6. Claim 8: The method of claim 7, wherein each of the unique tagged nucleic acid molecules subjected to the hybrid capture method has a nucleic acid sequence which is complementary to a capture probe on an array or on a microarray.

For the same reason as explained above with respect to claim 7, this claim is rendered obvious by the disclosure of Lo in view of the knowledge of the skilled artisan. *See* EX1002 ¶¶ 218-220.

As Dr. Cooper explains, the hybrid capture method described in claim 7 and disclosed by Lo works by isolating and enriching specific target DNA sequences of a sample through binding with probes that are complementary to the sequences of interest. *Id.* ¶ 219. Further, as noted above in connection with claim 7, Lo explicitly contemplates that this be performed through "hybridization based techniques such

as oligonucleotide array." *See* EX1010 ¶ 72. This is hybrid capture using an array, as recited in claim 8.

7. Claim 11: The method of claim 1, wherein each of the tagged nucleic acid molecules comprise more than one tag.

As Dr. Cooper explains, Lo in view the knowledge of the skilled artisan, as reflected in McCloskey, Chee, Brenner, and Porreca renders this claim obvious. *See* EX1002 ¶¶ 221-229. First, each of McCloskey, Chee, Brenner, and Porreca teach the use of multiple tags:

- McCloskey teaches the use of an "encoding oligonucleotide" containing multiple types of tags that are included in 4 distinct "informational regions" attached to each sequence of interest: EX1008 at 762. *See also* McCloskey, P. 762.
- Chee explains that the "use of two or even more unique identifiers can allow the use of shorter individual identifiers and/or two or more smaller 'subsets' of constructs due to the uniqueness conferred by the combination of the two tags." EX1015 at 20:4-15.
- Brenner uses a method described as "indexing" in conjunction with his counting approach. Indexing sis used to "analyze multiple samples using high throughput DNA sequencing instruments, where tags from different patients have different indexing sequences." EX1014 at 21:20-32. Brenner's teaching of using "indexing" would be understood by the skilled artisan as a disclosure of using multiple tags (*i.e.*, one tag distinguishes

molecules and is used for counting, and a second tag is the "index" that tracks the sample source). EX1002 ¶ 225.

- Porreca, citing McCloskey, states that "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.)." EX1017 at 31:5-9. Thus, Porreca, like both Brenner and McCloskey, contemplates using a second tag to track sample source in addition to a tag for distinguishing identical molecules. *See* EX1002 ¶ 226.

In light of Lo, a POSA would be motivated to more than one tag for several reasons. *See id.* ¶ 227. For instance, a POSA would be motivated to use multiple tags so that one can not only distinguish fragments prior to amplification, but also to distinguish DNA arising from different samples. This would enable one to combine multiple samples into a single run on the sequencing instrument. *See id.* Further, the use of multiple tags allows one to effectively increase the number of tags by enabling the possibility of combining individual tags in combinations, as explained by Chee. This allows one to better ensure that sample molecules receive tags that can truly distinguish among identical molecules and improve the counting accuracy. *Id.*

For the same reason as explained above for claim 1, there would be a reasonable expectation of being able to successfully use multiple tags, because the

basic machinery for tagging was well known and had been documented in several publications. *See supra* § IX.A.1.b; EX1002 ¶ 228.

8. Claim 17: The method of claim 1, wherein the genomic nucleic acids are obtained from blood or other bodily fluids.

Lo teaches, for example, an experiment wherein blood was collected from subjects after which the maternal plasma was harvested, and the DNA extracted from the samples. EX1010 ¶ 92. Further, Lo discloses using plasma, urine, or serum as a sample from which DNA is obtained. *Id.* ¶¶ 13-14, 106. Indeed, the examples and embodiments in Lo are generally based on the analysis of samples from blood or other bodily fluids. *See* EX1002 ¶¶ 230-232.

9. Claim 18: The method of claim 1, wherein the genomic nucleic acids are obtained from tumor cell DNA in a bodily fluid

Lo teaches an embodiment wherein "the cutoff value is determined at least in part on the percentage of tumor sequences in a biological sample, such as plasma, serum, saliva or urine, which contains a background of nucleic acid sequences derived from the non-malignant cells within the body." EX1010 ¶ 52. As Dr. Cooper explains, the analysis of tumor sequences in samples such as "plasma, serum, saliva or urine," refers to the analysis of genomic nucleic acids from a tumor cell DNA in a bodily fluid. EX1002 ¶¶ 233-235.

B. Ground 2: Claim 9 is Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA In Further View Of Specific Disclosure In Brenner Or McCloskey

1. Claim 9: The method of claim 1, wherein each of the tagged nucleic acid molecules differs at more than one nucleotide.

A POSA would understand that Lo in view of the knowledge of the skilled artisan renders obvious claim 1. Claim 9 is rendered obvious in view of specific disclosure in Brenner or McCloskey. EX1002 ¶¶ 236-241.

As Dr. Cooper explains, Brenner teaches that tags should be selected "to be maximally different" so that they can be "distinguished even in the presence of sequencing errors." EX1014 at 21:14-18; *see also id.* at 25; 37:48-52; EX1002 ¶ 237. Calling for the tags to be "maximally different," Brenner unambiguously discloses tagged nucleic acid molecules that differ at more than one nucleotide. Indeed, the claimed difference of merely one nucleotide is the minimum possible difference between nucleic acid sequences, and Brenner calls for the maximum possible difference.

McCloskey teaches that "[e]ach encoding oligonucleotide is unique because of distinct batch-stamp and random barcode regions." EX1008 at 762. Calling for each tag to be "unique" due to the combination of "distinct" batch-stamp and random barcode, McCloskey contemplates nucleic acid molecules that are different by more than one nucleotide, particularly because the possibility of the combined batch stamp and random barcode being identical save for a one-nucleotide difference is exceedingly slim. EX1002 ¶ 238.

A POSA would have been motivated to use tagged nucleic acid molecules that differ by more than one nucleotide because by making the tags differ by more than a single nucleotide, one is more likely to ensure that the tags can be distinguished from one another despite potential sequencing errors. *Id.* ¶ 239. Brenner, for instance, teaches precisely this, stating that "[p]referably, sequences of oligonucleotide tags are selected so that they can be distinguished even in the presence of sequencing errors. Thus, oligonucleotide tags (4120) are selected to be maximally different." EX1014 at 21:14-18.

Further, a POSA would have easily had a reasonable expectation of success in making the tagged nucleic acid molecules differ by more than one nucleotide. EX1002 ¶ 240. Indeed, Brenner teaches that there were well known ways of making the tags "maximally different." Brenner explains, for instance, that there were "distance measures" known in the art for designing such sequences. Brenner cites several literature references to this effect, stating "exemplary distance measures for sequences applicable to the invention are well known and are widely disclosed in the literature, as exemplified by the [disclosed] references. *Id.* at 25:58-66.

C. Ground 3: Claims 12 and 14-16 Are Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA In Further View Of Specific Disclosure In Brenner, McCloskey, Or Porreca.

1. Claim 12: The method of claim 1, wherein the tags further comprise a sample tag.

As Dr. Cooper explains, as of the '589 patent priority date, "sample tags" were well-known and used regularly to keep track of which DNA molecules came from which sample. EX1002 ¶¶ 242-249. Each of Brenner, McCloskey, and Porreca disclose this:

- Brenner discloses "indexing," explaining that "[s]uch indexing can also be used to analyze multiple samples using high throughput DNA sequencing instruments, where tags from different patients have different indexing sequences." EX1014 at 21:29-32; EX1002 ¶ 245.
- McCloskey teaches using a "batch stamp" alongside the "barcode" to identify specific samples. As McCloskey explains, the "assigned batch stamp is registered for a specific sample run on a specific date and is used only once." EX1008 at 764; *see also id.* at 761, 763; EX1002 ¶ 246.
- Porreca teaches that "[i]n some embodiments, one or more of the techniques described herein may be combined in a single assay protocol for evaluating multiple patient samples in parallel." EX1017 at 21:4-6. To this end, Porreca explains that "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.)." *Id.* at 31:5-9.

A POSA would be motivated to utilize sample tags with Lo's method. Lo's approach seeks to identify fetal chromosomal defects in samples of maternal blood through nucleic acid amplification, sequencing and subsequent counting of fragments. Providing a means to distinguish samples from each other through the use of tagging would permit someone to more efficiently analyze multiple samples at the same time on the sequencer. EX1002 ¶ 248. As reflected in Brenner, McCloskey, and Porreca the use of sample tags in this manner was a standard technique at the time and, accordingly, a POSA would have had a reasonable expectation of successfully using sample tags with Lo's method. *Id.*

2. Claim 14: The method of claim 1, wherein the unique tagged nucleic acid molecules are pooled with another plurality of unique tagged nucleic acid molecules having a different sample tag prior to the PCR or prior to said sequencing of the amplified tagged nucleic acid molecules of step (c).

As Dr. Cooper explains, claim 14 recites nothing more than the standard manner of using a "sample tag" with sequencing, as explained above in connection with claim 12. *Id.* ¶¶ 250-251. For the same reasons that claim 12 would have been obvious, so too would claim 14 have been obvious. *Id.*

3. Claim 15: The method of claim 14, further comprising deconvoluting the tag associated sequence reads by grouping the tag associated sequence reads.

Claim 15 recites nothing more than the standard manner of using a "sample tag" with sequencing, as Dr. Cooper explains above in connection with claim 12. *Id.* ¶¶ 252-253. For the same reasons that claims 12 and 14 would have been obvious, so too would claim 15 have been obvious. *Id.*

4. Claim 16: The method of claim 1, further comprising obtaining copy number information from genomic nucleic acids of different species in a population using the method of claim 1, and determining a relative count of the genomic nucleic acids of the different species in a population by comparing the copy number information obtained from the genomic nucleic acids of the different species.

As Dr. Cooper explains, in a population that includes different species, one may wish to determine the relative genomic copy number of multiple species in the population and compare the copy numbers in those species. *Id.* ¶¶ 254-256. A skilled artisan would have been motivated to perform this task and would have had a reasonable expectation of successfully doing so given the skilled artisan's knowledge regarding sample tagging, as reflected by the disclosure of Brenner, McCloskey, and Porreca.

Specifically, given a POSA's knowledge of sample tagging, a POSA confronted with a multi-species population would have understood that he or she could easily analyze multiple species in the population at the same time using the available sequencing technology by using sample tagging. *Id.* This would have made the process more efficient and less costly, and a POSA would have had a

reasonable expectation of successfully using tags for this purpose because it was a standard approach that had been widely used. In short, claim 16 is obvious for the same reason that claims 12 and 14-15 are obvious. *Id.*

D. Ground 4: Claim 13 is Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA In Further View Of Specific Disclosure In Chee Or Porreca

1. Claim 13: The method of claim 1, wherein the tagged nucleic acid molecules generated from step (b) and that differ by a single nucleotide are ignored during or after the counting step.

As discussed above, Lo in view of the knowledge of the skilled artisan renders obvious claim 1. As Dr. Cooper explains, claim 13 is further obvious in further view of specific disclosure in Chee or Porreca. *Id.* ¶¶ 257-266.

Claim 13 generally pertains to the concept of insisting upon a high degree of consensus in deciding which sequence reads to count. As the '589 patent explains, "[t]hrough sequence error during processing, the tag may very occasionally mutate, creating the appearance of a new tagged nucleic acid molecule. But if the set of tags is sufficiently large, the tagged nucleic acid molecules will generally differ at more than one nucleotide, and so tagged nucleic acid molecules that differ by a single nucleotide can (optionally) be ignored when counting." EX1001 at 23:19-25.

In other words, when there is sequencing error, two sequences that ought to be the same can nonetheless appear different. The '589 patent contemplates eliminating sequences that are out of the ordinary relative to other sequences. This

concept of generating a consensus sequence in this manner was previously taught in both Porreca and Chee.

Porreca teaches that specificity in detecting mutant alleles may be increased by ignoring small differences between sequenced molecules, i.e. a method of tuning the level of stringency in deciding whether to keep a sequence depending on how many reads must match each other. Porreca describes algorithms for stringency tuning based on knowledge of "whether the given position has any known disease-associated alleles" and reducing stringency in such positions by "***decreasing the minimum number of observed mutant reads necessary to make a consensus base-call***." EX1017 at 65:62-66:2. *See also id*. at 77:66-67; 78:1-8. As Dr. Cooper explains, Porreca discloses a protocol for stringency tuning in which one decides whether to keep certain sequence reads based on how they differ from other reads in a set of pre-defined parameters. EX1002 ¶¶ 260-261.

As Dr. Cooper further explains, Chee also discloses methods for tuning the stringency to allow identification of correct sequences from a master set or the correct ROIs from those that contain "errors due to technical realities of chemical synthesis, amplification, and the like" and identification of amplification bias in a sample. EX1015 at 10:33-46; EX1002 ¶¶ 262-263. Thus, Chee, like Porreca, teaches deciding whether to keep certain sequence reads based on how they differ from other reads. EX1002 ¶ 264.

A POSA would have been motivated to apply these methods in Chee and Porreca with tagging and ignore certain reads that differ from one another by a single nucleotide because it would improve the accuracy of the counting process. *Id.* ¶ 265. Specifically, where sequence information that ought to be the same is nonetheless different, it indicates that a sequencing error may have occurred and calls into question the veracity of the read. Eliminating reads that differ by a single nucleotide is a stringent standard that would allow one to only include reads that are most likely to be true and correct, a process that would improve the overall accuracy of the counting process. Those of skill in the art would have had no trouble implementing such a procedure because it amounts to nothing more than comparing sequence reads and eliminating reads that differ from what is expected. Accordingly, there would have been a reasonable expectation of successfully implementing Chee and Porreca's error correction method with Lo. *Id.*

E. Ground 5: Claims 4 And 5 Are Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA In Further View Of Quake

1. Claim 4: The method of claim 1, further comprising g) summing counts obtained in step (f) for locations on a first region of the reference genome, wherein the first region comprises more than one location; h) summing counts obtained in step (f) for locations on a second region of the reference genome, wherein the second region comprises a number of locations which is comparable to the number of locations of the first region; i) comparing the counts obtained in step (g) to the counts obtained in step (h), thereby estimating a relative copy number at the first region of the reference genome to the copy number at the second region of the reference genome.

Lo in view of the knowledge of the skilled artisan renders obvious claim 1. Claim 4 would have been obvious in view of further disclosure in Quake.

As Dr. Cooper explains, the embodiments and examples in the Lo reference focus primarily on approaches where one assesses copy number variation in a sample of interest by comparison to information from normal samples. *See id.* ¶¶ 87-88, 191-193, 268. Claim 4 merely contemplates using an internal standard to assess the copy number variation. Specifically, the claim contemplates comparing sequence count information from locations of a genome region to sequence count information from locations of another genome region ***in the same sample*** and assessing copy number information by checking whether the first set of information is markedly different from the second set of information. As Dr. Cooper explains, Quake teaches this. *Id.* ¶¶ 267-277.

More specifically, Quake divides the genome into "windows" or "bins," and computes the median number of sequence reads mapping to all the bins across the chromosomes. Quake discloses summing sequence read counts at "locations on a first region" in a genome (*e.g.*, the bins on the chromosomes that one is interested in testing) and "locations on a second region in a genome" (*e.g.*, the bins on the other chromosomes that are not being tested and are presumed normal). EX1019 at 8:61-9:4; EX1002 ¶ 269-272. Quake then computes an internal normalization constant N, which is the "median M of all 22 autosomes," and which can be "used to correct for differences in sequences obtained in different runs." EX1019 at 7:44-54. Nevertheless, the method next involves determining "a first number of sequence tags mapped to at least one normally distributed chromosome portion and a second number of sequence tags mapped to the specified chromosome portion, both chromosomes being in one mixed sample" and then "calculating a differential between the first number and the second number which is determinative of whether or not the abnormal distribution exists," i.e., whether there is a copy number variation, in exact correspondence with claim 4. *Id.* at 4:40-67.

Thus, rather than using an external control, Quake looks at large numbers of regions across the genome in an individual sample, most of which will not include a copy number variation of any sort and will be normal, and then computes normalized densities. *Id.* at FIG. 1; *see also id.* at Abstract; EX1002 ¶¶ 271-274. The normalized

densities that are presumed normal can then be compared to the regions of interest to assess whether there is a copy number change. *Id.* ¶¶ 272-273.

A Dr. Cooper explains, to the extent Quake's examples generally involved computing a median and not a sum of the counts of locations of first and second region, this is a minor distinction. *Id.* ¶ 275. A POSA would have found it obvious to, for instance, compute an alternative identifier of central tendency across the bins, such as an average, which would involve computing a sum across the bins (i.e., "locations") across the chromosomes (i.e., "regions"). As Quake explains, both median and mean values are considered a form of average number under certain conditions. *See* EX1019 at 4:59-61; EX1002 ¶ 275. A POSA would have thus found it obvious to try using the average instead of a median.

A POSA would be motivated to use Quake's internal standard method as a form of Lo's method for determination of CNV, because it would obviate the need to use any sort of external samples. *Id.* ¶ 277. Instead, one could confidently determine from just a single sample whether there is a copy number variation in a particular region of the genome by looking for outlier regions within a single genome. By avoiding the need to also analyze external normal samples, one can use more power of the sequencing instrument for testing unknown samples. *Id.* A POSA would have a reasonable expectation of successfully using this approach because

Quake proved that it would work, including with actual experimental data in his patent filing. *Id.*

2. Claim 5: The method of claim 4, wherein step (h) further comprises j) summing counts obtained in step (f) for locations at a third region of the reference genome, wherein the third region comprises a number of locations which is comparable to the number of locations of the first region; and k) obtaining an average of the sum of the counts obtained in step (f) for locations at the second region and the sum of the counts obtained in step (j) for locations at the third region.

Quake further discloses or renders obvious claim 5. Claim 5 adds little relative to claim 4. Claim 4 requires that one use two regions of a reference genome and claim 5 simply requires that one uses three regions, averaging the counts in these regions. Quake discloses this. *Id.* ¶¶ 278-282.

To begin Quake discloses step (j) of obtaining counts from a third region (*i.e.*, a chromosome). Specifically, the method in Quake obtained sequence tag counts not just for two chromosomes, but for all autosomes. EX1019 at 22:39-41, FIG. 12. Although Quake refers to generating a "median sequence tag density among autosomes," as explained above in connection with claim 4, it would have nonetheless been obvious to alternatively compute an average, which would involve computing a sum. EX1002 ¶¶ 275, 280.

Quake describes his method in more details as follows. As Quake explains, "a sliding window of 50 kb was applied across each chromosome, except in regions of assembly gaps and microsatellites, and the number of sequence tags falling within

each window was counted and the median value was chosen to be the representative of the chromosome. Because the total number of sequence tags for each sample was different, for each sample, we normalized the sequence tag density of each chromosome (except chromosome Y) to the median sequence tag density among autosomes. The normalized values were used for comparison among samples in subsequent analysis." EX1019 at 22:32-43. Thus, Quake again describes determining median numbers of sequence counts across different autosomes, which satisfies steps (j) and (k) of this claim., or at a minimum renders obvious the determination of other well-known indicators of central tendency, such as an average (which requires a sum). *See id.* at 4:1-5, 25:1-5; EX1002 ¶¶ 281-282. Given humans have 22 autosomal chromosomes, the method in Quake describes determining copy number by comparing a target region to an average of at least two other regions in the reference genome.

As Dr. Cooper explains, a POSA would be motivated to use this approach and have a reasonable expectation of successfully doing so for the same reasons explained above in connection with claim 4. *Id.* ¶ 282.

F. Ground 6: Claim 10 Is Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA In Further View Of Specific Disclosure In Chee, Porreca, Or Brenner

1. Claim 10: The method of claim 1, wherein said tagging the segments with nucleic acid tags comprises ligation of adaptors comprising the tags to at least one end of the segments of the genomic nucleic acids.

As Dr. Cooper explains, a skilled artisan would know that tagging of nucleic acid molecular fragments can be accomplished using multiple tags in various ways. *Id.* ¶¶ 283-290. As of the earliest priority date of the '589 patent, it was well-known and routine in the art to ligate adaptors comprising tags to at least one end of the segments of the genomic nucleic acids, as even indicated by the specification of the '589 patent. EX1001 at 2:12-22.

Because the use of ligation was a standard technique in the toolkit of the skilled artisan, other references that describe tagging also use ligation:

- Chee teaches attaching tagging sequences "via PCR primers or by ligation" and assembling oligonucleotides using, e.g., "ligation followed by PCR; [and] ligation followed by cloning in a biological vector." EX1015 at 11:18-20, 23:28-35.
- Porreca discloses the use of ligation to incorporate random tag differentiator sequences, which may be accomplished at one or both ends of a nucleic acid sequence. EX1017 at 31:10-21.
- Brenner discloses that one technique for providing polynucleotides with unique nucleotide tags is by ligation: "For

example, polynucleotides of a population to be labeled are each associated or linked, e.g. by ligation, to an oligonucleotide tag from a population that has a much larger size than that of the target polynucleotide population." EX1014 at 17:60-64.

A POSA would have been motivated to use ligation for several reasons. First, there are only¶ a small handful of ways to even introduce tags. Besides ligation, primer extension is the only other realistic method that one would use to introduce tags. EX1002 ¶ 289. Because there are only a small number of ways to introduce tags, a POSA would be immediately motivated to consider ligation as an option for doing this, putting aside any considerations as to its difficulty or effectiveness. Second, a POSA would have been particularly motivated to use ligation because it was such a well-known, routine, and conventional approach for introducing tags that was known to work well, as shown by its use in Brenner, Porreca, and Chee. *Id.* ¶¶ 284-289. Finally, because ligation was a conventional technique that was tried and true, a POSA would have had a reasonable expectation of successfully using ligation to introduce tags. *Id.* ¶ 290.

X. CONCLUSION

The challenged claims should be canceled.

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APPENDIX: CHALLENGED CLAIM LISTING

No.	Limitation
1	A method comprising: a) obtaining segments of genomic nucleic acids from a sample; b) tagging the segments with nucleic acid tags, thereby generating a plurality of unique tagged nucleic acid molecules, such that each of the unique tagged nucleic acid molecules comprises a segment of the segments of the genomic nucleic acids from step (a) and a nucleic acid tag of the nucleic acid tags; c) subjecting the plurality of the unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating amplified tagged nucleic acid molecules; d) generating tag associated sequence reads by sequencing the amplified tagged nucleic acid molecules of step (c); e) assigning each of the amplified tagged nucleic acid molecules to a location on a reference genome from the same species from which the sample has been obtained by mapping each of the tag associated sequence reads of step (d) to a location of the reference genome; and f) at a plurality of locations on the reference genome, counting the number of unique tagged nucleic acid molecules that have been assigned to the same location on the reference genome based on the mapping step to obtain a count for each location of the plurality of locations on the reference genome, thereby obtaining copy number information from genomic nucleic acids in the sample.
2	The method of claim 1, further comprising estimating a copy number at multiple locations within a region of the reference genome.
3	The method of claim 1, further comprising comparing a count from counts obtained in step (f) for a location on the reference genome to a count for the same location on the reference genome obtained from a reference sample, thereby estimating a relative copy number at the location.
4	The method of claim 1, further comprising g) summing counts obtained in step (f) for locations on a first region of the reference genome, wherein the first region comprises more than one location; h) summing counts obtained in step (f) for locations on a second region of the reference genome, wherein the second region comprises

	a number of locations which is comparable to the number of locations of the first region; i) comparing the counts obtained in step (g) to the counts obtained in step (h), thereby estimating a relative copy number at the first region of the reference genome to the copy number at the second region of the reference genome.
5	The method of claim 4, wherein step (h) further comprises j) summing counts obtained in step (f) for locations at a third region of the reference genome, wherein the third region comprises a number of locations which is comparable to the number of locations of the first region; and k) obtaining an average of the sum of the counts obtained in step (f) for locations at the second region and the sum of the counts obtained in step (j) for locations at the third region.
6	The method of claim 1, further comprising (l) summing the counts from the counts obtained in step (f) for locations at a region of the reference genome; (m) summing the counts obtained from a reference sample for the same region of the reference genome; and comparing the sums of the counts obtained in step (l) and step (m), thereby estimating a relative copy number at the region of the reference genome.
7	The method of claim 1, wherein the unique tagged nucleic acid molecules are subject to a hybrid capture method prior to PCR in step (c) or the amplified tagged nucleic acid molecules produced in step (c) are subject to a hybrid capture method prior to step (d).
8	The method of claim 7, wherein each of the unique tagged nucleic acid molecules subjected to the hybrid capture method has a nucleic acid sequence which is complementary to a capture probe on an array or on a microarray.
9	The method of claim 1, wherein each of the tagged nucleic acid molecules differs at more than one nucleotide.
10	The method of claim 1, wherein said tagging the segments with nucleic acid tags comprises ligation of adaptors comprising the tags to at least one end of the segments of the genomic nucleic acids.
11	The method of claim 1, wherein each of the tagged nucleic acid molecules comprise more than one tag.
12	The method of claim 1, wherein the tags further comprise a sample tag.

13	The method of claim 1, wherein the tagged nucleic acid molecules generated from step (b) and that differ by a single nucleotide are ignored during or after the counting step.
14	The method of claim 1, wherein the unique tagged nucleic acid molecules are pooled with another plurality of unique tagged nucleic acid molecules having a different sample tag prior to the PCR or prior to said sequencing of the amplified tagged nucleic acid molecules of step (c).
15	The method of claim 14, further comprising deconvoluting the tag associated sequence reads by grouping the tag associated sequence reads.
16	The method of claim 1, further comprising obtaining copy number information from genomic nucleic acids of different species in a population using the method of claim 1, and determining a relative count of the genomic nucleic acids of the different species in a population by comparing the copy number information obtained from the genomic nucleic acids of the different species.
17	The method of claim 1, wherein the genomic nucleic acids are obtained from blood or other bodily fluids.
18	The method of claim 1, wherein the genomic nucleic acids are obtained from tumor cell DNA in a bodily fluid.

CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24 et seq., the undersigned certifies that this document complies with the type-volume limitations. This document contains 13,998 words as calculated by the "Word Count" feature of Microsoft Word for Microsoft 365, the word processing program used to create it.

Dated: July 25, 2025

Respectfully submitted,

JONES DAY

By: */Derek C. Walter/*
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CERTIFICATE OF SERVICE

I hereby certify that a true and correct copy of **PETITION FOR INTER PARTES REVIEW OF U.S. PATENT NO. 10,947,589 WITH GUARDANT HEALTH, INC'S EXHIBIT LIST, POWER OF ATTORNEY, AND EXHIBITS 1001-1042** are being served on July 25, 2025, via Priority Express Mail (Fed Ex) pursuant to 37 C.F.R. § 42.105 and § 42.6(e) to the Patent Owner at the address below:

Gary J. Gershik John P. White Paul Teng Amster Rothstein & Eberstein LLP 405 Lexington Avenue, 48th Floor New York NY 10174	

Dated: July 25, 2025

By: */Derek C. Walter/*
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