

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA INC.,
Petitioner,

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner.

Case No. IPR2024-00965
Patent No. 11,768,200

PETITION FOR *INTER PARTES* REVIEW

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GLOSSARY

'200 Patent	U.S. Patent No. 11,768,200
'218 Patent	U.S. Patent No. 9,163,281
<i>Bold and italicized</i>	Emphasis added unless otherwise indicated
IPR	<i>Inter partes</i> review
POSA	Person of ordinary skill in the art

TABLE OF EXHIBITS

Exhibit	Description
1001	U.S. Patent No. 11,768,200
1002	Declaration of Gregory B. Gloor, Ph.D.
1003	Declaration of Paul T. Spellman, Ph.D.
1004	P. Parameswaran, et al., <i>A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing</i> , 35 NUCLEIC ACIDS RSCH. 19(e130) (2007).
1005	G.B. Gloor, et al., <i>Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products</i> , Cornell University Library arXiv:1007.5075v1 (2010).
1006	D.R. Bentley, et al., <i>Accurate whole human genome sequencing using reversible terminator chemistry</i> , 456 NATURE 6, 53-59 (2008).
1007	File History of U.S. Patent No. 11,768,200
1008	U.S. Provisional Application No. 61/426,817
1009	F. Sanger, et al., <i>DNA sequencing with chain-terminating inhibitors</i> , 74 PROC. NAT'L. ACAD. SCI. USA 12, 5463-5467 (1977).
1010	M. Kircher & J. Kelso, <i>High-throughput DNA sequencing – concepts and limitations</i> , 32 BIOESSAYS 6, 524-536 (2010).
1011	M.L. Metzker, <i>Sequencing technologies—the next generation</i> , 11 NATURE REVIEWS GENETICS 31-46 (2010).
1012	J. Shendure & H. Ji, <i>Next-generation DNA sequencing</i> , 26 NATURE BIOTECH. 10, 1135-1145 (2008).
1013	G. Wiley, et al., <i>Methods for generating shotgun and mixed shotgun/paired-end libraries for the 454 DNA sequencer</i> , 61 CURRENT PROTOCOLS IN HUMAN GENETICS 1, 18.1.1-18.1.21 (2009).
1014	M. Meyer, et al., <i>Parallel tagged sequencing on the 454 platform</i> , 3 NATURE PROTOCOLS 2, 267-278 (2008).

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1015	M. Meyer & M. Kircher, <i>Illumina sequencing library preparation for highly multiplexed target capture and sequencing</i> , 2010, COLD SPRING HARBOR PROTOCOL 6 (2010).
1016	U.S. Application Publication No. 2012/0028814
1017	S.J.G. Lahr & L.A. Katz, <i>Reducing the impact of PCR-mediated recombination in molecular evolution and environmental studies using a new-generation high-fidelity DNA polymerase</i> , 47 BIOTECHNIQUES 4, 857-866 (2009).
1018	A. Meyerhans, et al., <i>DNA recombination during PCR</i> , 18 NUCLEIC ACIDS RSCH. 7, 1687-1691 (1990).
1019	S. Paabo, et al., <i>DNA damage promotes jumping between templates during enzymatic amplification</i> , 265 J. OF BIOL. CHEM. 8, 4718-4721 (1990).
1020	S.J. Odelberg, et al., <i>Template-switching during DNA synthesis by <i>Thermus aquaticus</i> DNA polymerase I</i> , 23 NUCLEIC ACIDS RSCH. 11, 2049-2057 (1995).
1021	D.W. Craig, et al., <i>Identification of genetic variants using bar-coded multiplexed sequencing</i> , 5 NATURE METHODS 10, 887-893 (2008).
1022	R. Li, et al., <i>SNP detection for massively parallel whole-genome resequencing</i> , 19 GENOME RSCH. 1124-1132 (2009).
1023	M. Choi, et al., <i>Genetic diagnosis by whole exome capture and massively parallel DNA sequencing</i> , 106 PNAS 45, 19096-19101 (2009).
1024	G.B. Gloor, et al., <i>Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products</i> , 5 PLoS ONE 10(e15406) (2010).
1025	<i>DNA Sequencing With Solexa® Technology</i> , Technology Spotlight, Illumina® Systems & Software, Pub. No. 770-2007-002 (2007).
1026	File History of U.S. Patent No. 9,163,281
1027	Illumina Press Release, <i>Illumina Announces New TruSeq™ SBS Sequencing Kit for the Genome Analyzer</i> , ILLUMINA (July 26, 2010), accessed at https://emea.illumina.com/cmopany/news-center/press-releases/2010/1451638.html on June 1, 2024.

Exhibit	Description
1028	<i>Genome Sequencer 20 System First to the Finish</i> , Roche Diagnostics®, Pub. No. 04866452990 (2006).
1029	J. Binladen, et al., <i>The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing</i> , 2 PLoS ONE 2:e197 (2007).
1030	U.S. Patent No. 9,163,281
1031	Bruce Alberts, et al., <i>Molecular Biology of the Cell</i> 4, GARLAND SCIENCE (2002).
1032	<i>Polymerase Chain Reaction (PCR)</i> , NATIONAL HUMAN GENOME RESEARCH INSTITUTE (updated June 26, 2024), https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction .
1033	M. Dai, et al., <i>NGSQC: cross-platform quality analysis pipeline for deep sequencing data</i> , 11 BMC GENOMICS 11(Suppl 4):S7 (2010).
1034	S. Creer, et al., <i>Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises</i> , 19 MOLECULAR ECOLOGY (Suppl. 1) 4-20 (2010).
1035	The Cancer Genome Atlas Research Network, <i>Comprehensive genomic characterization defines human glioblastoma genes and core pathways</i> , 455 NATURE 23 (2008).
1036	U.S. Application Publication No. 2009/0226975
1037	International Patent Publication No. WO 2010/056728
1038	P. Maughan, et al., <i>Single-Nucleotide Polymorphism Genotyping in Mapping Populations via Genomic Reduction and Next-Generation Sequencing: Proof of Concept</i> , 3 PLANT GENOME (3) 166-178 (2010).

LIST OF CHALLENGED CLAIMS

Claim	Element
1[pre]	A method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction, the method comprising:
1[a]	i) detecting the presence of two or more identifier sequences that are uniquely associated with the nucleic acid analyte of interest,
1[b]	wherein at least a first identifier sequence is incorporated into a 5' portion of the nucleic acid analyte of interest and wherein at least a second identifier sequence is incorporated into a 3' portion of the nucleic acid analyte of interest, and
1[c]	wherein the first and second identifier sequences have four or more nucleotides and are different;
1[d]	ii) sequencing the first identifier sequence, the nucleic acid analyte of interest and the second identifier sequence; and
1[e]	iii) validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers and excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an incorrect pair of identifiers from sequence analysis of the multiplex sequencing reaction.
2	The method of claim 1, wherein the identifier sequences are nucleic acid sequence tags.
3	The method of claim 2, wherein the identifier sequences are barcode sequences.
4	The method of claim 3, wherein the barcode sequences are from 4 to 20 nucleotides.
5	The method of claim 4, wherein the barcode sequences are designed to have no homopolymer regions.
6	The method of claim 1, wherein the nucleic acid analyte of interest is on the surface of a flow cell.

I. INTRODUCTION

Petitioner Illumina, Inc. respectfully requests *Inter Partes* Review of U.S. Patent No. 11,768,200 (EX1001; the “Patent” or “’200 Patent”), assigned to Molecular Loop Biosciences, Inc., and cancellation of claims 1-6. Those claims are unpatentable as anticipated by two prior-art references and would also have been obvious over the combination of those references and other not-of-record references.

As explained in the accompanying Declaration of Dr. Paul Spellman (EX1003, ¶¶1-17, 21-32, 260-261), an expert in the sequencing field, the Patent is directed to validating nucleic acid sequences obtained from multiple samples in a single multiplex sequencing run by tagging nucleic acids with a pair of identifier sequences uniquely associated with nucleic acids from that sample. Specifically, it claims incorporating two or more identifier sequences that differ from each other, one into each end of the nucleic acid, and excluding sequences containing “only one identifier” or “an incorrect pair of identifiers,” which reflect errors in the sequencing process. Because these “identifier sequences” (sometimes called “indexes,” “barcodes,” or “tags”) are unique, detecting the presence of both identifiers validates the nucleic acid’s sequence.

The challenged claims are unpatentable. By the earliest possible priority date of December 23, 2010, using distinct pairs of “different” identifier sequences for this precise purpose was already well-known. *Gloor*, which was not considered by the

Examiner, discloses it. *Binladen*, which also was not considered by the Examiner, discloses it. And *Parameswaran*, which was disregarded, also discloses it. The challenged claims are therefore anticipated and rendered obvious by this art.

Statements made during prosecution confirm that the challenged claims are unpatentable. During prosecution of a parent application with substantially similar claims to the '200 Patent, the applicant distinguished *Parameswaran* by arguing that it used “different” identifier sequences, and amended the claims in that parent application to require use of the “same” identifier sequences. In the '200 Patent, Patent Owner *claims the very feature, “different” identifiers, admittedly taught by Parameswaran*, confirming that the challenged claims are unpatentable. Accordingly, this IPR should be instituted, and the challenged claims canceled.

II. BACKGROUND AND ASSERTED PRIOR ART

A. Next-Generation Sequencing Techniques Were Known as of December 2010

Until the early 2000s, DNA sequencing relied upon variations of traditional Sanger-based sequencing, a method first developed in the 1970s. EX1009; EX1003, ¶¶44-48. These methods could not be used to simultaneously sequence a mixture of samples. EX1011, 1; EX1010, 2-3; EX1035; EX1003, ¶¶49-50. By 2005, however, “multiplex sequencing” permitted sequencing many samples, mixed together, in a single run. Multiplex sequencing permitted the sequencing of more samples, over a

shorter time, than traditional Sanger-based sequencing, at a fraction of the cost. EX1011, 1; EX1010, 1; EX1003, ¶¶51, 81-83.

Because multiplex techniques sequenced mixtures of samples simultaneously, nucleic acids isolated from each sample needed to be tagged in some way so that the sequencing data could be matched to the correct sample. EX1003, ¶67. Thus, to sequence multiple samples (e.g., samples from different patients) during a single run, sample-specific “identifier sequences” (also referred to as “barcodes,” “tags,” or “indexes”) were added to the nucleic acids before sequencing them, such that the nucleic acid analytes could be associated with their appropriate source. EX1010, 9. After sequencing, the identifier sequences were used to assign the associated sequences of the nucleic acids to their corresponding samples, a process referred to as “demultiplexing.” EX1003, ¶68.

Illumina and Roche 454 were two of the three major sequencing platforms suitable for multiplex sequencing used as of December 2010. EX1004, 1; EX1013; EX1025; EX1027; EX1003, ¶52. Both sequencing techniques used forms of sequencing-by-synthesis, which involves determining the sequence of a single-stranded template nucleic acid attached to a substrate by synthesizing a nucleic acid strand complementary to that single-stranded template nucleic acid. EX1003, ¶¶53-54. As nucleotides are incorporated to build the complementary strands, methods are used to simultaneously detect each nucleotide that is incorporated (A, C, G, or T),

revealing the sequence of the complementary strand. EX1003, ¶¶55-58; EX1010, 3-6; EX1031, 38.

Both Illumina and Roche 454 sequencing involved preparing DNA sequencing libraries by extracting DNA sequences from their source, and then flanking the sequences of interest with adapters. EX1010, 3-6. The adapters were short nucleotide sequences used to tether the analyte to the surface where nucleic acid amplification and sequencing occurred. EX1010, 3-6. In both platforms, amplification was necessary to make more copies of each analyte being sequenced, which in turn produced a stronger signal to more reliably detect nucleotides being added in the sequencing-by-synthesis reaction. EX1011, 2; EX1032; EX1003 ¶¶61-66.

For sequencing, the templates containing the DNA sequences of interest and adaptors were placed into flow cells, where fluids containing sequencing reagents can flow over the templates in a controlled manner. EX1003, ¶¶59-60. In Illumina sequencing, the templates were copied directly to flow cells using a process called bridge amplification. EX1006, 1-2 (Fig.1); EX1010, 4-5. In Roche 454 sequencing, the templates were copied to beads via PCR amplification, and beads with amplified templates were then placed into flow cells. EX1028, 6-8.

B. Dual-Indexing Methods Were Known as of December 2010

Initially, multiplex sequencing for Roche 454 and Illumina sequencers used a single identifier sequence to associate a template nucleic acid with a specific sample. EX1003, ¶¶67-69. But by December 2010, dual-indexing methods—in which *two* identifier sequences were added to each template nucleic acid before multiplex sequencing (e.g., one identifier sequence at each end of the template)—were well known. EX1003, ¶¶70-80; EX1016, [0091]-[0092]; EX1038, 3-4 (attaching “barcode primer *pairs*” to “DNA fragments for each DNA sample” during Roche 454 and Illumina multiplex sequencing).

Dual-indexing methods improve the accuracy of multiplex sequencing. EX1003, ¶¶73-80. For example, the amplification reactions necessary for sequencing can terminate prematurely due to technical errors (such as limited PCR reaction capacity or suboptimal reaction conditions). *See* EX1004, 7; EX1029, 6. Alternatively, the adapters or primers used for amplification could be incorrectly synthesized. *See* EX1004, 7; EX1029, 6. In some instances, sequences resulting from these technical errors would include only one correct identifier sequence. *See* EX1004, 7. By requiring the presence of correct identifier sequences at *both* ends of the analyte, dual-indexing allowed researchers to identify and eliminate incomplete sequences produced by these technical errors. *See* EX1004, 7; EX1029, 5; EX1003, ¶¶73-80.

Relative to single indexing, dual-indexing also was known to improve the process of sample identification. EX1003, ¶¶73-80; EX1004, 8; EX1005, 1. For example, nucleic acids from different samples can anneal together during PCR amplification, creating erroneous “chimeric” combinations. *See e.g.*, EX1017, 1 (“Chimeras are formed when incompletely extended DNA fragments anneal to closely related sequences generating recombinants between starting templates.”); EX1018, 1; EX1019, 1; EX1020, 7; EX1003, ¶¶70-72. These chimeric molecules were particularly problematic for multiplex sequencing, causing misassignment of sequences to incorrect samples and compromising sequencing accuracy. EX1004, 7-8; *see* EX1034, 10, 13 (next-generation sequencing was “the ideal ‘breeding ground’” for chimeric sequences); EX1033, 8-9 (reporting about 10% of sequence reads as potential chimeric sequences generated during sequencing workflow); EX1017, 1 (chimeric sequences were “worrisome” because it can be difficult to differentiate closely related sequences from chimeras); EX1003, ¶72.

As of December 2010, there were two general forms of dual-indexing used for multiplex sequencing. EX1003, ¶¶73-80. The first, ***unique dual-indexing***, attached unique sequence identifiers at opposite ends of nucleic acids of interest obtained from a single sample. EX1003, ¶¶74-76. In unique dual-indexing, each pair of identifier sequences is specific to nucleic acids obtained from a single sample, and no single identifier sequence is used for any other sample. *See, e.g.*, EX1038, 3

(Table 1). Detection of a sequence with “mismatched” barcodes—pairs of barcodes that were not assigned to any sample—may indicate the presence of a chimeric molecule that erroneously formed during amplification. As discussed below, unique dual-indexing was used in *Parameswaran*. *Infra* §III.A.

The second form of dual-indexing was called combinatorial indexing, or ***non-unique dual-indexing***. EX1003, ¶¶77-80. In non-unique dual-indexing, pairs of identifiers are again attached at opposite ends of the nucleic acids obtained from each sample. While each ***pair*** of identifier sequences is used for nucleic acids from only one sample, the distinction from *unique* dual-indexing is that individual identifier sequences can be reused across nucleic acids from multiple samples. Non-unique dual-indexing cannot necessarily identify chimeric molecules without additional data processing, particularly if the pair of identifiers attached to the sequenced chimeric molecule matches one of the pairs already assigned to nucleic acids from a given sample. EX1003, ¶¶78-79. As discussed below, *Gloor* discloses the use of non-unique dual-indexing. *Infra* §III.B.

III. THE ASSERTED PRIOR ART

A. *Parameswaran* (EX1004)

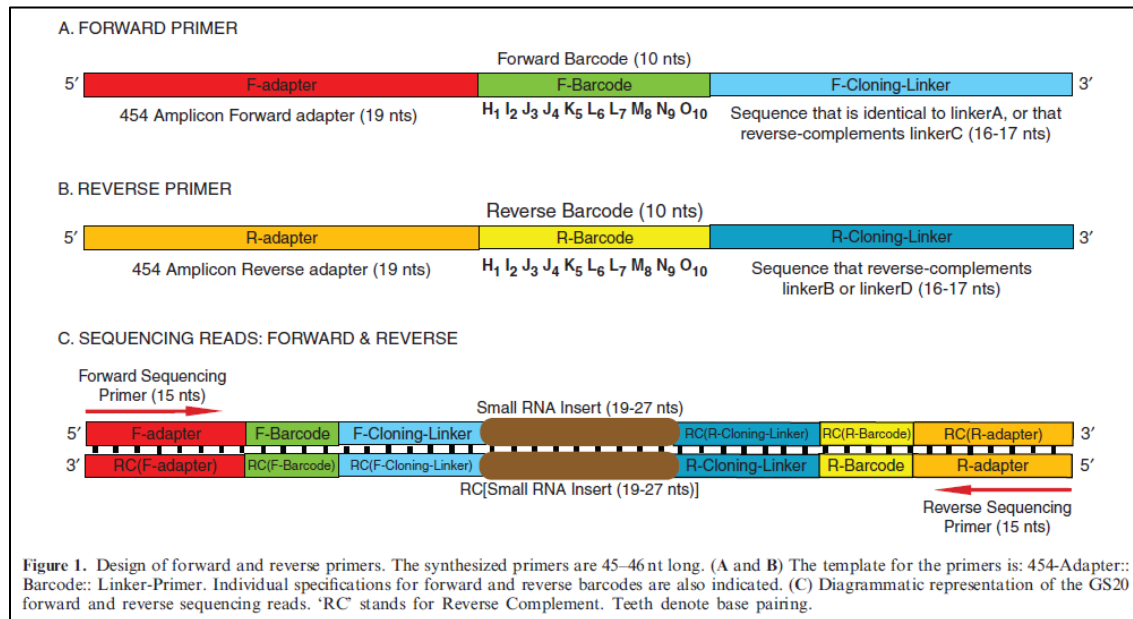
Parameswaran (EX1004), titled “A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing” and published October 11, 2007, is prior art under pre-AIA 35 U.S.C. §102(b).

EX1003, ¶84. *Parameswaran*'s prior-art public accessibility is evidenced at least by its citation in papers published over one year before December 23, 2010. *Bruckelmyer v. Ground Heaters*, 445 F.3d 1374, 1379 (Fed. Cir. 2006) (finding reference "publicly accessible" because cited in "research aid"). *Parameswaran* was cited in *Meyer*, published January 31, 2008, and *Craig*, published September 14, 2008. EX1014, 12 (reference 20); EX1021, 7 (reference 5).

Parameswaran describes a barcoding approach that assigned two uniquely designed, 10-nucleotide barcodes to the opposite ends of nucleic acids obtained from each sample, thereby enabling multiple samples to be pooled together and sequenced simultaneously. EX1004, 2-4; EX1003, ¶85. Each pair of barcodes included one "forward" barcode and one "reverse" barcode that differed from each other "by at least 4 nt positions." EX1004, 2-3, Abstract ("unique with respect to at least 4 nt positions"). The barcoding approach was used to sequence RNA-derived inserts of 19-27 nucleotides in length with a Roche 454 sequencer. EX1004, 5; EX1003, ¶86.

As shown in the excerpt below, the barcoded primers used in *Parameswaran* comprised three portions. EX1004, 2-3, 14-17 (Supplementary Table 1A) (reciting full sequences of forward and reverse primers); EX1003, ¶86. Forward primers were composed of the Roche-specific forward adapter ("F-adapter"), the forward barcode ("F-Barcode"), and the forward cloning linker ("F-Cloning-Linker"). Reverse primers were composed of the Roche-specific reverse adapter ("R-adapter"), the

reverse barcode (“R-Barcode”), and the reverse cloning linker (“R-Cloning-Linker”).
EX1004, 3 (Fig.1).



EX1004, 3 (Fig.1).

To maximize the uniqueness of dual-indexing and facilitate definitive sample assignments, *Parameswaran* expanded the 10-nucleotide barcodes to “barcode motifs,” which included three additional nucleotides from linkers adjoining the barcodes. EX1004, 7; EX1003, ¶87. Following sequencing, *Parameswaran* reports that about 94% of the obtained sequences had perfect forward and reverse barcode motifs, while about 5-6% of sequences had perfect barcode motifs only at one end. EX1004, 7; EX1003, ¶88. *Parameswaran* categorized the sequences that had a perfect match to only one of the expected barcode motifs into three subcategories: (1) those having perfect, unexpected pairings of unmatched forward and reverse

barcode motifs (“mismatched” barcodes); (2) those having a partial match to an expected barcode motif; and (3) those with no strong match to any barcode motif. EX1004, 7; EX1003, ¶89. In other words, 5-6% of *Parameswaran*’s sequences contained a pair of sequence identifiers different from any of the pairs assigned to the samples, or a pair that contained one correct sequence identifier and one incomplete or incorrect sequence identifier. EX1003, ¶89.

Parameswaran reports a “false-discovery” rate, defined as sequences containing unexpected, mismatched pairings of forward and reverse barcode motifs (i.e., pairs of identifiers not assigned to any template). EX1004, 8, Abstract; EX1003, ¶91. *Parameswaran* states that the “low false-discovery rate for perfect matches strengthens the argument that ***any sequence with both 5’ and 3’ barcode motifs*** [barcode motifs at both ends] will be placed with reasonable accuracy into the correct bin.” EX1004, 8. *Parameswaran* explains that sequences with mismatched barcode motifs can arise from various errors, for example, cross-contamination of DNA templates. EX1004, 7; EX1003, ¶90.

Parameswaran explains that its barcoding approach “may also be compatible with other sequencing platforms.” EX1004, 8; *see also* EX1004, 2 (“We also speculate on the use of these pyrosequencer-tailored barcodes with other sequencing platforms.”). *Parameswaran* identifies Illumina sequencers as one of the “major high-throughput sequencing platforms.” EX1004, 1; EX1003, ¶92.

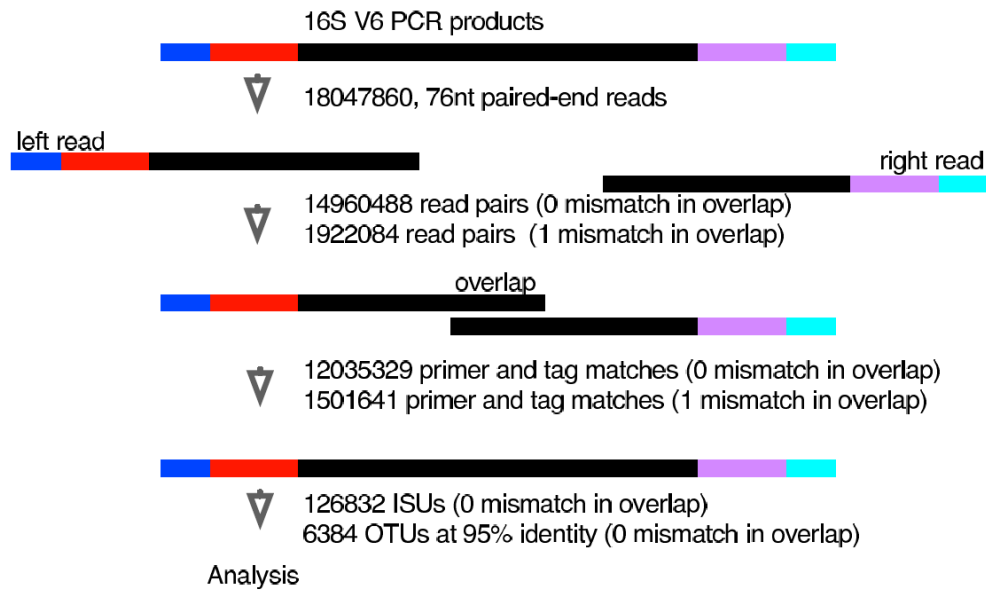
B. *Gloor* (EX1005)

Gloor (EX1005), titled “Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products” and published July 29, 2010, is prior art under at least pre-AIA 35 U.S.C. §102(a). EX1003, ¶93; *see also* EX1024. *Gloor*’s prior art public accessibility is evidenced by the Declaration of Gregory Gloor, Ph.D. (EX1002, ¶¶1-11). Dr. Gloor, the first named author and submitter of *Gloor* to arXiv.org, received confirmation from the archive that *Gloor* was published on July 29, 2010, recalls his paper being published promptly after submission, and recalls discussing the publication with other scientists at the Human Microbiome Research Conference in St. Louis, Missouri, in September 2010. EX1002, ¶¶12-19.

Gloor describes a low-cost, high-throughput Illumina sequencing method that was used to sequence the V6 region of bacterial 16S rRNA genes from 272 clinical samples. EX1005, 2; EX1003, ¶95. When preparing the sequencing library, *Gloor* used pairs constituting a “left primer” and a “right primer,” wherein each primer contained both a “sequence tag” (identifier sequence) and a sequence annealing to the specific gene region of interest. EX1005, 1-3. These primer pairs were used to amplify the region of interest, incorporating the sequence tags in the left and right primers into each end of the amplicons. EX1003, ¶95. *Gloor* used the left and right primers in a “combinatorial” manner, such that templates from each sample contained the specific gene region flanked by a unique pair of sequence identifiers.

EX1005, 1-3; EX1003, ¶94. *Gloor* discloses the sequences of the identifier sequences, each of which were between 3-6 nucleotides long. EX1005, 5-6 (Table 2). The templates were sequenced using the Illumina sequencer, which required Illumina-specific adaptors for tethering the templates. EX1005, Abstract, 23-24; *infra* §III.D; EX1003, ¶¶96-97.

Figure 2 in *Gloor*, reproduced below, depicts the workflow used. EX1003, ¶98. *Gloor* generated sequence reads that were 76 nucleotides long from each end of the PCR-amplified region. EX1005, 6-7. Because the analytes of interest produced amplified products of about 110-130 nucleotides long, each sequence read, if correctly amplified and sequenced, included either the left or right tag (shown in blue and light blue, respectively). EX1005, 3, 6-7 (Fig.2); EX1003, ¶98. *Gloor* then combined sequence reads that substantially overlapped one another, thereby identifying a complete sequence of each analyte containing both the left and right sequence tags. EX1005, 7; EX1003, ¶98.



EX1005, 7 (Fig.2).

Putting this workflow into practice, *Gloor* collected about 18 million sequence reads that were 76 nucleotides long. EX1005, 6-7; EX1003, ¶¶99. *Gloor* combined sequence reads that substantially overlapped to identify complete sequences. EX1003, ¶¶98-99. *Gloor* then “extract[ed] overlapping reads with ***proper primer sequences and correct sequence tags***.” EX1005, 7. *Gloor* explains that only “[t]he sequences derived from the perfectly overlapping reads form the basis of the remainder of the analysis.” EX1005, 7; *see also* EX1005, 6 (“Only reads with full-length sequence tag sequences were used in this analysis.”); EX1003, ¶¶99.

Gloor examined the sequences excluded from the subsequent analysis. EX1005, 7-8. *Gloor* explains that these excluded sequences contained “incorrect sequence tags,” for example, completely “lack[ing] the left or right end sequence tag”

or containing sequence tags that were truncated or showed addition or misincorporation of bases. EX1005, 7-8; EX1003, ¶100.

Gloor states that “the Illumina sequencing method has an exceedingly low error rate and that the majority of errors arise during the PCR amplification step.” EX1005, 2. *Gloor* explains that “[t]he Illumina sequencing method generated ~12 million useable reads at a cost of ~0.03 cents per read, an approximate order of magnitude cheaper than the per-read cost of pyrosequencing.” EX1005, 2 (“The cheaper per-read costs allows economical experiments on large numbers of samples at very large sequencing depths.”). Finally, *Gloor* states that the Illumina sequencing method can be easily adapted for paired-end sequencing of the microbial V3, V5, V6 and the eukaryotic V9 rRNA regions. EX1005, 2; EX1003, ¶101.

C. *Binladen* (EX1029)

Binladen (EX1029), titled “The Use of Coded Primers Enables High-Throughput Sequencing of Multiple Homolog Amplification Products by 454 Parallel Sequencing” and published in February 2007, is prior art under pre-AIA 35 U.S.C. §102(b). EX1003, ¶102. *Binladen*’s prior-art public accessibility is evidenced by its citation in papers published over one year before December 23, 2010, including *Parameswaran*. EX1004, 9 (reference 10).

Binladen used dual-indexing to address the problem of matching sequence reads in a multiplex reaction to a sample. EX1029, 1-2; EX1003, ¶¶103-104.

Binladen describes a dual-indexing approach “where initial PCR primers are 5’-tagged with short nucleotide sequences (tags) in such a way that a **unique** tagged primer combination can be applied to each specific DNA template source.” EX1029, 2. *Binladen* prepared two sets of tags: (1) a first set containing dinucleotide tags (2 nucleotides each) and (2) a second set containing tetranucleotide tags (4 nucleotides each). EX1029, 3; EX1003, ¶105. Following PCR with these tagged primers, the nucleic acid analytes from each DNA sample were flanked with unique forward and reverse sequence tags. EX1029, 3; EX1003, ¶105.

Using Roche 454 sequencing, *Binladen* sequenced mammalian mitochondrial DNAs from thirteen different species. EX1029, 3; EX1003, ¶106. *Binladen* generated sequence reads that were about 120 nucleotides long from each end of the nucleic acid analyte. EX1029, 3; EX1003, ¶106. Because the analytes were 133-141 nucleotides long, each sequence read, if correctly amplified and sequenced, included either the forward or reverse tag. EX1029, 3; EX1003, ¶106. *Binladen* explains that because “the correct association of tags and sequences is crucial to the approach,” “all sequence reads without an exact match to any of the primers used in the studies” were “discarded.” EX1029, 5; EX1003, ¶107. The discarded sequences included those that “failed” to show any match to the primer sequences in general, those that lacked a sequence identifier, and those with mismatches, deletions, and/or insertions. EX1029, 6; EX1003, ¶107. *Binladen* further explains that these discarded sequences

may have arisen through technical errors such as incorrect primer synthesis or errors in the sequencing-by-synthesis process. EX1029, 6; EX1003, ¶107. *Binladen* confirms that nearly 90% of the remaining sequences “diverged by no more than 1bp from one of the reference sequences, and could thus be assigned to one of the taxa analysed in the study.” EX1029, 6; EX1003, ¶108.

Binladen also reports that while primers with 4-nucleotide tags showed lower PCR efficiency, “overall the rate of sequence miss-assignment for these primers was lower than for the dinucleotide tags.” EX1029, 7, 8 (“the apparent reduced miss-assignment rates of the tetranucleotide tags”); EX1003, ¶108. *Binladen* concludes that its dual-indexing approach “will be of importance to a broad range of research areas where large-scale comparisons of homologous DNA sequences from multiple sources are needed.” EX1029, 8; EX1003, ¶108.

D. *Bentley* (EX1006)

Bentley (EX1006), titled “Accurate whole human genome sequencing using reversible terminator chemistry” and published on November 6, 2008, is prior art under at least pre-AIA 35 U.S.C. §102(b). EX1003, ¶109. *Bentley*’s prior-art public accessibility is evidenced by its citation in papers published over one year before December 23, 2010. *Bentley* was cited in *Li*, published May 6, 2009, and *Choi*, published November 10, 2009. EX1022, 9; EX1023, 6 (reference 16).

Bentley provides a detailed description of Illumina paired-end sequencing. EX1003, ¶¶110-112. Specifically, *Bentley* describes a “massively parallel synthetic sequencing approach” that involved shearing DNA strands into double-stranded DNA fragments for sequencing and ligating (joining) Illumina-specific adapters to both ends. EX1006, 2 (Fig.1a); *see also* EX1006, 8, 13-14; EX1003, ¶110. The DNA fragments were then denatured into single strands, and the Illumina-specific adapters on the ends of the templates were annealed to complementary oligonucleotides on the surface of the flow cell. EX1006, 2 (Fig.1b); *see also* EX1006, 8, 13-15; EX1003, ¶110.

Via bridge amplification, the single-stranded DNA fragments were amplified on the surface of the flow cell to form “clusters” of up to 1,000 identical copies of the single-stranded DNA fragments within close proximity ($\sim 1\mu\text{m}$) for sequencing-by-synthesis. EX1006, 2 (Fig.1b); *see also* EX1006, 8, 14-15. EX1003, ¶¶111-112.

IV. THE '200 PATENT

The Patent claims priority to U.S. Provisional Application No. 61/426,817, filed December 23, 2010. EX1001. For the purposes of this Petition, December 23, 2010, is used as the effective priority date for the Patent. EX1003, ¶¶18, 21.

A. Person of Skill in the Art (“POSA”)

A POSA would have had (i) a Ph.D. in molecular biology, genetics, bioinformatics, or a related field and at least two years of experience in high-throughput sequencing technologies or (ii) a Master’s degree in one of the same fields with at least four years of experience in high-throughput sequencing technologies. EX1003, ¶¶19-20.

B. Disclosure of the ’200 Patent

The Patent purports to disclose “methods for maintaining the integrity and identification of a nucleic acid template in a multiplex sequencing reaction” by incorporating “a pair of sequence identifiers into the template, and sequencing the template.” EX1001, Abstract; EX1003, ¶¶113, 117.

The Patent includes Figures 1-3. Figures 1 and 2 are conceptual. EX1003, ¶¶118-122. The Patent states that Figure 1 shows “how molecular overlap can lead to apparent high quality, but incorrect sequence data.” EX1001, 4:18-20. Figure 1 depicts nucleic acid analytes from different samples, each of which includes one barcode. EX1001, Fig.1; EX1003, ¶¶118-119. One of the analytes is partially amplified and combined with the amplified product of the other analyte, resulting in “improper molecular overlap.” EX1001, Fig.1, 4:17-63. Specifically, gene A (“GA”) from Patient A has improperly been combined with barcode B. EX1001, Fig.1.

Figure 2 depicts two nucleic acid analytes of interest, this time each including two different barcodes (with gene A flanked by barcodes A1 and A2, and gene B flanked by barcodes B1 and B2). EX1001, Fig.2; EX1003, ¶¶120-122. Figure 2 also depicts sequence products resulting from “improper molecular overlap.” EX1001, Fig.2, 5:41-6:6. Specifically, gene A is flanked by barcodes A1 and B2. The Patent states that, “[w]hile each barcode sequence on its own is a valid barcode sequence, taken together, the two barcode sequences do not form a valid barcode sequence pair.” EX1001, 5:62-65.

Figure 3 purports to show “that methods of the invention dramatically reduce false positives in a multiplex sequencing reaction.” EX1001, 6:7-9; EX1003, ¶¶123-124. It compares two sample preparations, one containing “only a single barcode sequence” and the other containing “two barcode sequences.” EX1001, Fig.3, 6:11-16. The Patent states that, as compared to the preparation with a single barcode sequence, using two barcode sequences allowed identification of products resulting from “crossover.” EX1001, 6:53-7:38. The Patent, however, provides no detailed information on how the experiment in Figure 3 was conducted. EX1003, ¶125. For example, the Patent is silent on the source of the samples, the identity of and design rationale for the barcode sequences used, and the sequencing platform used for the study. EX1003, ¶125. Figure 3 is not found in the 2010 priority application. *See generally* EX1008.

The Patent contains about 17 columns of text in the “Detailed Description.” Of those 17 columns, 11 appear to be copied nearly verbatim from Petitioner’s own patent publication (*compare* EX1001, 10:3-20:49 *with* EX1036, ¶¶20, 21, and 23-78), and 2 additional columns appear largely copied from another prior art patent publication (*compare* EX1001, 7:45-10:2 *with* EX1037, 9-11). Other portions of the ’200 Patent were also copied from that patent publication. *Compare* EX1001, 1:25-31, 3:37-43, 20:52-67 *with* EX1037, 1, 3, 18; *see also* EX1003, ¶126.

C. The Challenged Claims

Independent claim 1 recites:

A method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction, the method comprising:

i) detecting the presence of two or more identifier sequences that are uniquely associated with the nucleic acid analyte of interest,

wherein at least a first identifier sequence is incorporated into a 5’ portion of the nucleic acid analyte of interest and wherein at least a second identifier sequence is incorporated into a 3’ portion of the nucleic acid analyte of interest, and

wherein the first and second identifier sequences have four or more nucleotides and are different;

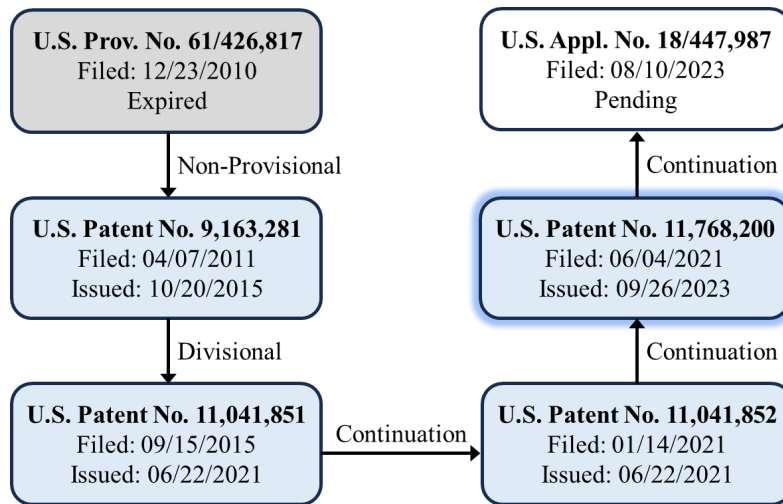
ii) sequencing the first identifier sequence, the nucleic acid analyte of interest and the second identifier sequence; and

iii) validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers and excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an incorrect pair of identifiers from sequence analysis of the multiplex sequencing reaction.

EX1001, 21:2-22:6; EX1003, ¶¶114-115. Challenged dependent claims 2-6 further recite features of the claimed method, such as identifier sequence type. EX1001, 22:7-16; EX1003, ¶116.

D. Prosecution History

The '200 Patent is descends from, and shares a specification with, U.S. Patent No. 9,163,281 ("the '281 Patent"). EX1003, ¶127. The '200 Patent claims similar subject matter as the '281 Patent, except that the "first and second identifier sequences" in the '200 Patent must be "different" whereas the "first and second barcode sequences" in the '281 Patent are "the same." EX1003, ¶127.



As detailed below, during prosecution of the parent '281 Patent, the applicant characterized *Parameswaran* as disclosing precisely what was later claimed in the '200 Patent.

1. '281 Patent

The '281 Patent was filed with claims directed to a “method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction” by “incorporating at least two sequence identifiers into the template.” EX1030, cl. 1, 20; EX1003, ¶128. The claims were amended to require an additional step of “analyzing the sequence reads to determine if the two incorporated sequence identifiers remain associated with the template, thereby validating the integrity of the nucleic acid template in a multiplex sequencing reaction.” EX1026, 146-148, 165-176; EX1003, ¶128.

The Examiner rejected the amended claims as anticipated by *Parameswaran*. EX1026, 184-185; EX1003, ¶129. In response, applicant further amended the claims

to require “incorporating at least two of *the same* sequence identifiers into the template.” EX1026, 262-274; EX1026, 394-395 (applicant arguing that *Parameswaran* “requires attaching barcodes that are not the same”) (emphasis in original); EX1003, ¶129.

The Examiner rejected the amended claims, noting that at least one pair of barcode sequences used in *Parameswaran* shared three identical nucleotides. EX1026, 415-417; EX1003, ¶130. In response, the applicant amended the claims to further require “at least two of *the same* sequence identifiers,” which “comprise *four or more nucleotides*.” EX1026, 444-456; EX1003, ¶130. Based on these amendments, the applicant argued that *Parameswaran* “does not teach incorporating into a nucleic acid template two of the same sequence identifiers or barcodes of four or more nucleotides” because it “reports a method of sequencing that involves attaching a forward barcode and a reverse barcode to a nucleic acid template, the forward and reverse barcodes having *different* designs from each other.” EX1026, 452-453; EX1003, ¶130.

The Examiner allowed the claims, stating that the “amendments overcame the previously presented rejections.” EX1026, 458-464; EX1003, ¶131.

2. '200 Patent

In the first and only Office Action during prosecution of the '200 Patent, the Examiner rejected then-pending claims 66-71 on the ground of nonstatutory double

patenting over certain claims of its parent, U.S. Patent No. 11,041,852, in view of *Parameswaran*. EX1007, 270-277; *see* EX1003, ¶132. The claims recited methods “for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction” using “first and second identifier sequences” that “have *four or more nucleotides* and are *different*.” EX1007, 267, 229-230.

In response, Patent Owner filed a terminal disclaimer over the ’852 Patent. EX1007, 378-382. The Examiner allowed the claims, stating that “[s]ubmission and acceptance of the terminal disclaimer obviated the previously presented non-statutory double patenting rejection.” EX1007, 395-401. Claims 66-71 issued as claims 1-6 of the ’200 Patent. EX1003, ¶132.

E. Claim Construction

Petitioner applies the plain and ordinary meaning to each claim term. EX1003, ¶133.

V. STATEMENT OF PRECISE RELIEF REQUESTED

Petitioner requests cancellation of Patent claims 1-6 on the following grounds.

Ground	References	Basis	Claims
1	<i>Gloor</i> (EX1005)	§102	1-6
2	<i>Parameswaran</i> (EX1004)	§102	1-6
3	<i>Parameswaran</i> (EX1004) and <i>Gloor</i> (EX1005)	§103	1-6
4	<i>Parameswaran</i> (EX1004) and <i>Binladen</i> (EX1029)	§103	1-6

5	<i>Parameswaran</i> (EX1004), <i>Binladen</i> (EX1029), and <i>Bentley</i> (EX1006)	§103	6
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VI. GROUND 1: CLAIMS 1-6 ARE ANTICIPATED BY *GLOOR*

A. Claim 1 Is Anticipated by *Gloor*

Gloor, which was not submitted during prosecution of the '200 Patent, anticipates claim 1 because it discloses each and every claim element. EX1003, ¶134.

1. *Gloor* discloses a “method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction”

Gloor discloses this element. EX1003, ¶¶135-140; *supra* §III.B. *Gloor* sequenced nucleic acid analytes of interest derived from 272 clinical samples that had been amplified using “combinatorial sequence tags attached to PCR primers.” EX1005, Abstract. Each nucleic acid analyte of interest was attached to a pair of sequence tags (i.e. the “identifier sequences” used in claim 1[a]). EX1005, 7 (Fig.2); EX1003, ¶136. These combinatorial pairs of sequence tags were unique, i.e., nucleic acid analytes of interest from each sample had their own pairs of identifier sequences, and no two samples shared the same pair. EX1005, 6 (Table 2) (listing sequence tags used to create combinatorial pairs); *see also* EX1005, 2 (“[w]e used paired-end sequencing in combination with unique sequence tags”); EX1003, ¶136.

These pairs of sequence tags were used to validate the sequences of the nucleic acid analytes. EX1005, 6-8; EX1003, ¶137. Specifically, *Gloor* compiled the

sequences of the analytes based on overlapping sequence reads, detected the presence of both tags in those sequences, and extracted sequences “with proper primer sequences and correct sequence tags.” EX1005, 7; EX1003, ¶138. *Gloor* explains that the sequences with “***two valid sequence tags*** and both primer sequences” were used in “the remainder of the analysis.” EX1005, 7; EX1003, ¶138.

Gloor also explains that the sequences excluded from further analysis had “incorrect sequence tags,” for example, completely “lack[ing] the left or right end sequence tag,” or containing sequence tags that were truncated or showed addition or misincorporation of bases. EX1005, 7-8; *see also* EX1005, 22 (explaining that many unwanted sequences were removed “because of the strict requirement for proper sequence tag and primer sequences on the left and right ends”); EX1003, ¶139. *Gloor* concludes that by imposing “the strict requirement for proper sequence tag and primer sequences on the left and right ends,” many erroneous sequences were eliminated, and that its methods achieved “more accurate” sequence assignments. EX1005, 22-23; EX1003, ¶139.

In sum, *Gloor* used Illumina sequencing to sequence nucleic acid analytes of interest from multiple samples simultaneously (i.e., multiplex sequencing), validated the sequences of those analytes by detecting the presence of two identifier sequences in each sequence, and excluded sequences that contained only one tag or incorrect tags. EX1003, ¶140. *Gloor* therefore discloses a method for validating the sequence

of a nucleic acid analyte of interest in a multiplex sequencing reaction. EX1005, 22-23; EX1003, ¶140.

2. *Gloor* discloses a method comprising “detecting the presence of two or more identifier sequences that are uniquely associated with the nucleic acid analyte of interest” (claim 1[a])

Gloor discloses claim 1[a]. EX1003, ¶¶141-143; *supra* §III.B. As discussed above, *Gloor* sequenced nucleic acid analytes derived from 272 clinical samples. EX1005, 2; *supra* §III.B. In *Gloor*, the nucleic acid analytes from each sample contained a pair of two identifier sequences that was not shared by any other sample, i.e., the pair was “unique” to that sample. EX1005, Abstract, 2 (“[w]e used paired-end sequencing in combination with unique sequence tags”); *see also* EX1005, 6 (Table 2) (providing sequences of left- and right-side identifier sequences); EX1003, ¶141.

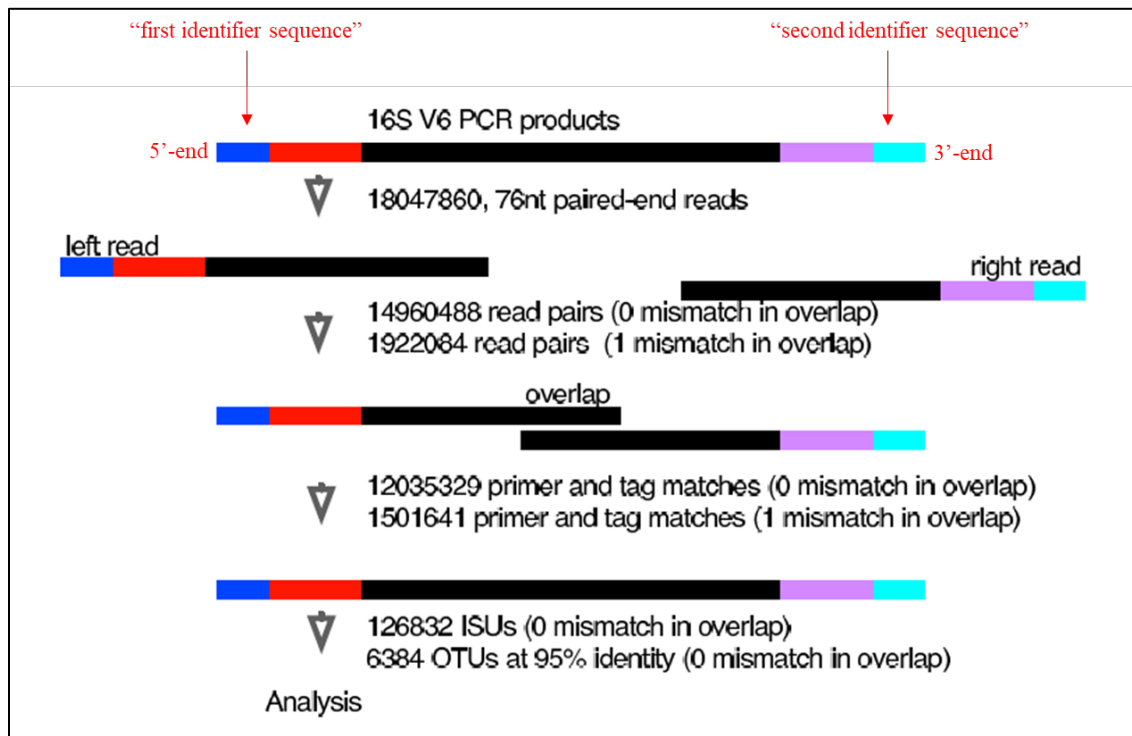
Gloor sequenced the amplified nucleic acid analytes and detected the presence of the identifier sequences at both ends of each nucleic acid analyte of interest. EX1005, 7 (identifying “sequences contain[ing] two valid sequence tags and both primer sequences”); *see also* EX1005, 7 (Fig.2) (illustrating detection of sequences each containing two identifier sequences); EX1003, ¶142. Thus, *Gloor* discloses detecting the presence of at least two identifier sequences, where the pair of the

identifiers is uniquely associated with the nucleic acid analyte of interest.

EX1003, ¶143.

3. ***Gloor* discloses a method in which “at least a first identifier sequence is incorporated into a 5’ portion of the nucleic acid analyte of interest and wherein at least a second identifier sequence is incorporated into a 3’ portion of the nucleic acid analyte of interest” (claim 1[b])**

Gloor discloses claim 1[b]. EX1003, ¶144; *supra* §III.B. In Figure 2, *Gloor* depicts the arrangement of two identifier sequences and a nucleic acid analyte of interest. EX1005, 7; EX1003, ¶144. As shown below in an annotated version of Figure 2, *Gloor* discloses incorporating a first identifier sequence into the 5’ portion of the nucleic acid analyte of interest and incorporation of a second identifier sequence into the 3’ portion of the analyte. EX1005, 2 (disclosing a “combination of sequence tags at each *end* of the overlapped reads”), 5-6 (describing “left-side” and “right-side” sequence tags incorporated into the “*end* of the PCR primers”); EX1003, ¶144.



EX1005, 7 (Fig.2 annotated with red text and arrows). The left tag constitutes a first identifier sequence and the right tag a second identifier sequence. EX1003, ¶144.

4. *Gloor* discloses a method in which “the first and second identifier sequences have four or more nucleotides and are different” (claim 1[c])

Gloor discloses claim 1[c]. EX1003, ¶¶145-147; *supra* §III.B. *Gloor*’s identifier sequences are “between 3 and 6 nucleotides long.” EX1005, 5. The majority of these identifier sequences have four or more nucleotides and are different. EX1005, 6 (Table 2); EX1003, ¶145. For example, the “0-v6L” left tag and the “1-v6R” right tag have six and five nucleotides, respectively, and are different from one another. EX1005, 6 (Table 2); EX1003, ¶146. Thus, many of the left and right sequence tags used in *Gloor* constitute “first and second identifier sequences [that]

have four or more nucleotides and are different,” such that *Gloor* satisfies this limitation. EX1003, ¶147; *ModernaTx, Inc. v. Arbutus Biopharma Corp.*, 18 F.4th 1352, 1363-64 (Fed. Cir. 2021) (a prior art reference disclosing at least one composition falling within the scope of the challenged claims anticipates the claims).

5. *Gloor* discloses a method comprising “sequencing the first identifier sequence, the nucleic acid analyte of interest and the second identifier sequence” (claim 1[d])

Gloor discloses claim 1[d]. EX1003, ¶¶148-149; *supra* §III.B. As shown in Figure 2, each sequence read obtained in *Gloor* contained the nucleic acid analyte of interest and a left or right tag. EX1005, 7 (Fig.2); EX1003, ¶148. *Gloor* compiled the sequences of each analyte based on sequence reads with substantial overlap. EX1005, 7. Thus, the sequences of the analytes each contained: (1) the left tag (i.e., the first identifier sequence); (2) the nucleic acid analyte of interest; and (3) the right tag (i.e., the second identifier sequence). EX1005, 7 (identifying “sequences [that] contained two valid sequence tags and both primer sequences”); EX1003, ¶148. Thus, *Gloor* discloses “sequencing the first identifier sequence, the nucleic acid analyte of interest and the second identifier sequence.” EX1003, ¶149.

6. *Gloor* discloses a method comprising “validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers and excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an

**incorrect pair of identifiers from sequence analysis of the
multiplex sequencing reaction” (claim 1[e])**

Gloor discloses claim 1[e]. EX1003, ¶¶150-151; *supra* §III.B. *Gloor* disclosed a method of sequencing a nucleic acid analyte of interest wherein each analyte was flanked by a uniquely associated pair of left and right tags. EX1005, 6, 22; EX1003, ¶150. *Gloor* used an “Illumina paired-end protocol to generate millions of overlapping reads.” EX1005, 1. *Gloor* compiled those sequence reads based on substantial overlap and “extract[ed] overlapping reads with proper primer sequences and correct sequence tags.” EX1005, 7. Of the about 18 million sequence reads collected, *Gloor* identified about 12 million sequences that “contained two valid sequence tags and both primer sequences,” which “form[ed] the basis of the remainder of the analysis.” EX1005, 7. *Gloor* concludes that its dual-indexing method led to “more accurate” sequence assignments. EX1005, 22-23; EX1003, ¶150. Thus, *Gloor* discloses “validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers.” EX1003, ¶150.

Gloor also examined sequences with “incorrect sequence tags.” EX1005, 7-8; EX1003, ¶151. These sequences completely “lack[ed] the left or right end sequence tag” (i.e., sequences with only one identifier) or contained sequence tags that were truncated or included additional or misincorporated bases (i.e., sequences with identifiers that were incorrect). EX1005, 7-8; *see also* EX1005, 22 (explaining that

many unwanted sequences were removed “because of the strict requirement for proper sequence tag and primer sequences on the left and right ends”); EX1003, ¶151. *Gloor* therefore discloses validating the sequence of the nucleic acid analyte of interest by “excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an incorrect pair of identifiers from sequence analysis of the multiplex sequencing reaction.” EX1003, ¶151.

Gloor therefore anticipates claim 1. EX1003, ¶152.

B. Dependent Claims 2-6 Are Anticipated by *Gloor*

Claims 2-6 depend directly or indirectly from claim 1 and are anticipated for the reasons described above (§VI.A) and for the additional reasons detailed below. EX1003, ¶153.

1. Claims 2-3

Claim 2 recites the “method of claim 1, wherein the identifier sequences are nucleic acid sequence tags.” EX1001, 22:7-8. Claim 3 recites the “method of claim 2, wherein the identifier sequences are barcode sequences.” EX1001, 22:9-10. The Patent states that nucleic acid barcodes can be used as identifier sequences. EX1001, 2:16-20; EX1003, ¶154.

Gloor anticipates claims 2-3 because its identifier sequences are nucleic acid sequence tags and barcode sequences. EX1005, 2 (disclosing a “combination of sequence tags at each end of the overlapped reads”), 5 (stating identifier sequences

are “between 3 and 6 nucleotides long”), Table 2 (providing nucleic acid sequences of barcode sequences); EX1003, ¶155.

2. Claim 4

Claim 4 recites the “method of claim 3, wherein the barcode sequences are from 4 to 20 nucleotides.” EX1001, 22:11-12. *Gloor* anticipates claim 4 because the majority of its barcode sequences were 4 to 20 nucleotides in length. EX1005, 6 (Table 2) (providing sequences of left- and right-side barcode sequences); EX1003, ¶¶156-157; *see ModernaTx*, 18 F.4th at 1363-64.

3. Claim 5

Claim 5 recites the “method of claim 4, wherein t [*sic*] barcode sequences are designed to have no homopolymer regions.” EX1001, 22:13-14. Homopolymeric regions are stretches of nucleotides with repeating bases. EX1012, 4; EX1003, ¶86 n.1. For example, the sequence TAGGGGCG has a homopolymeric run of four consecutive “G” bases. EX1003, ¶86 n.1.

Gloor anticipates claim 5 because its barcode sequences lack homopolymer regions. EX1005, 6 (Table 2) (showing that the barcodes contained no consecutively repeated nucleotides); EX1003, ¶¶158-159.

4. Claim 6

Claim 6 recites the “method of claim 1, wherein the nucleic acid analyte of interest is on the surface of a flow cell.” EX1001, 22:15-16. *Gloor* anticipates claim

6 because it uses Illumina sequencing, which was known to involve attaching nucleic acid analytes on the surface of a flow cell (*supra* §§III.B, II.A). EX1005, Abstract; EX1003, ¶¶160-161.

VII. GROUND 2: CLAIMS 1-6 ARE ANTICIPATED BY *PARAMESWARAN*

As detailed below, *Parameswaran* discloses each and every element of the '200 Patent claims and thus anticipates the claims. EX1003, ¶162. This is underscored by the applicant's description of *Parameswaran* during prosecution of the '281 Patent, a parent to the '200 Patent. *Supra* §IV.D.1. The applicant argued that the '281 Patent claims were not anticipated by *Parameswaran* because they required the "same" identifier sequences on each end of a nucleic acid analyte of interest, whereas *Parameswaran* used "different" first and second identifier sequences. *Supra* §IV.D.1. But in the '200 Patent, as in *Parameswaran*, Patent Owner claims the use of "different" first and second identifier sequencers. *Supra* §IV.D.2.

A. Claim 1 Is Anticipated by *Parameswaran*

1. *Parameswaran* discloses a "method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction"

Parameswaran discloses this element. EX1003, ¶¶163-167; *supra* §III.A. Using Roche 454 multiplex sequencing, *Parameswaran* sequenced "DNA from two independent sets of 25 barcoded libraries" prepared from multiple samples. EX1004,

7; EX1003, ¶163. To facilitate “unambiguous assignment of nucleic acid sequences” to their originating samples, the nucleic acids from each sample were attached to “two uniquely designed, 10-nucleotide barcodes” via nucleotide linkers. EX1004, 2; EX1003, ¶163. Following sequencing, the sequences were evaluated using “barcode motifs” (the “identifier sequences” recited in claim 1[a]), which were a combination of the 10-nucleotide barcodes and 3 nucleotides from the linkers. EX1004, 7-8; EX1003, ¶163.

Parameswaran reports that around 94% of sequences retained a correct pair of forward and reverse barcode motifs—the sequences of both the forward and reverse sequence identifiers were correct *and* the forward identifier was paired with the correct reverse identifier. EX1004, 7; EX1003, ¶164. *Parameswaran* discloses that these sequences were accurately and unambiguously assigned to their originating sample, i.e., their sequences were validated. EX1004, 8 (“any sequence with both 5’ and 3’ barcode motifs will be placed with reasonable accuracy into the correct bin”); EX1003, ¶164.

Parameswaran reports that the remaining 5-6% of sequences contained either only a single identifier sequence or a pair of *mismatched* identifier sequences. EX1004, 7; EX1003, ¶165. In mismatched or “misassigned” sequences, the forward identifier was incorrectly paired with the wrong reverse identifier. EX1003, ¶165. *Parameswaran* characterizes these erroneous misassigned sequences as “false-

discover[ies],” and describes various errors that can produce them. EX1004, 7-8. *Parameswaran* states that a “remarkably low misassignment rate (0.00042% and 0.0044% for two independent experiments) was obtained by requiring a match to the 10-base barcodes and additional three bases of the fixed linker” (i.e., the forward and reverse barcode motifs). EX1004, 8; EX1003, ¶166. *Parameswaran* also teaches that lowering the false-discovery (misassignment) rate allows sequences to be assigned to correct samples more accurately. EX1004, 8; EX1003, ¶166.

In sum, *Parameswaran* sequenced nucleic acid analytes of interests using Roche 454 sequencing and validated the sequences of the analytes by analyzing *both* the forward and reverse identifiers. EX1003, ¶167. *Parameswaran* characterized each sequence with “unexpected perfect pairings of unmatched forward and reverse barcodes” as a “misassignment.” EX1004, 8, Abstract; EX1003, ¶167. *Parameswaran* therefore discloses a method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction. EX1003, ¶167.

2. *Parameswaran* discloses a method that comprises “detecting the presence of two or more identifier sequences that are uniquely associated with the nucleic acid analyte of interest” (claim 1[a])

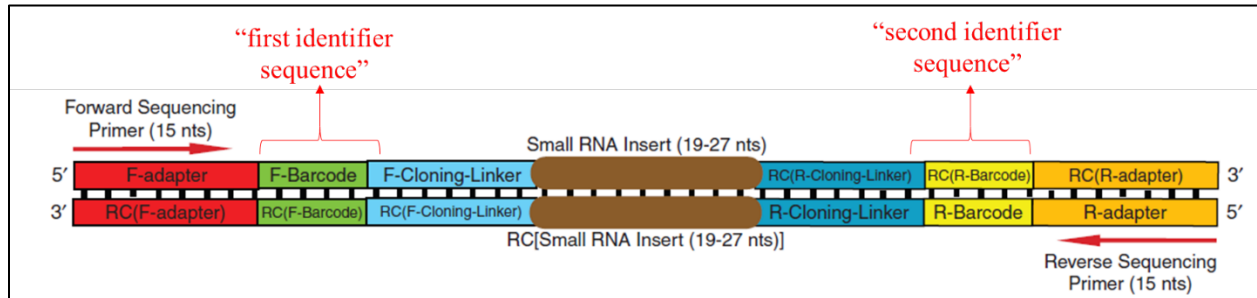
Parameswaran discloses claim 1[a]. EX1003, ¶¶168-170; *supra* §III.A. As discussed above (*supra* §III.A), *Parameswaran* sequenced pooled nucleic acid analytes from multiple samples, each of which was associated with a pair of

13-nucleotide “barcode motifs” (i.e., “identifier sequences”). EX1004, 2, 7. Each pair of identifier sequences was unique to the nucleic acid analytes from each sample. EX1004, 14-17 (Supplementary Table 1) (providing a complete list of primers in which identifier sequences are not repeated across samples); EX1003, ¶168. *Parameswaran* amplified and sequenced the analytes and detected the presence of forward and reverse identifier sequences associated with each sample. EX1004, 7 (reporting that about 94% of sequences had perfect forward and reverse barcode motifs associated with the nucleic acid analytes of interest); EX1003, ¶169. Thus, *Parameswaran* discloses detecting the presence of at least two identifier sequences, each of which is uniquely associated with the nucleic acid analyte of interest. EX1003, ¶170.

3. ***Parameswaran* discloses a method in which “at least a first identifier sequence is incorporated into a 5’ portion of the nucleic acid analyte of interest and wherein at least a second identifier sequence is incorporated into a 3’ portion of the nucleic acid analyte of interest” (claim 1[b])**

Parameswaran discloses claim 1[b]. EX1003, ¶¶171-172; *supra* §III.A. *Parameswaran* states that each nucleic acid analyte was attached to a pair of 13-nucleotide “barcode motifs,” each of which was made of a 10-nucleotide barcode and three nucleotides from an adjacent linker. EX1004, 2, 7. As annotated below, the forward and reverse identifier sequences for each nucleic acid analyte of interest

are incorporated into the 5' and 3' portions of the analyte, respectively.
EX1003, ¶171.



EX1004, 3 (Fig.1C annotated with red text and vertical arrows). The forward barcode motif (forward barcode plus three nucleotides from the adjoining linker) constitutes a first identifier sequence and the reverse barcode motif (reverse barcode plus and three nucleotides from the adjoining linker) constitutes a second identifier sequence. EX1003, ¶¶171-172.

4. *Parameswaran* discloses a method in which “the first and second identifier sequences have four or more nucleotides and are different” (claim 1[c])

Parameswaran discloses claim 1[c]. EX1003, ¶¶173-174; *supra* §III.A. *Parameswaran* explains that each nucleic acid analyte of interest was attached to a pair of “10-nucleotide” barcodes, which “differ[ed] from each other at multiple positions (by at least 4 nt [nucleotides]).” EX1004, 2; EX1003, ¶173. To ensure that all samples were uniquely dual-indexed, *Parameswaran* combined three nucleotides from the linkers with the 10-nucleotide barcodes to create 13-nucleotide “barcode motifs.” EX1004, 7; *see also* EX1004, 8 (discussing combining barcodes and

portions of linkers “for definitive sample assignment”); EX1003, ¶173. Supplementary Table 1 provides a complete list of barcoded primers, in which identifier sequences were not repeated across samples. EX1004, 14-17 (Supplementary Table 1); EX1003, ¶173.

During prosecution of the '281 patent, the applicant admitted that the forward and reverse barcodes in *Parameswaran* have “*different* designs from each other.” EX1026, 452-453; *supra* §IV.D.1. Thus, the forward and reverse barcode motifs used in *Parameswaran* are “first and second identifier sequences [that] have four or more nucleotides and are different.” EX1003, ¶174.

5. *Parameswaran* discloses a method comprising “sequencing the first identifier sequence, the nucleic acid analyte of interest and the second identifier sequence” (claim 1[d])

Parameswaran discloses claim 1[d]. EX1003, ¶175; *supra* §III.A. *Parameswaran* discloses a multiplex sequencing reaction that sequenced the first identifier sequence, the nucleic acid analyte of interest, and the second identifier sequence. EX1004, 7. *Parameswaran* also depicts in Figure 1C a schematic of the sequence reads comprising the forward barcode motif, the nucleic acid analyte of interest, and the reverse barcode motif. EX1004, 3 (Fig.1C); EX1003, ¶175.

6. *Parameswaran* discloses a method comprising “validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers and excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an

**incorrect pair of identifiers from sequence analysis of the
multiplex sequencing reaction” (claim 1[e])**

Parameswaran discloses claim 1[e]. EX1003, ¶¶176-180; *supra* §III.A. As discussed above (*supra* §III.A), *Parameswaran* sequenced pooled nucleic acid analytes from multiple samples, each of which was associated with unique forward and reverse barcode motifs. EX1003, ¶176. *Parameswaran* analyzed the resulting sequences, and reported that about 94% of sequences had perfect forward and reverse barcode motifs. EX1004, 7; EX1003, ¶176. *Parameswaran* discloses that these sequences with perfect forward and reverse barcode motifs “will be placed with reasonable accuracy into the correct bin.” EX1004, 8 (explaining that its barcoding approach allowed for “the unambiguous assignment of nucleic acid sequences”); EX1003, ¶176.

Parameswaran reports that the remaining 5-6% of sequences contained only one barcode motif or a pair of “mismatched” barcode motifs. EX1004, 7; EX1003, ¶177. *Parameswaran* explained that these sequences may have arisen from various errors such as cross-contamination of DNA analytes and other errors that occurred during sequencing-by-synthesis and emulsion PCR. EX1004, 7; EX1003, ¶177.

Parameswaran states “for our purposes, all sequences with a match to one of the two expected sequence motifs were considered as true members of the

corresponding dataset.” EX1004, 8; EX1003, ¶178. But *Parameswaran* characterizes sequences with mismatched barcodes as “false-discover[ies]” arising due to various errors that result in “misassignment” of sequences to incorrect samples. EX1004, 7-8; EX1003, ¶¶178-179. And *Parameswaran* expressly links sequencing accuracy with the presence of both barcode motifs, stating that “any sequence with *both 5’ and 3’ barcode motifs* will be placed with reasonable accuracy into the correct bin.” EX1004, 8; EX1003, ¶179. Thus, *Parameswaran* discloses that lowering the misassignment rate allows sequences to be assigned to correct samples more accurately. EX1004, 8.; EX1003, ¶179.

Parameswaran therefore discloses “validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers and excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an incorrect pair of identifiers from sequence analysis of the multiplex sequencing reaction.” EX1003, ¶180.

Parameswaran therefore anticipates claim 1. EX1003, ¶181.

B. Dependent Claims 2-6 Are Anticipated by *Parameswaran*

Claims 2-6 depend directly or indirectly from claim 1 and are anticipated for the reasons described above (§VII.A) and for the additional reasons detailed below. EX1003, ¶182.

1. Claims 2-3

Claim 2 recites the “method of claim 1, wherein the identifier sequences are nucleic acid sequence tags.” EX1001, 22:7-8. Claim 3 recites the “method of claim 2, wherein the identifier sequences are barcode sequences.” EX1001, 22:9-10. The Patent states that nucleic acid barcodes can be used as identifier sequences. EX1001, 2:16-20; EX1003, ¶183.

Parameswaran anticipates claims 2-3 because its identifier sequences are nucleic acid sequence tags and barcode sequences. EX1004, 7 (describing nucleic acid “barcode motifs,” each made of nucleotides); *see also* EX1004, 14-15 (Supplementary Table 1A) (reciting full nucleotide sequences of the barcode sequences); EX1003, ¶184.

2. Claim 4

Claim 4 recites the “method of claim 3, wherein the barcode sequences are from 4 to 20 nucleotides.” EX1001, 22:11-12. *Parameswaran* anticipates claim 4 because its barcode motifs include “10-nucleotide barcodes” and 3 nucleotides from adjoining linkers (i.e., a total of 13 nucleotides in length). EX1004, 7; EX1003, ¶¶185-186.

3. Claim 5

Claim 5 recites the “method of claim 4, wherein t [sic] barcode sequences are designed to have no homopolymer regions.” EX1001, 22:13-14. *Parameswaran*

anticipates claim 5 because its “barcode design facilitated unambiguous assignment, with care taken to avoid homopolymeric runs.” EX1004, 4 (“This design avoids significant problems due to error rate at homopolymeric runs of nucleotides.”); EX1003, ¶¶187-188.

4. Claim 6

Claim 6 recites the “method of claim 1, wherein the nucleic acid analyte of interest is on the surface of a flow cell.” EX1001, 22:15-16. Claim 6 does not require attaching the analyte to the surface of a flow cell—it simply requires the analyte to be “on the surface of a flow cell.” EX1003, ¶189. *Parameswaran* anticipates claim 6 because it uses Roche 454 sequencing, where bead-bound nucleic acid analytes of interest are placed into a flow cell for sequencing. EX1004, 4; *supra* §II.A; EX1028, 6-8 (depicting bead-bound nucleic acid analytes placed in wells of a flow cell); EX1003, ¶190. Thus, *Parameswaran* teaches a method where “the nucleic acid analyte of interest is on the surface of a flow cell.” EX1003, ¶190.

VIII. GROUND 3: CLAIMS 1-6 WOULD HAVE BEEN OBVIOUS OVER PARAMESWARAN AND GLOOR

Parameswaran and *Gloor* both disclose every claim limitation of the challenged claims. *Supra* §§VI-VII (incorporated herein); EX1003, ¶191. As explained below, their combination also renders the claims obvious. EX1003, ¶192. A POSA would have been motivated to combine these references, with a reasonable

expectation of success, because both use dual barcoding strategies for multiplex sequencing; both are directed to reducing sequencing error; and both emphasize the compatibility of dual indexing for Roche 454 and Illumina sequencing to reduce sequencing error. EX1003, ¶192.

That the challenged claims would have been obvious over *Parameswaran* and *Gloor* is clear from the anticipation grounds above. *Realtime Data, LLC v. Iancu*, 912 F.3d 1368, 1373 (Fed. Cir. 2019) (explaining that “anticipation is the epitome of obviousness”).

A. Claim 1 Would Have Been Obvious Over *Parameswaran* and *Gloor*

1. *Parameswaran* and *Gloor* disclose a “method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction”

As detailed above, each of *Parameswaran* and *Gloor* discloses this element. The combination of the references therefore discloses it. EX1003, ¶193; *supra* §§VI.A.1, VII.A.1 (discussing *Gloor* and *Parameswaran*, respectively).

2. *Parameswaran* and *Gloor* disclose a method comprising “detecting the presence of two or more identifier sequences that are uniquely associated with the nucleic acid analyte of interest” (claim 1[a])

As detailed above, each of *Parameswaran* and *Gloor* discloses claim 1[a]. The combination of the references therefore discloses it. EX1003, ¶194; *supra* §§VI.A.2, VII.A.2 (discussing *Gloor* and *Parameswaran*, respectively).

3. ***Parameswaran* and *Gloor* disclose a method in which “at least a first identifier sequence is incorporated into a 5’ portion of the nucleic acid analyte of interest and wherein at least a second identifier sequence is incorporated into a 3’ portion of the nucleic acid analyte of interest” (claim 1[b])**

As detailed above, each of *Parameswaran* and *Gloor* discloses claim 1[b].

The combination of references therefore discloses it. EX1003, ¶195; *supra* §§VI.A.3, VII.A.3 (discussing *Gloor* and *Parameswaran*, respectively).

4. ***Parameswaran* and *Gloor* disclose a method in which “the first and second identifier sequences have four or more nucleotides and are different” (claim 1[c])**

As detailed above, each of *Parameswaran* and *Gloor* discloses claim 1[c].

The combination of the references therefore also discloses it. EX1003, ¶195; *supra* §§VI.A.4, VII.A.4 (discussing *Gloor* and *Parameswaran*, respectively).

While *Gloor* included several identifier sequences that were three nucleotides in length, it would have been obvious to use sequence identifiers that were four or more nucleotides in length and different—as was done in *Parameswaran* and with the majority of tags in *Gloor*. EX1005, 5-6 (Table 2); EX1003, ¶¶196-197. As explained in *Parameswaran*, using longer sequence identifiers allowed for “definitive assignment.” EX1004, 4; EX1003, ¶197.

5. ***Parameswaran* and *Gloor* disclose a method comprising “sequencing the first identifier sequence, the nucleic acid**

analyte of interest and the second identifier sequence” (claim 1[d])

As detailed above, each of *Parameswaran* and *Gloor* discloses claim 1[d]. The combination of the references therefore also discloses it. EX1003, ¶198; *supra* §§VI.A.5, VII.A.5 (discussing *Gloor* and *Parameswaran*, respectively).

6. *Parameswaran* and *Gloor* disclose a method comprising “validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers and excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an incorrect pair of identifiers from sequence analysis of the multiplex sequencing reaction” (claim 1[e])

As detailed above, *Parameswaran* discloses claim 1[e]. EX1003, ¶¶199-200; *supra* §VII.A.6. *Parameswaran* describes a method that determined that about 94% of sequences had perfect forward and reverse barcodes, thereby validating the sequences for these nucleic acid analytes of interest. EX1004, 7; *see also* EX1004, 8 (“any sequence with both 5’ and 3’ barcode motifs will be placed with reasonable accuracy into the correct bin”); EX1003, ¶199.

A POSA reading *Parameswaran* would have understood that it discloses sequencing methods configured to identify erroneous sequences that have only one perfect barcode motif, which can be kept or discarded depending on the goals of the project. EX1003, ¶200. *Parameswaran* detected sequences lacking any match to the expected barcode motifs, and “discarded” them from further analysis. EX1004, 7-8;

EX1003, ¶200. While *Parameswaran* indicates that “[f]or our purposes, all sequences with a match to one of the two expected sequence motifs were considered as true members of the corresponding dataset,” a POSA would have understood that *Parameswaran* identifies these sequences as resulting from errors that lead to undesirable misassignment of sequences to incorrect samples. EX1003, ¶200. A POSA would have understood that such sequences could be excluded to increase accuracy. EX1003, ¶200. Indeed, *Parameswaran* states that “any sequence with ***both 5’ and 3’ barcode motifs*** will be placed with reasonable accuracy into the correct bin.” EX1004, 8; EX1003, ¶200.

Gloor also discloses this claim limitation. EX1003, ¶201; *supra* §VI.A.6. As detailed above, *Gloor* discloses “extraction of overlapping reads” with “correct sequence tags” (i.e., first and second sequence identifiers), identification of sequences that “contained two valid sequence tags,” and exclusion of sequences not containing two correct sequence tags from “the remainder of the analysis.” EX1005, 7; EX1003, ¶201. *Gloor* further explains that these excluded sequences contained “incorrect sequence tags,” including sequences that lacked the first or second sequence identifier or that had errors in the first or second sequence identifiers. EX1005, 7-8; EX1003, ¶201. *Gloor* concludes that its methods achieved “more accurate” sequence assignment. EX1005, 22-23; EX1003, ¶201.

In sum, both references teach dual-index multiplex sequencing methods that identify erroneous sequences having only one identifier or an incorrect pair of identifiers. Both references teach that these erroneous sequences can be excluded and that the remaining sequences with correct pairs of identifiers have been validated. EX1005, 22-23 (emphasizing that “the strict requirement for proper sequence tag and primer sequences on the left and right ends” resulted in the removal of erroneous sequences). As such, each of the references discloses and suggests claim 1[e], and the combination of the references therefore also discloses and suggests it.

B. A POSA Would Have Been Motivated to Combine *Parameswaran* and *Gloor* to Arrive at the Subject Matter of Claim 1

A POSA would have been motivated to combine *Parameswaran* and *Gloor*. EX1003, ¶202. *First*, a POSA would have been motivated to combine these references to reduce technical errors that occur during multiplex sequencing. EX1003, ¶202. Both references report that multiplex sequencing can produce various types of erroneous sequences due to technical errors. *Supra* §VIII.A.6; EX1003, ¶202. Both references report that these errors undermine sequencing accuracy, and can lead to “misassignment” of sequences to incorrect samples. *Supra* §VIII.A.6; EX1004, Abstract; EX1005, 7-8, 22-23. Consistent with other prior art publications such as *Binladen* (EX1029) (*infra* §IX.A.6), both references demonstrate that dual-indexing methods with unique barcodes allow a POSA to

identify and discard these erroneous sequences. *Supra* §VIII.A.6; EX1003, ¶202. A POSA would have been motivated to combine the dual indexing methods disclosed in *Parameswaran* and *Gloor* to reduce technical errors that occur during multiplex sequencing. EX1003, ¶202.

Second, a POSA would have been motivated to combine *Parameswaran* and *Gloor* because doing so would allow the POSA to efficiently identify and discard chimeric sequences. EX1003, ¶203. As of December 2010, a POSA would have known that chimeric sequences were problematic for next-generation sequencing platforms that used PCR amplification, including Illumina sequencing. *See supra* §II.B; EX1003, ¶203. Because *Gloor* repeated individual sequence identifiers across different samples, additional bioinformatic analyses were needed to identify and discard chimeric sequences. EX1003, ¶203; EX1005, 8-9. In contrast, *Parameswaran*'s unique dual-indexing method made it possible to directly compare pairs of identifiers assigned to nucleic acid samples versus those actually detected in the sequences, allowing the POSA to readily identify and discard chimeric sequences containing barcode pairs that differed from any of the uniquely assigned barcode pairs. EX1003, ¶203. Thus, to improve sequencing accuracy, a POSA would have been motivated to use *Parameswaran*'s unique dual-indexing on *Gloor*'s Illumina sequencing platform to efficiently detect and exclude erroneous chimeric sequences. EX1003, ¶203.

Third, a POSA would have been particularly motivated to combine *Parameswaran*'s unique dual-indexing method with *Gloor*'s Illumina sequencing because of methodologic advantages. EX1003, ¶¶204-205. By December 2010, Illumina sequencing was understood to be more efficient, less expensive, and faster than Roche 454 sequencing. EX1003, ¶¶204-205. For example, the throughput of Illumina sequencers was approximately 5,000 Mb/day, compared to Roche 454's throughput of only 750 Mb/day. EX1010, 10 (Table 1); EX1003, ¶204. The speed and efficiency of Illumina sequencers equated to a lower cost: Illumina sequencing cost an order of magnitude less than Roche 454 sequencing—approximately \$0.50/Mb compared to approximately \$20/Mb for Roche 454 sequencing. EX1010, 10 (Table 1); *see also* EX1005, 2; EX1003, ¶204.

Fourth, the express disclosures of *Parameswaran* and *Gloor* would have motivated a POSA to combine these references. *Parameswaran* teaches that its barcodes “may also be compatible with other sequencing platforms,” and specifically identifies Illumina sequencing as a major high throughput sequencing platform. EX1004, 1, 8; EX1003, ¶206. *Gloor* identifies “454 pyrosequencing” as a multiplex sequencing platform, but explains that Illumina sequencing has a “substantially lower error rate.” EX1005, 12; EX1003, ¶206.

Given that there were only three major high-throughput platforms, it would at the very least have been obvious for a POSA to try *Parameswaran*'s unique dual-

indexing method with one of the other two sequencing platforms in place of Roche 454 sequencing, as *Parameswaran* suggests. EX1003, ¶207; *KSR Int'l Co. v. Teleflex, Inc.*, 550 U.S. 398, 421 (2007). Moreover, the choice of sequencing platform was agnostic to dual-indexing methods, and instead related to other design considerations such as cost and accessibility. *See* EX1003, ¶¶207, 204-205, 83. Indeed, researchers in the field had used unique dual-indexing with the same barcodes on both Roche 454 and Illumina sequencers. EX1038, Abstract; EX1003, ¶207. There was nothing inventive about switching from Roche 454 sequencing (used in *Parameswaran*) to Illumina sequencing (used in *Gloor*). *See* EX1003, ¶207. Thus, a POSA would have been motivated to combine *Parameswaran* and *Gloor*. EX1003, ¶208.

C. A POSA Would Have Had a Reasonable Expectation of Success in Combining *Parameswaran* and *Gloor* to Arrive at the Subject Matter of Claim 1

A POSA would have had a reasonable expectation of success in combining *Parameswaran* and *Gloor*. EX1003, ¶209. Both *Parameswaran* and *Gloor* provide detailed methods for multiplex sequencing nucleic acid analytes of interest, detecting first and second identifier sequences, and validating the sequences of analytes of interest by analyzing the presence of both identifiers and excluding sequence reads containing only one identifier or incorrect pairs of identifiers. *Supra* §§VIII.A, III.A-B; EX1003, ¶209. Following *Parameswaran* and *Gloor*, a POSA

would have reasonably expected that the sequences of nucleic acid analytes of interest in a multiplex sequencing reaction would be successfully validated. EX1003, ¶209.

A POSA would have reasonably expected that the claimed methods would be compatible with the Roche 454 and Illumina sequencing platforms. EX1003, ¶210. Using Roche 454 sequencing, *Parameswaran* identifies multiple groups of erroneous sequences, including those containing only one identifier or mismatched pairs of identifiers. *See* EX1004, 7-8; *supra* §§VIII.A.6, III.A. *Parameswaran* also states that its barcoding approach is compatible “with other sequencing platforms” (EX1004, 2, 8) and specifically identifies Illumina sequencers as one of the “major high-throughput sequencing platforms” (EX1004, 1). *Gloor* discloses using Illumina sequencing to identify and exclude sequences having only one identifier or incorrect identifiers. EX1005, Abstract, 6-8; *supra* §§VIII.A.6, III.B. The proven success of dual-indexing strategies for multiplex sequencing on both platforms strongly supports the expectation of success. EX1003, ¶210.

To perform *Parameswaran*’s method with Illumina sequencing (or *Gloor*’s method with Roche 454 sequencing), a POSA would have needed to simply swap Roche 454 adapters for Illumina-specific adapters (or vice versa) and make other minor modifications to the sequencing process. EX1003, ¶¶211, 214; EX1015, 1 (“By exchanging adapter sequences, removing and shortening several reaction steps,

and introducing an amplification scheme, the [Roche 454] protocol has been redesigned for rapid preparation of Illumina multiplex sequencing libraries using a 96-well plate format.”). Notably, the prior art disclosed detailed methods for performing unique dual-index sequencing on both Roche 454 and Illumina sequencers, including using the same barcodes on both platforms. *See generally* EX1038; EX1003, ¶212.

None of these minor modifications change the fundamental aspect of the dual barcoding methods disclosed in *Parameswaran* or *Gloor*. EX1003, ¶211. Tellingly, no novel or non-obvious chemistry was needed to make the claimed subject matter, and the Patent admits that “the exact means for detecting and compiling sequence data does not affect the function of the invention.” EX1001, 3:44-48, 7:45-20:49 (referring a POSA to the prior art to implement the claimed subject matter and acknowledging that “essentially any sequencing methodology” can be used). EX1003, ¶213. That the Patent’s “Detailed Description” section copies 13 of its 17 columns from other references (including Petitioner’s patent publication), further confirms that no special implementation would have been required. *Supra* §IV.B.

As addressed in Section XI below, Petitioner is aware of no objective evidence of nonobviousness for the Patent. EX1003, ¶¶258-259. As such, claim 1 would have been obvious over *Parameswaran* and *Gloor*. EX1003, ¶215.

**D. Dependent Claims 2-6 Would Have Been Obvious Over
Parameswaran and *Gloor***

Claims 2-6 ultimately depend from claim 1 and would have been obvious for the reasons described above (§§VIII.A-C) and the additional reasons described here. EX1003, ¶216.

1. Claims 2-3

Claim 2 recites the “method of claim 1, wherein the identifier sequences are nucleic acid sequence tags.” EX1001, 22:7-8. Claim 3 recites the “method of claim 2, wherein the identifier sequences are barcode sequences.” EX1001, 22:9-10. Both *Parameswaran* and *Gloor* disclose these limitations. *Supra* §§VI.B.1, VII.B.1 (discussing *Gloor* and *Parameswaran*, respectively); EX1003, ¶217. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VIII.B-C; EX1003, ¶217.

2. Claim 4

Claim 4 recites the “method of claim 3, wherein the barcode sequences are from 4 to 20 nucleotides.” EX1001, 22:11-12. *Parameswaran* and *Gloor* both disclose barcode sequences that are 4 to 20 nucleotides. *Supra* §§VI.B.2, VII.B.2 (discussing *Gloor* and *Parameswaran*, respectively); EX1003, ¶218. A POSA would have been motivated, with a reasonable expectation of success, to combine

Parameswaran and *Gloor* for the reasons set forth above. *Supra* §§VIII.B-C; EX1003, ¶218.

3. Claim 5

Claim 5 recites the “method of claim 4, wherein t [sic] barcode sequences are designed to have no homopolymer regions.” EX1001, 22:13-14. *Parameswaran* and *Gloor* both disclose barcode sequences that are designed to have no homopolymer regions. *Supra* §§VI.B.3, VII.B.3 (discussing *Gloor* and *Parameswaran*, respectively); EX1003, ¶219. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VIII.B-C; EX1003, ¶219.

4. Claim 6

Claim 6 recites the “method of claim 1, wherein the nucleic acid analyte of interest is on the surface of a flow cell.” EX1001, 22:15-16. *Parameswaran* discloses this limitation. *Supra* §VII.B.4; EX1003, ¶220.

Even if Patent Owner were to argue, and the Board were to accept, that Roche sequencing does not occur on the surface of a flow cell, *Gloor* teaches this limitation because *Gloor* uses Illumina sequencing. Illumina sequencing involved attaching nucleic acid analytes to the surface of a flow cell. *Supra* §VI.B.4; EX1003, ¶221. Further, as of December 2010, a POSA would have been motivated to use Illumina sequencing, as it was understood to be more efficient, more accurate, less expensive,

and faster than Roche 454 sequencers. EX1003, ¶221. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VIII.B-C; EX1003, ¶221.

IX. GROUND 4: CLAIMS 1-6 WOULD HAVE BEEN OBVIOUS OVER *PARAMESWARAN* AND *BINLADEN*

Claims 1-6 of the '200 Patent also would have been obvious over *Parameswaran* and *Binladen*. EX1003, ¶¶222-223. As discussed below, *Parameswaran* studied the dual-indexing methods used in *Binladen*, developed an improved dual-indexing method using longer and unique identifier sequences (like those claimed by the '200 Patent), and confirmed that its method was beneficial “for a more definitive assignment of samples and/or for enhanced multiplexing capabilities.” EX1004, 2; EX1029, 3, 5-6; EX1003, ¶223. *Parameswaran* and *Binladen* illustrate that a POSA would have been able to “implement a predictable variation” of a known dual-indexing method to arrive at the claimed subject matter, rendering it obvious. *See KSR*, 550 U.S. at 417 (“If a person of ordinary skill can implement a predictable variation, §103 likely bars its patentability.”); EX1003, ¶223.

A. Claim 1 Would Have Been Obvious Over *Parameswaran* and *Binladen*

1. *Parameswaran* and *Binladen* disclose a “method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction”

Parameswaran discloses this element. EX1003, ¶224; *supra* §VIII.A.1.

Binladen also discloses it. EX1003, ¶¶225-227; *supra* §III.C. Using Roche 454 sequencing, *Binladen* sequenced DNA samples from thirteen species. EX1029, Abstract. *Binladen* labeled each nucleic acid analyte using a pair of forward and reverse primers that each contained an identifier sequencer at the 5'-end. EX1029, 3; *see also* EX1029, 2 (Fig.1) (depicting dual-indexed nucleic acid analytes of interest); EX1003, ¶225. Because “the correct association of tags and sequences” was “crucial to the approach,” *Binladen* “discarded all sequence reads without an exact match to any of the primers used in the studies,” including those with incorrect identifiers or lacking an identifier. EX1029, 6; EX1003, ¶226. Only the remaining sequences were aligned to the reference sequences. EX1029, 6; EX1003, ¶226. *Binladen* therefore discloses a method for validating the sequence of a nucleic acid analyte of interest in a multiplex sequencing reaction. EX1003, ¶227.

2. *Parameswaran* discloses a method comprising “detecting the presence of two or more identifier sequences that are uniquely associated with the nucleic acid analyte of interest” (claim 1[a])

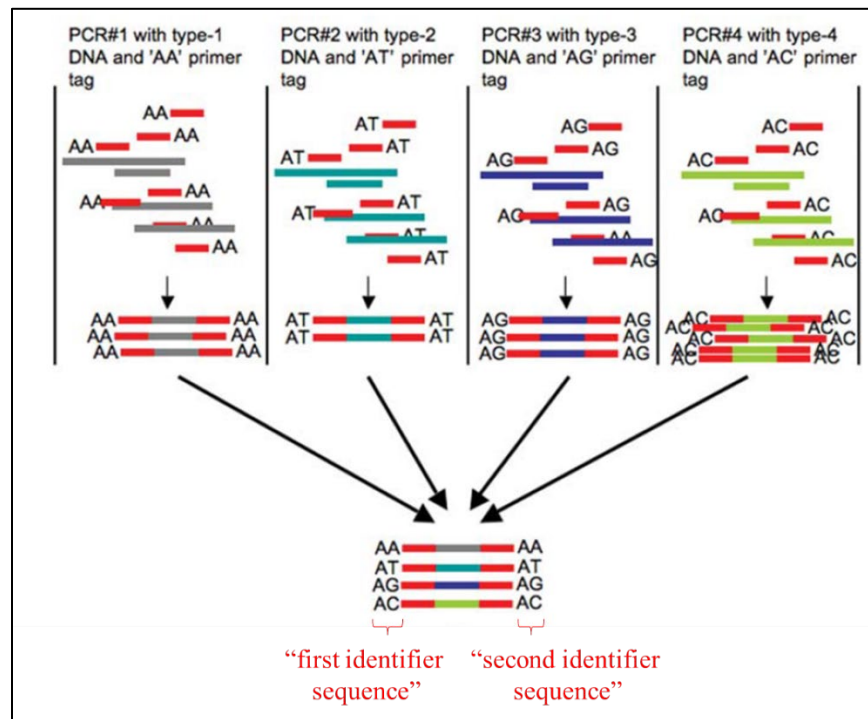
Parameswaran discloses claim 1[a]. EX1003, ¶228; *supra* §VIII.A.2.

Binladen discloses a method where the nucleic acid analytes of interest were labeled with pairs of two identifier sequences. EX1003, ¶229; *supra* §III.C. *Binladen*, however, repeated several pairs of identifier sequences for nucleic acid analytes from different samples. EX1029, 3 (“we used individual primer pairs on several different extractions each”); EX1003, ¶229.

3. ***Parameswaran* and *Binladen* disclose a method in which “at least a first identifier sequence is incorporated into a 5’ portion of the nucleic acid analyte of interest and wherein at least a second identifier sequence is incorporated into a 3’ portion of the nucleic acid analyte of interest” (claim 1[b])**

Parameswaran discloses claim 1[b]. EX1003, ¶230; *supra* §VIII.A.3.

Binladen also discloses this limitation. EX1003, ¶¶231-232; *supra* §III.C. Each forward and reverse primer used in *Binladen* contained an identifier sequence EX1029, 2 (Fig.1); EX1003, ¶231. As shown below, *Binladen* incorporated these identifier sequences into the 5’- and 3’-ends of each nucleic acid analyte of interest. EX1003, ¶232.



EX1029, 2 (Fig.1 annotated with red text and brackets); *see also* EX1029, 3 (it was “necessary to label both the forward and reverse end of each PCR product”). *Binladen* therefore discloses a nucleic acid analyte of interest containing a first identifier sequence in the 5'-end portion and a second identifier sequence in the 3'-end portion. EX1003, ¶232.

4. *Parameswaran* and *Binladen* disclose a method in which “the first and second identifier sequences have four or more nucleotides and are different” (claim 1[c])

Parameswaran discloses claim 1[c]. EX1003, ¶233; *supra* §VIII.A.4. *Binladen* also discloses this element. EX1003, ¶¶234-235; *supra* §III.C. *Binladen* discloses 19 pairs of first and second identifier sequences. EX1029, 3 (Table 1), 7 (Table 3). Three of those pairs were made of identifier sequences that are 4

nucleotides in length and different from each other. EX1003, ¶234. While *Binladen* also disclosed the use of identifier sequences that were two nucleotides in length, it explains that the rate of misassignment was lower for tags with four nucleotides. EX1029, 7; EX1003, ¶235. As observed in *Binladen* and further explained in *Parameswaran*, using longer sequence identifiers allows for “definitive assignment.” EX1004, 4; EX1029, 7; EX1003, ¶235.

5. *Parameswaran* and *Binladen* disclose a method comprising “sequencing the first identifier sequence, the nucleic acid analyte of interest and the second identifier sequence” (claim 1[d])

Parameswaran discloses claim 1[d]. EX1003, ¶236; *supra* §VIII.A.5.

Binladen also discloses this limitation. EX1003, ¶¶237-238; *supra* §III.C. As explained in *Binladen*, approximately 50% of PCR products were sequenced from the orientation of the forward primer, and 50% from the orientation of the reverse primer. EX1029, 3; EX1003, ¶237. These sequence reads collectively contained: (1) the forward tag (i.e., the first identifier sequence), (2) the nucleic acid analyte of interest, and (3) the reverse tag (i.e., the second identifier sequence). EX1029, 2 (Fig.1), 5. EX1003, ¶¶237-238.

6. *Parameswaran* and *Binladen* disclose a method comprising “validating the sequence of the nucleic acid analyte of interest by analyzing both identifiers and excluding the sequences of those nucleic acid analytes of interest containing only one identifier or an incorrect pair of identifiers from

**sequence analysis of the multiplex sequencing reaction”
(claim 1[e])**

Parameswaran discloses claim 1[e]. EX1003, ¶239; *supra* §VIII.A.6.

Binladen also discloses this limitation. EX1003, ¶¶240-241; *supra* §III.C. Following sequencing, *Binladen* “discarded all sequence reads without an exact match to any of the primers used in the studies.” EX1029, 5; EX1003, ¶240. Because the primers included the 5’ and 3’ sequence identifiers, only sequence reads with an appropriate sequence identifier were further analyzed. EX1029, 5-6; EX1003, ¶240.

Binladen necessarily excluded nucleic acid analytes of interest containing only one identifier or an incorrect pair of identifiers. EX1003, ¶241. Each analyte in *Binladen* generated two sets of sequence **reads**—each containing either the forward or reverse tag. EX1029, 3; EX1003, ¶241. By discarding reads without an exact forward or reverse tag, *Binladen* necessarily excluded the sequences of nucleic acid analytes having only one identifier or an incorrect pair of identifiers. EX1003, ¶241. Thus, *Binladen* discloses this claim element. EX1003, ¶241.

In sum, both references teach dual-index multiplex sequencing methods that identify erroneous sequences having only one identifier or an incorrect pair of identifiers. Both references teach that these erroneous sequences can be excluded and that the remaining sequences with correct pairs of identifiers have been validated.

As such, each of the references discloses and suggests claim 1[e], and the combination of the references therefore also discloses and suggests it.

B. A POSA Would Have Been Motivated to Combine, with a Reasonable Expectation of Success, *Parameswaran* and *Binladen* to Arrive at the Subject Matter of Claim 1

A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Binladen* to improve multiplex sequencing accuracy. EX1003, ¶¶242-246. As reported in *Parameswaran* and *Binladen*—and other prior art references including *Gloor* (EX1005) (*supra* §VIII.A.6), a POSA would have known that multiplex sequencing resulted in incorrect sequences derived from various technical errors, which could lower the accuracy of sequencing. *See* EX1004, 7; EX1029, 6; EX1003, ¶242. To improve accuracy, a POSA would have been motivated to adopt the dual-indexing methods used in *Parameswaran* and *Binladen* to identify and exclude erroneous sequences. EX1003, ¶243. Indeed, both *Parameswaran* and *Binladen* used dual identifiers to differentiate and validate correct sequences from sequences resulting from errors. *See* EX1004, 7; EX1029, 5-6; EX1003, ¶243. By excluding erroneous sequences that failed to match the expected identifiers, both *Parameswaran* and *Binladen* significantly lowered the rates of misassignment. *See* EX1004, Abstract; EX1029, Abstract; EX1003, ¶243.

In combining *Parameswaran* and *Binladen*, a POSA would have known how to make minor modifications to further improve the accuracy of sequencing. EX1003,

¶244. For example, *Binladen* used 2-nucleotide and 4-nucleotide long sequence identifiers, and observed that the longer identifiers lowered the misassignment rate. EX1029, 7; EX1003, ¶244. *Parameswaran* made this explicit, characterizing *Binladen* as “valuable” but explaining that some applications “call for a more definitive assignment of samples and/or for enhancing multiplexing capabilities,” and that this could be achieved through the use of “10-base barcodes that differ from each other at multiple positions (by at least 4 nt).” EX1004, 2; EX1003, ¶244. A POSA similarly motivated to improve sequence accuracy would have been motivated to combine *Parameswaran* and *Binladen* to practice the subject matter of claim 1. EX1003, ¶244; *Scanner Tech. Corp. v. ICOS Vision Sys. Corp.*, 528 F.3d 1365, 1381-82 (Fed. Cir. 2008) (“the relatively small logical gap between the prior art and the claim in this case is closed by a person of ordinary skill in the art ‘pursuing known options within his or her technical grasp’”).

A POSA would have had a reasonable expectation of success in combining *Parameswaran* and *Binladen*. EX1003, ¶245. Both *Parameswaran* and *Binladen* provide detailed methods for multiplex sequencing, detecting first and second identifier sequences, and excluding sequence reads containing only one identifier or incorrect pairs of identifiers. *Supra* §§IX.A, III.A, III.C. Both used Roche 454 multiplex sequencing. *Supra* §§III.A, III.C. Following *Parameswaran* and *Binladen*, a POSA would have reasonably expected that the sequences of nucleic acid analytes

of interest in a multiplex sequencing reaction would be successfully validated by practicing the claimed subject matter. EX1003, ¶245.

As addressed in Section XI below, Petitioner is aware of no objective evidence of nonobviousness. EX1003, ¶¶258-259. Claim 1 therefore would have been obvious over *Parameswaran* and *Binladen*. EX1003, ¶46.

C. Dependent Claims 2-6 Would Have Been Obvious Over *Parameswaran* and *Binladen*

Claims 2-6 ultimately depend from claim 1 and would have been obvious for the reasons described above (§§IX.A-C) and the additional reasons described here. EX1003, ¶247.

1. Claims 2-3

Claim 2 recites the “method of claim 1, wherein the identifier sequences are nucleic acid sequence tags.” EX1001, 22:7-8. Claim 3 recites the “method of claim 2, wherein the identifier sequences are barcode sequences.” EX1001, 22:9-10. *Parameswaran* discloses this element. *Supra* §VII.B.1; EX1003, ¶248. *Binladen* also used nucleic acid sequence tags that function as barcode identifier sequences. EX1029, 3, 6; EX1003, ¶248. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Binladen* for the reasons set forth above. *Supra* §IX.B; EX1003, ¶248.

2. Claim 4

Claim 4 recites the “method of claim 3, wherein the barcode sequences are from 4 to 20 nucleotides.” EX1001, 22:11-12. *Parameswaran* discloses barcode sequences that are 4 to 20 nucleotides. *Supra* §VII.B.2; EX1003, ¶249. Likewise, *Binladen* discloses the use of barcode sequences having 4 nucleotides. EX1029, 3; *see also* EX1029, 3, 7 (Tables 1, 3); EX1003, ¶249. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Binladen* for the reasons set forth above. *Supra* §IX.B; EX1003, ¶249.

3. Claim 5

Claim 5 recites the “method of claim 4, wherein t [sic] barcode sequences are designed to have no homopolymer regions.” EX1001, 22:13-14. *Parameswaran* discloses barcode sequences that are designed to have no homopolymer regions. *Supra* §VII.B.3; EX1003, ¶250. *Binladen*’s pairs of 4-nucleotide tags also do not include any homopolymer region. *See* EX1029, 3, 7 (Tables 1, 3); EX1003, ¶250. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Binladen* for the reasons set forth above. *Supra* §IX.B; EX1003, ¶250.

4. Claim 6

Claim 6 recites the “method of claim 1, wherein the nucleic acid analyte of interest is on the surface of a flow cell.” EX1001, 22:15-16. Claim 6 does not require

attaching the analyte to the surface of a flow cell—it simply requires the analyte to be “on the surface of a flow cell.” EX1003, ¶251. *Parameswaran* and *Binladen* both use Roche 454 sequencing, which involved placing bead-bound nucleic acid analytes into a flow cell for sequencing. EX1004, 4; *supra* §§II.A, III.A, III.C; EX1028, 6-8 (depicting bead-bound nucleic acid analytes placed in wells of a flow cell); EX1003, ¶251. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Binladen* for the reasons set forth above. *Supra* §IX.B; EX1003, ¶251.

X. GROUND 5: CLAIM 6 WOULD HAVE BEEN OBVIOUS OVER *PARAMESWARAN*, *BINLADEN*, AND *BENTLEY*

Even if Patent Owner were to argue, and the Board were to accept, that Roche 454 sequencing as used in *Parameswaran* and *Binladen* did not occur on the surface of a flow cell, *Bentley* teaches this limitation. EX1003, ¶¶252-253. *Bentley* discloses Illumina sequencing of template nucleic acids after bridge amplification on the surface of the flow cell. EX1006, 1-2 (“Single molecules of DNA are attached to a flat surface, amplified *in situ* and used as templates for synthetic sequencing with fluorescent reversible terminator deoxyribonucleotides.”); EX1003, ¶253.

A POSA would have been motivated to combine these references, with a reasonable expectation of success, for the reasons discussed above. *Supra* §§IX.A-C, VIII.B-C; EX1003, ¶254. For example, to increase accuracy and decrease cost, a

POSA would have been motivated to use the dual index strategies of *Parameswaran* and *Binladen* with Illumina sequencing as disclosed in *Bentley*. EX1003, ¶254; *supra* §§VIII.B, III.D. *Parameswaran* states that its barcoding approach is compatible “with other sequencing platforms” (EX1004, 2, 8) and specifically identifies Illumina sequencers as one of the “major high-throughput sequencing platforms” (EX1004, 1); EX1003, ¶255. To perform dual-indexing with Illumina sequencing as taught in *Bentley*, a POSA would have needed to simply swap Roche 454 adapters for Illumina-specific adapters and make other minor modifications to the sequencing process, none of which involved changing the dual barcoding approach disclosed in *Parameswaran* or *Binladen*. EX1003, ¶255; *see supra* §VIII.C.

Although *Bentley*’s read lengths of 35 bases were shorter than the read lengths of about 70 to 141 bases used for *Parameswaran*’s and *Binladen*’s dual-indexing methods, a POSA would have known that read lengths for Illumina sequencing had significantly increased as of December 2010, exceeding the read lengths needed to sequence the nucleic acid analytes sequenced in *Parameswaran*. EX1003, ¶256; EX1027, 1 (reporting that Illumina sequencers were capable of “high-quality paired end reads of 150 base pairs (bp)). A POSA as of December 2010 would therefore have been motivated, with a reasonable expectation of success, to combine *Parameswaran*, *Binladen*, and *Bentley* to practice the subject matter of claim 6. EX1003, ¶257. Claim 6 therefore would have been obvious. EX1003, ¶257.

XI. GROUNDS 3-5: THERE IS NO OBJECTIVE EVIDENCE OF NONOBVIOUSNESS

The Patent provides no evidence of unexpected results or other secondary considerations of non-obviousness, and Petitioner is aware of none. EX1003, ¶¶258-259. Nor could any such evidence justify a non-obviousness finding given that there is nothing novel or inventive about the challenged claims over *Parameswaran*, *Gloor*, *Binladen*, and *Bentley*. See *In re Huai-Hung Kao*, 639 F.3d 1057,1068 (Fed. Cir. 2011) (the offered secondary consideration must result from “what is both claimed and *novel* in the claim”); *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1372 (Fed. Cir. 2007) (holding objective evidence insufficient where the record established “a strong case of obviousness”).

XII. DISCRETIONARY DENIAL IS NOT WARRANTED

This IPR should be instituted under the *Advanced Bionics* §325(d) test because the Examiner did not consider directly relevant prior art (i.e., *Gloor*, *Binladen*, and *Bentley*) and, moreover, overlooked applicant’s prior concession that the claimed subject matter here, including the use of “different” barcodes, was disclosed in *Parameswaran*. *Supra* §IV.D.1; *Advanced Bionics, LLC v. MED-EL Elektromedizinische Gerate GmbH*, IPR2019-01469, Paper 6 at 6-11 (PTAB Feb. 13, 2020) (precedential); *Becton, Dickinson & Co. v. B. Braun Melsungen AG*,

IPR2017-01586, Paper 8 at 17-18 (PTAB Dec. 15, 2017) (precedential as to §III.C.5, first paragraph).

Becton factors (a)-(b) favor institution because the asserted art is materially different from, and not cumulative of, the art evaluated by the Office. Specifically, *Gloor*, *Binladen*, and *Bentley* were not considered, let alone relied upon as the basis of a rejection, during prosecution of the '200 Patent or any of its parent applications. *Supra* §IV.D; see *Netflix, Inc. v. DivX, LLC*, IPR2020-00558, Paper 10 at 40 (PTAB Aug. 26, 2020) (granting institution and explaining “[b]ecause the combination... was not considered by the examiner, we find consideration of the references and arguments based thereon to be materially different than the examiner’s previous consideration[.]”).

Becton factors (c)-(f) also favor institution. The '200 Patent issued without any prior art rejection. *Supra* §IV.D.2. While the Examiner rejected the claims in the parent '281 Patent over *Parameswaran*, the argument made to overcome that rejection **supports** Petitioner’s challenge. Specifically, to distinguish *Parameswaran*, applicant argued that *Parameswaran* discloses “different” sequence identifiers, whereas the then pending claims required the “same” identifiers. EX1026, 452-453; *Supra* §IV.D.1. Yet in the '200 Patent, Patent Owner claims the very feature (i.e., “different” identifiers) taught by *Parameswaran*. *Supra* §§IV.B, IV.D.1. The Examiner therefore erred by not reinstating the earlier anticipation rejection.

Supra §IV.D.2; *Advanced Bionics*, IPR2019-01469, Paper 6 at 8 n.9 (“An example of a material error may include misapprehending or overlooking specific teachings of the relevant prior art[.]”).

XIII. MANDATORY NOTICES

A. Real Party-in-Interest

Petitioner certifies that the real party-in-interest is Illumina, Inc. No other party exercised control or could exercise control over Illumina’s participation in this proceeding, the filing of this Petition, or the conduct of any ensuing trial.

B. Related Matters

On June 10, 2024, Molecular Loop filed a Complaint in the U.S. District Court for the District of Delaware, seeking a declaration that Illumina and Verinata Health, Inc. (“Verinata”) infringe at least claim 1 of the ’200 Patent (1:24-cv-00680). On the same day, Illumina and Verinata filed a declaratory judgment action in the U.S. District Court for the District of Massachusetts, seeking a declaration that neither Illumina nor Verinata infringes any claim of the ’200 Patent (1:24-cv-11501).

The ’200 Patent is a continuation of the ’852 Patent, which is being challenged in co-pending IPR2024-00964.

C. Lead and Backup Counsel, and Service Information

Lead Counsel	Backup Counsel
William B. Raich	Charles T. Collins-Chase

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Please address correspondence to counsel at the email addresses above.

Petitioner consents to service by e-mail.

XIV. GROUNDS FOR STANDING

Petitioner certifies that the Patent is available for IPR and that Petitioner is not barred or estopped from requesting IPR challenging the patent claims on the grounds identified herein.

XV. CONCLUSION

For the reasons above, Petitioner requests institution of *inter partes* review and cancellation of each challenged claim. The Office may charge any required fees to Deposit Account No. 06-0916.

Dated: July 2, 2024

By: /William B. Raich/
William B. Raich, Lead Counsel
Reg. No. 54,386

CERTIFICATE OF COMPLIANCE

The undersigned certifies that the foregoing Petition for *Inter Partes* Review contains 13,915 words, excluding those portions identified in 37 C.F.R. §42.24(a), as counted by the word-processing software used to prepare this paper.

Dated: July 2, 2024

By: /William B. Raich/
William B. Raich, Lead Counsel
Reg. No. 54,386

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§42.6(e) and 42.105(a), the undersigned certifies that on July 2, 2024, a copy of the foregoing Petition for *Inter Partes* Review of U.S. Patent No. 11,768,200, the associated power of attorney, and Exhibits 1001-1038 were served by FedEx Priority Overnight on the correspondence address of record indicated in the Patent Office's public Patent Center system for U.S. Patent No. 11,768,200:

Cooley LLP
ATTN: IP Docketing Department
1299 Pennsylvania, Avenue, NW
Suite 700
Washington, DC 20004

Dated: July 2, 2024

By: /William Esper/
William Esper
Legal Assistant and PTAB Coordinator

Finnegan, Henderson, Farabow,
Garrett & Dunner, LLP