

Case IPR2024-00964
U.S. Patent No. 11,041,852

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA INC.,
Petitioner

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner

Case IPR2024-00964

U.S. Patent No. 11,041,852

PATENT OWNER'S RESPONSE

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<i>Profectus Tech. v. Huawei Techs. Co.</i> , 823 F.3d 1375 (Fed. Cir. 2016)	19
<i>Purdue Pharma L.P. v. Depomed, Inc.</i> , 643 F. App'x 960 (Fed. Cir. 2016)	27, 28, 35, 37
<i>Samsung Elecs. Col., Ltd. v. Infobridge Pte. Ltd.</i> , 929 F.3d 1363 (Fed. Cir. 2019)	46, 47
<i>Virtek Vision Int'l ULC v. Assembly Guidance Sys., Inc.</i> , 97 F.4th 882 (Fed. Cir. 2024)	37, 38

All citations to 35 U.S.C. §§102 and 103 in this paper refer to the pre-AIA statute.

All emphases are added unless otherwise noted.

PATENT OWNER'S EXHIBIT LIST

Ex. #	DESCRIPTION
2001	Declaration of Viktor Adalsteinsson, Ph.D.
2002	Deposition Transcript of Paul T. Spellman, Ph.D., April 9, 2025
2003	Illumina White Paper – Effects of Index Misassignment on Multiplexing and Downstream Analysis, Illumina (2018), available at https://www.illumina.com/content/dam/illumina-marketing/documents/products/whitepapers/index-hopping-white-paper-770-2017-004.pdf
2004	Kircher et al., <i>Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform</i> , Nucleic Acids Research 40(1):e3 (2011).
2005	Lefrancois et al., <i>Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing</i> , BMC Genomics 10:37 (2009).
2006	Illumina Application Note – High-Speed Multiplexed 16S Microbial Sequencing on the MiSeq® System, Illumina (2011), available at https://www.illumina.com/documents/products/appnotes/appnote_miseq_16S.pdf
2007	Illumina Sequencing - Multiplexed Sequencing with the Illumina Genome Analyzer System (2008), available at https://www.illumina.com/documents/products/datasheets/datasheet_sequencing_multiplex.pdf
2008	<i>Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products</i> , arxiv.org/abs/1007.5075 (as archived December 12, 2020, at https://web.archive.org/web/20201212073547/https://arxiv.org/abs/1007.5075)
2009	Maughan et al., <i>SNP Discovery vis Genomic Reduction, Barcoding, and 454-Pyrosequencing in Amaranth</i> , The Plant Genome 2:260-270 (2009).
2010	Meyer et al., <i>Targeted high-throughput sequencing of tagged nucleic acid samples</i> , Nucleic Acids Research 35(15): e97 (2007).
2011	Wang et al., <i>A Low-Cost Library Construction Protocol and Data Analysis Pipeline for Illumina-Based Strand-Specific Multiplex RNA-Seq</i> , PLoS One 6(1): e26426 (2011)
2012	Kenny et al., <i>Multiplex Target Enrichment Using DNA Indexing for Ultra-High Throughput SNP Detection</i> , DNA Research 18:31-38 (2010).

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All citations to Exhibits refer to stamped exhibit page numbers.

I. Introduction

U.S. Patent No. 11,041,852 (the “’852 Patent”) covers significant advances in next-generation sequencing (“NGS”) and multiplexing technologies. Multiplexing involves pooling nucleic acid templates from many different samples and amplifying the pooled nucleic acid templates, improving efficiency by allowing simultaneous sequencing of amplicons from multiple samples and eliminating the need for individual sequencing reactions for each sample. Prior to the ’852 Patent, errors were often introduced during multiplexing after the samples were pooled together due to amplification mechanisms that formed chimeric molecules. However, the errors from these chimeric molecules were not recognized at the time of the ’852 Patent invention, leading to incorrect sample assignment and causing flawed analysis of some sequences in multiplexing reactions.

The ’852 Patent inventors discovered this issue and worked against conventional wisdom to develop a novel sequencing approach that would reduce these errors and ensure accurate sample assignment while still being scalable and cost-effective, contributing to the commercial appeal of NGS and its later widespread adoption in the industry. Specifically, the inventors found that adding identifier sequences unique to each sample prior to pooling and amplification allowed downstream errors to be more easily recognized and corrected during data analysis, increasing sample assignment accuracy. *See, e.g.*, EX1001, 2:12-29. For

example, with their method the inventors were able to detect and discard chimeric molecules formed during cluster amplification before performing subsequent analyses. In the years following the advancement of the '852 Patent, its methods have proven to be an efficient solution to a problem that was unrecognized throughout the industry at the time of the invention.

Petitioner cannot prevail on any of its grounds. For Ground 1 that relies on Parameswaran in view of Gloor, Petitioner fails to show that a POSA would have been motivated to combine Parameswaran's pyrosequencing method with Gloor's flow-cell sequencing method, or that a POSA would have had a reasonable expectation of success of achieving the claimed invention. And, even if they were combined, Petitioner fails to show that the combination would include the claimed "discarding" step. Furthermore, Petitioner fails to show that Gloor was prior art as of the priority date used in the Petition (Paper 1, "Pet.") for the '852 Patent.

For Ground 2 that relies on Parameswaran in view of Bentley, Petitioner again fails to show that a POSA would have been motivated to combine Parameswaran's pyrosequencing method with Bentley's flow-cell sequencing method, or that a POSA would have had a reasonable expectation of success of achieving the claimed invention with this combination. And again, Petitioner fails to show that the combination would meet the claimed "discarding" step even if the references were combined.

Accordingly, Petitioner cannot prevail on Ground 1 or 2, and claims 1-8 (“Challenged Claims”) are patentable.

II. Background

A. Overview Of The ’852 Patent Technology

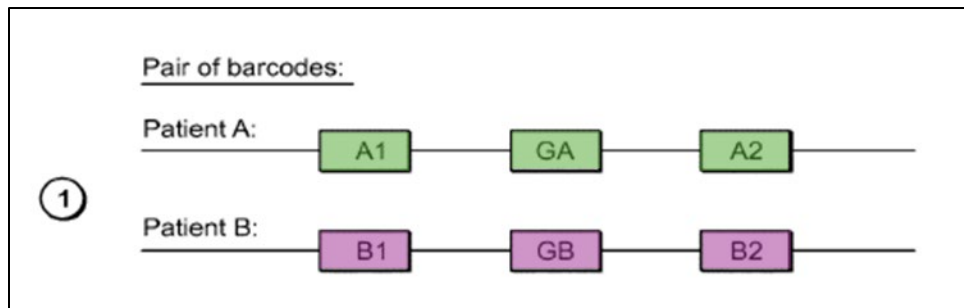
The ’852 Patent describes “maintaining the integrity and identification of a nucleic acid template in a multiplex sequencing reaction.” EX1001, 1:19-21; EX2001, ¶32. As the ’852 Patent recognizes, “molecular overlap can lead to apparent high quality, but incorrect sequence data” during amplification after the samples have been pooled together in multiplex sequencing, leading to error. EX1001, 4:17-20, 4:46-52; EX2001, ¶40. For example, the overlap and resulting high-quality signal can cause data to “be assigned to the wrong sample,” an issue that was not remedied by earlier approaches. EX1001, 4:60-63; EX2001, ¶¶40-41. At the time, others did not fully appreciate these problems, including the prevalence of sequence misassignment caused by chimeric molecules created during cluster amplification, nor were existing methods sufficient for accurate data analysis. EX1001, 4:53-63, 6:17-20, 7:20-23, Figure 3; EX2001, ¶40.

However, as others eventually came to recognize after the ’852 Patent, chimeric molecules formed after sample pooling during amplification in NGS workflows can have a significant impact on accurately tracing sequences back to the correct sample. EX2004; EX2003; EX2001, ¶¶41-44. Thus, finding a solution was

crucial to improving multiplexing, and the '852 Patent provided that solution by recognizing that downstream sample identification would be more accurate if sequences were identified with multiple identifier sequences that were unique to each starting template. EX1001, 7:24-38, EX2001, ¶35.

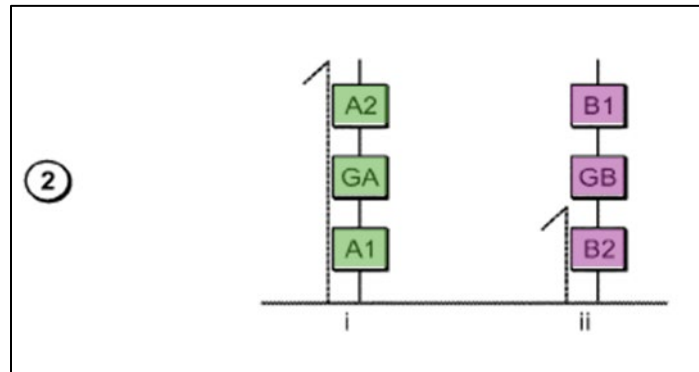
The '852 Patent provided its novel solution for addressing this issue with a method to identify and discard invalid sequence reads attributable to the chimeric molecules that arise during amplification. *See* EX1001, 5:55-58, 6:53-55, EX2001 ¶¶44-48. As the '852 Patent explains, although previous barcode identifiers were used in an attempt to assign sequencing data to samples, they still could not accurately detect the cross-over errors from chimeric sequences between different templates that formed during amplification, especially when formed during an early amplification cycle. EX1001, 4:53-63; EX2001, ¶¶42-43. By requiring detection of the multiple identifier sequences, the solution reduced “false positive and/or false negative results.” EX1001, 2:4-11, 2:16-18 (“The invention is based upon using ***two or more identifiers***, such as nucleic acid barcodes that are uniquely associated with an analyte to be detected.”), 7:37-38. And by using at least two identifiers for each template (e.g., for each sample), the '852 Patent increased the ability to detect and eliminate error because a “template containing only one identifier or an incorrect pair (i.e., a pair or more of identifiers not associated with the same template) will be excluded from analysis.” *Id.*, 2:23-25; EX2001, ¶41.

The ability to detect cross-over error by using multiple identifiers is shown in Figure 2 of the '852 Patent. As illustrated, when samples have been collected from a patient A and a patient B, each template from each patient will have a pair of sequence identifiers specific to that patient (e.g., A1 and A2 for patient A; B1 and B2 for patient B) that flank a gene fragment (e.g., GA for patient A; GB for patient B). *Id.*, 5:22-27; EX2001, ¶¶34, 45.



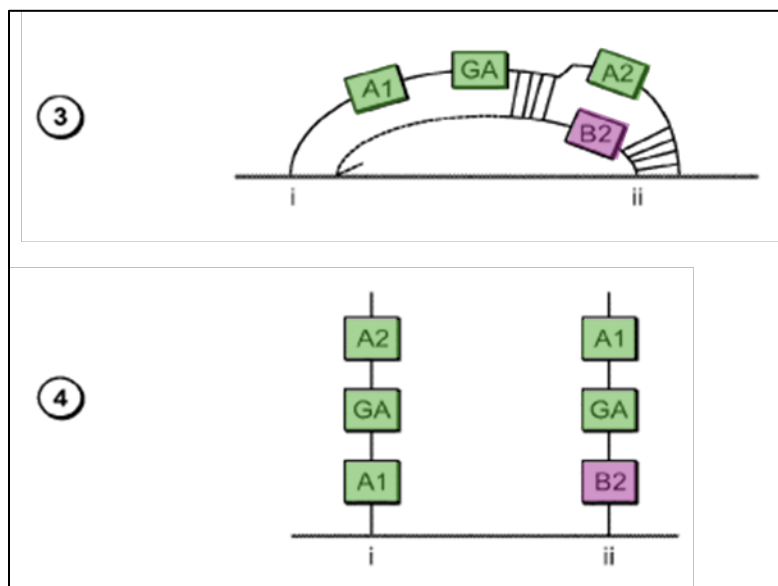
EX1001, FIG. 2(1) (annotated).

After various tagged nucleic acid samples are pooled together, “the samples are then hybridized to primers at discrete locations on a flow cell” for subsequent amplification and sequencing. *Id.* at 5:28-29; EX2001, ¶¶36-38, 46. In some instances, “the nucleic acid from patient A and the nucleic acid from patient B land within a cluster radius” on the surface of the flow cell, and the proximity permits improper overlap between them when the primer of one template (e.g., from patient B) is only partially extended and thus incorporates one identifier sequence but not the other. EX1001, 5:28-40; EX2001, ¶46.



EX1001, FIG. 2(2) (annotated).

After extension, the template-primer duplex will be denatured and re-annealed for another amplification cycle on the surface of the flow cell. Subsequent polymerase extension of the template amplicons will result in a chimeric molecule containing one of patient B's barcodes (e.g., B2 in Fig. 2(3)-(4) below), one of patient A's barcodes (e.g., A1 in Fig. 2(3)-(4) below), and the gene fragment from patient A. *Id.*, 5:41-55; EX2001, ¶¶38, 47.



EX1001, FIG. 2(3)-(4) (annotated).

The chimeric molecule that forms on the surface of the flow cell includes an incorrect pair of barcodes (i.e., one from patient A and one from patient B), allowing the chimeric molecule to be identified as error and discarded instead of being incorrectly attributed to patient B in the analysis. *Id.*, 5:55-58; EX2001, ¶¶39, 47-48. “While each barcode sequence [A1 and B2] 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; EX2001, ¶48. Even though “[d]uring the sequencing process, this would yield a high-quality signal,” sequencing data resulting from the improper molecule that was formed during amplification can still be detected and excluded because “during data analysis the molecules would be recognized as invalid” due to their invalid barcode pair. EX1001, 5:65-6:6; EX2001, ¶48.

By increasing the ability to detect and exclude errors, the inventive methods of the '852 Patent “dramatically reduce false positives in a multiplex sequencing reaction” and provide a solution to problems that previously hampered next-generation multiplex sequencing reactions and were not recognized by others until several years later. EX1001, 6:53-57, 7:24-38, Figure 3; *see also* EX2004; EX2003; EX2001, ¶¶41-44.¹

¹ Petitioner’s assertions that the inventions in the '852 Patent were copied from others are not well taken. *See* Pet., 19. Petitioner does not contend that the detailed

B. Overview Of The '852 File History

The '852 Patent's application was filed on January 14, 2021, as Application No. 17/149,504, claiming priority to December 23, 2010. EX1001, Cover. Petitioner applies December 23, 2010, as the effective priority date of the '852 Patent for purposes of the Petition. Pet., 17; EX2001, ¶50.

In an Office Action dated February 26, 2021, the Examiner rejected pending claims as anticipated by Parameswaran (EX1004). EX1007, 195-196; EX2001, ¶51. In response, in an Amendment dated April 13, 2021, and following an interview with the Examiner, Applicant amended the pending claims to recite amplifying nucleic acid templates “on a surface of a flow cell.” EX1007, 302; EX2001, ¶52. Without conceding that Parameswaran actually disclosed any previous claim limitations, Applicant explained that “the claims as amended include a limitation not present in Parmeswaran, namely that templates are amplified ‘to form clusters in a surface of

description and figures showing the invention are copied from any other publications. *Id.* (citing EX1001, 1:25-31; 3:37-43; 7:45-20:62), *id.*, 42-43. And Petitioner's overstated accusations of “copying” refer only to text discussing general considerations relevant to the invention that are incorporated into the '852 Patent from prior publications that were cited to the Patent Office, including some that are incorporated by reference in their entirety into the patent's specification.

a flow cell.” EX1007, 302, 305. During the interview, the Examiner noted that the claims could be distinguished from the prior art if they were limited to flow cell-based mechanisms of amplification to generate a cluster comprising a chimeric sequence. *Id.*, 311.

Following Applicant’s response, the Examiner deemed the claims allowable. A Notice of Allowance was issued on April 27, 2021, stating that “Applicant’s claim amendments overcame the previously presented claim rejections.” EX1007, 312, 316-317; EX2001, ¶53.

III. Overview Of The References

A. Parameswaran (EX1004)

Parameswaran presented pyrosequencing-based methods for multiplex sequencing. EX1004, 1 (“A *pyrosequencing-tailored nucleotide barcode design* unveils opportunities for large-scale sample multiplexing.”). Parameswaran explained that constraints in its sequencing, such as physical space segregation, previously limited the maximum number of samples that could be multiplexed to 16 independent samples. *Id.*, 1-2. Parameswaran introduced a method that pooled samples together for sequencing instead of “relying on physical separators,” therefore “maximiz[ing] sequence space and multiplex capabilities.” *Id.*, 2. Parameswaran provided a method that allowed “up to 48 different samples” to be unambiguously assigned and highlighted the viability of sequencing even more

samples with combinatorial tagging. *Id.*, 1, 8 (“The low misassignment rate also indicates the potential utility of combinations of forward and reverse barcodes to generate up to 48^2 (= 2304) combination barcodes for this set of sequences.”); EX2001, ¶¶65, 69.

Parameswaran designed its “barcoding approach to substantially enhance the scope and capacity of multiplexed high-throughput pyrosequencing.” EX1004, 4. Because Parameswaran identified known errors in pyrosequencing technologies, it designed its barcodes to minimize those pyrosequencing-specific errors. *Id.*; EX2001, ¶¶54-61. For example, Parameswaran “expected to minimize errors related to pyrosequencing” with its barcoding method and claimed to minimize the “errors most frequently observed for pyrosequencing.” EX1004, 7; EX2001, ¶104.

Parameswaran’s methods isolated RNA from individual samples and converted it to cDNA for sequencing. EX1004, 2. Parameswaran then amplified the DNA with two rounds of PCR with barcoded primers creating a library of barcoded PCR products. *Id.*, 3-4; EX2001, ¶62. Parameswaran pooled barcoded libraries from 25 samples and ran the pooled samples through pyrosequencing. EX1004, 4. Parameswaran analyzed “all sequences with a match to *one of the two expected*” barcodes, which it considered as “true members of the corresponding dataset.” *Id.*, 8. Parameswaran found it could “unambiguously assign 99.8% of obtained barcoded

sequences by bioinformatically probing for an error-free barcode *at either end of the sequence of interest.*” *Id.*, 2; EX2001, ¶¶56-57, 63-68, 136.

B. Gloor (EX1005)

Gloor discusses a “low-cost, high-throughput microbiome profiling method that uses combinatorial sequence tags.” EX1005, 1; EX2001, ¶70. Gloor developed a “low-cost high throughput” method that allowed it to sequence “272 clinical samples by a single Illumina paired-end sequencing run.” EX1005, 1, 3; EX2001, ¶72. Gloor explained a recent study had reported that sequences reported as “rare microbiota” were actually caused by pyrosequencing errors. EX1005, 12; EX2001, ¶71. Gloor found that with Illumina sequencing and combinatorial dual indexes (“CDIs”), it could sequence “much greater depth at a much lower cost than is possible with current pyrosequencing technology.” EX1005, 23; EX2001, ¶73. Because Gloor averaged “20-fold greater sequence coverage” using Illumina sequencing platforms, Gloor concluded that the “higher fidelity Illumina sequencing may have resulted in fewer [species] because of a lower error rate contributing to fewer ‘rare microbiome’ [species].” EX1005, 23.

Gloor explained that “[c]ombinatorial sequence tagging can be used to examine hundreds of samples with far fewer primers than is required when sequence tags are incorporated at only a single end.” *Id.*, 1. Such combinatorial sequence tagging uses fewer primers to tag a greater number of samples than single indexes

or unique dual indexes (“UDIs”) because each unique tag requires its own primer. EX2001, ¶¶74, 95-96, 99-100. Gloor reported that only 34 unique barcodes (and thus 34 primers) were needed to sequence 272 samples when using combinatorial sequence tagging. EX1005, 6 (Table 2); EX2001, ¶¶95-96, 99-100.

C. Bentley (EX1006)

Bentley sought to develop an approach “for accurate, rapid and economical whole-genome” sequencing. EX1006, 1. Bentley explains that previous methods, such as capillary sequencing, took multiple years and millions of dollars to sequence one individual human genome. *Id.* However, Bentley used massively parallel sequencing on Illumina Genome Analyzers to sequence one human genome and “determined an accurate whole human genome sequence in 8 weeks” with an approximate cost of \$250,000. *Id.*, 3, 5.

Bentley sequences a single sample and therefore does not sequence, or suggest sequencing, more than one sample at once in a multiplex reaction, or using any sample indexes to do so. EX2001, ¶¶85-89, 149.

IV. The Level Of Ordinary Skill In The Art

Petitioner proposes a level of ordinary skill in the art as a person having either “(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.” Pet., 17. Patent Owner² adopts Petitioner’s proposal for a POSA for purposes of this proceeding. EX2001, ¶¶19-22.

V. Claim Construction

A. “Template Cross-Over Error” And “Cross-Over Error” (Claim 1)

Claim 1 recites “template cross-over error” in its preamble and “cross-over error” in its last limitation. The Board should construe these terms to mean “error from sequences of chimeric molecules created during cluster amplification and made from template nucleic acids derived from different samples.” Petitioner proposes a similar construction (Pet., 22-23), but Petitioner’s proposal improperly (1) redefines “error” as “sequences” as opposed to error from such sequences and (2) improperly broadens from the consistent descriptions in the ’852 Patent explaining that the invention addresses error caused by chimeric sequences *created during cluster amplification*. Patent Owner’s construction is better supported by the claims and intrinsic evidence, and correctly recognizes the problem and inventive solution provided in the ’852 Patent. EX2001, ¶¶23-30.

First, “cross-over error” should be construed as “*error from* sequences of chimeric molecules...” as Patent Owner proposes, rather than the sequences

² As indicated in the Updated Mandatory Notices filed February 27, 2025 (Paper 11), the Patent Owner in this proceeding is Molecular Loop Innovations LLC.

themselves as Petitioner proposes (Pet., 22). Patent Owner's proposal is true to the specification's repeated description of identifying and reducing error for more accurate analysis, and it fits into the language of the claims. In both the preamble and final limitation, Patent Owner's proposal makes clear the claimed methods are for "reducing *error* from sequences of chimeric molecules...", as is described throughout the '852 Patent with examples of errors such as "false positives" and "false negatives." EX1001, 7:37-38. Petitioner's proposal, on the other hand, would require "reducing sequences" themselves, which is less clear. EX2001, ¶¶24-25, 28. Patent Owner's proposal provides the more logical reading and should be adopted.

Second, Patent Owner's proposal correctly recognizes that the '852 Patent's inventive solution addresses error caused by chimeric sequences arising after sample pooling *during amplification*, not at other points during sequencing workflow. *See supra* Section II.A; EX2001, ¶¶26-30. Indeed, the specification repeatedly describes chimeric sequences that give rise to cross-over error in a specific, consistent way: as artifacts that arise during cluster amplification in sequencing workflows. The '852 Patent repeatedly discusses such errors arising from the formation of chimeras during amplification after sample pooling. *See* EX1001, Figure 2, 5:1-5, 5:29-28, 5:41-43; EX2001, ¶¶26-30, 41-49. For example, it describes the formation of heteroduplexes resulting from "improper molecular overlap" during amplification. EX1001, 4:42-45, 5:43-46; EX2002, 99:15-100:10. It then explains how chimeric

sequences are multiplied from these heteroduplexes in amplification cycles. EX1001, 4:42-52, 5:41-58, 6:35-44; EX2001, ¶¶26-28, 40, 47-48. As the '852 Patent notes, these chimeric sequences may create high-quality signals, particularly if formed *early in the amplification process*, but cause sequencing data to be assigned to the wrong sample because of their association with an incorrect sequence identifier. EX1001, 4:60-63; EX2001, ¶¶26-28.

The '852 Patent also depicts sample identification issues causing error from the formation of chimeric molecules *during amplification* (and after pooling samples) when only single barcodes are used. EX1001, Figure 1, 4:17-20. The specification explains that when nucleic acids from two different patient samples are attached to single barcodes and pooled, chimeric molecules are formed *after* “the amplification process begins” as a result of improper overlap of two different templates. *Id.*, 4:20-23, 4:32-33, 4:40-45; EX2001, ¶¶26-30, 41-49. When the amplification process is complete, “[a] chimeric molecule has been formed” as a result of the overlap that occurred *during amplification*. EX1001, 4:49-52. The specification further explains that chimeric molecules formed early *during the amplification process* can create “a homogenous clonal chimeric cluster” leading to errors. *Id.*, 4:57-63; EX2001, ¶27.

The '852 Patent goes on to describe its inventive solutions that use dual barcodes to more accurately identify nucleic acids and exclude chimeric molecules

formed during amplification. EX2001, ¶¶28, 43-49. The specification explains that when dual barcodes are used, chimeric molecules are still formed *after* “the amplification process begins” as a result of template overlap during amplification. EX1001, Figure 2, 5:1-5, 5:32-33, 5:41-46. The resulting chimeric molecule formed during amplification contains two barcode sequences (one from patient A and one from patient B), allowing the inventive methods to more readily identify the chimeric sequence as invalid during data analysis. *Id.*, 5:55-58, 5:65-6:4 (explaining “a *crossover* has occurred” during amplification); EX2001, ¶¶44-48. And the specification discusses data demonstrating the effect of using single identifiers and dual identifiers to reduce errors from chimeric molecules formed during amplification. EX1001, 6:7-9, 6:29-34; EX2001, ¶¶35, 49. The specification again explains that formation of a chimeric molecule early *during the amplification process* leads to a homogenous clonal cluster of chimeric molecules that is better detected as invalid when dual barcodes are used. EX1001, 6:41-52. The patent highlights that “[u]nlike the single barcode amplification reaction, the chimeric molecule *formed in this amplification reaction* included one barcode sequence from sample A (A1) and one barcode sequence from sample B (B2),” and thus was detectable using the claimed inventive methods. EX1001, 7:20-23, 7:27-32; EX2001, ¶¶47-48.

The '852 Patent notes that its inventive methods can be performed on different sequencers (EX1001, 20:37-45) and that the particular mechanism that forms a chimeric molecule may vary (*id.*, 6:25-26), but in all instances describes any chimeric sequences that might be formed on such sequencers or by other mechanisms (e.g., other types of biomolecular interactions) as being ***formed during amplification***. *Id.*, Figures 1-3, 5:1-5, 5:28-29, 5:41-43; EX2001, ¶¶25-30, 42-48. For example, the '852 Patent explains that the formation of a heteroduplex between nucleic acids is one mechanism for forming a chimeric molecule during amplification. EX1001, 4:42-45, 5:43-46. That specific example provides one theory or mechanism of how a chimeric molecule might be formed during amplification after sample pooling, but other mechanisms might also arise during amplification to create a chimeric molecule. *Id.*, 6:25-29; EX2001, ¶29. That explanation is consistent with Patent Owner's construction, and with the understanding of Petitioner's expert. EX2002, 100:7-10 ("Q: So a heteroduplex is one mechanism for creating a chimeric sequence but not necessarily the only mechanism, correct? A: Correct."); EX2001, ¶¶24-30. Although the specific mechanism of action by which a chimeric molecule is created is not limited only to the formation of heteroduplexes, when read in context of the specification, any other mechanism of action that might form a chimeric molecule must still occur ***during amplification***. EX1001, 4:57-60, 5:41-58, 6:22-25, 6:35-44. Indeed, neither the specification nor the claims describe

any other cross-over error caused by a “chimeric sequence” created at any other point in a sequencing workflow other than amplification. EX2001, ¶¶25-30, 42-48.

Patent Owner’s construction is also consistent with the prosecution history. For example, the ’852 Patent claims were allowed after an amendment to recite amplifying nucleic acid templates “on a surface of a flow cell” to form at least one cluster comprising a chimeric sequence. EX1007, 302. And during an interview the Examiner noted that the claims could be distinguished from the prior art if they were limited to flow cell based mechanisms of amplifying to generate clusters. *Id.*, 311. Consistent with that history, Patent Owner’s construction requires that the chimeric sequences that cause cross-over error be formed **during amplification** (which occurs on the surface of a flow cell as claimed). EX2001, ¶¶25-30, 42-48.

Patent Owner’s construction of “template cross-over error” and “cross-over error” is thus consistent with the claims, the specification (including every disclosed embodiment), and the prosecution history, and should be adopted. *See, e.g., Groove Digital, Inc. v. United Bank*, 825 Fed. Appx. 852, 856-57 (Fed. Cir. 2020) (affirming construction based on a term’s consistent and repeated use in the specification and every embodiment, finding that “[a]n explicit definition is not required to inform a claim term’s meaning” because a patent’s specification can “define claim terms by implication”); *Profectus Tech. v. Huawei Techs. Co.*, 823 F.3d 1375, 1380-81 (Fed. Cir. 2016) (affirming construction of a term based on its meaning in repeated uses

in the patent—including “every embodiment described in the specification”); *Poly-America v. API Industries*, 839 F.3d 1131, 1136 (Fed. Cir. 2016) (affirming construction based on the specification and prosecution history, finding that disavowal of the full scope of a term in the specification or prosecution history “need not be explicit”).

Petitioner’s construction of “template cross-over error” is too broad. Under Petitioner’s construction, an event occurring between two template molecules of different samples at any time, including before a multiplex sequencing reaction workflow even begins (e.g., cross-contamination between sample tubes during sample-gathering or pipetting steps), could cause formation of a chimeric sequence giving rise to cross-over error. EX2001, ¶¶24-26. That broad read is not true to the problem identified and inventive solutions provided throughout the ’852 Patent. EX2001, ¶¶23-30. Patent Owner’s construction properly recognizes that a term cannot be construed in a way that departs from the problem identified and solved by the ’852 Patent. *On Demand Machine Corp. v. Ingram Industries, Inc.*, 442 F.3d 1331, 1340 (Fed. Cir. 2006) (reversing construction based on an out-of-context dictionary definition of a term instead of the specification’s consistent usage of it, finding “claims cannot be of broader scope than the invention that is set forth in the specification”).

Accordingly, the Board should adopt Patent Owner’s proposed construction.

VI. Ground 1: The Challenged Claims Are Patentable Over Parameswaran And Gloor.

A. Petitioner Fails To Show A Motivation To Combine. (All Claims)

1. Petitioner Improperly Cherry-Picks Portions Of Parameswaran and Gloor Without Considering The Full Context Of The References And The State Of The Art.

Petitioner argues that a POSA would have been motivated to combine Parameswaran and Gloor to “use unique dual-indexing, as taught in Parameswaran, with Illumina sequencers, as taught in Gloor.” Pet., 36-39; EX2001, ¶¶90. Petitioner consistently ignores context, fails to consider the references together, and fails to consider significant reasons why a POSA would not make that combination. *Arctic Cat Inc. v. Bombardier Recreational Prods. Inc.*, 876 F.3d 1350, 1363 (Fed. Cir. 2017) (“Evidence suggesting reasons to combine cannot be viewed in a vacuum apart from evidence suggesting reasons not to combine.”). A POSA would not have been motivated to combine Parameswaran’s unique dual indexes (“UDIs”) and pyrosequencing with Gloor’s CDIs and Illumina sequencers because a POSA (1) would appreciate that the pyrosequencing-specific problems for which Parameswaran’s barcodes were tailored had not hampered Illumina sequencers as used in Gloor, (2) would heed Gloor’s warning that pyrosequencing methods were often unsuitable for Illumina sequencers, and (3) would not have any reason to use the UDIs from Parameswaran to replace Gloor’s disclosed CDI sequencing method.

First, a POSA in December 2010 would not have thought that Parameswaran's barcoding methods would have utility in Gloor's Illumina sequencers because Illumina sequencers did not have the same needs of pyrosequencing that guided the design of Parameswaran. EX2001, ¶¶91-94. Parameswaran in 2007 expressed the view that "multiplexed high-throughput pyrosequencing is currently limited in complexity (number of samples sequenced in parallel), and in capacity (number of sequences obtained per sample)," and sought to allow for "pooling and sequencing of DNA from independent samples, and to facilitate subsequent segregation of sequencing capacity." EX1004, 1. Parameswaran explains that the utility of its barcoding approach was "to substantially enhance the scope and capacity of multiplexed high-throughput pyrosequencing" because it "allows for the unambiguous assignment of nucleic acid sequences from a mixture of libraries from up to 48 different samples." *Id.*, 4, 8.

But by the time of the invention in 2010, as Petitioner's expert recognizes, it was already "standard to sequence many samples in parallel referred to as 'multiplex' sequencing." EX1003, ¶64. Indeed, Gloor used Illumina sequencers for 272 clinical samples, over five times the sequencing capability taught in Parameswaran, and achieved about 12 million useable reads. EX1005, 2. Gloor also explains that it found "an exceedingly low error rate." *Id.* Accordingly, a POSA in 2010 would not have seen utility in applying Parameswaran's methods to Illumina

sequencers at least because Gloor claimed to achieve the high complexity and capacity at a low error rate that previously escaped pyrosequencing, and for which Parameswaran's barcoding method was tailored for pyrosequencing applications. EX2001, ¶¶91-94.

Second, a POSA would not have been motivated to use Parameswaran's "pyrosequencing-tailored nucleotide barcode design" (EX1004, 1 (Title)) with Gloor, because Gloor *warns against* applying pyrosequencing approaches to Illumina sequencers. Gloor stated "we found that methods used to analyze pyrosequencing microbiome data were *often unsuitable for reads generated by Illumina sequencing*, and we present a workflow that can be used for rapid and robust generation of the relative abundance of organisms in each sample." EX1005, 2. And Parameswaran emphasizes that its methods were specifically designed for pyrosequencing. EX1004, 1 ("A *pyrosequencing-tailored nucleotide barcode design* unveils opportunities for large-scale sample multiplexing."), 4 ("Specifications for the barcodes are *based on details of pyrosequencing error characteristics*."). A POSA would not seek to introduce Parameswaran's "often unsuitable" (in Gloor's words) pyrosequencing methods into a workflow specifically designed for Illumina sequencers, which was already thought to be "rapid and robust." EX2001, ¶¶91-94; *Arctic Cat*, 876 F.3d at 1363 (one must consider evidence

that “suggests reasons that a skilled artisan would be discouraged from pursuing such a combination,” even when not rising to the level of teaching away).

Third, a POSA would not have had a reason to use the UDI methods disclosed in Parameswaran to replace Gloor’s CDI method. *Medtronic, Inc. v. Teleflex Innovations S.a.r.l.*, 69 F.4th 1341, 1351 (Fed. Cir. 2023) (requiring motivation to incorporate a specific feature of one art into another based on what a POSA would have understood were the benefits of that feature). Parameswaran concluded that near-perfect assignment was achieved “based on the presence of *either* a perfect forward *or* a perfect reverse barcode.” EX1004, 1. Based on that conclusion, as Dr. Spellman admits, Parameswaran actually espoused CDIs for increasing sample capacity, with “the potential utility of combinations of forward and reverse barcodes to generate up to 48^2 (= 2304) combination barcodes for this set of sequences.” *Id.*, 8; EX2002, 147:9-148:3. And Gloor already used CDI methods, which Parameswaran encouraged, to sequence 272 samples and achieve an apparent “exceedingly low error rate.” EX1005, 2.

Neither Gloor nor Parameswaran provided any indication that UDIs would improve the sequencing using an Illumina sequencer over the CDI method disclosed in Gloor. EX2001, ¶¶95-97. To the contrary, Parameswaran suggests the opposite (EX1004, 8), and Gloor touts that its use of “the *combination* of sequence tags at each end of the overlapped reads *allowed us to use a small number of primers to*

uniquely tag a large number of samples” (EX1005, 2). A POSA would also recognize that using UDIs would require *more* primers and barcodes than CDIs. EX2001, ¶¶95-96. And Gloor taught that using combinatorial indexes on Illumina sequencers allowed “much greater depth at a much lower cost than is possible with current pyrosequencing technology.” EX1005, 23. Accordingly, a POSA viewing the references in full context would have no reason to use UDIs instead of Gloor’s CDIs in view of the increased complexity and cost required for essentially no reward. EX2001, ¶¶95-97; *see Arctic Cat*, 876 F.3d at 1363 (“evidence suggesting reasons to combine cannot be viewed in a vacuum apart from evidence suggesting reasons not to combine”).

2. Petitioner’s Proffered Reasons Fail To Show A Motivation To Combine Parameswaran’s Unique Dual Indexes With Gloor’s Illumina Sequencer

i. Petitioner Does Not Show That Efficiency And Cost Considerations Would Have Motivated The Proposed Combination.

Petitioner argues “efficiency and cost advantages” would have motivated a POSA “to use Illumina sequencers” (Pet., 36-37), but in doing so does not sufficiently explain why a POSA would implement UDIs to achieve those alleged advantages. When evaluating efficiency and cost considerations, a POSA would have understood that Gloor’s CDIs—*not* Parameswaran’s UDIs—provided the cheaper and more efficient approach. EX2001, ¶¶98-101. Indeed, both

Parameswaran and Gloor highlighted that benefit of CDIs. EX1004, Abstract (“[C]ombinations of forward and reverse barcodes may be used to sequence from as many as n^2 independent libraries for each set of ‘n’ forward and ‘n’ reverse barcodes, for each defined set of cloning-linkers.”); EX1005, 1 (“Combinatorial sequence tagging can be used to examine hundreds of samples with far fewer primers than is required when sequence tags are incorporated at only a single end.”).

In view of Gloor touting the cost-effective nature of its approach, a POSA would not be motivated to replace its CDIs with UDIs because such modification would run counter to Gloor’s touted benefits. EX2001, ¶¶99-101. In particular, a POSA would appreciate that using UDIs with Gloor would be *more costly* than the CDIs Gloor was already using. For example, UDIs require more barcodes per sample than CDIs. EX2001, ¶100. Parameswaran noted it could sequence more samples with fewer unique barcodes if it used combinatorial tagging. EX1004, 1, 8; EX2001, ¶100. Indeed, Gloor was able to sequence 272 samples with only 34 unique barcodes (18 left barcodes and 16 right barcodes). EX1005, 6. But for Petitioner’s combination, a POSA would be required to design and use 544 unique barcodes (272 pairs)—*16 times more* barcodes than Gloor used—to sequence the same sample set. EX2001, ¶100. Thus, a cost-sensitive and efficiency-focused POSA would steer clear of the combination because UDIs would be more expensive and less efficient

than combinatorial indexes, which provide economies of scale with increased simplicity and savings as the number of samples increases.

A POSA would further understand that the unnecessary introduction of additional unique barcodes could lead to more errors. EX2001, ¶101. For example, Parameswaran observed errors related to barcode design that resulted in sequences with error-free mismatched barcode motifs. EX2001, ¶101. Some of Parameswaran's forward barcodes were reverse complements of reverse barcodes, or vice versa, and Parameswaran attributed the most sequences with mismatched barcode motifs to those reverse complement barcodes, requiring the analysis of additional nucleotides from the cloning linkers. EX1004, 7 ("Since six of the forward barcodes are exact reverse complements of six reverse barcodes, we added a number of adjoining nucleotides in the Cloning-Linker (0-17) to the barcode query to reduce ambiguity while segregating the sequences into independent datasets."); EX2001, ¶101. Thus, a POSA would understand that barcode design, particularly when the number of barcodes requires designing the barcodes to be too similar to one another, could lead to more errors, and thus that challenges and potential errors related to barcode design increase with the number of unique barcodes required to be designed. EX2001, ¶101.

Accordingly, cost and efficiency considerations would have led a POSA away from implementing unique dual indexing in Gloor's Illumina sequencing, not toward such combination. *Arctic Cat*, 876 F.3d at 1363.

ii. Petitioner Does Not Show That A Desire To “Reduce Sequencing Error” Would Have Motivated The Proposed Combination.

Petitioner incorrectly argues that a POSA would have combined Parameswaran and Gloor due to a general desire to “reduce sequencing error,” particularly alleging that a POSA would want to “identify and discard chimeric sequences and thus reduce crossover error.” Pet., 37-38. Petitioner's argument fails because “it improperly rel[ies] on hindsight in formulating the problem to be solved,” as a POSA at the time of the invention would not have understood cross-over error to be a problem in Illumina sequencers. *Purdue Pharma L.P. v. Depomed, Inc.*, 643 F. App'x 960, 965 (Fed. Cir. 2016).

a. Parameswaran And Gloor Both Claimed A Lack Of Sequencing Errors.

The general assertion that Illumina sequencers have less sequencing error (Pet., 37) does not provide motivation to move Parameswaran's *pyrosequencing-tailored* UDI methods over to Illumina sequencers to reduce error. Parameswaran reports that “over 99.8% of obtained sequences could be assigned,” and that “false discovery rate ... was estimated to be <0.005%.” EX1004, 1 (Abstract). Parameswaran “expected to *minimize errors related to pyrosequencing*,” and

indeed found that the “observed error spectrum appeared most closely to match that *expected as a result of known imprecisions in the chemical synthesis of DNA primers used in standard PCR reactions.*” EX1004, 7. Parameswaran thus touted its method’s ability to already address any appreciated “sequencing error” in pyrosequencing. EX2001, ¶102.

To the extent a POSA might have sought to further reduce sequencing error on Gloor’s Illumina sequencers, the references suggest any such error came from PCR methods, not from sequencing. EX2001, ¶¶102-104. Parameswaran and Gloor, after touting their near perfection, both attribute any miniscule remaining error to steps separate from sequencing, such as PCR amplification. EX1004, 7 (“rare coincidental sequence matches (due to multiple fortuitous errors during barcode synthesis or sequencing)”, “contamination in PCR primer stocks”, and “cross-contamination of template DNAs in the second round of PCR”); EX1005, 2 (“the majority of errors arise during the PCR amplification step”). A POSA following those threads certainly would not have looked to Parameswaran’s method, which included a second round of PCR that potentially led to cross-contamination of template DNA. EX1004, 7; EX2001, ¶¶102-104. Accordingly, a POSA would not have motivation to use Parameswaran’s UDIs on Illumina sequencers as disclosed in Gloor to reduce sequencing error.

b. Petitioner's Arguments Regarding A Purported Desire To Discard Chimeric Sequences And Thus Reduce Cross-Over Error Are Unsupported And Infected With Hindsight.

Petitioner incorrectly argues that a POSA would seek “to identify and discard chimeric sequences and thus reduce cross-over error.” Pet., 37-38. The assertion that a POSA would have been concerned with cross-over error on next generation sequencers, and particularly on flow cell sequencers as claimed, fails because it is quintessential “hindsight in formulating the problem to be solved.” *Purdue Pharma.*, 643 F. App'x 960, 965. A POSA would not have known about cross-over error on NGS machines such as those in Parameswaran and Gloor, and would not have had any reason to take unique dual indexing from Parameswaran to replace Gloor's combinatorial indexing as Petitioner proposes. EX2001, ¶¶105-130; *Leo Pharm. Prods., Ltd. v. Rea*, 726 F.3d 1346, 1354 (Fed. Cir. 2013) (no motivation to combine where POSA would have had to recognize the problem solved by combining two references in order to be motivated to combine them).

Petitioner overstates the awareness of cross-over error problems prior to the invention. At the time of the '852 Patent, a POSA would not have known that cross-over error was problematic for NGS, particularly for sequencers that amplify on a flow cell like Illumina's. EX2001, ¶¶105-115. Petitioner cites references that generally discuss chimeric molecules (Pet., 6-7), but none of those actually discusses

any issues of cross-over error caused by chimeric sequences formed between samples. EX2001, ¶¶105-115. Instead, they discuss only potential chimeras formed *within samples* (e.g., during standard PCR), do not utilize any tagged nucleic acids, and/or generally provide only potential mechanisms that could create chimeras in standard PCR. EX2001, ¶¶105-110; EX1017, 5 (“[c]apturing the full diversity *within a sample*”); EX1018, 3-4; EX1019, 1; EX1020, 1. And those references addressing standard PCR do not discuss NGS systems like Parameswaran’s pyrosequencer or Gloor’s Illumina sequencer; most of the references predate NGS by *more than a decade*. See EX1018 (dated 1990); EX1019 (dated 1989); EX1020 (dated 1995). The two references Petitioner cites that actually address NGS do not discuss the claimed cross-over error or chimeric sequences giving rise to such error. EX2001, ¶¶111-115; EX1033, 9 (hypothesizing chimeras are “caused by problems in library preparation” or “the result of poor sequencing library preparation”); EX1034, 10. To the contrary, literature at the time of the patented invention taught that cross-over error for NGS, and particularly on flow-cell based machines like Illumina’s, was not a problem. EX2005 (“Thus, analysis of different ChIP samples with distinct barcodes indicates that *signal from one barcoded library does not crossover to those of other barcoded libraries.*”).

Petitioner argues that a POSA would have known that Parameswaran’s UDIs would allow a POSA “to identify and discard chimeric sequences and thus reduce

crossover error” (Pet., 38), but that argument incorrectly presupposes the POSA was aware of the cross-over problem. A POSA would understand that Parameswaran’s mismatched barcode motifs were **not** cross-over error. EX2001, ¶¶116-123. Parameswaran reported sequencing runs in which the perceived “mismatched” motifs were corrected by adding nucleotides from the cloning linkers to the analysis. EX1004, 7 (Table 3). A POSA would not have appreciated from that disclosure that there was any remaining cross-over error in need of correcting. EX2001, ¶¶116-117. Instead, as Dr. Adalsteinsson explains, and Dr. Spellman agreed, Parameswaran’s data suggested that the mismatched motifs were caused by “sequencing error” that the linker nucleotides solved (leading Parameswaran to claim a resulting lack of sequencing error), **not** by actual barcode swapping leading to cross-over error. EX2001, ¶¶116-117; EX2002, 168:6-17. If the mismatched barcode motifs were a source of cross-over error in Parameswaran, a POSA would not expect the number of mismatched barcode motifs to decrease at all with the inclusion of additional nucleotides from the cloning linkers in the “search motif” used to distinguish sequences from various independent experiments or samples. EX2001, ¶¶116-117; EX1004, 7 (Table 3). Instead, a POSA would expect cross-over error to remain steady no matter how many cloning linkers are included in the search motif. EX2001, ¶¶116-117. But in Parameswaran, the inclusion of additional cloning linkers in the search motif significantly reduced the instances of “error-free mismatched barcodes”

to the point where Parameswaran was comfortable highlighting that its approach might not even need UDIs and could sequence far more samples (“ n^2 ” rather than $n/2$) with CDIs. EX1004, 1; *id.*, 8.

Petitioner further argues that a POSA would be motivated to use Parameswaran’s unique dual indexing because Parameswaran “permitted ‘unambiguous assignment’ and a ‘remarkably low misassignment rate.’” Pet., 38. But a POSA would not have made any such connection because Parameswaran itself does not attribute its unambiguous assignment or remarkably low misassignment rate to unique dual indexing. EX2001, ¶¶67-69, 115. Instead, Parameswaran concludes a single barcode motif—*not both*—is needed for “unambiguous assignment.” EX1004, 2 (stating the methods could “unambiguously assign 99.8% of obtained barcoded sequences by bioinformatically probing for *an error-free barcode at either end of the sequence of interest*”), 1 (“[W]e found that over 99.8% of obtained sequences could be assigned to 25 independent, uniquely barcoded libraries based on the presence of *either a perfect forward or a perfect reverse barcode*.”). EX2001, ¶¶67-69, 115.

Parameswaran instead posits three potential sources of its mismatched barcode motifs, none of which are cross-over error. EX1004, 7 ((1) “rare coincidental sequence matches (due to multiple fortuitous errors during barcode

synthesis or sequencing)”, (2) “contamination in PCR primer stocks”, and (3) “cross-contamination of template DNAs in the second round of PCR”).

First, Parameswaran’s mention of rare coincidental sequence matches due to errors in barcode synthesis and sequencing cannot be template cross-over error because they are errors in the barcodes themselves (or reads of the barcodes) that would cause all template DNA from one sample to have barcodes corresponding to two different samples from the start. That is not a cross-over error because it is not caused by a chimeric molecule with template nucleic acids from two different samples, much less one formed during amplification. EX2001, ¶¶66, 101, 120-121. A POSA would have understood that the coincidental sequence matches are a product of barcode design itself, not any potential cross-over error during the sequencing workflow. EX2001, ¶¶66, 101, 120-121.

Second, Parameswaran’s mention of contamination in PCR primer stock cannot be cross-over error because PCR primers are added *prior to* any pooling, amplification, or sequencing step in a nucleic acid workflow. EX2001, ¶¶122-123. Contamination in Parameswaran’s PCR primers would lead to mis-labeling, with the wrong primer permeating all template nucleic acid from one sample, creating template nucleic acid molecules with barcodes associated with two different samples. EX2001, ¶¶122-123. A POSA concerned with contamination in PCR primer stocks would thus have no inkling of cross-over errors.

Third, Parameswaran’s mention of cross-contamination in the second round of PCR occurs *before any samples are pooled together*, amplified, or sequenced. Petitioner provides no explanation how a POSA could thus understand cross-contamination in the second round of PCR to create chimeric sequences between two template nucleic acids from *different samples*. EX2001, ¶¶122-123. And Dr. Spellman explained that contamination in Parameswaran would result from problems such as “pipette errors” or “aerosols” occurring prior to amplification—*not* cross-over error. EX2002, 137:22-139:9. Furthermore, a POSA could simply avoid the second round of PCR to deal with that contamination. EX2009, 2, 3, 5; EX2001, ¶¶102, 122-123.

Gloor similarly does not instruct a POSA that it suffered from cross-over error. As Petitioner recognizes, Gloor’s use of combinatorial indexes meant that its method could not have unearthed the cross-over error that Petitioner alleges a POSA would seek to reduce. Pet., 38; EX2001, ¶¶74-84, 124. To the extent chimeric sequences are discussed in Gloor, Gloor expressed no concern about them. Gloor found such a low rate of chimeric sequences through alternative testing, that “the dataset was used *without further regard to chimeric sequences*.” EX1005, 8. Petitioner provides no reason why a POSA implementing Gloor’s sequencing would seek to reduce any issue caused by chimeric sequences when such an occurrence was determined to be insignificant and not worthy of further consideration.

Researchers in the field around the time of the invention determined a single error-free index was sufficient to address any false assignment rate of next generation sequencers, further confirming that a POSA would not have known that cross-over error was an issue for next generation sequencers, and specifically flow-cell based sequencers, even with the existence of dual-indexing methods such as Parameswaran's and Gloor's. EX2010, 3; EX1029, 1; EX2011, 2, 6; EX2012, 1; EX2001, ¶¶125-130. Indeed, Illumina's own documents at the time touted the use of single indexes with its sequencing systems. EX2006; EX2007. It was not until around 2017 that Illumina acknowledged cross-over error on its sequencers, and moved toward UDIs. EX2004; EX2003; EX2001, ¶¶128-129.

Petitioner's attempt to read the cross-over problem addressed by the '852 Patent into the prior art is the type of hindsight that the Federal Circuit has warned against. *Mintz v. Dietz & Watson*, 679 F.3d 1372, 1377-78 (Fed. Cir. 2012) (impermissible hindsight arises when motivation is based on a problem a POSA would not have recognized, because "when someone is presented with the identical problem and told to make the patented invention, it becomes virtually certain that the artisan will succeed in making the invention"); *Purdue Pharma.*, 643 F. App'x at 963 ("[T]o the extent that [Petitioner] relied on the nature of the problem to be solved to supply a reason to combine the prior art, it improperly used hindsight by defining the problem with a recitation of the challenged claims."). Because there was

no awareness that cross-over error was an issue for NGS systems like Parameswaran's and Gloor's at the time of the invention, a POSA would not have recognized it as a problem nor sought to solve such a problem through the use of UDIs.

iii. Petitioner Does Not Show That Parameswaran's Speculation Regarding Other Sequencers Would Have Motivated The Proposed Combination.

Petitioner argues that Parameswaran provides “the express suggestion ... to apply its dual-indexing method to other sequencing platforms.” Pet., 38. But Petitioner mischaracterizes Parameswaran, which only states that its “pyrosequencer-tailored *barcodes*”—and specifically its individual “barcode specification”—might be compatible with other platforms, not the entire pyrosequencing-tailored unique dual indexing approach. EX1004, 8. Parameswaran thus suggests that its individual barcode blueprint might provide a starting point for barcodes to enable multiplexing in other sequencing platforms, and that “the precise structure of the barcodes might need to be revised to reflect sequencing error profiles of various systems.” *Id.*

Moreover, Parameswaran only *speculates* that its barcodes *might* be compatible with other sequencing platforms. Such mere speculation, without explaining why a POSA would *want* to add one part of Parameswaran's barcode method (specifically the unique dual indexes) to Gloor is insufficient. Even if

Parameswaran's speculation were to lead one to *consider* other sequencing platforms, the Federal Circuit requires more than just consideration and possibility. *Adidas AG v. Nike, Inc.*, 963 F.3d 1355, 1359 (Fed. Cir. 2020) (“The obviousness inquiry *does not merely ask whether a skilled artisan could combine the references*, but instead asks whether ‘they would have been motivated to do so.’”).

Petitioner further argues 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.” Pet., 39 (citing *KSR Int’l Co. v. Teleflex, Inc.*, 550 U.S. 398, 421 (2007)). But “KSR did not create a presumption that all experimentation in fields where there is already a background of useful knowledge is ‘obvious to try,’ without considering the nature of the science of technology.” *Leo Pharm.*, 726 F.3d at 1357 (quoting *Abbott Labs. V. Sandoz, Inc.*, 544 F.3d 1341, 1352 (Fed. Cir. 2008)); *Purdue Pharma.*, 643 F. App’x at 965 (“Indeed, it remains important to identify a *reason* that would have prompted a person of ordinary skill in the relevant field to combine the elements *in the way* the claimed new invention does.”) (emphasis in original). Instead, *KSR* requires “a design need or market pressure to *solve a problem*” and a “finite number of identified, predictable solutions” to satisfy the motivation to combine requirement. 550 U.S. at 421. The mere identification of other alternative methods in a prior art reference cannot itself satisfy the motivation to combine.

Virtek Vision Int’l ULC v. Assembly Guidance Sys., Inc., 97 F.4th 882, 888 (Fed. Cir. 2024) (“***It does not suffice to simply be known.*** A reason for combining must exist.”). Thus, Petitioner’s argument that it would have been obvious to try Parameswaran’s UDIs on Gloor’s sequencers simply because they were known fails.

Moreover, Petitioner fails to consider that there were downsides to simply adding UDIs to Gloor’s sequencing with CDIs on an Illumina sequencer. As just one example, and as explained above, using UDIs on Gloor’s 272 samples would have required the creation of far more unique barcodes, whereas Gloor was able to sequence all of its samples with “exceedingly low error” using “far fewer primers than is required for single-end bar-code sequencing.” *Supra*, Section VI.A.1. Petitioner provides no reason why a POSA would have gone through that trouble, and extra cost, without any suggestion that UDIs would reduce error in Gloor’s sequencing, simply because it was “obvious to try.” And although Petitioner claims other researchers had used unique dual-indexing (Pet., 29 (citing EX1038)), Petitioner merely points to an indexing method that sequenced and analyzed a single barcode with each sequence. EX2001, ¶131; EX1038, 5-6. Even ignoring the apparent downsides to UDIs, at best a POSA would have seen UDIs as simply another alternative, which does not create motivation to combine. *Virtek*, 97 F.4th at 886 (finding no motivation to combine where the prior art “disclosures ... do not provide any reason why a skilled artisan would use the claimed alternative instead

of another,” because “it does not suffice to meet the motivation to combine requirement to recognize that two alternative arrangements ... were both known in the art”).

B. Petitioner Fails To Show A Reasonable Expectation of Success. (All Claims)

Petitioner fails to show that a POSA would have reasonably expected combining Parameswaran with Gloor to successfully reduce template cross-over error as claimed. “The reasonable expectation of success requirement refers to the likelihood of success in combining references to *meet the limitations of the claimed invention.*” *Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1367 (Fed. Cir. 2016). Petitioner baldly asserts that “a POSA would have expected the unique dual-barcode approach described in Parameswaran to successfully reduce cross-over error on an Illumina sequencer.” Pet., 44. But a POSA, having no knowledge that cross-over error was even a problem for NGS, and particularly for Illumina sequencers like Gloor’s, would have no expectation to “reduce cross-over error” as claimed by incorporating Parameswaran’s UDIs into Gloor’s Illumina sequencer approach.

Petitioner first argues that a POSA would have expected (1) that “swapping the Roche 454 adapters for Illumina-specific adapters” could work and (2) that “Illumina sequencing enabled read lengths appropriate to sequence”

Parameswaran's nucleic acids. Pet., 41-44. But even if true, neither of those assertions relates to cross-over error or a POSA's expectation of reducing such error as claimed, and Petitioner does not argue otherwise. *See id.*

Petitioner additionally argues that “a POSA would have expected the unique dual-barcode approach described in Parameswaran to successfully reduce cross-over error on an Illumina sequencer” (Pet., 42, 44-45), but Petitioner's scant evidence and argument on that point does not come close to supporting the assertion. Petitioner merely notes Parameswaran's speculation regarding “other sequencing platforms” (citing EX1004, 1, 2, 8), but none of that speculation says anything about cross-over error on any such platforms, much less reducing cross-over error. EX2001, ¶¶132-138. And Petitioner's implication that the '852 Patent is generic regarding the exact sequencing mechanism used (Pet., 45), fails to appreciate the core of the figures, description of the invention, and claims that specifically addresses cross-over error, its origins, and the inventive methods for reducing it.

None of Parameswaran, Gloor, or the prevailing views in the art at the time of the invention, identified *template cross-over error* as a source of error that Parameswaran's indexes could address or reduce at the time of the patented invention. *See supra* Section VI.A. To the contrary, Parameswaran identified sequences of “perfect mismatched barcodes” but attributed those errors to PCR contamination, *not* cross-over error. EX1004, 7; EX2001, ¶136. Indeed, rather than

discard those sequences for fear of cross-over error, Parameswaran included those reads in its data set. EX1004, 8; *see infra* Section VI.C. Thus, nothing in Parameswaran suggests cross-over error at all, let alone that its methods could do anything to reduce cross-over error. EX2001, ¶¶116-123, 135-137. Gloor similarly does not suggest that Illumina sequencers could generate cross-over error that a POSA would expect to be reduced with Parameswaran. Indeed, as Petitioner acknowledges, Gloor's CDI methods rendered Gloor unable to detect any such cross-over error because Gloor used identifiers across multiple samples and thus was unable to detect whether a given identifier jumped from one sample to another. Pet., 8. Gloor does not suggest that any different barcoding method could further reduce any rate of error, nor suggest that it would be possible or helpful to do so. EX2001, ¶¶74-84, 102, 124-125, 138. And the prevailing views at the time were wholly silent on any perceived cross-over error that could be reduced with UDIs. *See supra*, Section VI.A. Thus, nothing that Petitioner puts forth shows any reason why a POSA would have a reasonable expectation of success of reducing cross-over error by combining Parameswaran and Gloor.

C. Petitioner Fails To Show That The Combination Discloses Or Renders Obvious “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)

Petitioner argues that Parameswaran discloses the claimed discarding (Pet., 34-35) and that Gloor also discloses the claimed discarding (Pet., 35-36). But Petitioner fails to point to any such disclosure in either reference. And to the extent Petitioner’s argument is interpreted as merely arguing this step would have been obvious to add to the combination (despite offering only the repeated assertion that both references disclosed the step), Petitioner fails there too.

Petitioner argues that Parameswaran discloses the discarding step because it “*distinguished*” sequences having “combinations of identifier sequences that were different than any of assigned combinations of identifier sequences” (Pet. 34), but “distinguishing” is not “discarding.” And while Petitioner argues that a POSA *could* exclude error-free mismatched barcodes because “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’” (Pet., 35 (quoting EX1004, 8)), Parameswaran never states that it excluded those mismatches. EX2001, ¶¶139-141. Parameswaran explained that “[t]he low false-discovery rate for perfect matches strengthens the argument that any sequence with both 5’ and 3’ barcode motifs will be placed with reasonable accuracy into the correct bin.”

EX1004, 8. Because it observed a low false discovery rate, Parameswaran *included* any sequence with two error-free barcodes in its analysis; it did not discard any such sequences as claimed. EX1004, 8 (“*[A]ll sequences with a match to one of the two expected sequence motifs*” were considered as true members of the corresponding dataset.”); EX2001, ¶¶139-144.

Petitioner also argues that chimeric sequences “*could* be discarded” or “*could* be excluded” in Parameswaran (Pet., 35), but to the extent Petitioner argues adding the claimed discarding step to the combination would have been obvious, any such theory fails. As explained above, none of Parameswaran, Gloor, or researchers in the field were aware of the cross-over error occurring in the NGS systems used in the prior art. *Supra*, Section VI.A. And Parameswaran aimed to *maximize* the samples and sequences used in multiplex analysis. EX1004, 2; EX2001, ¶¶91-93, 140-143. Parameswaran thus included samples with only one barcode or with two barcodes, specifically stating it discarded *only sequences having no recognizable barcodes at all*. EX1004, 8 (“Run I (0.13%) and run II (0.17%) of sequences had *no perfect matches to any of the sequence motifs (forward or reverse)*, and were discarded.”); EX2001, ¶140. Parameswaran further suggests moving on to sequencing even more samples using combinatorial indexes (which would be unable to identify “mismatched barcode motifs”), leading a POSA even further away from discarding such sequences. EX1004, 8; EX2001, ¶142. Petitioner fails to show why adding the

claimed discarding step would have been obvious in view of those disclosures in Parameswaran. EX2001, ¶¶139-144.

Petitioner argues that Gloor “also identified and discarded chimeric sequences” (Pet., 35-36), but cannot show any evidence of Gloor discarding sequences containing a “combination of identifier sequences that are different than any of said distinct pairs” as claimed. EX2001, ¶¶74-84, 145-148. Petitioner acknowledges that Gloor could not detect or discard such reads to reduce cross-over error because Gloor’s combinatorial or “non-unique” indexing approach would include cross-over error. Pet., 8 (“Non-unique dual-indexing cannot necessarily identify ‘cross-over’ errors without additional data processing....”); *id.*, 33; EX2001, ¶147. Petitioner also cites Gloor’s statement that “chimeric sequences *may have been removed* because of the strict requirement for proper sequence tag and primer sequences” (Pet., 35 (citing EX1005, 22)), but provides no explanation how that speculation regarding what *might* have been removed meets the claimed discarding step. EX2001, ¶146-147. And to the extent Petitioner attempts to pivot to inherency, they cannot support such a theory because they assert only that “the chimeric sequences in Gloor *may contain* pairs of identifier sequences assigned to template nucleic acids from another sample.” Pet., 36.

D. Petitioner Did Not Prove That Gloor Is Prior Art. (All Claims)

Petitioner fails to carry its burden to prove by a preponderance of the evidence that Gloor qualifies as a prior art printed publication. *See Hulu, LLC, v. Sound View Innovations, LLC*, IPR2018-01039, Paper 66, 22 (PTAB Oct. 5, 2021). To demonstrate that Gloor is a printed publication, Petitioner must show that Gloor was “sufficiently accessible to the public interested in the art’ before the critical date.” *Align Tech., Inc. v. Dental Monitoring SAS*, IPR2023-01369, Paper 42, 25 (PTAB Mar. 3, 2025) (quoting *In re Cronyn*, 890 F.2d 1158, 1160 (Fed. Cir. 1989)). Petitioner fails to show that Gloor was publicly accessible before December 23, 2010 for three reasons. **First**, the scant evidence Petitioner relies upon to show public accessibility—a declaration and exhibits submitted by Dr. Gloor (EX1002), one of the authors of the Gloor reference—shows at best that Gloor may have been **submitted** to arXiv.org by July 28, 2010, but does not show that Gloor was actually disseminated or accessible to the public on July 29, 2010. **Second**, Petitioner does not point to any evidence to support Dr. Gloor’s proclaimed speculative belief that Gloor was accessible on arXiv.org or by any other means at any point in 2010. **Third**, there is no reliable evidence regarding arXiv.org’s policies or business practices at the time Gloor was allegedly submitted that could make up for the deficient direct evidence regarding Gloor’s alleged accessibility on arXiv.org. Because Petitioner’s

evidence fails to show that Gloor was publicly accessible, Petitioner fails to show that Gloor qualifies as a prior art printed publication.

First, Petitioner’s evidence shows, at best, that Gloor was **submitted** on July 28, 2010, but does not show that Gloor was disseminated or otherwise made available at that time. *Jazz Pharm., Inc. v. Amneal Pharms., LLC*, 895 F.3d 1347, 1355-56 (Fed. Cir. 2018) (“A reference is considered publicly accessible upon a satisfactory showing that such document **has been disseminated or otherwise made available** to the extent that persons interested and ordinarily skilled in the subject matter or art, exercising reasonable diligence, can locate it.”). For example, Dr. Gloor states that he **submitted** his paper on July 28, 2010 (EX1002, ¶16), with “the **intention** of making its findings accessible to the public” (*id.*, ¶18), but does not show that he or anyone else could confirm that arXiv.org actually published it on July 29, 2010.

The exhibits to the Gloor declaration similarly show that Dr. Gloor might have submitted the article on July 28, 2010, but they do not show that it was ever actually disseminated or otherwise made available to the public. Exhibit A is a copy of the Gloor paper itself—it lacks any disclosure or date showing when it was **published**. Exhibits B and C show the “**Submission History**” of Gloor showing it was submitted on July 28, 2010, but do not provide any disclosure or date when it was **published**. Exhibit E is an email Dr. Gloor received that states his paper was submitted and

provides a link to Dr. Gloor himself where the article might be available, but neither Exhibit E nor Dr. Gloor says that the link was actually disseminated or accessible to the public on July 29, 2010. And Exhibit E is insufficient in any event because it shows only that Dr. Gloor might have been able to access the paper. A reference is “*not* publicly accessible if the only people who know how to find it are the ones who created it.” *Samsung Elecs. Col., Ltd. v. Infobridge Pte. Ltd.*, 929 F.3d 1363, 1372 (Fed. Cir. 2019).

Second, there is no evidence that Gloor actually was disseminated or made available in 2010. Both the Gloor declaration and the accompanying exhibits lack any actual announcement, email, webpage capture, or any other evidence showing that the arXiv.org link was actually announced and made available to the public in 2010. For example, Exhibit E states that the Gloor *abstract*—not the full paper—“*will* appear in today’s mailing.” EX1002, Ex. E, 1. Petitioner does not provide that mailing. Similarly, Exhibit F, an email Dr. Gloor received on March 21, 2024, not in 2010, states that the Gloor paper was submitted on July 28, 2010, and states (with no supporting explanation) that “[i]t *would* have been announced on Thursday, July 29, 2010.” Petitioner does not provide that announcement. What is left is Dr. Gloor’s uncorroborated speculation that he “*believe[s]* arXiv.org sent email alerts to subscribers on July 29, 2010, announcing that *Gloor* was publicly accessible,” with no evidence supporting that speculative belief. That is insufficient; “the Board is not

obligated to accept a witness’s speculation as true.” *See Samsung Elecs. Co., Ltd. v. Infobridge Pte. Ltd.*, 929 F.3d 1363, 1372 n.3 (Fed. Cir. 2019) (affirming Board finding that a reference published on a website was not publicly accessible where the petitioner “did not offer evidence, apart from the *speculation* of [the author], that a person of ordinary skill, exercising reasonable diligence, would have located the [allegedly public website] or even known to look for it.”).

Further, Dr. Gloor’s alleged recollection that he presented *findings* from his paper to attendees of the Human Microbiome Research Conference in August and September 2010 does not demonstrate that the Gloor *reference* was publicly accessible before December 23, 2010. Critically, Dr. Gloor does not even claim he presented all details (e.g., background, methods, results, discussion) of the Gloor reference itself. Instead, he merely claims to have given a poster presentation summarizing findings: “I presented a *poster describing the findings* reported in Gloor (Exhibit H) to attendees of the Human Microbiome Research Conference held between August 31 and September 2, 2010.” EX1002, ¶18. And that poster merely cited the Gloor reference and another paper. EX1002, Ex. H (excerpted below):

References

- Hummelen, R., Fernandes, A.D., Macklaim, J.M., Dickson, R.J., Changalucha, J., Gloor, G.B., and Reid, G. (2010). Deep sequencing of the vaginal microbiota of women with HIV. *PLoS One* 5, e12078.
- Gloor, G.B., Hummelen, R., Macklaim, J.M., Dickson, R.J., Fernandes, A.D., MacPhee, R., and Reid, G. (2010). Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products. *ArXiv e-Prints*. arXiv:1007.5075v1 [q-bio.GN]

But a presentation made *after* the alleged date of public accessibility that merely refers to Gloor does not show that Gloor itself was publicly accessible. *See In re Klopfenstein*, 380 F.3d 1345, 1349 (Fed. Cir. 2004) (“[T]he mere presentation of slides accompanying an oral presentation at a professional conference is not per se a ‘printed publication’ for the purposes of § 102(b).”).

Third, Petitioner fails to provide evidence of arXiv.org’s policies or business practices *in 2010* to support Dr. Gloor’s speculation that the Gloor reference was made available before December 23, 2010. For example, Exhibits C and D to the Gloor declaration detail arXiv.org’s submission policies *in 2024*—the date of their capture—but there is no evidence tying those policies to the alleged publication *fourteen years earlier* in 2010. And Dr. Gloor cannot provide authoritative testimony regarding arXiv.org’s publication policies or practices in 2010. Though Dr. Gloor claims he is “familiar with arXiv.org as a user of the system and a registered author of seven articles available from the system” (EX1002, ¶12), he admits that he did not become a registered member of arXiv.org until July 28, 2010—the same day he allegedly submitted his paper. Dr. Gloor describes no personal experience or firsthand knowledge with arXiv.org, let alone its policies and practices, at the time he submitted his paper (as a brand new user) on July 28, 2010. Accordingly, Dr. Gloor cannot backfill the lack of evidence showing that the Gloor

reference was actually announced, disseminated, or otherwise made available to the public in 2010.

Petitioner's deficient evidentiary record here falls far short of the kind of evidence necessary to prove public accessibility. For example, in *Align Tech. Inc. v. Dental Monitoring SAS*, the petitioner similarly relied on a paper published via arXiv.org before a July 21, 2017 priority date. But there, unlike here, the petitioner provided corroborative evidence establishing public accessibility, including (a) a declaration providing "personal knowledge of arXiv.org's ***routine business practice in the 2017 timeframe***"; (b) WayBack Machine archived copies of arXiv.org ***from 2016 and 2017*** reflecting arXiv.org's publication policies at that time; (c) a declaration reviewing metadata of the reference in question showing it was created and last modified before the priority date; and (d) a declaration from the Records Request Processor at the Internet Archive explaining the functioning of the WayBack Machine. *Align Tech.*, IPR2023-01369, Paper 42, 29-32 (PTAB Mar. 3, 2025). Based on the entirety of the evidentiary record presented in *Align Tech.*, the Board found Petitioner had carried its burden to prove public accessibility. *Id.*, 33.

Petitioner provided none of that evidence here. Dr. Gloor did not have personal knowledge of arXiv.org's business practices and policies before July 29, 2010, because he admits that he only became a registered user the day before. EX1002, ¶12. Petitioner provided no archived copies of arXiv.org's policies in

2010—Petitioner relies on evidence from **2024**. EX1002, Exs. C, D, F. Petitioner fails to show that the arXiv.org link was created or last modified in July 2010—and in fact, the earliest WayBack Machine capture of that link dates only to 2020. EX2008. And Petitioner provides no declarations from any employee of arXiv.org or any other archival organization showing that the Gloor reference was actually published on arXiv.org in 2010.

Accordingly, Petitioner has failed to show that Gloor was publicly accessible before December 23, 2010 to qualify as a prior art printed publication.

VII. Ground 2: The Challenged Claims Are Patentable Over Parameswaran And Bentley.

A. Petitioner Fails To Show A Motivation To Combine. (All Claims)

Petitioner’s motivation to combine analysis for Ground 2 tracks its analysis for Ground 1, offering the same reasons for motivation to combine and citing back to the mirrored analysis in Ground 1. Pet., 55-56. Specifically, Petitioner offers the same three reasons for combining Parameswaran with Bentley’s Illumina sequencers as it offered for combining Parameswaran with Gloor’s Illumina sequencers. *Id.* Petitioner’s assertions for Ground 2 fail for the same reasons as explained above for the proposed combination for Ground 1. *See supra* Section VI.A; EX2001, ¶149.

Furthermore, Petitioner argues that a POSA would have been motivated to combine Parameswaran and Bentley because “[i]t would have been obvious to try both sequencing platforms” at the time of the invention given the alleged efficiency

of Illumina sequencers and Parameswaran's unique dual indexing methods. Pet., 55-56. However, Petitioner fails to explain why a POSA would be motivated to even consider combining Parameswaran's unique dual indexing method, which was used for sample identification purposes in multiplexing, with Bentley's whole-genome sequencing project, which did not even involve multiplexing or sample pooling. EX2001, ¶¶85-89, 149-150.

For at least these reasons, Petitioner fails to explain why a POSA would be motivated to combine Parameswaran with Bentley.

**B. Petitioner Fails To Show A Reasonable Expectation Of Success.
(All Claims)**

Petitioner's arguments for reasonable expectation of success for the combination of Parameswaran and Bentley in Ground 2 again mirror its arguments for the combination of Parameswaran and Gloor in Ground 1, citing back to the same analysis. Pet., 56-58. However, for the same reasons that a POSA would not have reasonably expected the combination of Parameswaran with Gloor's Illumina sequencer to successfully reduce cross-over error as claimed, Petitioner's repeated argument for the combination of Parameswaran with Bentley's Illumina sequencer in Ground 2 again fails. *See supra*, Section VI.B.

C. Petitioner Fails To Show That The Combination Discloses Or Renders Obvious “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)

Bentley does not perform sample pooling, does not disclose chimeric sequences or cross-over error, and does not disclose identifying and discarding chimeric sequence reads. EX2001, ¶152. Petitioner relies exclusively on Parameswaran for allegedly disclosing each of these limitations. Pet., 55. However, Petitioner fails to point to any persuasive evidence that Parameswaran discloses the discarding step, or that the combination would render the missing step obvious. *See supra*, Section VI.C; EX2001, ¶¶139-144, 148, 152. Thus, the proposed combination of Parameswaran and Bentley does not disclose or render obvious the “discarding” step.

VIII. Conclusion

For the foregoing reasons, the Challenged Claims are patentable.

Case IPR2024-00964
U.S. Patent No. 11,041,852

Dated: April 17, 2025

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U.S. Patent No. 11,041,852

CERTIFICATION UNDER 37 C.F.R. § 42.24(d)

I hereby certify that this paper, excluding the portions exempted under 37 C.F.R. § 42.24(a), has 11,451 words as counted by Microsoft Word 365, the word-processing system used to prepare this paper.

Dated: April 17, 2025

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Case IPR2024-00964
U.S. Patent No. 11,041,852

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), the undersigned certifies that on April 17, 2025, a complete copy of the foregoing was served on counsel of record for Petitioner by filing this document through P-TACTS and by sending this document via electronic mail to the following addresses:

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