

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.

Petitioner,

v.

COLD SPRING HARBOR LABORATORY

Patent Owner

Case No. IPR2025-_____
U.S. Patent No. 12,234,510

**PETITION FOR *INTER PARTES* REVIEW
OF
U.S. PATENT NO. 12,234,510**

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PETITIONER’S EXHIBIT LIST

Exhibit #	Description
EX1001	U.S. Patent No. 12,234,510 (“the ’510 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, <i>Parse Biosciences, Inc., v. 10X Genomics, Inc.</i> , IPR2023-00958 (February 3, 2025) (Paper 48)
EX1006	Petition for <i>Inter Partes</i> Review of U.S. Patent No. 10,697,013, <i>Parse Biosciences, Inc., v. 10X Genomics, Inc.</i> , IPR2023-00958 (May 25, 2023) (Paper 1)
EX1007	Brooks E. Miner et al., <i>Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR</i> , 32 Nucleic Acids Research 1-4 (2004) (“Miner”)
EX1008	Megan L McCloskey et al., <i>Encoding PCR Products with Batch-Stamps and Barcodes</i> , 45 Biochemical Genetics, 761-767 (2007) (“McCloskey”)
EX1009	Prosecution File History of U.S. Patent No. 12,234,510
EX1010	U.S. Patent Application 2009/0029377 (“Lo”)
EX1011	Rossa W. K. Chiu et al., <i>Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma</i> , PNAS (December 23, 2008) (“Chiu”)
EX1012	Tian-Li Wang et al., <i>Digital karyotyping</i> , PNAS (December 10, 2002) (“Wang”)
EX1013	Derek Y. Chiang et al., <i>High-resolution mapping of copy-number alterations with massively parallel sequencing</i> , 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	Intentionally left blank
EX1020	Plaintiff’s Disclosure of Asserted Claims and Infringement Contentions for U.S. Patent Nos. 10,947,589 and 12,234,510, <i>Cold Spring Harbor Laboratory v. Guardant Health, Inc.</i> , 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 <i>Inter Partes</i> Review, <i>Parse Biosciences, Inc., v. 10X Genomics, Inc.</i> , 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 <i>Inter Partes</i> Review, <i>Parse Biosciences, Inc., v. 10X Genomics, Inc.</i> , 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

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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., <i>Population Analysis of Large Copy Number Variants and Hotspots of Human Genetic Disease</i> , The American Journal of Human Genetics 84, 148-161 (February 13, 2009)
EX1036	Heather C. Mefford et al., <i>A method for rapid, targeted CNV genotyping identifies rare variants associated with neurocognitive disease</i> , Genome Research (June 8, 2009)
EX1037	U.S. Patent No. 7,794,984
EX1038	Jingdong Tian et al., <i>Advancing high-throughput gene synthesis technology</i> , Molecular BioSystems 5, 714-722 (2009)
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 1-12, 15, 17-23, 26, and 28-36 of U.S. Patent No. 12,234,510 (“the ’510 patent”), which is assigned to Cold Spring Harbor Laboratory (“Patent Owner” or “PO”).

The ’510 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 general method recited in the claims 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, “obtaining relative 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 ’510 patent never suggests that determining copy number information is new or novel.

What, then, is supposedly new in the '510 patent? According to the patent, it is the ability to overcome 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 '510 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.” EX1001 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 the use of “tags” to overcome amplification distortion was a standard technique that had long been used by POSAs. If anything, by characterizing the use of tagging to overcome amplification distortion as the alleged invention, the '510 patent admits invalidity. 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 '510 patent by as much as six years. *Id.*

At bottom, and as Dr. Wigler confirmed, the '510 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 had even expressly suggested this combination long before the '510 patent. As such, the case for obviousness here is unusually compelling. It is the rare case where four different prior art references include express teaching for a particular combination of prior art features.

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 ¶¶ 38-42. Critically, the '013 patent has a substantially *earlier* priority date than the '510 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. *Id.* at 36-37, 51. Notably, the IPR against the '013 patent relied upon one of the very references relied upon here as disclosing tagging for counting—the McCloskey reference. If

anything, because the '510 patent came after the '013 patent, it is even more invalid 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 '510 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. 10,947,589 in a co-pending IPR.

C. Grounds for Standing

Petitioner certifies that the '510 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, 2, 9-11, 22, 26, 28, and 33-34 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 3 is unpatentable as obvious over Lo in view of the knowledge of a POSA as reflected in specific disclosure in McCloskey, Brenner, or Chee.
- **Ground 3:** Claim 4 is unpatentable as obvious over Lo in view of the knowledge of a POSA as reflected in specific disclosure in Brenner or Porreca.
- **Ground 4:** Claims 5-8 are unpatentable as obvious over Lo in view of the knowledge of a POSA as reflected in specific disclosure in McCloskey, Brenner, or Porreca.
- **Ground 5:** Claims 12, 15, 17, 23, 35-36 are unpatentable as obvious over Lo in view of the knowledge of a POSA as reflected in specific disclosure in Brenner, Chee, or Porreca.
- **Ground 6:** Claims 18-21 and 29-32 are unpatentable as obvious in further view of the knowledge of the skilled artisan.

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 ¶ 49-52. The four nucleotides are adenine (“A”), thymine (“T”), guanine, (“G”), and cytosine (“C”). *Id.* ¶ 51. 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. EX1002 ¶ 50. 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.* ¶¶ 72-78. In the 2000s, so-called next-generation sequencing (“NGS”) methods became commercially available. *Id.* ¶ 73. These methods were distinguished from prior sequencing methods, because they allow scientists and researchers to sequence millions of DNA molecules simultaneously. *Id.* ¶ 74.

Once the sequences of such molecules have been obtained, they can be further analyzed using a so-called “reference genome.” *See id.* ¶ 75. 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 genome of the species. 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.* ¶ 53, 74. Accordingly, before sequencing, DNA samples must often first be “amplified,” particularly in situations where the samples include limited amounts of DNA. *Id.* ¶ 53. 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.* ¶¶ 53-59.

The amplification of DNA samples presented a significant problem for NGS sequencing analysis. *Id.* ¶¶ 67-71. Specifically, not all DNA sequences amplify equally. *Id.* ¶ 67. 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.* ¶¶ 67-68. 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 to 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 ’510 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.* ¶ 60-66, 69-71.

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.* ¶ 69. 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.* ¶¶ 69-71. 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 '510 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 '510 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 discloses the concept discussed in this section wherein tags are used to confer uniqueness prior to amplification to overcome any effects of distortion.

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 ¶¶ 79-81.

V. OVERVIEW OF THE ’510 PATENT

The ’510 Patent, which was filed on February 7, 2019, identifies two provisional applications, the earlier of which was filed on October 22, 2010. EX1001 at (60). Thus, the earliest priority date of the ’510 Patent is October 22, 2010. During prosecution, the examiner rejected the ’510 Patent claims as obvious over two prior art references not used in this Petition. *See* EX1009. 434. But the

distinctions cited by the Patent Owner from those prior art references are obvious based on the references of Lo, McCloskey, Brenner, Chee, and Porreca described below. The examiner did not give meaningful scrutiny to the prior art references used herein.

Claim 1 of the '510 patent, which is listed 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 “relative 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 Lo was published at least one year before the earliest claimed priority date of the ’510 patent. Lo is analogous art to the ’519 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 ¶¶ 82-95. 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 of 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 ’510 patent. *Id.*

Lo’s method begins with a biological sample from, for instance, a pregnant female. *Id.* ¶ 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.

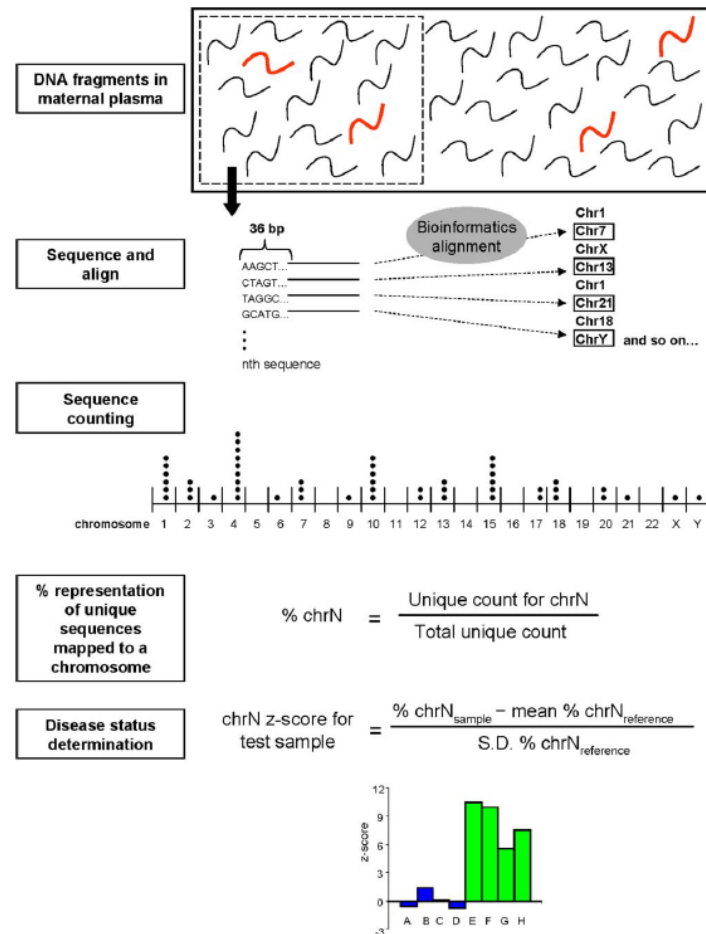
Lo discloses several ways to assess this percentage. *Id.* ¶ 60. These can include “counting the number of sequenced tags, the number of sequenced nucleotides (basepairs) or the accumulated lengths of sequenced nucleotides (basepairs) originating from particular chromosome(s) or chromosomal regions.” *Id.* In one embodiment, Lo teaches aligning the sequenced tags to a human reference genome sequence to determine chromosomal origin. EX1010 ¶ 70. This process of

“aligning” is nothing more than the process of determining which particular chromosome a DNA sequence came from, and long predates the priority date of the ’510 patent. EX1002 ¶ 88.

Following alignment, the total number of “sequenced tags” aligned to each chromosome can be tabulated. *Id.* 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 relative copy number change exists for the first chromosome or chromosomal region compared to other regions of the genome. *Id.* ¶¶ 64, 66. Lo discloses that the cutoff values are determined using an external standard based on normal genomes. As Lo states, 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.

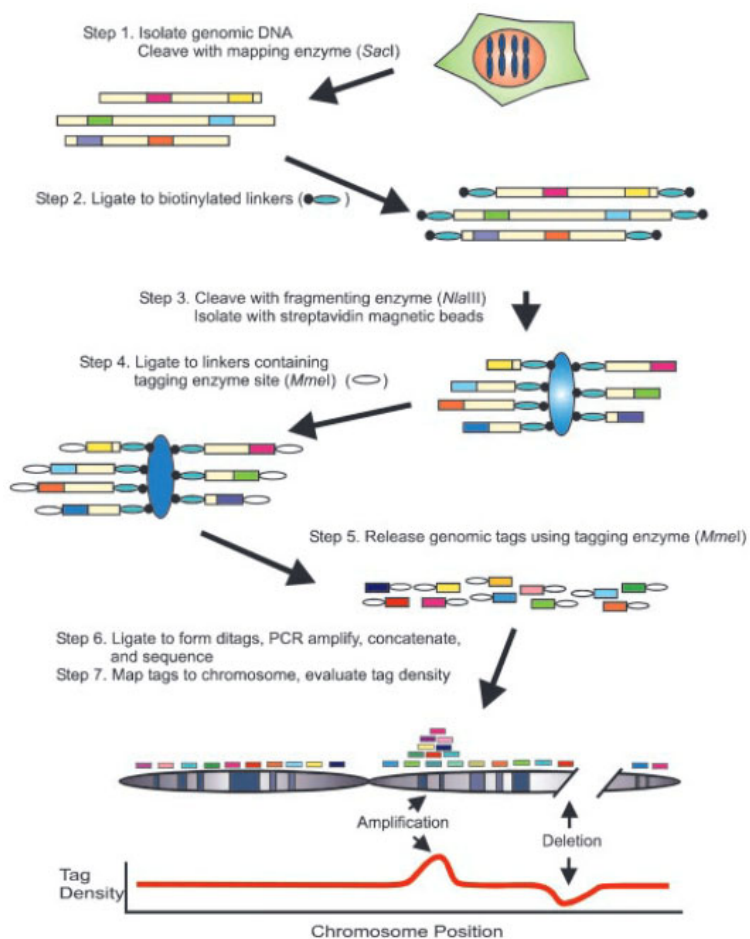
FIG. 1 of a related 2008 publication by Lo (EX1011) neatly explains the Lo method as applied to a sample of DNA from a pregnant woman, which has DNA from both the mother and fetus:



EX1011 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 embodiments in Lo focus 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 a standard method that had been previously applied straightforwardly to other sources of DNA to detect copy number information. EX1002 ¶¶ 91-95. 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).” EX1012 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” most 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. EX1013 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 the Lo publication focuses primarily on applications where the sample was cell-free DNA from pregnant women, there is nothing about the method in Lo that would limit it to such applications. EX1002 ¶¶ 91-95. 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 ¶¶ 96-127. 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 confirmed lead inventor Michael Wigler. *See id.* ¶¶ 66, 96-127; EX1004 at 1. Each of these references are analogous to the ’510 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 to the ’510 patent 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 to the ’510 patent 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. Chee is therefore prior art to the ’510 patent 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).

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 '510 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'"). PO's infringement contentions are cited herein where appropriate to illustrate its interpretations.

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 '510 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 ¶¶ 45-46. 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, and (5) aligning and counting sequence reads. *Id.* ¶ 47.

IX. IDENTIFICATION OF HOW THE CHALLENGED CLAIMS ARE UNPATENTABLE

Lo teaches the same general approach set forth in claim 1 of the '510 patent, wherein one determines copy number information by sequencing genomic segments, mapping the sequence reads to a reference genome, and then finally determining copy number information by counting sequence reads for the genomic region of interest.

To the extent any aspect of the independent claims is missing in Lo, it is the process of tagging the DNA sequences to generate unique tagged nucleic acid molecules followed by amplification of these tagged nucleic acid molecules. Yet, this process 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 '510 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 ¶¶ 128-131.

A. Ground 1: Claims 1, 2, 9-11, 22, 26, 28, and 33-34 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 containing the genomic nucleic acids;**

Lo teaches the use of “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 ¶¶ 132-135.

- b) [1B] randomly tagging the segments of genomic nucleic acids with different nucleic acid tags, thereby generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids, 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 random tagging to generate “unique tagged nucleic acid molecules,” it would nonetheless have 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 ¶¶ 136-159. *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 ’510 patent, it was well-known and routine in the art to randomly attach tag sequences to nucleic acid fragments prior to amplification to obtain accurate copy number information and minimize bias and distortion. EX1002 ¶¶ 29-31, 67-71, 137. Indeed, the ’510 patent admits as much in its discussion of Miner, McCloskey, and Brenner. *See* EX1001 at 2:1-28. Consistent with this, and as documented below, each of Brenner, Chee, McCloskey, and Porreca describe random tagging for molecular counting in

conjunction with amplification, reflecting the general knowledge of the skilled artisan as to tagging for molecular counting. *See* EX1002 ¶¶ 138-147.

- 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 ’510 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 ¶¶ 140-141. 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

¹ Emphasis supplied unless otherwise noted.

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 ¶¶ 142-143.

- Chee teaches tagging “regions of interest coupled with **unique identifiers**,” and hence teaches “unique tagged nucleic acid 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 ¶¶ 144-145.
- 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 ¶¶ 146.

As Dr. Cooper further explains, each of McCloskey, Porreca, Chee, and Brenner teach not just tagging, but random tagging because they all teach the use of random sequences that are paired up with target nucleic acids through random stochastic processes during solution-phase reactions, such as primer extension or ligation, wherein the tag that is added to a target will be determined based on which tag happened to interact with a particular target. *Id.* ¶¶ 140, 143, 145-146. At bottom, each of these four references demonstrate that it was well-known and routine in the art to randomly tag nucleic acid fragments to generate unique tagged nucleic acid molecules. *Id.* ¶¶ 137, 147. As Dr. Cooper further explains, a POSA would have been motivated to use this technique with Lo for several reasons.

First, the method of tagging for counting molecules was well-known and part of the standard tool kit of a POSA as of the priority date of the '510 patent. Tagging 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 set forth 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. EX1002 ¶¶ 148-149. 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 the tagging feature to Lo would be a natural and obvious choice for a POSA. Lo is an approach based on counting, and therefore a POSA would naturally think to include tagging as an extension of Lo's method of molecular counting because this was a method that was specifically intended for improving the accuracy of molecular counting. EX1002 ¶ 150. *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 of the references mentioned above expressly contemplate using tagging to determine copy number information. For example, Brenner discusses using tagging and subsequent amplification 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; *see also id.* at 18:30-49 (explaining that the disclosed method can be used to “increase the confidence in calling copy number variations”); 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 combine tagging with an approach for determining copy number variation based on molecular counting, as is taught in Lo. EX1002 ¶ 151.

Fourth, adding the tagging features described above would help solve the signal-to-noise challenge described in Lo. Lo explains that “fetal DNA is present in

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

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 ¶ 152.

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.* ¶¶ 153-159. A POSA would know 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 achieving of this, including through “distance measures” known in the art for designing such sequences. Brenner cites several literature 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; *see also* EX1015 at 11:26-38. Thus, methods were available for preparing a large number of different oligonucleotide tags.

So too were methods for attaching the tags. *See* EX1002 ¶¶ 156-157. For example, Porreca describes methods for combining tags with target sequences. *See, e.g.,* EX1017 at FIG. 4A-4C. Brenner details a general 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 '510 patent admits that various methods of tagging existed in the art, there is no question as to 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 '510 patent characterizes the Miner and McCloskey methods based on tagging and amplification as successfully implemented prior art methods, a point that further 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, confirming a reasonable expectation of success with respect to not just tagging, but also amplification of tagged molecules, as discussed in the claim element immediately below. *Id.*

- c) **[1C] subjecting the unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating copies of the unique 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 ¶¶ 160-170.

As an initial matter, the use of 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 set forth in Brenner, McCloskey, Chee, and Porreca, which is all reflective of the knowledge of the skilled artisan. EX1002 ¶¶ 165. As Dr. Cooper explains, there is no reason to utilize tagging without subsequent amplification because the whole point of tagging is to permit one to subsequently perform amplification and 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 ¶ 166; *see also* EX1010 ¶¶ 13, 16, 18. Therefore, a POSA would have naturally been motivated to perform amplification to increase the overall amount of DNA for testing.

Yet additional motivation to utilize amplification exists within Lo itself. Specifically, Lo teaches multiple methods of 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 of 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.*

- Lo also teaches the use of hybridization techniques to accomplish enrichment of potentially aneuploid chromosomes and one or more normal chromosomes, “for example onto oligonucleotide microarrays,” followed by random sequencing. *Id.* ¶ 79. This cost-saving technique was known to POSAs. EX1002 ¶ 167.
- 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 an additional increase in accuracy by virtue of the tagging. *See* EX1002 ¶¶ 167-169.

Further, a POSA would have had a reasonable expectation of success in amplifying the tagged nucleic acids because amplification was so routine and widely used at the time. EX1002 ¶ 170. The '510 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 '510 patent characterizes PCR as a well-known prior art method that people of skill in the art had no trouble utilizing. EX1002 ¶ 170.

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

As discussed above for 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 in connection with Lo's method. *See* EX1002 ¶¶ 171-176.

As Dr. Cooper explains, there can be no question that Lo's method is a sequencing-based method for molecular counting. *Id.* ¶¶ 172-174. 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 set forth 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 have been 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 the sequencing method of Lo for all the reasons stated above for those 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 ¶¶ 175-176. 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 unique tagged nucleic acid molecules to a location on a genome by mapping each of the tag associated sequence reads of step (d) to a location in the 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 ¶¶ 177-182. 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 use tagging and amplification with Lo's method. Having been motivated to modify Lo's method, 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 ¶¶ 182. There can be no question that a POSA would have had a reasonable expectation of success 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] in a plurality of locations in the genome, obtaining relative copy number information of the genomic nucleic acids from the sample based on the number of unique tagged nucleic acid molecules that have been assigned to each of the plurality of locations in the genome.**

As Dr. Cooper explains, Lo teaches this claim element. *See* EX1002 ¶¶ 183-191. 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 ¶ 70. 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 the Patent Owner’s broad reading of “location” in the parallel district court litigation. *See* EX1020 (interpreting “locations” as any of “genes,” “genetic loci,” or “start/stop positions”).

Lo, however, further discloses how to use the counted sequence tags to derive yet additional copy number information, specifically an estimation of relative copy number. EX1002 ¶¶ 184-187. Lo teaches taking 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 by these means is “copy number variations.” *Id.* ¶¶ 37, 50; *see also id.* ¶ 70 (“The total number of individual sequenced tags aligned to each chromosome were tabulated and compared with the relative size of each chromosome as expected from the reference human genome or non-disease representative specimens. Chromosome gains or losses were then identified.”). Thus, there is no question that Lo’s method

is based on counting nucleic acid molecules to estimate relative copy number variations reflecting deviations from the normal two copiers per genome that is standard.

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 ¶¶ 189-190. 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, wherein the polymerase chain reaction is performed in the presence of region specific PCR primers.

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 ¶¶ 192-199. First, each of McCloskey, Chee, Brenner, and Porreca teach the use of region specific PCR primers:

- McCloskey used region-specific primers that targeted the FMR1 gene. A portion of the tags used in McCloskey hybridize to a

locus in the FMR1 gene. After a first extension, PCR is performed using a primer that anneals to the primer binding site of the tags and a second primer that anneals to the FMR1 sequence. EX1008 at 762; *see also* EX1002 ¶ 193.

- Chee teaches the use of “subset-specific primers” to amplify defined groups of DNA molecules. Chee explains that large oligonucleotide sequences can be organized into smaller “subsets” which can be selectively amplified with subset-specific primers. EX1015 at 16:27-30; *see also* EX1002 ¶ 195. Chee also discloses ROI-specific primers which can convert single stranded regions of interest into double-stranded molecules. *Id.* at 17:48-50.
- Brenner teaches multiple variations of PCR, including “quantitative PCR,” which routinely “require[e] the use of locus-specific primers.” EX1014 at 38:58-60; EX1010 ¶ 82; *see also* EX1002 ¶ 194.
- Porreca discloses region-specific primers in its discussion of “amplification of multiple target nucleic acids in parallel.” EX1017 at 6:22-23. Multiplex PCR typically uses “distinct sets of primers...for each sequence being amplified.” *Id.* at 39:19-24.

In light of Lo’s teaching of enrichment of target sequences prior to sequencing and counting, a POSA would have been motivated to use region specific primers. *See* EX1002 ¶ 197. Lo even discusses that digital PCR uses “sequence-specific

primers” to amplify “specific nucleic acid targets.” EX1010 ¶ 10. Accordingly, a POSA would recognize that region-specific primers were a well-established way to selectively enrich a sample for sequencing and counting. EX1002 ¶ 197.

For the same reason as explained above for claim 1, there can be no question of a reasonable expectation of being able to successfully use region-specific primers, because targeted amplification was well known and had been successfully documented in the publications above.

3. Claim 9: The method of claim 1, wherein the unique tagged nucleic acid molecules are subjected to hybrid capture prior to the PCR or the amplified tagged nucleic acid molecules produced in step (c) are subjected to hybrid capture prior to step (d).

As Dr. Cooper explains, Lo further teaches the features of claim 7. *See* EX1002 ¶¶ 200-203. 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 have been motivated to carry out the hybrid capture of claim 7, and that there would have been 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.” EX1010 ¶ 77; *see also id.*

¶ 79. As Lo makes clear, a POSA would have been motivated to perform advance enrichment because it would reduce the cost of the sequencing. There would have been a reasonable expectation of success because this would have simply involved the use of existing oligonucleotide array technologies.

4. Claim 10: The method of claim 1, wherein the genomic nucleic acids are from a single species, two or more organisms, two or more species, or from a population of microbes.

Lo teaches the additional features of claim 10, or, at a minimum, renders them obvious.

Lo discloses collecting and sequencing genomic DNA from pregnant women, which are humans and therefore a “single species.” EX1010 ¶ 92. Additionally, maternal plasma samples contain genomic DNA of both fetal and maternal origin. *Id.* ¶ 6; *see also* EX1002 ¶ 204-206.

5. Claim 11: The method of claim 1, wherein the sample is a blood sample or bone marrow sample.

Lo teaches the additional features of claim 11, or, at a minimum, renders them obvious.

Lo's method begins with a blood draw. EX1010 ¶ 92 ("Twenty milliliters of peripheral venous blood was collected from each subject....DNA was then extracted from 5-10 mL of each plasma sample."); *see also* EX1002 ¶ 207-209.

6. Claim 22: The method of claim 1, wherein the nucleic acid tags comprise a sufficiently large number of distinct nucleic acid tags.

As Dr. Cooper explains, Lo in view the knowledge of the skilled artisan, as reflected in McCloskey, Chee, Brenner, and Porreca, renders claim 22 obvious. *See* EX1002 ¶¶ 210-216. First, each of McCloskey, Chee, Brenner, and Porreca teach the use of a sufficiently large number of distinct nucleic acid tags:

- McCloskey's tags include a seven-base "barcode" that are "random sequence[s] of the four deoxyribonucleotides." EX1008 at 764. As McCloskey explains, the "number of distinguishable barcodes in a population of oligonucleotides...is determined by the number of random bases, *n*." *Id.* The number of unique barcodes is 4^n or 16,384 possibilities for a barcode seven nucleotides long. *Id.*; *see also* EX1002 ¶ 211.
- Brenner teaches that the "size of mutually discriminable sets of oligonucleotide tags may [be]...50 to 1.6×10^7 ." EX1014 at 37:52-55. Brenner also explains that a "sufficiently large" population of tags might be "at least 100 times the number of fragments to be counted." *Id.* at 7:23-29.
- Chee teaches that "[t]he diversity of the identifiers needs to be *sufficient* so that molecules that are not related won't be mistaken

as descendent ROIs.” EX1015 at 11:20-22. Chee further discloses the use of an identifier with roughly 10^{12} possible sequences. *Id.* at 11:35-36; *see also* EX1002 ¶ 213.

- Porreca also explains that the “total number of unique differentiator tag sequences” is 4^n where n is the “[l]ength in nucleotides of the differentiator tag sequence.” EX1017 at 73:10-17. Porreca further gives a formula to calculate the probability, given the number of unique possible tags and target sequence copies, that two sequence copies would receive an identical tag. *Id.* at 73:17-30. In one example, a tag length of 15 nucleotides is chosen for a less than 5% chance of two target sequence copies receiving the same tag sequence. *Id.* at 73:31-38; *see also* EX1002 ¶ 214.

As Porreca explains, a POSA would have been motivated to use a large number of unique tags because, a larger population of tags means a lower likelihood that DNA fragments with the same original sequence will end up with the same sequence when combined with a tag. *See also* EX1002 ¶ 216. A POSA would have had an expectation of success because using a large number of tags was a well-known, conventional technique, as described above. *Id.*

7. Claim 26: The method of claim 22, wherein the nucleic acid tags comprise nucleotide sequences with constant portions and variable portions.

As Dr. Cooper explains, Lo in view the knowledge of the skilled artisan, as reflected in McCloskey, Chee, Brenner, and Porreca, renders claim 26 obvious. *See* EX1002 ¶¶ 217-223. First, each of McCloskey, Chee, Brenner, and Porreca teach the use of tags with constant portions and variable portions:

- McCloskey discloses tags with a fixed batch-stamp and a fixed primer-binding site alongside a variable random barcode. EX1008 at 762.
- Chee describes embodiments with “two or more amplification sites flanking the ROI and the unique identifier.” EX1015 at 2:65-67. The amplification sites are constant, but the unique identifier varies between tags.
- Brenner discloses tags comprising constant primer-binding sites and binary tags. EX1014 at 8:31-33. The primer binding site “has the same, or substantially the same, sequence whenever it is present.” *Id.* at 43:45-53. The binary tag is variable and could be one of roughly 65,000 different sequences. *Id.* at 14:30-33.
- Porreca also teaches using tag sequences that contain both a variable differentiator tag sequence alongside a constant common adaptor sequence, which may contain the sequencing primer site. EX1017 at 31:21-27.

A POSA would have been motivated to use tags with both variable sequence portions and constant sequence portions because variable portions allow for the unique tagging and counting described in step [1B] above while constant portions facilitate other workflow steps including PCR amplification and sequencing described in steps [1C] and [1D]. As Dr. Cooper explains, combining both portions into a single tag simplifies the workflow and was commonplace. EX1002 ¶¶ 222-223. A POSA would have had a reasonable expectation of success combining both constant and variable tag portions not only because doing so was well-known and commonplace but also because it simplified the workflow. *Id.*

8. Claim 28: The method of claim 26, wherein the length of the constant portions and the length of the variable portions are different.

As Dr. Cooper explains, Lo in view the knowledge of the skilled artisan, as reflected in McCloskey, Chee, Brenner, and Porreca, renders claim 28 obvious. *See* EX1002 ¶¶ 224-230. Each of McCloskey, Chee, Brenner, and Porreca teach the use of tags with constant and variable portions of different lengths:

- McCloskey uses a tag with a constant 8-nucleotide batch-stamp; a variable 7-nucleotide random barcode, a constant 21-nucleotide primer-binding site; and a constant 22-nucleotide genomic target-binding site. *See* EX1008 at 764.
- Chee provides an example of a 20-nucleotide long variable identifier. EX1015 at 11:30-36. As explained above for claim

22, Chee teaches that the length of this identifier varies based on the number of target ROIs. *Id.* at 11:20-22. In contrast, the length of the amplification sites depends on the length of the amplification primers. EX1002 ¶ 227.

- Brenner discloses using a forward primer site with a constant 9-nucleotide long sequence. EX1014 at 46:40-45. The variable binary tags, however, “may vary widely” and in example “comprise from 14 to 17 dinucleotide subunits.” *Id.* at 10:39-41, 44-45.
- Porreca explains that variable “[d]ifferentiator tag sequences may be...up to 25, or more nucleotides in length.” EX1017 at 29:17-21. The constant adapter for PCR primer sites would be chosen based on PCR and sequencing considerations and of a different length. *Id.* at 31:3-5; *see also* EX1002 ¶ 228.

As explained for claim 26 above, a POSA would have been motivated to use tags with variable sequence portions and constant sequence portions of different lengths because using both constant and variable tag portions simplifies the tagging, amplification, and sequencing workflows. The prior art teaches that the variable portion length should be chosen to provide a large enough number of unique tags as explained in claim 22. The constant portion length would be chosen based on the length of PCR or sequencing primers. In practice, these lengths will be different as seen above in McCloskey and Brenner. *See* EX1002 ¶ 230. A POSA would have a

reasonable expectation of success for the same reasons as claim 26—the methods were well-known and simplified the workflow. *Id.*

9. Claims 33 and 34: The method of claim 22, wherein the nucleic acid tags comprise [nucleotide sequences that are not known before the tagged nucleic acid molecules are generated/random nucleotide sequences].

Claims 33 and 34 recite tags having “nucleotide sequence that are not known before the tagged nucleic acid molecules are generated” and “random nucleotide sequences,” respectively. In its infringement contentions, PO contends that both of these claim elements may be satisfied by the use of tags with random sequences. *See* EX1020.325, .331. As explained above in connection with claim element [1B], each of Brenner, Chee, Porreca, and McCloskey teach the use of random tags, and the use of these random tags in combination with Lo would have been obvious. *See supra* § IX.A.1.b; EX1002 ¶ 231.

B. Ground 2: Claim 3 Is Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA, As Reflected In McCloskey, Brenner, Or Chee.

- 1. Claim 3: The method of claim 1, wherein the unique tagged nucleic acid molecules differ at more than one nucleotide or wherein the sequences of the nucleic acid tags are known prior to said generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids.**

A POSA would understand that Lo in view the knowledge of the skilled artisan renders obvious claim 1. Claim 3 is rendered obvious in view of specific disclosure in McCloskey, Brenner, or Chee. *See* EX1002 ¶¶ 232-239.

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 ¶ 235. 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 ¶¶ 233-234. Furthermore, the sequence of a batch stamp “specifies the DNA source...and the date of reaction” and “is registered for a specific sample run,” meaning that the sequence of any batch stamp is known prior to sequencing the unique tagged nucleic acid molecules. EX1008 at 764.

Chee teaches attaching “a diverse but *pre-defined* set of sequences” to ROIs, including via PCR primers or by ligation. EX1015 at 11:18-20. Chee further describes dividing tagged constructions into subsets and selectively amplifying them with “subset-specific primers” described in claim 2, which presumes prior knowledge of the tag sequences. *Id.* at 16:27-30; *see also* EX1002 ¶ 236.

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.* ¶ 237. 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. Additionally, pre-defined tag

sequences—taught by Chee and McCloskey—simplify data interpretation, improve error correct, and support traceability of samples. EX1002 ¶ 238.

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 and using tags with known sequences. EX1002 ¶¶ 237-238. 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. EX1014 at 25:58-66.

C. Ground 3: Claim 4 Is Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA, As Reflected In Brenner Or Porreca.

1. Claim 4: The method of claim 1, wherein the segments of the genomic nucleic acids are produced by mechanical shearing, heating, or sonicating the genomic nucleic acids from the sample.

As Dr. Cooper explains, mechanical shearing, heating, and sonication were all known methods of DNA fragmentation. EX1002 ¶¶ 240-243. When working with a sample of already fragmented DNA, such as when using Lo’s method with cell-free DNA, further fragmentation would reduce the amount of an already limited the

DNA sample. *Id.* ¶ 241. Nevertheless, Lo’s counting method for determining copy number had also been applied to less fragmented DNA samples, which may need to be sheared, heated, or sonicated prior to DNA sequencing. *Id.* These fragmentation methods are taught in Brenner and Porreca.

Brenner discloses generating target polynucleotide from source DNA “by shearing or by cleavage with one or more nucleases.” EX1014 at 18:33-34. Porreca states that genomic DNA can be “[m]echanically shear[ed], using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument.” EX1017 at 71:16-17. Porreca explains that “DNA should be sheared into fragments several hundred basepairs in length” in order to prepare the sample for sequencing. *Id.* at 71:17-19.

A POSA would understand that some sequencing systems require DNA fragments of a specific size and would use the known methods of mechanical shearing, heating, and sonication to adapt larger DNA molecules for sequencing systems that required smaller fragments. EX1002 ¶¶ 242-243. If smaller fragments were required, employing these fragmentation techniques would have been an obvious design choice with a reasonable expectation of success. *Id.*

D. Ground 4: Claims 5-8 Are Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA, As Reflected In McCloskey, Brenner Or Porreca.

1. Claim 5: The method of claim 1, wherein the nucleic acid tags comprise a sample tag or a sample tag set.

The use of sample tags was well-known and regular as of the priority date and would have been obvious in view of knowledge of the skilled artisan. *See* EX1002 ¶¶ 244-249. As Dr. Cooper explains, “sample tags” are tags of a known sequence that identify DNA as sourced from a specific sample. *Id.* ¶ 245. Once tagged, DNA from multiple samples can be pooled and sequenced simultaneously. After sequencing, the sample origin can be determined by identifying the sample tag sequences within the tagged molecule sequences. *Id.* Such sample tags, Dr. Cooper explains, were commercially available for use with sequencing technologies that were available as of the priority date. *Id.* The use of sample tags is also disclosed by Brenner, McCloskey, and Porreca.

- Brenner discloses using oligonucleotide tags for “indexing” and explains that “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 21:39-32.
- McCloskey teaches using a “batch-stamp” tag alongside the variable “barcode” sequence. EX1008 at 761 (“Batch-stamps specify the date and sample identity, and barcodes detect

template redundancy.”); *id.* at 763 (“[T]he batch-stamp, unique to each experiment, specifies the DNA source such as the patient or sample identification”).

- Porreca explains that “one or more techniques described herein may be combined in a single assay protocol for evaluating multiple patient samples in parallel.” EX1017 at 21:3-5. Porreca uses “barcodes,” here meaning sample-specific tags, “to uniquely identify individual samples (e.g. patient samples) that may be mixed together.” *Id.* at 31.5-9.

A POSA would have been motivated to use sample tags with Lo’s method because sample tags provide a means of distinguishing sample sources after sequencing and permit pooling the samples in a single sequencing run. Pooling and sequencing samples together would improve efficiency and reduce costs. EX1002 ¶ 249. As reflected in Brenner, McCloskey, and Porreca, sample tags could easily be included alongside the unique distinct tag portions described in claim 1, step [1B], that facilitate counting after amplification. *See, supra*, § IX.A.1.b. For the same reasons as explained above for claim 1, there can be no question of a reasonable expectation of success because tagging was well known and successfully documented in the publications above. EX1002 ¶ 249.

2. **Claim 6: The method of claim 5, wherein the unique tagged nucleic acid molecules are pooled with a 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 product of step (c).**

As Dr. Cooper explains, claim 6 recites nothing more than the standard manner of using a “sample tag” with sequencing, as explained above in connection with claim 5. EX1002 ¶¶ 250-251. For the same reasons that claim 5 would have been obvious, so too would claim 6 have been obvious. *Id.*

3. **Claim 7: The method of claim 6, further comprising deconvoluting the tag associated sequence reads by grouping the tag associated sequence reads.**

Claim 7 recites nothing more than the standard manner of using a “sample tag” with sequencing, as Dr. Cooper explains above in connection with claim 5. EX1002 ¶¶ 252-253. For the same reasons that claims 5 and 6 would have been obvious, so too would claim 7 have been obvious. *Id.*

4. **Claim 8: The method of claim 1, wherein the nucleic acid tags are double-stranded.**

As Dr. Cooper explains, using double-stranded tags was well-known and would have been obvious in view of the knowledge of a skilled artisan, as reflected by the disclosure of Brenner, McCloskey, and Porreca. EX1002 ¶¶ 254-258.

- Brenner discloses “a set of 64 double stranded composite tags” and specifies that “each composite tag contains a double stranded metric tag.” EX1014 at 28:37-39.

- McCloskey discloses a double-stranded hairpin loop tag. EX1008 at 764.
- Porreca also discloses double-stranded oligonucleotide tags which ligate to both strands of a double-stranded “molecule of interest.” EX1017 at FIG. 4C.

A POSA would have been motivated to use double-stranded tags as one of only two options for tagging and the option that is most used for ligation. EX1002 ¶ 258. Double-stranded DNA would thus have been obvious to try, in part because DNA exists in nature in double-stranded form. *Id.* A POSA would have a reasonable expectation of success because Brenner, McCloskey, and Porreca collectively demonstrate double stranded tags’ utility, compatibility with PCR, and prevalence in molecular tagging protocols. *Id.* Those of skill in the art would have had no trouble implementing double-stranded DNA tags as successfully implemented by Brenner, McCloskey, and Porreca in tagging, amplifying, sequencing, and counting workflows.

E. Ground 5: Claims 12, 15, 17, 23, And 35-36 Are Unpatentable As Obvious Over Lo In View Of The Knowledge Of A POSA, As Reflected In Brenner, Chee, Or Porreca.

- 1. Claim 12: The method of claim 1, wherein the unique tagged nucleic acid molecules are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids.**

A POSA would understand that Lo in view of the knowledge of the skilled artisan renders obvious claim 1. Claim 12 is rendered obvious in view of specific disclosure in Brenner, Chee, or Porreca. EX1002 ¶¶ 259-264.

- Brenner teaches that “substantially every molecule to be counted in a sample...is associated with a probe having a different tag.” EX1014 at 5:17-21. Brenner further teaches that “a size of tag population is selected that ensures with *high probability* that substantially every target polynucleotide will have a unique tag.” *Id.* at 18:2-4. Brenner makes clear that the population of tags need not exceed the number of targets if the targets are diverse. In such cases, the combination of tag and information in the genomic nucleic acid provides uniqueness. EX1002 ¶ 260.
- Chee explains that “a feature of the invention [is] that the different constructs have unique identifiers, including constructs that may have the same ROI.” EX1015 at 5:27-29. Chee teaches that “sets of constructs are designed so there is a large excess of possible identifiers relative to the ROIs in a given set. Thus, the chances of having constructs with given identifiers associated

with more than one ROI is very low, and the chances of the same identifier being associated with the same ROI in different constructs is extremely low.” *Id.* at 9:54-60. Chee makes clear that some tags might be associated with more than one ROI, so uniqueness is conferred not solely by tags but by the combination of the tag and genomic nucleic acid.

- Porreca teaches a method “wherein the isolated nucleic acids are identified as independently isolated if they comprise unique ***combinations*** of target nucleic acid and differentiator tag sequences.” EX1017 at 5:41-43. Porreca explains that the length of the differentiator tag “is selected to control the likelihood of producing unique target:differentiator tag sequences.” *Id.* at 29:13-14. Ultimately, Porreca counts the unique combinations of target nucleic acid and differentiator tag. *Id.* at 72:67-73:2.

A POSA would have been motivated to adopt the approach where uniqueness is conferred by the combination of target sequence and tag because, although a large population of tags is beneficial, it may not be cost-effective in all cases to generate a large enough number of targets to give each target molecule a unique tag. EX1002 ¶ 263. As shown in Brenner, Chee, and Porreca, a POSA would have the combination of tag and target nucleic acid could establish uniqueness, allowing a smaller population of tags to be used. Given the wide diversity of sample molecules in a typical DNA sample and the ease of increasing the tag population by increasing

tag length, there would have been no question of a POSA's reasonable expectation of success here. *Id.*

Additionally, as Dr. Cooper explains, during the sequencing process both tags and genomic nucleic acid segments are sequenced. EX1002 ¶ 260. It was commonplace and straightforward to analyze both portions together for uniqueness. *Id.*

2. Claim 15: The method of claim 12, wherein the nucleic acid tags comprise nucleotide sequences with constant portions and variable portions.

As Dr. Cooper explains, claim 15 is rendered obvious in view of specific disclosure in Brenner, Chee, or Porreca. EX1002 ¶¶ 265-270. Claim 15 recites the same additional limitations as claim 26. As described above for claim 26, each of Brenner, Chee, and Porreca teach using tags with constant and variable portions. *See supra* § IX.A.7. Brenner teaches tag structures that incorporate constant regions, such as primer binding sites, alongside variable regions for indexing or sample identification. EX1014 at 43:51-53, 14:30-33. Chee describes some embodiments with a variable unique identifier which distinguishes between constructs and constant amplification sites EX1015 at 2:65-67. Porreca likewise teaches adding tag sequences that contain both a variable differentiator sequence alongside a constant adaptor sequence. EX1017 at 31:21-27.

As described above for claim 26, a POSA would have been motivated to use constant and variable portions and would have reasonable expectation of success because doing so simplifies the tagging and sequencing workflow. *See supra* § IX.A.7; EX1002 ¶276.

3. Claim 17: The method of claim 15, wherein the length of the constant portions and the length of the variable portions are different.

As discussed above, Lo in view of the knowledge of the skilled artisan renders obvious claim 15. The limitation of claim 17 is the same as that of claim 28. As explained above, each of Brenner, Chee, and Porreca disclose tags with constant and variable portions and render obvious this limitation. *See supra* §§ IX.A.8, IX.E.2; EX1002 ¶¶ 271-276.

4. Claim 23: The method of claim 22, wherein the unique tagged nucleic acid molecules are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids.

As discussed above, Lo in view of the knowledge of the skilled artisan renders obvious claim 22. The limitation of claim 23 is the same as that of claim 12. As explained above, each of Brenner, Chee, and Porreca disclose uniqueness based on the combination of tag and sequence information and render obvious this limitation. *See supra* § IX.E.1; EX1002 ¶¶ 277-279.

5. Claims 35-36: The method of [claim 22/claim 1] wherein the nucleic acid tags are substantially unique nucleic acid tags.

In its infringement contentions, PO contends that this claim element is satisfied by “nonredundant” tags and tag sets that are “non-unique.” See EX1020.340, .342, .345, .347. To the extent the specification defines “substantially unique tags,” PO does not apply this definition. While any such definition ought to control, for the purposes of this Petition, the interpretation reflected in PO’s infringement contentions is applied. There, PO treats this claim element as merely encompassing tag sets that are “non redundant” in that not all the tags are the same (*i.e.*, “non-unique”). *Id.*

As documented above in connection with claim 12, Brenner teaches that “a size of tag population is selected that ensures with high probability that substantially every target polynucleotide will have a unique tag. In one aspect, such probability is at least 90 percent; in another aspect, such probability is at least 95 percent; and in another aspect, such probability is at least 99 percent.” EX1014 at 18:2-7. Likewise, Chee teaches that “sets of constructs are designed so there is a large excess of possible identifiers relative to the ROIs in a given set.” EX1015 at 9:54-56. And Porreca teaches that the total number of tag sequences is 4^n , where n is the number of nucleotides in the tag. EX1017 at 73:14-17. Porreca goes on to contemplate using more than a billion different tags. *Id.* at 73:31-32. Thus, each of Chee, Brenner, and

Porreca contemplate using large numbers of tags that will all be different from one another and, in that regard, are nonredundant. *See also* EX1002 ¶¶ 280-282.

As Dr. Cooper explains, a POSA would be motivated to use tags having this property because it would help ensure overall uniqueness of different the tag-target constructs in advance of amplification and would hence increase the accuracy of the counting. EX1002 ¶ 282. Moreover, a POSA would have reasonably expected success in pursuing this approach because it would not require any changes to the normal process for creating tags and would have simply required manufacturing a large set of tags as needed compared the number of sample molecules. *Id.*

F. Ground 6: Claims 18-21 And 29-32 Are Obvious In Further View Of The Knowledge Of The Skilled Artisan

Claims 18-21 and 29-32 recite tags with variable portions that are constructed from particular palettes of nucleotides, specifically, using only “two species of nucleotides” (claims 18 and 29), using only “three species of nucleotides” (claims 19 and 30), using “a set of dinucleotides” (claims 20 and 31), and using “a set of trinucleotides” (claims 21 and 32).

As shown above, the claims from which these eight claims depend are all invalid as obvious. As Dr. Cooper further explains, the use of tags with variable portions constructed from the claimed palettes of oligonucleotides also would have been obvious approaches that a POSA would have been motivated to explore. *See*

EX1002 ¶¶ 283-284. Using only a limited species of oligonucleotides would simplify synthesis and data analysis because variable portions of tags could be distinguished from other tag regions by virtue of only including a limited repertoire of nucleotides. *Id.* ¶ 284. Similarly, variable tag portions constructed from dinucleotides and trinucleotides because would allow one to effectively have an expanded set of units for synthesis of tags without introducing any new nucleotides. *Id.* This is because while there are only four nucleotides, there are 16 possible dinucleotides and 64 possible trinucleotides. To simplify the design of unique tags, a POSA would be motivated to use di- and trinucleotides as the basic building blocks. *See Id.*; EX1037 at 2:61-3:44. Further, a POSA would have had a reasonable expectation of successfully pursuing these options because the technology for synthesis of oligonucleotides was well-developed as of 2010. *See, e.g.*, EX1002 ¶ 284; EX1038.

X. CONCLUSION

The challenged claims should be canceled.

Dated: July 21, 2025

Respectfully submitted,

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APPENDIX: CHALLENGED CLAIM LISTING

No.	Limitation
1	A method comprising: a) obtaining segments of genomic nucleic acids from a sample containing the genomic nucleic acids; b) randomly tagging the segments of genomic nucleic acids with different nucleic acid tags, thereby generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids, 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 unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating copies of the unique tagged nucleic acid molecules; d) generating tag associated sequence reads by sequencing the copies of the unique tagged nucleic acid molecules of step (c); e) assigning each of the unique tagged nucleic acid molecules to a location on a genome by mapping each of the tag associated sequence reads of step (d) to a location in the genome; and f) in a plurality of locations in the genome, obtaining relative copy number information of the genomic nucleic acids from the sample based on the number of unique tagged nucleic acid molecules that have been assigned to each of the plurality of locations in the genome.
2	The method of claim 1, wherein the polymerase chain reaction is performed in the presence of region specific PCR primers.
3	The method of claim 1, wherein the unique tagged nucleic acid molecules differ at more than one nucleotide or wherein the sequences of the nucleic acid tags are known prior to said generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids.
4	The method of claim 1, wherein the segments of the genomic nucleic acids are produced by mechanical shearing, heating, or sonicating the genomic nucleic acids from the sample.

5	The method of claim 1, wherein the nucleic acid tags comprise a sample tag or a sample tag set.
6	The method of claim 5, wherein the unique tagged nucleic acid molecules are pooled with a 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 product of step (c).
7	The method of claim 6, further comprising deconvoluting the tag associated sequence reads by grouping the tag associated sequence reads.
8	The method of claim 1, wherein the nucleic acid tags are double-stranded.
9	The method of claim 1, wherein the unique tagged nucleic acid molecules are subjected to hybrid capture prior to the PCR or the amplified tagged nucleic acid molecules produced in step (c) are subjected to hybrid capture prior to step (d).
10	The method of claim 1, wherein the genomic nucleic acids are from a single species, a single cell, two or more organisms, two or more species, or from a population of microbes.
11	The method of claim 1, wherein the sample is a blood sample or bone marrow sample.
12	The method of claim 1, wherein the unique tagged nucleic acid molecules are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids.
15	The method of claim 12, wherein the nucleic acid tags comprise nucleotide sequences with constant portions and variable portions.
16	The method of claim 15, wherein the length of the constant portions and the length of the variable portions is the same.
17	The method of claim 15, wherein the length of the constant portions and the length of the variable portions are different.
18	The method of claim 15, wherein the variable portions consist of only two species of nucleotides.
19	The method of claim 15, wherein the variable portions consist of only three species of nucleotides.
20	The method of claim 12, wherein the nucleic acid tags comprise variable portions constructed by a set of dinucleotides.

21	The method of claim 12, wherein the nucleic acid tags comprise variable portions constructed by a set of trinucleotides.
22	The method of claim 1, wherein the nucleic acid tags comprise a sufficiently large number of distinct nucleic acid tags.
23	The method of claim 22, wherein the unique tagged nucleic acid molecules are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids.
26	The method of claim 22, wherein the nucleic acid tags comprise nucleotide sequences with constant portions and variable portions.
27	The method of claim 26, wherein the length of the constant portions and the length of the variable portions is the same.
28	The method of claim 26, wherein the length of the constant portions and the length of the variable portions are different.
29	The method of claim 26, wherein the variable portions consist of only two species of nucleotides.
30	The method of claim 26, wherein the variable portions consist of only three species of nucleotides.
31	The method of claim 22, wherein the nucleic acid tags comprise variable portions constructed by a set of dinucleotides.
32	The method of claim 22, wherein the nucleic acid tags comprise variable portions constructed by a set of trinucleotides.
33	The method of claim 22, wherein the nucleic acid tags comprise nucleotide sequences that are not known before the tagged nucleic acid molecules are generated.
34	The method of claim 22, wherein the nucleic acid tags comprise random nucleotide sequences.
35	The method of claim 22, wherein the nucleic acid tags are substantially unique nucleic acid tags.
36	The method of claim 1, wherein the nucleic acid tags are substantially unique nucleic acid tags.

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 13,930 words as calculated by the “Word Count” feature of Microsoft Word for Microsoft 365, the word processing program used to create it.

Respectfully submitted,

JONES DAY

Dated: July 25, 2025

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. 12,234,510 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, 48 th Floor New York NY 10174	
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