

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. IPR2024-00964
Patent No. 11,041,852

PETITION FOR *INTER PARTES* REVIEW

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Statutes

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GLOSSARY

'852 Patent	U.S. Patent No. 11,041,852
<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. 11,041,852
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. 11,041,852
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	(Intentionally left blank)
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	(Intentionally left blank)
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 reducing template cross-over error introduced during sequencing workflow, the method comprising the steps of
1[a]	incorporating at least two members of a plurality of identifier sequences into template nucleic acids obtained from at least two different samples, wherein said two members constitute a distinct pair associated with said template;
1[b]	combining said nucleic acid templates into a single sample;
1[c]	amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters,
1[d]	at least one of which comprises a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs;
1[e]	sequencing amplicons obtained in said amplifying step; and
1[f]	discarding sequence reads obtained in said sequencing step that contain said combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce cross-over error.
2	The method of claim 1, wherein the identifier sequences are nucleic acid sequence tags.
3	The method of claim 1, wherein the identifiers are first and second barcode sequences.
4	The method of claim 1, wherein a first identifier sequence is incorporated into a 5' portion of the template, and a second identifier sequence is incorporated into a 3' portion of the template.

Claim	Element
5	The method of claim 4, wherein after incorporation of the identifier sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.
6	The method of claim 1, wherein a first identifier sequence is incorporated into a 5' end of the template, and a second identifier sequence is incorporated into a 3' end of the template.
7	The method of claim 1, wherein prior to said sequencing step, the template is attached to a substrate.
8	The method of claim 7, wherein the template is directly attached to the substrate.

I. INTRODUCTION

Petitioner Illumina, Inc. respectfully requests *Inter Partes* Review of U.S. Patent No. 11,041,852 (EX1001; the “Patent” or “’852 Patent”), assigned to Molecular Loop Biosciences, Inc., and cancellation of claims 1-8. Those claims are unpatentable as obvious over two separate prior-art combinations—*Parameswaran* and *Gloor* (Ground 1) and *Parameswaran* and *Bentley* (Ground 2). Neither combination was considered by the Office during prosecution of the Patent.

As explained in the accompanying Declaration of Dr. Paul Spellman (EX1003, ¶¶1-17, 21-30, 223-224), an expert in the sequencing field, the Patent is directed to methods of reducing error when sequencing nucleic acids obtained from multiple samples. Specifically, it claims methods for reducing errors during sequencing by adding a “distinct pair” of identifier sequences for nucleic acids from a given sample. During data analysis, sequences with incorrect pairs of identifier sequences are discarded, as they reflect errors in the sequencing process. The use of “distinct pairs” of identifier sequences (sometimes called “indexes,” “barcodes,” or “tags”) is the focus of the Patent.

The challenged claims are unpatentable. By the earliest possible priority date of December 23, 2010, there was nothing inventive about a method of reducing errors by adding a “distinct pair” of identifier sequences during sequencing—as confirmed by the prosecution history. The Patent issued after a single anticipation

rejection over *Parameswaran*, which describes reducing error by assigning “two uniquely designed” identifier sequences to nucleic acids from multiple samples before amplification and sequencing on a Roche 454 sequencer. Without disputing that *Parameswaran* met all limitations of the then-pending claims, Patent Owner amended the claims to require that amplification occurs “on a surface of a flow cell”—known at the time to be used with Illumina sequencers—and persuaded the Examiner that *Parameswaran* is “limited to [Roche 454] pyrosequencing” and “does not mention forming clusters in a flow cell.”

But it was well known that pairs of identifier sequences could be used to improve sequencing accuracy with either Roche 454 *or* Illumina sequencers, which were two of the three major sequencing platforms used as of December 2010. Choosing one sequencing platform over another was a matter of design choice—not an invention. *Gloor*, which was not of record during prosecution, performed a dual-indexing method similar to *Parameswaran*’s method *using an Illumina sequencer*—tagging template nucleic acids with distinct pairs of identifier sequences before amplification and sequencing on the surface of a flow cell. *Bentley*, which also was not of record during prosecution, also provides a detailed description of Illumina sequencing. And *Parameswaran* expressly contemplates the possibility of using its dual-indexing methods on other sequencing platforms, mentioning Illumina sequencing specifically. A POSA in December 2010 would have

recognized that, by simply swapping the Roche 454 adapters for Illumina-specific adapters, as described in both *Gloor* and *Bentley*, the method described by *Parameswaran* could be adapted to Illumina sequencers. Thus, all challenged claims are rendered obvious by (i) *Parameswaran* in combination with *Gloor* and, alternatively, (ii) *Parameswaran* in combination with *Bentley*.

Accordingly, this IPR should be instituted, and the challenged claims canceled pursuant to the “significant public policy interest in removing invalid patents from the public arena.” *SmithKline Beecham Corp. v. Apotex Corp.*, 403 F.3d 1331, 1354 (Fed. Cir. 2005).

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, ¶¶41-45. These methods could not be used to simultaneously sequence nucleic acids from a mixture of samples. EX1011, 1; EX1010, 2-3; EX1035; EX1003, ¶¶46-47. 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, ¶¶48, 78-80.

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, ¶64. 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 template nucleic acids could be associated with the sample from which they came. EX1010, 9. After sequencing, the identifier sequences were used to assign the associated sequences of the template nucleic acids to the corresponding sample, a process referred to as “demultiplexing.” EX1003, ¶65.

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, ¶49. 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, ¶¶50-51. 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, ¶¶52-55; EX1010, 3-6; EX1031, 38.

Both Illumina and Roche 454 sequencing involved preparing DNA sequencing libraries (containing “template” nucleic acids) 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 template amplification and sequencing occurred. EX1010, 3-6. In both platforms, template 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, 1; EX1003, ¶¶58-61, 62-63.

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, ¶¶56-57. 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, ¶¶64-66. 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, ¶¶67-77; EX1016, [0091]-[0092]; EX1038, 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, ¶¶70-77. 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 PCR 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, ¶¶70-77.

Relative to single indexing, dual-indexing also was known to improve the process of sample identification. EX1003, ¶¶70-77; EX1004, 8; EX1005, 1. For example, template nucleic acids from different samples can anneal together during PCR amplification, creating erroneous “chimeric” combinations—a process that the

Patent calls “cross-over” error. *Infra* §IV.B; *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, ¶¶67-69. 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, ¶69.

As of December 2010, there were two general forms of dual-indexing used for multiplex sequencing. EX1003, ¶¶70-77. The first, ***unique dual-indexing***, attached unique sequence identifiers at opposite ends of template nucleic acids obtained from a single sample. EX1003, ¶¶71-73. 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, ¶¶74-76. In non-unique dual-indexing, pairs of identifiers are again attached at opposite ends of template nucleic acids obtained from each sample. While each ***pair*** of identifier sequences is used for template nucleic acids from only one sample, the distinction from *unique* dual-indexing is that individual identifier sequences can be reused across template nucleic acids from multiple samples. Non-unique dual-indexing cannot necessarily identify “cross-over” errors without additional data processing, particularly if the pair of identifiers attached to the sequenced chimeric molecule matches one of the distinct pairs assigned to nucleic acids from a given sample. EX1003, ¶¶75-76. As discussed below, *Gloor* discloses the use of non-unique dual-indexing. *Infra* §§III.B, VI.A.5.

III. THE ASSERTED PRIOR ART

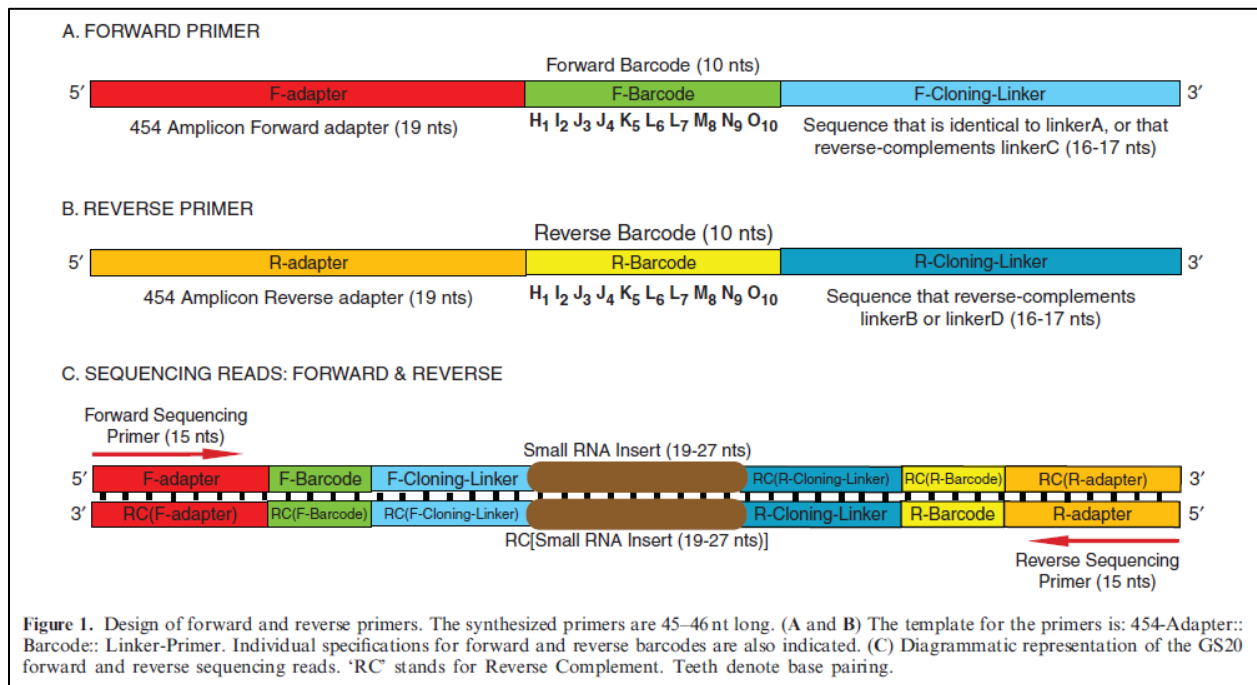
A. *Parameswaran* (EX1004)

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, ¶81. *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, ¶82. 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, ¶83.

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, ¶83. 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, ¶84. 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, ¶85. *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, ¶86. 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, ¶86.

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, ¶88. *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, ¶87.

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, ¶89.

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, ¶90. *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, ¶92. 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.¹ EX1005, 1-3. These primer pairs were used to

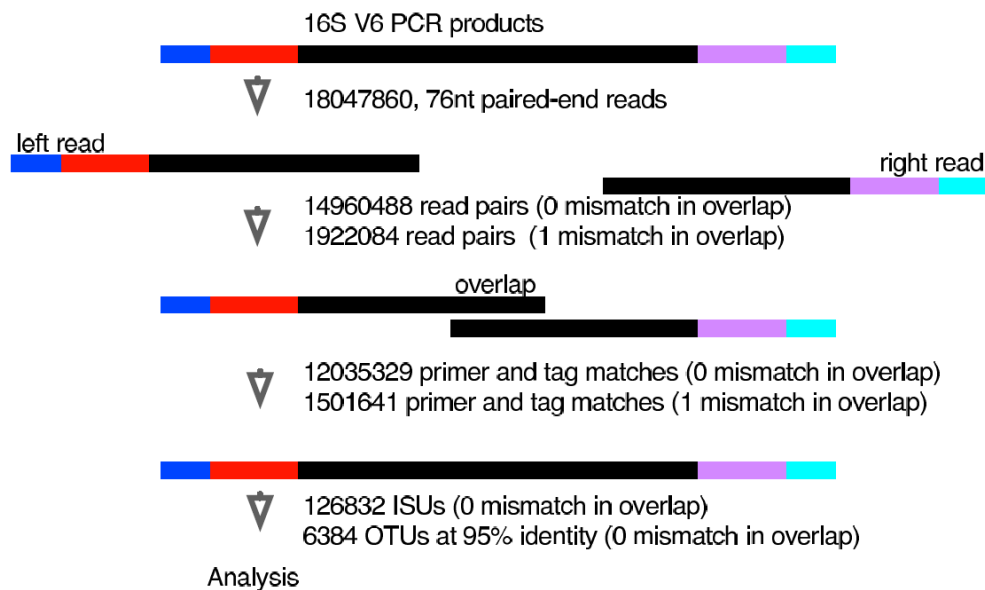
¹ Although drawn correctly in *Gloor* Figure 2, the paper states that unique sequence tags were added at the “3’” end of each primer, i.e., closer to the nucleic acid template. EX1005, 2, 6, 22. Consistent with Figure 2, a POSA would have

amplify the region of interest, incorporating the sequence tags in the left and right primers into each end of the amplicons. EX1003, ¶92. *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, ¶91. *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.C; EX1003, ¶93.

Figure 2 in *Gloor*, reproduced below, depicts the workflow used. EX1003, ¶95. *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

understood that the paper should have stated “5’,” i.e., further from the nucleic acid template, as a sequence of interest cannot be amplified with a primer having a barcode immediately adjacent to the template. EX1003, ¶94. This was corrected in an October 2010 republication of *Gloor*. Compare EX1005, 2, 6, 22, with EX1024, 1, 3, 11.

blue and light blue, respectively). EX1005, 3, 6-7 (Fig. 2); EX1003, ¶95. *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, ¶95.



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, ¶96. *Gloor* combined sequence reads that substantially overlapped to identify complete sequences. EX1003, ¶95. *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, ¶¶96-97.

Gloor compared these 12 million sequences to one another and identified “putative chimeric” sequences, which *Gloor* explains “can arise during PCR.” EX1005, 8; EX1003, ¶98. *Gloor* evaluated these sequences “for chimerism” by comparing them against bacterial reference sequences available in a public database. EX1005, 8; EX1003, ¶98. *Gloor* explains that “many chimeric sequences may have been removed because of the strict requirement for proper sequence tag and primer sequences on the left and right ends.” EX1005, 22; *see also* EX1005, 22 (explaining that “inspection of a fraction of the read pairs that failed to overlap, or that failed to pass the sequence tag and primer requirements showed that many of these were chimeric or deleted at one or both ends”); EX1003, ¶98.

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, ¶99.

C. *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, ¶100. *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, ¶¶101-103. 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, ¶101. 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, ¶101.

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, ¶¶102-103.

IV. THE '852 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 '852 Patent

The Patent is directed to reducing “cross-over error introduced during sequencing workflow.” EX1001, cl. 1. Specifically, 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, ¶¶104,

108. By incorporating a “pair of sequence identifiers” into the template nucleic acid, the Patent purports to identify instances of “crossover error” in which chimeric molecules of two samples are formed during amplification. *See* EX1001, 7:24-38; EX1003, ¶¶104, 108.

The Patent includes Figures 1-3. Figures 1 and 2 are conceptual. EX1003, ¶¶109-113. 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, ¶¶109-110. 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, ¶¶111-113. 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, ¶¶114-115. 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, ¶116. 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, ¶116. 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, 10:3-20:46 *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, 20:47-62 *with* EX1037, 9-11). Other portions of the ’852 Patent were also copied from that patent publication. *Compare* EX1001, 1:25-31, 3:37-43, 20:47-62 *with* EX1037, 1, 3, 18; *see also* EX1003, ¶117.

C. The Challenged Claims

Independent claim 1 recites:

A method for reducing template cross-over error introduced during sequencing workflow, the method comprising

the steps of incorporating at least two members of a plurality of identifier sequences into template nucleic acids obtained from at least two different samples, wherein said two members constitute a distinct pair associated with said template;

combining said nucleic acid templates into a single sample;

amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters, at least one of which comprises a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs;

sequencing amplicons obtained in said amplifying step;

and discarding sequence reads obtained in said sequencing step that contain said combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce cross-over error.

EX1001, 20:65-21:18; EX1003, ¶¶105-106. Challenged dependent claims 2-8 further recite features of the claimed method of reducing template cross-over error,

such as template configuration and identifier sequence type. EX1001, 21:19-22:20; EX1003, ¶107.

D. Prosecution History of the '852 Patent

In the first Office Action, the Examiner issued a §102 rejection over *Parameswaran* (EX1005). EX1007, 192-197; EX1003, ¶118. The Examiner explained that *Parameswaran* discloses all limitations of the claimed methods for reducing template cross-over error, including “reducing template cross-over error introduced during sequencing workflow” and “incorporating at least two members of a plurality of identifier sequences into template nucleic acid obtained from at least two different samples, wherein said two members constitute a distinct pair associated with said template.” EX1007, 195-196; EX1003, ¶118.

Without disputing that *Parameswaran* discloses all limitations of the rejected claims, Patent Owner amended the claims to require amplification “on a surface of a flow cell.” EX1007, 301-306; EX1003, ¶119. Patent Owner persuaded the Examiner that *Parameswaran* is “limited to pyrosequencing” and fails “to disclose amplifying nucleic acids to form clusters,” as would occur in standard Illumina sequencing. EX1007, 301-306; EX1003, ¶119. Patent Owner also argued that *Parameswaran* “is directed to overcoming a problem unique to pyrosequencing in which bead-bound DNA must be physically separated on a sequencing medium, thus limiting the number of output sequences.” EX1007, 305; EX1003, ¶119. As Dr.

Spellman explains, however, the problem addressed in *Parameswaran* was not “unique” to pyrosequencing. EX1003, ¶120. Rather, it applied to multiplex sequencing generally, because the sequences needed to be both demultiplexed and accurately assigned. EX1003, ¶120; *supra* §II.B.

Nevertheless, the Examiner issued a Notice of Allowance, stating that Patent Owner’s “claim amendments overcame the previously presented claim rejections” and “[s]ince no new references were found teaching or suggesting the claims as amended, they are allowed.” EX1007, 312-318; EX1003, ¶121.

E. Claim Construction

Solely for the purposes of this proceeding, Petitioner provides the following construction for “template cross-over error.” The plain and ordinary meaning is applied for all other terms. EX1003, ¶123.

The claims of the Patent are directed to a method of reducing “template cross-over error” introduced during sequencing workflow. EX1003, ¶122. The specification mentions the term “crossover” four times, each describing the formation of a chimeric molecule made from template nucleic acids derived from different samples. EX1001, 5:41-6:6 (“a crossover has occurred” when a “chimeric molecule formed” between template nucleic acids derived from patient A and patient B due to “improper molecular overlap”), 6:53-7:38 (“a crossover has occurred” when “template from sample A and template from sample B hybridized, forming a

bridge”). EX1003, ¶122. Thus, for purposes of this proceeding, Petitioner construes “template cross-over error” as sequences of chimeric molecules made from template nucleic acids derived from different samples. EX1003, ¶122.

V. STATEMENT OF PRECISE RELIEF REQUESTED

Petitioner requests cancellation of Patent claims 1-8 on the following grounds.

Ground	References	Basis	Claims
1	<i>Parameswaran</i> (EX1004) and <i>Gloor</i> (EX1005)	§103	1-8
2	<i>Parameswaran</i> (EX1004) and <i>Bentley</i> (EX1006)	§103	1-8

VI. GROUND 1: CLAIMS 1-8 WOULD HAVE BEEN OBVIOUS OVER *PARAMESWARAN* AND *GLOOR*

The claimed method of reducing cross-over error would have been obvious over the combination of *Parameswaran* and *Gloor*. EX1003, ¶124. As the Examiner explained during prosecution, *Parameswaran* discloses a method of reducing cross-over error that met the limitations of the then-pending claims. *Supra* §IV.D. Patent Owner did not dispute that *Parameswaran*’s unique dual-indexing method disclosed all limitations of the rejected claims, but instead narrowed the claims to Illumina sequencers by adding the limitation “on the surface of a flow cell.” *Supra* §IV.D. As explained herein (*infra* §IX), the Examiner did not consider *Gloor* and *Bentley*, and, moreover, failed to appreciate the interchangeability of Roche 454 and Illumina sequencers in the claimed methods (*infra* §§VI.B-C, VII.B-C).

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

Claim 1 would have been obvious over *Parameswaran* and *Gloor*. EX1003, ¶125. As discussed below, *Parameswaran* and *Gloor* disclose each and every element of claim 1, including amplification “on a surface of a flow cell”—the sole alleged difference between *Parameswaran* and claim 1. EX1003, ¶¶126-155. Thus, the combined scope of *Parameswaran* and *Gloor* is identical to that of claim 1, and the combination of *Parameswaran* and *Gloor* renders claim 1 obvious. EX1003, ¶¶156-173.

1. *Parameswaran* and *Gloor* disclose a “method for reducing template cross-over error introduced during sequencing workflow”

Parameswaran describes this element. EX1003, ¶¶126-131; *supra* §III.A. Using Roche 454 sequencing, *Parameswaran* discloses a barcoding approach in which “each sample is associated with two uniquely designed, 10-nucleotide barcodes.” EX1004, 2; EX1003, ¶126. To facilitate “unambiguous assignment of nucleic acid sequences from a mixture of libraries from up to 48 different samples,” *Parameswaran* discloses “requiring a match to the 10-base barcodes and additional three bases of the fixed linker”—called a “barcode motif.” EX1004, 7-8; EX1003, ¶126. *Parameswaran* reports that about 94% of 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, ¶127.

Parameswaran reports that a subset of this 5-6% of sequences contained a pair of **mismatched** barcode motifs. *See* EX1004, 7. In mismatched or “misassigned” sequences, the forward identifier was incorrectly paired with the wrong reverse identifier. EX1004, 8, Abstract; EX1003, ¶¶128-129. Indeed, *Parameswaran* reports the “false-discovery” rate, defined as sequences that contain mismatched pairs of forward and reverse barcode motifs (i.e., pairs of identifiers that were not assigned to any template). EX1004, Abstract, 8; EX1003, ¶131. *Parameswaran* explains that “any sequence with both 5’ and 3’ barcode motifs will be placed with reasonable accuracy into the correct bin.” EX1004, 8; EX1003, ¶131. *Parameswaran* explains that sequences with mismatched barcode motifs can arise from various errors, for example, cross-contamination of DNA templates. EX1004, 7; EX1003, ¶130.

A POSA would have understood that these sequences with mismatched barcode motifs include chimeric sequences resulting from cross-over error (sequences of chimeric molecules made from template nucleic acids derived from different samples). EX1003, ¶130. As detailed above (*supra* §II.B), when using unique dual-indexing methods, like the one disclosed in *Parameswaran*, chimeric sequences resulting from cross-over events contain a pair of identifiers different from any of the distinct pairs assigned to the templates—exactly what is reported in *Parameswaran*. EX1004, Abstract (“The false-discovery rate, as measured by the percentage of sequences with unexpected perfect pairings of unmatched forward and

reverse barcodes, was estimated to be <0.005%.”); EX1003, ¶¶130, 67-69, 71-73; *Adasa Inc. v. Avery Dennison Corp.*, 55 F.4th 900, 910 (Fed. Cir. 2022) (holding that “the reference need not disclose the elements in the very same terms used by the patent”).

Parameswaran characterizes each sequence with “unexpected perfect pairings of unmatched forward and reverse barcodes” as a “misassignment.” EX1004, 8, Abstract; EX1003, ¶131. *Parameswaran* explains that the misassignment rate was remarkably low due to “requiring a match to the 10-base barcodes and additional three bases of the fixed linker.” EX1004, 8; EX1003, ¶131. *Parameswaran* further discloses that lowering the misassignment rate would allow sequences to be assigned to correct samples more accurately. EX1004, 8; EX1003, ¶131. Thus, *Parameswaran* discloses a method for reducing template cross-over error introduced during sequencing workflow. EX1003, ¶131.

Gloor likewise describes a method for reducing template cross-over introduced during sequencing workflow. EX1003, ¶¶132-136; *supra* §III.B. *Gloor* sequenced nucleic acids derived from 272 clinical samples using an Illumina sequencer. EX1005, 2; EX1003, ¶132. The nucleic acids were amplified using “combinatorial sequence tags attached to PCR primers.” EX1005, Abstract. These combinatorial sequence tags (i.e., identifier sequences) constituted unique pairs of

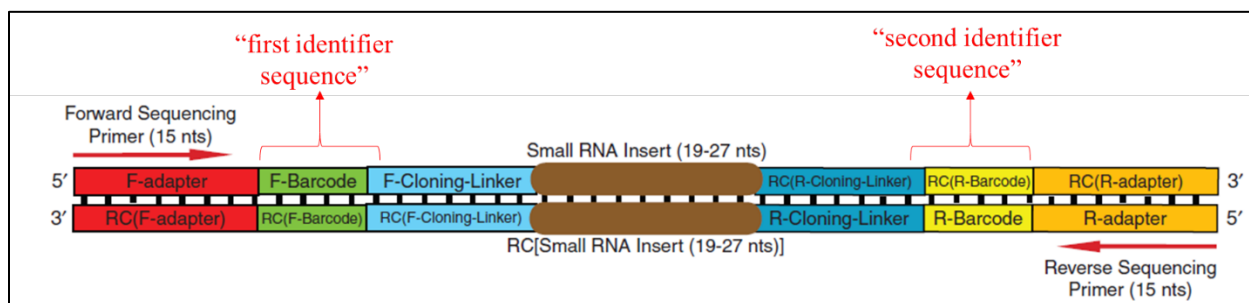
two sequence tags, one incorporated into each end of a nucleic acid analyte of interest. EX1005, 2-3, 6 (Table 2); EX1003, ¶132.

Gloor compiled the sequences of the nucleic acids from 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, ¶¶133-134. *Gloor* compared these extracted sequences and identified “putative chimeric” sequences, which “can arise during PCR.” EX1005, 8 (citing EX1017); EX1017, 1 (chimeras are formed via PCR-mediated recombination “when incompletely extended DNA fragments anneal to closely related sequences generating recombinants between starting templates”); EX1003, ¶134. *Gloor* evaluated these putative chimeric sequences “for chimerism” by comparing the sequences against bacterial reference sequences available in a public database. EX1005, 8; EX1003, ¶135. *Gloor* reports that “putative chimeric sequences composed a miniscule fraction of reads” likely because “many chimeric sequences may have been removed” in part based on “the strict requirement for proper sequence tag and primer sequences on the left and right ends.” EX1005, 22-23; EX1003, ¶135. By imposing this “strict requirement” and removing chimeric sequences (EX1005, 22-23), *Gloor* discloses a method of reducing cross-over error introduced during sequencing workflow. EX1003, ¶136.

Because each of *Parameswaran* and *Gloor* discloses a method of reducing cross-over error introduced during sequencing workflow, the combination of *Parameswaran* and *Gloor* likewise discloses the method.

2. *Parameswaran* and *Gloor* disclose methods that comprise “incorporating at least two members of a plurality of identifier sequences into template nucleic acids obtained from at least two different samples, wherein said two members constitute a distinct pair associated with said template” (claim 1[a])

Parameswaran discloses claim 1[a]. EX1003, ¶¶137-139; *supra* §III.A. Specifically, *Parameswaran* discloses a method in which “two uniquely-designed, 10-nucleotide barcodes” are attached to template nucleic acids from “each sample” and, along with three nucleotides from an adjacent linker sequence, comprise a 13-nucleotide “barcode motif.” EX1004, 2, 7; EX1003, ¶¶137-138. Figure 1C of *Parameswaran* provides a diagram of forward and reverse sequencing reads, showing two identifier sequences incorporated into the opposite ends of the template nucleic acids (called the 5’ and 3’ ends). EX1005, 3 (copied below).



Parameswaran's unique dual-indexing method also meets the limitation “wherein said two members constitute a distinct pair associated with said template.” EX1003, ¶139. To ensure that all samples were uniquely dual-indexed, *Parameswaran* combined three nucleotides from 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”). Supplementary Table 1 of *Parameswaran* provides a complete list of barcoded primers, in which identifier sequences were not repeated across samples EX1004, 14-17; EX1003, ¶139. Thus, template nucleic acids from different samples contained distinct pairs of identifier sequences. EX1003, ¶139.

Gloor also meets this limitation. EX1003, ¶140; *supra* §III.B. Like *Parameswaran*, *Gloor* discloses using a pair of identifier sequences, one on the left end and one on the right end of each target sequence. EX1003, ¶140. Unlike *Parameswaran*, some of the templates in *Gloor* shared a common left identifier sequence or a common right identifier sequence, but not both. EX1005, Abstract (explaining that 272 clinical samples were attached using “combinatorial sequence tags”), 6 (Table 2) (listing 18 left identifier sequences and 16 right identifier sequences). In every instance, however, the template nucleic acids from a given sample were associated with a distinct pair of identifier sequences. EX1003, ¶140. In other words, each pair of identifier sequences associated with template nucleic

acids from one sample was “distinct” from other pairs of identifier sequences used for template nucleic acids from other samples. EX1003, ¶140.

Because each of *Parameswaran* and *Gloor* discloses a method in which “at least two members of a plurality of identifier sequences” are incorporated “into template nucleic acids obtained from at least two different samples, wherein said two members constitute a distinct pair associated with said template,” the combination of *Parameswaran* and *Gloor* likewise discloses this method.

3. *Parameswaran* and *Gloor* disclose methods that comprise “combining said nucleic acid templates into a single sample” (claim 1[b])

Parameswaran and *Gloor* disclose claim 1[b]. EX1003, ¶¶141-142; *supra* §§III.A-B. For example, *Parameswaran* discloses that its barcoding approach facilitated “pooling and sequencing of DNA from independent samples.” EX1004, Abstract; *see also* EX1004, 4 (“Approximately 50 ng of the purified pool was set aside for each pyrosequencing run.”). A POSA would have understood that the “pooling” of nucleic acid templates described in *Parameswaran* constitutes “combining said nucleic acid templates into a single sample.” EX1003, ¶141.

Likewise, *Gloor* states that 272 clinical samples were analyzed “by a single Illumina paired-end sequencing run.” EX1005, 3; *see also* EX1005, 23-24 (stating that “PCR products were mixed together to give the final sample sent for Illumina sequencing”). Because the nucleic acid templates from these 272 clinical samples

were sequenced in a single run, they therefore were “combin[ed] . . . into a single sample.” EX1003, ¶142.

Because each of *Parameswaran* and *Gloor* discloses a method in which the “nucleic acid templates” are combined “into a single sample,” the combination of *Parameswaran* and *Gloor* likewise discloses this method.

4. *Gloor* discloses a method that comprises “amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters” (claim 1[c])

Parameswaran discloses a method that comprises amplifying nucleic acid templates. See EX1004, 4 (disclosing conditions used for “emulsion PCR reaction” for “high-throughput sequencing”); EX1003, ¶143. Because *Parameswaran* used pyrosequencing on a Roche 454 sequencer, amplification occurred on beads, rather than on the surface of a flow cell. *Supra* §§II.A, III.A; EX1003, ¶¶143, 56-57, 61. *Parameswaran* states, however, that its method “may also be compatible with other sequencing platforms” and specifically identifies Illumina sequencers as one of the “major high-throughput sequencing platforms.” EX1004, 1-2, 8; EX1003, ¶144.

Although not empirically tested in *Parameswaran*, a method comprising amplifying nucleic acid templates on a surface of a flow cell to form clusters was used in *Gloor*. EX1003, ¶¶145-146; *supra* §III.B. *Gloor* discloses that the nucleic acid templates are “attached to the solid surface” and that “[a]mplified PCR products are sequenced using an Illumina paired-end protocol to generate millions of

overlapping reads.” EX1005, Abstract, 22, 5 (referring to “Illumina solid support”); EX1003, ¶145. The Illumina paired-end sequencing used by *Gloor* necessarily involved “amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters.” EX1003, ¶¶145-146, 57, 60; EX1025, 2 (describing bridge amplification on the surface of Illumina flow cell). Thus, *Gloor* discloses a method that comprises “amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters.” EX1003, ¶146.

5. *Parameswaran* discloses methods in which “at least one” nucleic acid template “comprises a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs” (claim 1[d])

Parameswaran discloses claim 1[d]. EX1003, ¶¶147-148; *supra* §III.A. As discussed above (*supra* §III.A), *Parameswaran* describes sequences containing either only a single identifier sequence or a pair of *mismatched* identifier sequences. *See* EX1004, 7; EX1003, ¶147. In mismatched or “misassigned” sequences, the forward identifier was incorrectly paired with the wrong reverse identifier. EX1004, 8, Abstract; EX1003, ¶147. *Parameswaran* explains that sequences with mismatched barcode motifs can arise from various errors, for example, cross-contamination of DNA templates. EX1004, 7; EX1003, ¶147. As discussed above, sequences with mismatched barcode motifs include chimeric sequences resulting from cross-over events. *Supra* §§II.B, VI.A.1; EX1003, ¶148. Thus, *Parameswaran*

discloses at least one amplified nucleic acid template comprising “a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs.” EX1003, ¶148.

Gloor also discloses chimeric sequences arising from cross-over error. EX1005, 8 (citing EX1017); EX1003, ¶149; *supra* §III.B. But because *Gloor*’s pairs of identifiers were prepared in a combinatorial manner (i.e., non-unique dual-indexing), the pair of identifiers found in these chimeric sequences may nevertheless match one of the pairs used for other nucleic acid templates. EX1003, ¶149; *supra* §§II.B, III.B, VI.A.1.

6. *Parameswaran* and *Gloor* disclose methods that comprise “sequencing amplicons obtained in said amplifying step” (claim 1[e])

Parameswaran and *Gloor* disclose claim 1[e]. EX1003, ¶¶150-151; *supra* §§III.A-B. Specifically, *Parameswaran* states that the use of unique barcode motifs allowed them to pool and “simultaneously pyrosequence” DNA in each of the two independent sets of 25 barcoded libraries using Roche 454 sequencing. EX1004, 2; EX1003, ¶150. The reference further states that “[a]mplicon libraries prepared as described . . . may be entered directly into the 454/Roche sequencing pipeline.” EX1004, 4; EX1003, ¶150.

Gloor states that “[a]mplified PCR products are sequenced using an Illumina paired-end protocol to generate millions of overlapping reads.” EX1005, 1; EX1003,

¶151. *Gloor* states that “PCR products were mixed together to give the final sample sent for Illumina sequencing” (EX1005, 23-24), which yielded “18047860 reads that were 76 nt long from each end of the PCR amplified region” (EX1005, 6-7). EX1003, ¶151. Accordingly, *Gloor* discloses a method that involves “sequencing amplicons obtained in said amplifying step.” EX1003, ¶151.

Because each of *Parameswaran* and *Gloor* discloses a method in which “amplicons obtained in said amplifying step” are sequenced, the combination of *Parameswaran* and *Gloor* likewise discloses this method.

7. *Parameswaran* discloses methods that comprise “discarding sequence reads obtained in said sequencing step that contain said combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce cross-over error” (claim 1[f])

Parameswaran discloses claim 1[f]. EX1003, ¶¶152-154; *supra* §III.A. As discussed above (*supra* §§III.A, VI.A.1), after sequencing two independent sets of 25 barcoded libraries, *Parameswaran* reports that, while the majority of sequences retained a pair of perfect forward and reverse identifiers, some sequences contained combinations of identifier sequences that were different than any of the distinct pairs previously assigned to the template nucleic acids. EX1004, 7; EX1003, ¶152. Thus, *Parameswaran* distinguished sequences obtained in the sequencing step that contained combinations of identifier sequences that were different than any of assigned combinations of identifier sequences. EX1003, ¶153.

A POSA would have understood that *Parameswaran* disclosed a method in which these chimeric sequences could be discarded, and thus, reduce cross-over error. EX1003, ¶154. While *Parameswaran* states 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,” *Parameswaran* also calls them “false-discover[ies]” arising due to various errors that result in “misassignment” of sequences to incorrect samples. EX1004, 7-8; EX1003, ¶154. A POSA would have understood that these false discoveries could be excluded to increase accuracy. EX1003, ¶154. Indeed, *Parameswaran* states that, by lowering the false-discovery rate, “any sequence with both 5’ and 3’ barcode motifs will be placed with reasonable accuracy into the correct bin.” EX1004, 8; EX1003, ¶154. As such, a POSA would have understood that *Parameswaran* disclosed a method that involves “discarding sequence reads obtained in said sequencing step that contain said combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce cross-over error.” EX1003, ¶154.

Gloor also identified and discarded chimeric sequences, extracting only “overlapping reads with proper primer sequences and correct sequence tags.” EX1005, 7; EX1003, ¶155; *supra* §III.B. *Gloor* reports that “many chimeric sequences may have been removed because of the strict requirement for proper sequence tag and primer sequences on the left and right ends.” EX1005, 22

(explaining that “inspection of a fraction of the read pairs that failed to overlap, or that failed to pass the sequence tag and primer requirements showed that many of these were chimeric or deleted at one or both ends”). EX1003, ¶155. As discussed above (*supra* §§II.B, VI.A.5), because *Gloor* disclosed non-unique dual-indexing, the chimeric sequences in *Gloor* may contain pairs of identifier sequences assigned to template nucleic acids from another sample. EX1005, Abstract; EX1003, ¶155.

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* for several reasons. EX1003, ¶¶156-164. *First*, a POSA would have been motivated to use unique dual-indexing, as taught in *Parameswaran*, with Illumina sequencers, as taught in *Gloor*. EX1003, ¶¶156-157. There were numerous advantages offered by Illumina sequencers, which were faster, more efficient, and less expensive than Roche 454 sequencers as of December 2010. EX1003, ¶¶156-157. The throughput of Illumina sequencers was approximately 5,000Mb/day, far higher than the 750Mb/day throughput of Roche 454 sequencers. EX1010, 10 (Table 1); EX1003, ¶156. And Illumina sequencing cost an order of magnitude less than Roche 454 sequencing—approximately \$0.05/Mb compared to \$20/Mb with Roche 454 sequencers. EX1010, 10 (Table 1); EX1003, ¶156. As *Gloor* states, the “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; EX1003, ¶156. These efficiency and cost advantages would have been strong motivators for anyone working in DNA sequencing to use Illumina sequencers. EX1003, ¶¶156-157; *DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1368 (Fed. Cir. 2006) (motivation to combine exists when “the combination of references results in a product or process that is more desirable,” for example because it is “cheaper” or “more efficient.”).

Second, a POSA would have been motivated to combine *Parameswaran* and *Gloor* to reduce sequencing error. EX1003, ¶¶158-159. A POSA would have been motivated to use *Parameswaran*’s unique dual-indexing method with Illumina sequencing because, as of December 2010, Illumina sequencers were known to have lower error rates, particularly with respect to homopolymer runs (consecutive instances of the same base, such as AAA or GGG). EX1004, 4; EX1012, 4; EX1003, ¶158. Notably, *Gloor* expressly addresses the higher error rate of Roche 454 sequencing compared to Illumina sequencing. EX1005, 2. *Gloor* states, for example, that “pyrosequencing is constrained by cost limitations and a relatively high per-read error rate.” EX1005, 2; EX1003, ¶159.

A POSA’s desire to reduce sequencing error would also have provided a motivation to combine *Parameswaran* and *Gloor* because doing so would allow the

POSA to identify and discard chimeric sequences and thus reduce cross-over error. EX1003, ¶¶160-161. As of December 2010, a POSA would have known that chimeric sequences, including those resulting from cross-over errors, were problematic for next-generation sequencing platforms that used PCR amplification, including Illumina sequencing—and that *Parameswaran*’s indexing method would reduce these errors. *Supra* §§II.B, III.A, VI.A.7; EX1003, ¶160. Specifically, *Parameswaran*’s unique dual-indexing method permitted “unambiguous assignment” and a “remarkably low misassignment rate.” EX1004, 8; EX1003, ¶161. A POSA would have known that the unique dual-indexing method disclosed in *Parameswaran* made it possible to compare pairs of identifiers assigned to nucleic acid samples to those actually detected in the sequences, allowing the POSA to readily identify and discard chimeric sequences containing a pair of barcodes that are different from any of the intended barcode pairs. EX1003, ¶161. In contrast, because *Gloor* repeated individual sequence identifiers across different samples, additional bioinformatic analyses were needed to identify and discard chimeric sequences. EX1003, ¶161.

Third, a POSA would have been motivated to combine *Parameswaran* and *Gloor* based on the express suggestion in *Parameswaran* to apply its dual-indexing method to other sequencing platforms. EX1003, ¶162. *Parameswaran* states that its “barcodes may also be compatible with other sequencing platforms” and

“speculate[s] on the use of these pyrosequencer-tailored barcodes with other sequencing platforms.” EX1004, 2, 8; EX1003, ¶162. And *Parameswaran* identifies Illumina sequencing and Roche 454 sequencing as two of the “[t]hree major high-throughput sequencing platforms” in use at the time. EX1004, 1; EX1003, ¶162. In view of this express teaching, a POSA would have been motivated to apply *Parameswaran*’s dual-indexing methods to Illumina sequencing, as disclosed in *Gloor*. EX1003, ¶162.

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, ¶163. *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 421 (2007) (“When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp.”). 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, ¶¶156-157, 80. Indeed, researchers in the field had used unique dual-indexing with the same barcodes on both Roche 454 and Illumina sequencers. EX1038, Abstract; EX1003, ¶163. There

was nothing inventive about switching from Roche 454 sequencing (used in *Parameswaran*) to Illumina sequencing (used in *Gloor*). See EX1003, ¶163.

The Patent likewise does not suggest that the choice of sequencing platform is critical to the claimed subject matter, and in fact acknowledges that “essentially **any sequencing methodology** which relies on successive incorporation of nucleotides into a polynucleotide chain can be used.” EX1001, 20:37-41; EX1003, ¶163; see *Ex parte Spangler*, No. 2018-003800, slip op. 6-9 (PTAB Feb. 15, 2019) (affirming obviousness rejection when alleged differences from prior art “are an obvious matter of design choice” and the specification does not indicate that those differences are “critical”); *KSR*, 550 U.S. at 401 (“[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond that person’s skill.”); *Intel Corp. v. PACT XPP Schweiz AG*, 61 F.4th 1373, 1379-81 (Fed. Cir. 2023) (applying *KSR*’s “known technique” rationale in finding motivation to combine). The Patent’s lack of inventiveness is further underscored by the extensive copying from prior art publications. *Supra* §IV.B.

Patent Owner may argue that a POSA would not have been motivated to adapt *Parameswaran*’s unique dual-indexing methods for Illumina sequencing because *Gloor* used non-unique dual-indexing and *Parameswaran* contemplated

combinational pairing of indexes. Not so. Both references aimed to reduce various technical errors—like cross-over errors that occur during multiplex sequencing—using dual-indexing, and both references achieved that goal. EX1003, ¶160. A POSA would have been familiar with the respective benefits of unique and non-unique dual-indexing and would have chosen an appropriate indexing method depending on various factors, e.g., cost, sequencing scale, and desired level of accuracy. *See* EX1003, ¶¶161, 156-158, 71-77. A POSA would have known that *Parameswaran*’s unique dual-indexing conferred exceptional sequencing accuracy and a POSA therefore would have been motivated to combine that method with the most efficient, accurate, and low-cost high-throughput sequencing platform, as disclosed in *Gloor*. *See* EX1003, ¶¶161, 156-158, 71-77. *Elektá Ltd. v. ZAP Surgical Sys., Inc.*, 81 F.4th 1368, 1374 (Fed. Cir. 2023) (“The inquiry into the existence of a motivation to combine assumes that a skilled artisan is a person of ordinary creativity with common sense, common wisdom, and common knowledge.”). Thus, a POSA would have been motivated to combine *Parameswaran* and *Gloor*. EX1003, ¶164.

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

As further detailed below, a POSA would have had a reasonable expectation of success in combining *Parameswaran* and *Gloor* at least because a POSA would have recognized that (i) performing the unique dual-indexing method taught by

Parameswaran using Illumina sequencers and flow cells would simply require swapping the Roche 454 adapters for Illumina-specific adapters; (ii) Illumina sequencing enabled read lengths appropriate to sequence the template nucleic acids disclosed in *Parameswaran*; and (iii) *Parameswaran*'s unique dual-barcoding approach would desirably reduce cross-over error on an Illumina sequencer. EX1003, ¶¶165-176.

A POSA would have known that, to adapt the method disclosed in *Parameswaran* to Illumina sequencing on a flow cell, one would have needed to simply swap Roche 454 adapters for Illumina-specific adapters and make other minor modifications to the sequencing process. EX1003, ¶¶165-166, 168-170; 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.”). The prior art included publications with detailed methods for unique dual-index sequencing on both Roche 454 and Illumina sequencers, using the same barcodes. *See generally* EX1038; EX1003, ¶170.

None of these minor modifications change the fundamental aspect of the dual-barcoding methods disclosed in *Parameswaran* or *Gloor*. EX1003, ¶¶169-170. Indeed, the fact that even 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 is required to adapt *Parameswaran*'s unique dual-indexing to an Illumina sequencer. *Supra* §IV.B; *see* EX1003, ¶167. Framed another way, applying *Parameswaran*'s unique dual-indexing method to *Gloor*'s non-unique dual-indexing on an Illumina sequencer would require no more than modifying *Gloor* to use additional identifier sequences (such that no identifier was repeated across samples).

Designing adapters appropriate for Roche 454 and Illumina sequencing was well within the level of skill of a POSA, and moreover, any desired adapters were accessible via a commercial manufacturer. EX1003, ¶¶166, 168-170, 78-79; *see also* EX1038, 4 (purchasing barcoded primers from a commercial source and sequencing at university core facilities using standard protocols). As such, a POSA would have predictably applied the dual-indexing method disclosed in *Parameswaran* to the Illumina sequencers described in *Gloor*. EX1003, ¶¶166, 168-170; *Medtronic, Inc. v. Marital Deduction Trust*, IPR2014-00100, Paper 46 at 25 (PTAB Mar. 24, 2015) (finding a reasonable expectation of success for performing “substitution of a similar component for another in a similar device to yield a result one would have predicted from reading the references”). Indeed, *Gloor* expressly discloses doing just that—using dual-indexing with Illumina sequencing on a flow cell. A POSA therefore would have had a reasonable expectation of success in combining *Parameswaran* and *Gloor* at least because *Gloor* had already demonstrated that dual-indexing

methods are effective on Illumina sequencers. *Supra* §III.B; EX1003, ¶¶132-136, 172.

A POSA also would have understood that the read lengths of Illumina sequencing used in *Gloor* were adequate for the dual-indexing method disclosed in *Parameswaran*. EX1003, ¶171. Specifically, by December 2010, Illumina sequencers enabled “high-quality paired end reads of 150 base pairs (bp)” (i.e., nucleotides). EX1027, 1. Indeed, *Gloor* successfully conducted Illumina paired-end sequencing even with shorter read lengths of 76 nucleotides. EX1005, 6-7; EX1003, ¶171. The sequencing reads of each molecule in the *Parameswaran* DNA library include at least the F-Barcode (10 nucleotides), small RNA insert (19-27 nucleotides), R-Barcode (10 nucleotides), and optionally the F-Cloning Linker (16-17 nucleotides) and R-Cloning Linker (16-17 nucleotides)—totaling between 71 and 81 nucleotides. EX1003, ¶171. As such, Illumina sequencers could readily sequence the template nucleic acids described in *Parameswaran* and would have carried with them all the advantages discussed above (*supra* §VI.B). EX1003, ¶171.

Finally, a POSA would have expected the unique dual-barcode approach described in *Parameswaran* to successfully reduce cross-over error on an Illumina sequencer. EX1003, ¶172. *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, ¶172. Tellingly, 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, 7:45-20:46 (referring a POSA to the prior art to implement the claimed subject matter and acknowledging that “essentially any sequencing methodology” can be used); EX1003, ¶¶117, 163, 167.

In sum, *Parameswaran* and *Gloor* collectively teach every element of claim 1. EX1003, ¶¶126-155. A POSA would have been motivated to combine *Parameswaran* and *Gloor*, with a reasonable expectation of success, for many reasons, including because both used dual-indexing to reduce error during multiplex sequencing. EX1003, ¶¶156-172. As addressed in Section VIII below, the Patent provides no evidence of unexpected results or other secondary considerations of nonobviousness, and Petitioner is aware of none. EX1003, ¶¶221-222. As such, claim 1 would have been obvious over *Parameswaran* and *Gloor*. EX1003, ¶173.

D. Dependent Claims 2-8 Would Have Been Obvious Over *Parameswaran* and *Gloor*

Claims 2-8 ultimately depend from claim 1 and would have been obvious for the reasons described above (§§VI.A-C) and the additional reasons described here. EX1003, ¶174.

1. Claim 2

Claim 2 recites the “method of claim 1, wherein the *identifier sequences* are *nucleic acid sequence tags*.” EX1001, 21:19-20. *Parameswaran* and *Gloor* each disclose methods comprising identifier sequences that are nucleic acid sequence tags. EX1004, 7 (describing nucleic acid “barcode motifs,” each made of a 10-nucleotide barcode and three additional nucleotides from the adjoining linker); *see also* EX1004, 14-17 (Supplementary Table 1A) (reciting full nucleotide sequences of forward and reverse primers); EX1005, 5 (stating identifier sequences are “between 3 and 6 nucleotides long”), 6 (Table 2) (providing nucleic acid sequences of identifier sequences); EX1003, ¶176; *supra* §§III.A-B. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VI.B-C; EX1003, ¶¶175-177.

2. Claim 3

Claim 3 recites the “method of claim 1, wherein the *identifiers* are *first and second barcode sequences*.” EX1001, 22:1-2. *Parameswaran* and *Gloor* each disclose methods comprising “first and second barcode sequences.” EX1004, 7 (describing “barcode motifs,” each made of a 10-nucleotide barcode and three additional nucleotides from the adjoining linker); 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), 6 (Table 2) (providing sequences of left-

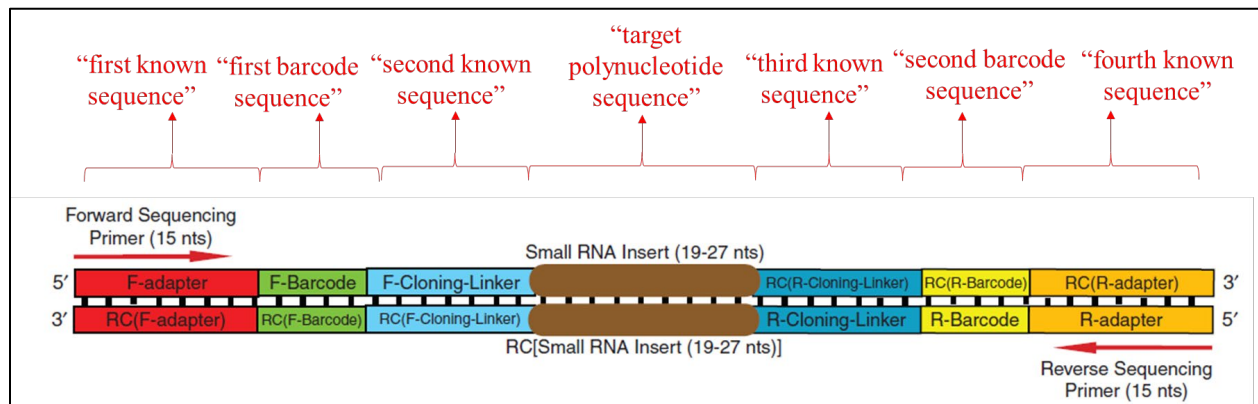
and right-side identifier sequences); EX1003, ¶179; *supra* §§III.A-B. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VI.B-C; EX1003, ¶¶178-180.

3. Claims 4 and 6

Claim 4 recites the “method of claim 1, wherein a first identifier sequence is incorporated into a **5’ portion of the template**, and a second identifier sequence is incorporated into a **3’ portion of the template**.” EX1001, 22:3-6. Claim 6 recites the “method of claim 1, wherein a first identifier sequence is incorporated into a **5’ end of the template**, and a second identifier sequence is incorporated into a **3’ end of the template**.” EX1001, 22:13-16. *Parameswaran* and *Gloor* render claims 4 and 6 obvious because their two identifier sequences are incorporated into the 5’ and 3’ ends of the template nucleic acid, respectively. EX1004, 3 (Fig.1C) (showing two identifier sequences incorporated into the 5’ and 3’ ends of template nucleic acid, respectively); EX1005, 2 (“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, ¶¶182-183; *supra* §§III.A-B. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VI.B-C; EX1003, ¶¶181-184.

4. Claim 5

Claim 5 recites the “method of claim 4, wherein after incorporation of the identifier sequences, the template has the following configuration from 5’ to 3’: a *first known sequence*; a *first barcode sequence*; a *second known sequence*; a *target polynucleotide sequence*; a *third known sequence*; a *second barcode sequence*; and a *fourth known sequence*.” EX1001, 22:7-12. As shown below, *Parameswaran* discloses a template having the claimed 5’ to 3’ configuration. EX1004, 3 (Fig.1C); EX1003, ¶¶186-187; *supra* §III.A.

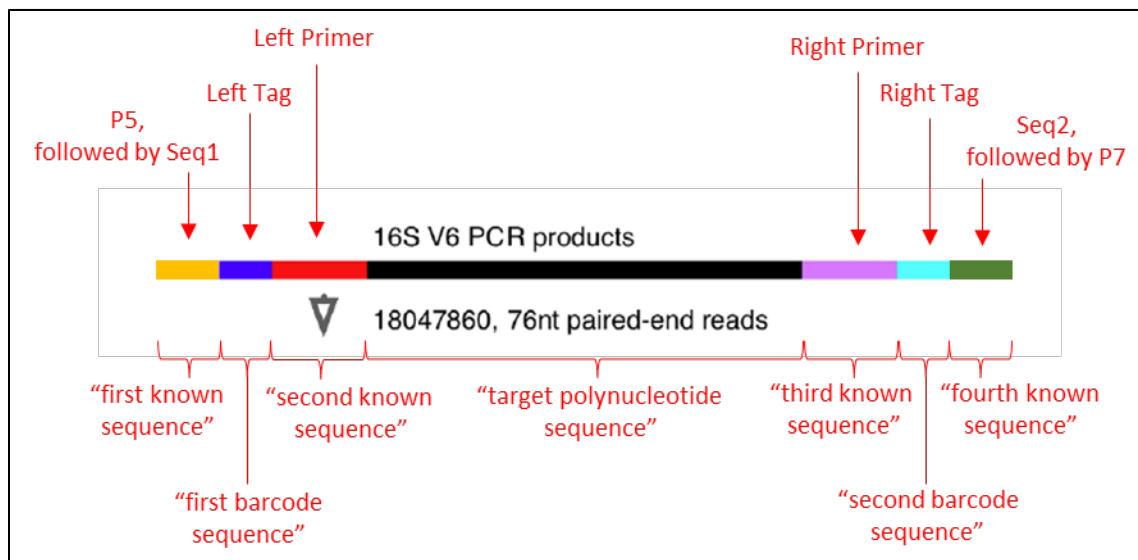


EX1004, 3 (Fig.1C) (annotated).

Gloor likewise discloses the claimed template configuration. EX1003, ¶¶188-189; *supra* §III.B. Specifically, *Gloor* uses a pair of left and right primers to amplify the target polynucleotide sequence wherein the sequence tags are incorporated in the 5’ end of the primers. EX1005, 3, 6; EX1003, ¶188. *Gloor*’s sequencing templates, therefore, contain at least the following components in succession: a left tag (“first barcode sequence”), a left primer (“second known sequence”), a target

polynucleotide sequence, a right primer (“third known sequence”), and a right tag (“second barcode sequence”). EX1003, ¶188.

A POSA would have understood that *Gloor*’s sequencing templates also include adapters at both ends (i.e., “first known sequence” and “fourth known sequence”). EX1003, ¶¶188-189. As of December 2010, Illumina sequencing required specific adapters (P5 and P7 adapters) at the 5’ and 3’ ends for immobilizing a template on the surface of an Illumina flow cell and sequencing primer-priming sites (Seq1 and Seq2) for sequencing from both directions. EX1006, 13-14; *supra* §§II.A, III.B; EX1003, ¶188. Because *Gloor* uses Illumina paired-end sequencing (EX1005, 2), the sequencing template included these additional known sequences at each end of the template. EX1003, ¶188. Figure 2 from *Gloor*, modified and annotated below, discloses the location of the barcode sequences, primers, and sequence of interest. EX1003, ¶¶188-189.



EX1005, 7 (Fig.2) (annotated with red text and modified to include Illumina adapters shown with yellow and green bars).

A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VI.B-C; EX1003, ¶¶185, 190.

5. Claims 7 and 8

Claim 7 recites the “method of claim 1, wherein prior to said sequencing step, the *template is attached to a substrate*.” EX1001, 22:17-18. Claim 8 recites the “method of claim 7, wherein the *template is directly attached to the substrate*.” EX1001, 22:19-20. Solely for purposes of this proceeding, Petitioner interprets “direct” attachment to encompass the covalent attachment of template nucleic acids to the substrate that occurs during the amplification processes used in Illumina and Roche 454 sequencing. *Supra* §II.A; EX1003, ¶191.

Parameswaran discloses a method that uses Roche 454 paired-end sequencing, which involves direct attachment of nucleic acid templates to beads. EX1004, 1-2 (referring to “bead-bound DNA template molecules”); EX1028, 6-9 (showing direct attachment of templates to beads before amplification and sequencing steps); EX1003, ¶192; *supra* §III.A.

Gloor’s nucleic acid templates are also directly attached to the substrate. EX1003, ¶193; *supra* §III.B. According to *Gloor*, “[a]mplified PCR products are

sequenced using an Illumina paired-end protocol to generate millions of overlapping reads.” EX1005, Abstract, 22; *see also* EX1005, 5 (referring to “Illumina solid support”). The Illumina paired-end sequencing used by *Gloor* involves direct attachment of nucleic acid templates to a substrate during amplification in accordance with the Illumina sequencing protocol. EX1003, ¶193; EX1025, 2 (describing bridge amplification on the surface of an Illumina flow cell).

A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Gloor* for the reasons set forth above. *Supra* §§VI.B-C; EX1003, ¶194.

VII. GROUND 2: CLAIMS 1-8 WOULD HAVE BEEN OBVIOUS OVER *PARAMESWARAN* AND *BENTLEY*

As explained above, *Parameswaran* discloses every limitation of claims 1-8 with the exception of the flow cell limitation. EX1003, ¶195. *Bentley* provides a detailed description of Illumina paired-end sequencing and would have equipped a POSA in December 2010 to modify the method taught by *Parameswaran* for use on an Illumina sequencer. EX1003, ¶195. Because *Parameswaran* discloses a method of unique dual-indexing and a POSA would have been motivated to conduct unique dual-indexing on an Illumina sequencer with a reasonable expectation of success, the combination of *Parameswaran* and *Bentley* render claims 1-8 obvious. EX1003, ¶196.

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

The method recited in claim 1 of reducing cross-over error would have been obvious over *Parameswaran* and *Bentley* for the reasons stated above (*supra* §VI) and the additional reasons described here. EX1003, ¶¶197-212. Petitioner’s descriptions of *Parameswaran* provided in Ground 1 are incorporated herein. During prosecution, Patent Owner did not dispute that *Parameswaran* meets all of the limitations of claim 1 but for amplification on the surface of a flow cell. *Supra* §IV.D. *Bentley* discloses that alleged missing limitation. *Supra* §III.C; EX1003, ¶¶200-202.

1. *Parameswaran* discloses a “method for reducing template cross-over error introduced during sequencing workflow”

As detailed above in Section VI.A.1, *Parameswaran* discloses this claim element. EX1003, ¶197.

2. *Parameswaran* discloses a method that comprises “incorporating at least two members of a plurality of identifier sequences into template nucleic acids obtained from at least two different samples, wherein said two members constitute a distinct pair associated with said template” (claim 1[a])

As detailed above in Section VI.A.2, *Parameswaran* discloses claim 1[a]. EX1003, ¶198.

3. *Parameswaran* discloses a method that comprises “combining said nucleic acid templates into a single sample” (claim 1[b])

As detailed above in Section VI.A.3, *Parameswaran* discloses claim 1[b].
EX1003, ¶199.

4. *Bentley* discloses a method that comprises “amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters” (claim 1[c])

As detailed above, *Parameswaran* reports data generated on a Roche 454 sequencer, which amplifies templates on beads as opposed to on the surface of a flow cell. *Supra* §§II.A, VI.A.4; EX1003, ¶¶143-144.

Bentley discloses a method that comprises “amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters.” EX1003, ¶¶200-202; *supra* §III.C. Specifically, *Bentley* discloses bridge amplification on the surface of an Illumina flow cell to form clusters. EX1006, 2 (Figs.1a-1b); EX1003, ¶200. In *Bentley*, DNA strands are sheared into double-stranded fragments, and Illumina-specific adapters were ligated (joined) to both ends. EX1006, 2; EX1003, ¶200. 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 EX1003, ¶201. A new strand was copied from the original strand in an extension reaction primed from the 3’ end of the surface-bound oligonucleotide, and the original strand was removed by

denaturation. EX1006, 2; EX1003, ¶201. The adapter sequence of the 3' end of the copied strand was then annealed to a new surface-bound complementary oligonucleotide, forming a bridge and generating a new site for synthesis of a second strand. EX1006, 2; EX1003, ¶201. The process was repeated until clusters were formed on the surface of the flow cell. EX1006, 2; EX1003, ¶201. *Bentley* thus expressly discloses a process for amplifying nucleic acid templates on a surface of a flow cell to form clusters. EX1003, ¶202.

5. ***Parameswaran* discloses a method in which “at least one” nucleic acid template “comprises a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs” (claim 1[d])**

As detailed above in Section VI.A.5, *Parameswaran* discloses claim 1[d]. EX1003, ¶203.

6. ***Bentley* and *Parameswaran* disclose methods that comprise “sequencing amplicons obtained in said amplifying step” (claim 1[e])**

Both *Bentley* and *Parameswaran* disclose claim 1[e]. *Parameswaran*’s disclosures are detailed above. *Supra* §VI.A.6; EX1003, ¶204. *Bentley* discloses paired-end Illumina sequencing of template nucleic acids after bridge amplification of the molecules 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, ¶205; *supra* §III.C.

7. ***Parameswaran* discloses a method that comprises “discarding sequence reads obtained in said sequencing step that contain said combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce cross-over error” (claim 1[f])**

As detailed above in Section VI.A.7, *Parameswaran* discloses claim 1[f]. EX1003, ¶206.

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

A POSA would have been motivated to combine *Parameswaran* and *Bentley*. EX1003, ¶207. The only alleged difference between *Parameswaran* and claim 1 of the Patent is the use of Illumina sequencing in place of Roche 454 sequencing. *Supra* §IV.D. It would have been obvious to try both sequencing platforms, which were two of the three major next-generation sequencing methods used as of December 2010. *KSR*, 550 U.S. at 421; *supra* §VI.B; EX1003, ¶163. Indeed, *Parameswaran* expressly contemplates this substitution. EX1007, 8; EX1003, ¶162. As detailed below, there were also other reasons for combining *Parameswaran* and *Bentley* to perform unique dual-indexing on a flow cell in Illumina sequencing.

First, a POSA would have been motivated to combine *Parameswaran* with *Bentley* because Illumina sequencers were known to be faster, less costly, and more

efficient than Roche 454 sequencers. *Supra* §VI.B; EX1003, ¶¶207, 156-157; *DyStar*, 464 F.3d at 1368. Second, a POSA would have been motivated to combine *Parameswaran* with *Bentley* because a POSA would have been motivated to use *Parameswaran*'s unique dual-indexing method to improve the accuracy of Illumina sequencing. *Supra* §VI.B; EX1003, ¶¶207, 158-161; *KSR*, 550 U.S. at 401; *Intel*, 61 F.4th at 1379-81. Third, a POSA would have been motivated to combine *Parameswaran* and *Bentley* because *Parameswaran* expressly suggests using its unique dual-indexing methods on other multiplex sequencing platforms and identifies Illumina sequencers as one of only two other such “major high-throughput sequencing platforms.” EX1004, 1, 8. *Supra* §VI.B; EX1003, ¶¶207, 162-163.

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

A POSA would have had a reasonable expectation of success in combining *Parameswaran* and *Bentley*. EX1003, ¶¶208-211. A POSA would have recognized that adapting the method taught by *Parameswaran* to use Illumina sequencers would have required merely exchanging the Roche-specific adapters for Illumina-specific adapters, and other minor modifications to the sequencing process. *Supra* §VI.C; EX1003, ¶¶208, 166-170. Further, a POSA would have recognized that Illumina sequencing provided sufficient read lengths to sequence the template nucleic acids disclosed in *Parameswaran*. *Supra* §VI.C; EX1003, ¶¶208, 171. A POSA also

would have recognized that *Parameswaran*'s unique dual-barcoding approach would desirably reduce cross-over error on an Illumina sequencer. *Supra* §VI.C; EX1003, ¶¶208, 172.

A POSA's reasonable expectation of success in combining *Parameswaran* with *Bentley* is similarly reinforced by *Parameswaran*'s identification of Illumina sequencers as a "major high-throughput platform" and disclosure that its barcoding approach "may also be compatible with other sequencing platforms." EX1004, 1, 8; EX1003, ¶209. *Parameswaran* thus contemplates performing its unique dual-indexing methods with other sequencing platforms and identifies Illumina sequencing as one such platform. EX1003, ¶209; *see also* EX1038. Tellingly, the Patent discloses no novel or nonobvious chemistry and states as follows: "Numerous techniques are known for detecting sequence and for identifying barcodes and some are exemplified below. However, the exact means for detecting and compiling sequence data does not affect the function of the invention." EX1001, 3:44-48; *see also* EX1001, 20:37-46; EX1003, ¶210.

Although *Bentley*'s read lengths of 35 bases were shorter than the read lengths of 71 to 81 bases used for *Parameswaran*'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 carry out the methods of *Parameswaran*. EX1003, ¶211; 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 have had a reasonable expectation of success in configuring *Parameswaran*’s dual-indexing methods to be suitable for Illumina sequencing disclosed in *Bentley* and achieving read lengths adequate for sequencing a template nucleic acid and a pair of identifier sequences as a whole. EX1003, ¶211.

Petitioner is not aware of any objective evidence of nonobviousness. *Infra* §VIII. As such, claim 1 would have been obvious over *Parameswaran* and *Bentley*. EX1003, ¶¶212, 221-222.

D. Dependent Claims 2-8 Would Have Been Obvious Over *Parameswaran* and *Bentley*

Claims 2-8 ultimately depend from claim 1 and would have been obvious for the reasons described above (§§VII.A-C) and the additional reasons described here. EX1003, ¶213.

1. Claim 2

Claim 2 recites the “method of claim 1, wherein the *identifier sequences* are *nucleic acid sequence tags*.” EX1001, 21:19-20. As explained above in Section VI.D.1, *Parameswaran* discloses a method in which the identifier sequences are nucleic acid sequence tags. EX1003, ¶214. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Bentley* for the reasons set forth above. *Supra* §§VII.B-C; EX1003, ¶214.

2. Claim 3

Claim 3 recites the “method of claim 1, wherein the *identifiers* are *first and second barcode sequences*.” EX1001, 22:1-2. As explained above in Section VI.D.2, *Parameswaran* discloses a method in which the identifiers are first and second barcode sequences. EX1003, ¶215. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Bentley* for the reasons set forth above. *Supra* §§VII.B-C; EX1003, ¶215.

3. Claims 4 and 6

Claim 4 recites the “method of claim 1, wherein a first identifier sequence is incorporated into a *5’ portion of the template*, and a second identifier sequence is incorporated into a *3’ portion of the template*.” EX1001, 22:3-6. Claim 6 recites the “method of claim 1, wherein a first identifier sequence is incorporated into a *5’ end of the template*, and a second identifier sequence is incorporated into a *3’ end of the template*.” EX1001, 22:13-16. As explained above in Section VI.D.3, *Parameswaran* discloses a method in which the first identifier sequence is incorporated into the 5’ end of the template, and the second identifier sequence is incorporated into the 3’ end of the template. EX1003, ¶216. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Bentley* for the reasons set forth above. *Supra* §§VII.B-C; EX1003, ¶216.

4. Claim 5

Claim 5 recites the “method of claim 4, wherein after incorporation of the identifier sequences, the template has the following configuration from 5’ to 3’: a *first known sequence*; a *first barcode sequence*; a *second known sequence*; a *target polynucleotide sequence*; a *third known sequence*; a *second barcode sequence*; and a *fourth known sequence*.” EX1001, 22:7-12. As explained above in Section VI.D.4, *Parameswaran* discloses a method in which the template nucleic acid has the claimed configuration. EX1003, ¶217. A POSA would have been motivated, with a reasonable expectation of success, to combine *Parameswaran* and *Bentley* for the reasons set forth above. *Supra* §§VII.B-C; EX1003, ¶217.

5. Claims 7 and 8

Claim 7 recites the “method of claim 1, wherein prior to said sequencing step, the *template is attached to a substrate*.” EX1001, 22:17-18. Claim 8 recites the “method of claim 7, wherein the *template is directly attached to the substrate*.” EX1001, 22:19-20. As explained above in Section VI.D.5, *Parameswaran* discloses these limitations. EX1003, ¶218. *Bentley* also discloses them. EX1003, ¶219; *supra* §III.C. Specifically, *Bentley* discloses that, prior to the sequencing step, the single-stranded DNA fragments are “annealed to complementary oligonucleotides on the flow-cell surface.” EX1006, 2; EX1003, ¶219. A POSA would have been motivated,

with a reasonable expectation of success, to combine *Parameswaran* and *Bentley* for the reasons set forth above. *Supra* §§VII.B-C; EX1003, ¶220.

VIII. GROUNDS 1-2: THERE IS NO EVIDENCE OF NONOBVIOUSNESS

The Patent provides no evidence of unexpected results or other secondary considerations of nonobviousness, and Petitioner is aware of none. EX1003, ¶¶221-222. Nor could any such evidence justify a nonobviousness finding given that there is nothing novel about the challenged claims over *Parameswaran* and *Gloor* and in view of the strong case of obviousness outlined above. *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 secondary considerations insufficient where the record established “a strong case of obviousness”).

IX. DISCRETIONARY DENIAL IS NOT WARRANTED

This IPR should be instituted under §325(d) because the Examiner did not consider directly relevant prior art (i.e., *Gloor* and *Bentley*) and, moreover, did not recognize that a POSA would have been motivated to adapt *Parameswaran* to Illumina sequencers with a reasonable expectation of success. *See 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). Although the Examiner cited *Parameswaran* in a §102 rejection, neither *Gloor*, *Bentley*, nor the combinations of *Parameswaran* and *Gloor* (Ground 1) and *Parameswaran* and *Bentley* (Ground 2) were considered or cited by the Examiner during prosecution of the Patent.

Regarding *Becton* factors (a) and (b), the asserted combinations are materially different from, and not cumulative of, the single prior art reference raised during examination of the challenged claims, *Parameswaran*. Although *Parameswaran* states that its barcoding approach “may also be compatible with other sequencing platforms” and specifically identifies Illumina sequencers as one of the “major high-throughput sequencing platforms” (EX1004, 1-2, 8), Patent Owner nevertheless alleged *Parameswaran* was “limited to pyrosequencing” and failed “to disclose amplifying nucleic acids to form clusters.” EX1007, 304; *supra* §IV.D. Even assuming for the sake of argument this is correct, *Gloor*, which was not disclosed, describes a method of reducing cross-over error on Illumina sequencers by a dual-indexing method. *Supra* §§III.B, VI.A.4; EX1003, ¶¶90-99, 145-146; *see Oticon Med. AB v. Cochlear Ltd.*, IPR2019-00975, Paper 15 at 15-16 (PTAB Oct. 16, 2019) (precedential) (concluding that an undisclosed reference provided “an advantage” over the art considered during prosecution and therefore is not cumulative). *Bentley*, moreover, which also was not disclosed, differs from *Parameswaran* in that it

provides a detailed description of Illumina paired-end sequencing on the surface of a flow cell. *Supra* §§III.C, VII.A.4; EX1003, ¶¶100-103, 200-202.

Regarding *Becton* factors (c) and (d), neither *Gloor* nor *Bentley* were considered—let alone relied upon as the basis of a rejection—during prosecution of the Patent. *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[.]”); *supra* §IV.D. The asserted combinations of *Parameswaran* and *Gloor* (Ground 1) and *Parameswaran* and *Bentley* (Ground 2), moreover, differ from the arguments made during prosecution because they rely upon a different statutory basis (obviousness under §103) and describe amplification “on a surface of a flow cell thereby to form clusters.” *Supra* §§IV.D, III.B-C, VI.A.4, VII.A.4; EX1003, ¶¶90-99, 100-103, 145-146, 200-202. This weighs strongly against any discretionary denial, particularly where the Examiner appears to have disregarded the statements in *Parameswaran* regarding the possibility of performing unique dual-indexing with other sequencing platforms, including Illumina sequencing. EX1004, 1-2. 8; EX1003, ¶¶143-144. The Examiner was also apparently not made aware that the vast majority of the Patent was derived from prior art patent publications from others, including Petitioner. *Supra* §IV.B.

Regarding *Becton* factors (e) and (f), the Examiner erred in allowing the asserted claims to issue at least because, as discussed above, “the Examiner was simply not aware” of the disclosures of *Gloor* and *Bentley* and “[t]he Petition ... presents different prior art than the Office was aware of.” *Oticon*, IPR2019-00975, Paper 15 at 19-20. The Examiner further erred by not recognizing that a POSA would have been motivated to adapt the disclosures of *Parameswaran* to an Illumina sequencer with a reasonable expectation of success by simply swapping the Roche 454 adapters for Illumina-specific adapters. *Supra* §§VI.B-C, VII.B-C; *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[.]”).

X. 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 Patent (1:24-cv-00680). On the

same day, Illumina and Verinata also 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 Patent (1:24-cv-11501).

The '852 Patent is also a parent case of the '200 Patent, which is being challenged in co-pending IPR2024-00965.

C. Lead and Backup Counsel, and Service Information

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Please address correspondence to counsel at the email addresses above.

Petitioner consents to service by e-mail.

XI. 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.

XII. 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 13,185 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. 11,041,852, the associated power of attorney, and Exhibits 1001-1025, 1027-1029, 1031-1038 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. 11,041,852:

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