

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 PRELIMINARY RESPONSE

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Patent Trial and Appeal Board
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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.

All citations to exhibits refer to stamped exhibit page numbers.

I. Introduction

U.S. Patent No. 11,041,852 (the “’852 Patent”) solved significant problems in the field of sequencing by improving efficiency and accuracy with scalable technologies. Prior to the ’852 Patent, errors were often introduced during sequencing workflows, such as during amplification and sequencing when many samples were pooled together. For example, nucleic acid molecules from different samples that were analyzed together could overlap during the process of amplifying the nucleic acid, creating erroneous molecules that were sequenced and assigned to incorrect samples as a result of the errors introduced during amplification. Thus, there was a need for sequencing approaches that could avoid these errors and ensure accuracy while providing scalable and cost-effective sequencing technology for widespread adoption, for example by maintaining accuracy during multiplex sequencing when many patient samples were pooled together to increase throughput. *See* EX1001, 1:35-62.

The inventors of the ’852 Patent discovered that preparing nucleic acid samples with certain identifier sequences before the nucleic acid was analyzed through multiplex sequencing reactions allowed for downstream errors to be recognized and corrected during data analysis to increase the accuracy of the process. The inventors developed methods that incorporated pairs of identifier sequences corresponding to specific individual samples so that the samples could be pooled

and sequenced together to increase throughput (e.g., increase the number of samples that can be analyzed in a single sequencing run). *See, e.g.*, EX1001, 2:12-29. The pairs of identifier sequences allowed for amplicons produced through amplification and then sequenced during a run to be accurately traced back to the original sample from which they originated, ensuring that different sequences were accurately identified and analyzed for their particular samples after the multiplexed sequencing run. *Id.*

Petitioner fails to show a reasonable likelihood that the inventive claimed methods were known or that they would have been obvious to a POSA. Petitioner thus fails to meet its burden, and the Petition should be denied because it is deficient in several respects.

First, Petitioner fails to establish a reasonable likelihood of prevailing on its obviousness ground that relies on combining Parameswaran with Gloor (Ground 1). For two different claim limitations (the “amplifying” and “discarding” steps), Petitioner fails to show that its proposed combination discloses or renders obvious the requirements of the challenged claims. Additionally for Ground 1, Petitioner fails to show there was a reasonable likelihood that Gloor was publicly accessible as of December 23, 2010, the priority date used in the Petition for the ’852 Patent, rendering null Petitioner’s argument that the challenged claims would have been obvious over Parameswaran and Gloor at the time of the invention.

Second, Petitioner fails to establish a reasonable likelihood of prevailing on its obviousness ground that relies on combining Parameswaran with Bentley (Ground 2). For similar reasons as its Ground 1, Petitioner also fails to show that its proposed combination of Ground 2 discloses or renders obvious the requirements of the challenged claims for two different claim limitations (the “amplifying” and “discarding” steps).

Due to these deficiencies, the Petition does not establish “a reasonable likelihood that the petitioner would prevail with respect to at least one of the claims challenged in the petition.” 35 U.S.C. § 314(a). While Patent Owner reserves the right to present additional arguments, the deficiencies of the Petition noted herein are sufficient for the Board to find that Petitioner has not met its burden and deny institution.

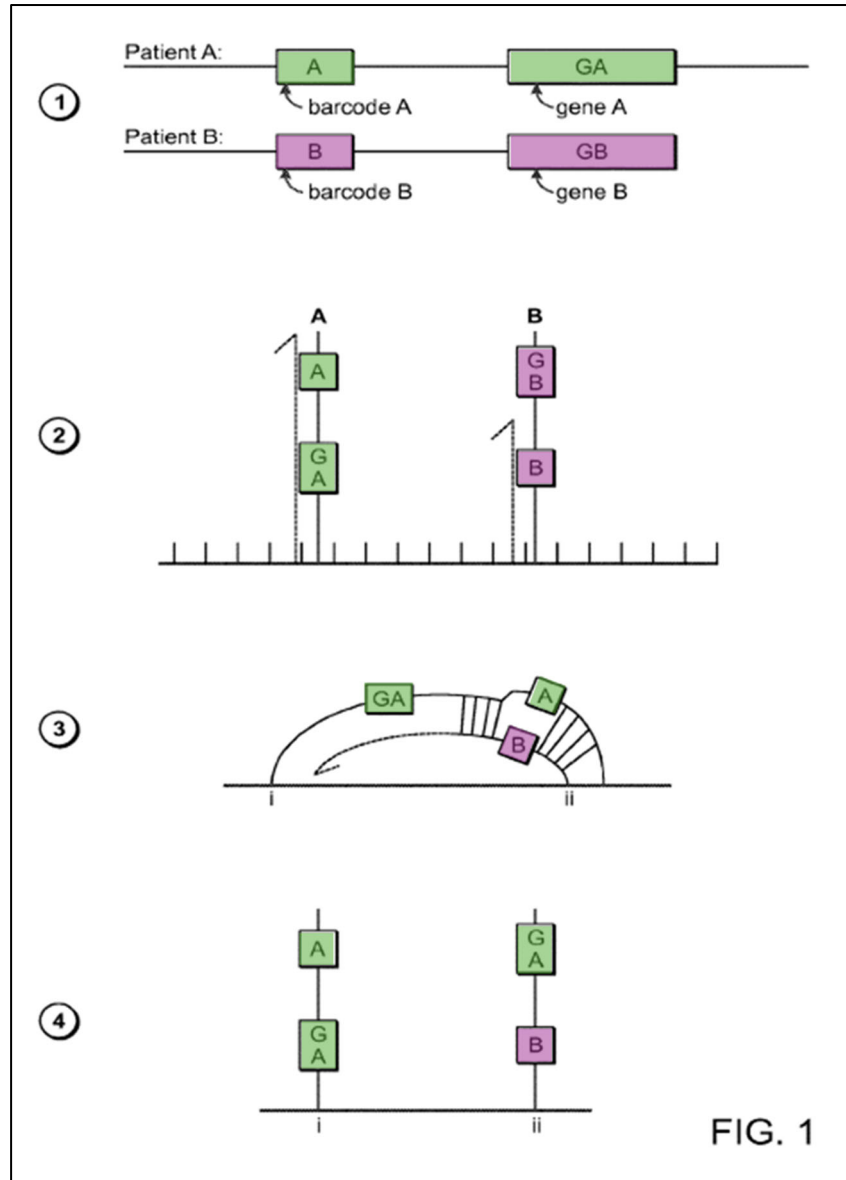
II. Background

A. Overview Of The ’852 Patent

The ’852 Patent relates to “maintaining the integrity and identification of a nucleic acid template in a multiplex sequencing reaction.” EX1001, 1:19-21; 1:35-37. During such multiplex sequencing reactions, “the sample preparation phase frequently introduce[d] errors into the subsequent sequencing reaction.” *Id.*, 1:37-39. The ’852 Patent addressed those problems in next-generation multiplex sequencing reactions, including addressing errors that could arise when “template

molecules may physically overlap on a substrate; and in some cases result in a detectable erroneous signal.” *Id.*, 1:39-41. As the ’852 Patent notes, “[i]n any sequencing process that requires high accuracy, such errors can have a significant impact on results.” *Id.*, 1:41-43.

An error that can be introduced during next-generation sequencing results from “improper pairing of template strands from different samples,” which can “form during the amplification of the multiplexed samples.” *Id.*, 1:52-54. As the ’852 Patent notes, “such errors can have a significant [e]ffect on the results.” *Id.*, 1:59-60. Some efforts had been taken to address these sources of error, for example by using single “barcodes” to identify samples from which nucleic acid was obtained prior to multiplexing, but those efforts had not sufficiently addressed the problems. The ’852 Patent explains that when single barcode identifiers were used to identify different samples, “improper molecular overlap” occurring between samples during amplification could lead to a high-quality signal being obtained and the overlap not being detected, meaning that ultimately “the data will be assigned to the wrong sample because it has become associated with the wrong barcode.” EX1001, 4:17-63. The ’852 Patent illustrates this phenomenon in Figure 1.



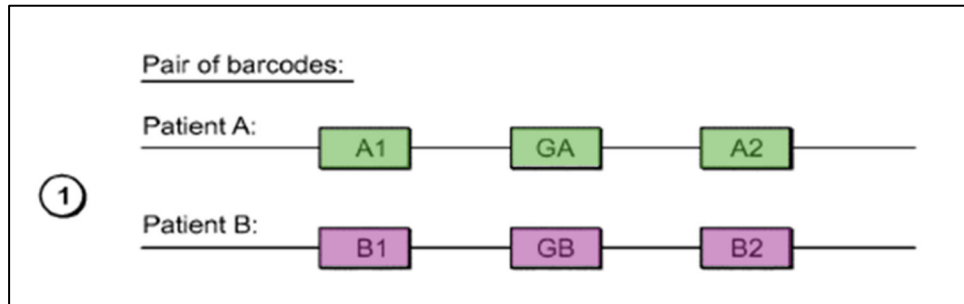
EX1001, FIG. 1 (annotated). As a result of the molecular overlap shown during amplification, an erroneous molecule is produced because “[s]trand ii contains the barcode for patient B but the gene fragment for patient A.” *Id.*, 4:53-55. The prior single-barcode approach was unable to detect this erroneous molecule, and thus “[d]uring the sequencing process, patient A’s sequence data will be improperly and incorrectly associated with patient B.” *Id.*, 4:55-57; 4:60-63.

The '852 Patent addressed the shortcomings of prior approaches by “utiliz[ing] two or more identifiers that are uniquely associated with an analyte of interest, such that the identifiers will only be present when the analyte of interest is detected” and “requiring concomitant detection of two or more independent markers associated with the analyte to be detected” to avoid “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.”). 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 “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.

For example, as shown in Figure 2 of the '852 Patent, the inventive methods include identifying template nucleic acids from different samples by incorporating pairs of identifiers before the samples are pooled for a multiplex sequencing reaction. *Id.*, FIG. 2. “Each template has a gene fragment from that patient (e.g., GA), and a pair of sequence identifiers (first and second) specific to that patient (e.g., A1 and A2).” *Id.*, 5:2-5. “[A] first identifier can be incorporated into a 5' end of the template and a second identifier can incorporated into a 3' end of the template. The first and second identifiers are designed such that each set of identifiers is correlated

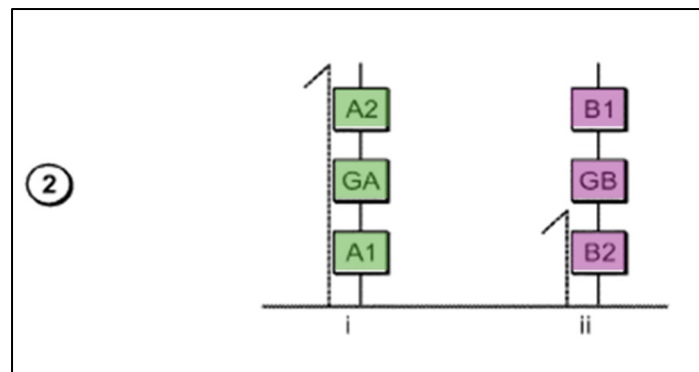
to a particular sample ... allowing samples to be distinguished and/or validated.”

Id., 3:12-19; 5:6-8.



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

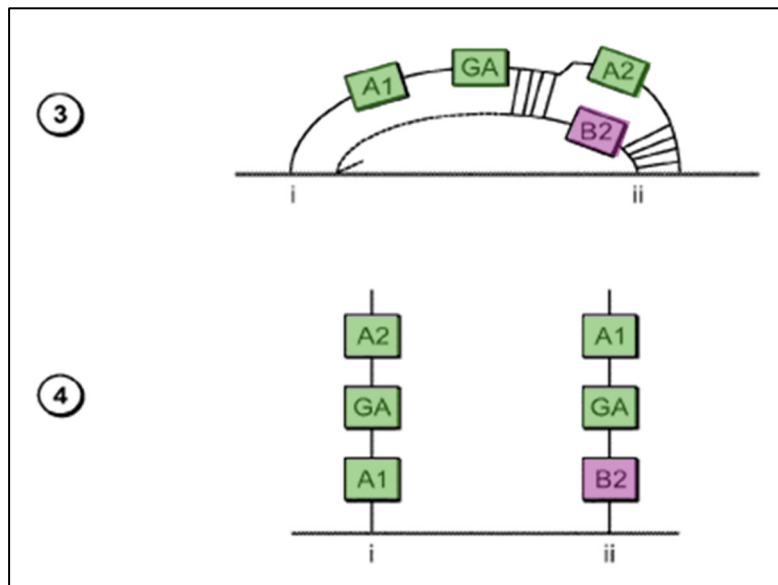
After nucleic acid samples are pooled together for multiplexed sequencing, “the samples are then hybridized to primers at discrete locations on a flow cell,” and in some instances “the nucleic acid from patient A and the nucleic acid from patient B land within a cluster radius on the solid support.” *Id.*, 5:28-40.



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

The pooled nucleic acid is then amplified to facilitate sequencing and analysis, and the amplification can again result in improper molecular overlap occurring between samples (e.g., between nucleic acid from patient A and nucleic acid from

patient B). “However, in the case of two barcodes, only one of patient B’s barcodes (B2) will be contained in the copied strand,” and “[t]he other barcode will be one of patient A’s barcode sequences (A1).” *Id.*, 5:41-55. Thus, “[u]nlike the example in FIG. 1, the chimeric molecule formed in this example includes one barcode sequence from patient A (A1) and one barcode sequence from patient B (B2).” *Id.*, 5:55-58.



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

Because two identifiers are now being used for each sample, the resulting chimeric molecule contains mismatched barcodes (i.e., “A1” paired with “B2” in strand ii above). “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.” *Id.*, 5:62-65. The erroneous molecule produced from amplification can thus be detected even though “[d]uring the sequencing process, this would yield a high-quality signal,” because “during data analysis the molecules

would be recognized as invalid” and “the data can then be excluded from final analysis.” *Id.*, 5:65-6:6.

By increasing the ability to detect and exclude errors, the inventive methods described in the ’852 Patent “dramatically reduce false positives in a multiplex sequencing reaction” and address the problems that previously hampered next-generation multiplex sequencing reactions. EX1001, 6:53-57; 7:24-38; FIG. 3.

Petitioner asserts that the inventions in the ’852 Patent were copied from others, including from Petitioner (*see* Pet., 19, 42-43), but that accusation is not well taken. Inventions in the ’852 Patent are shown in the three figures included in the patent and described in at least the corresponding detailed description of the figures. *See, e.g.*, EX1001, 3:63-7:37; FIGS. 1-3. Petitioner does not contend that any of those figures or corresponding description appear in any other publications, whether from Petitioner or third parties. Pet., 19 (citing EX1001, 1:25-31; 3:37-43; 7:45-20:62), 42-43. And Petitioner’s overstated accusations of “copying” refer only to text discussing general considerations relevant to the invention, including text that is 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. *See, e.g.*, EX1001, 3 (listing Steinmann et al. U.S. Patent Publication No. 2011/0301042, which is a national phase application that shares the same specification with WO2010/056728 that Petitioner cited as

EX1037); 7:39-44 (noting “[t]he following sections discuss general considerations” for concepts related to the inventions); 10:4-10 (identifying and incorporating by reference in its entirety Sabot et al. U.S. Patent Publication No. 2009/0226975, which Petitioner cited as EX1036); 10:34-41 (same); 20:50-54 (“References and citations to other documents ... have been made throughout this disclosure. *All such documents are hereby incorporated herein by reference in their entirety* for all purposes.”); *see also id.*, 8:6-13; 8:65-9:4; 9:17-20; 15:44-48; 16:30-40; 18:7-12; 19:24-29; 19:64-20:2.

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. EX1007, 11-13; EX1001, 1. Petitioner uses December 23, 2010, as the effective priority date of the ’852 Patent for purposes of the Petition. Paper 1 (“Pet.”), 17.

In an Office Action dated February 26, 2021, the Examiner rejected pending claims as anticipated by Parameswaran (EX1004). EX1007, 195-196. The Examiner asserted that Parameswaran taught the method claimed in then-pending claims 47-54. *Id.*

In response, following an interview with the Examiner, Applicant amended pending independent claim 47 to recite amplifying nucleic acid templates “on a surface of a flow cell.” EX1007, 302. Without conceding that Parameswaran actually

disclosed any other 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 a flow cell’.” EX1007, 305.

Following Applicant’s response, the Examiner deemed the claims to be allowable. A Notice of Allowance was issued on April 27, 2021. EX1007, 312, 316-317.

III. Overview Of The References¹

A. Parmeswaran (EX1004)

Parmeswaran is titled “A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing.” Parmeswaran, 1. The “pyrosequencing” Parmeswaran describes is sequencing performed on “454/Roche Genome Sequencers.” *Id.*, 1. Parmeswaran noted “[t]hree major high-throughput sequencing platforms” in use at the time and explained that “the Solexa and SOLiD systems provide many more sequence reads, but render much shorter read lengths than the 454/Roche Genome Sequencers.” *Id.* Parmeswaran determined that “[t]his *makes the 454/Roche platform appealing for use with barcoding technology*, as

¹ Patent Owner does not concede that the references cited in the Petition are prior art to the ’852 Patent. Patent Owner reserves its right to raise additional challenges to the prior art status of the references in future proceedings.

the enhanced read length facilitates the unambiguous identification of both complex barcodes and sequences of interest.” *Id.*

Parameswaran discusses using pyrosequencing with “a high-information-content barcoding approach in which each sample is associated with two uniquely designed, 10-nucleotide barcodes.” *Id.*, 2. Parameswaran compared its barcode approach with prior methods that required physical separation between nucleic acids from different samples, stating that “[b]y not relying on physical separators, this procedure maximizes sequence space and multiplexing capabilities.” *Id.*

In addition to barcodes, Parameswaran incorporated forward and reverse cloning linkers into nucleic acids. *Id.*, 2, 3 (Fig. 1). Parameswaran used the cloning linkers to increase the accuracy of its methods. In particular, during analysis, because “six of the forward barcodes are exact reverse complements of six reverse barcodes,” Parameswaran “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.” *Id.*, 7. Parameswaran refers to the combination of 10-nucleotide barcodes and additional nucleotides from the cloning linkers as “barcode motifs” (or “search motifs”) that were ultimately used for sample identification. *Id.* Parameswaran’s study aimed to determine “how much of the (fixed) linker sequence will be required in addition to the (variable) barcode for definitive sample

assignment,” and ultimately decided that “a search motif of 13 nt was determined to be ideal for our studies in minimizing false-discovery rate[.]” *Id.*, 7-8, Table 3.

When using 13-nt barcode motifs, Parameswaran found that approximately 94% of sequences had “perfect” forward and reverse barcode motifs. *Id.*, 7. Parameswaran attributed sequence reads that contained one perfect motif sequence unexpectedly paired with a second perfect motif sequence to “rare coincidental sequence matches (due to multiple fortuitous errors during barcode synthesis or sequencing), to contamination in PCR primer stocks, or to cross-contamination of template DNAs in the second round of PCR.” *Id.* The percentage of these “perfect and unexpected” motif pair occurrences was less than 0.005% when 13-nt motifs were used, and Parameswaran did not exclude any such occurrences from its analysis. *Id.*, 8. Instead, “***all sequences with a match to one of the two expected sequence motifs were considered as true members*** of the corresponding dataset.” *Id.*

Parameswaran was considered by the Examiner during prosecution of the ’852 Patent. Parameswaran was identified in an Information Disclosure Statement, and the Examiner signed the Statement indicating Parameswaran was considered on February 22, 2021. EX1007, 121, 279. The Examiner also referenced Parameswaran in substantive rejections, but the ’852 Patent was allowed after the Applicant overcame those rejections. *See supra* Section II.B.

B. Gloor (EX1005)

Gloor is titled “Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products.” Gloor, 1. Gloor discloses using “combinatorial sequence tagging” to analyze samples for microbiome profiling. *Id.* Gloor noted 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.* Gloor “suggest[s] that this method will be useful for the deep sequencing of any short nucleotide region that is taxonomically informative....” *Id.* Gloor used Illumina sequencing in its methods and noted that “[t]he Illumina sequencing platform is currently restricted to read lengths of ~100 nucleotides from each end of a DNA fragment, and was limited to ~75 nt at the time of experimental design.” *Id.*, 3.

Gloor added primers and combinatorial barcodes to nucleic acids prior to sequencing. For the barcodes, Gloor “chose sequence tags to ensure all 4 nucleotides were represented in each of the first four positions of the primers using parameters similar to those in the barcrawl program.” *Id.*, 5. For the barcode tags, Gloor “var[ied] the length of the tags between 3 and 6 nucleotides long.” *Id.*; *see also id.*, Table 2. “The tag length variation was expected to reduce the likelihood that adjacent spots on the Illumina solid support would be scored as one during the sequencing of the amplification primers.” *Id.*, 5.

After sequencing, Gloor identified sequences that contained “incorrect sequence tags” and inspected those sequences to determine that “the single largest contributor to the difference between the number of reads with proper primer sequences and the number of reads with proper sequence tag sequences was an N-1 truncation of the sequence tag, *which presumably arose during the primer synthesis.*” *Id.*, 7. “The next largest class of sequence tag error was complete lack of the left or right end sequence tag.” *Id.* For remaining errors, Gloor states that “remaining missing reads are composed of a large number of classes of sequence tag sequences each containing small numbers of errors including additional 5’ bases, misincorporated bases or *difficult to classify errors* that presumably arose during the PCR amplification.” *Id.*

C. Bentley (EX1006)

Bentley is titled “Accurate whole human genome sequencing using reversible terminator chemistry.” Bentley, 1. Bentley describes massively parallel synthetic sequencing used to analyze whole human genomes. *Id.*, 3. Bentley explained that it “generated high-density single-molecule arrays of genomic DNA fragments attached to the surface of the reaction chamber (the flow cell) and used isothermal ‘bridging’ amplification to form DNA ‘clusters’ from each fragment.” *Id.*, 1.

IV. The Level Of Ordinary Skill In The Art

Petitioner proposes a level of ordinary skill in the art. Pet., 17. For the purposes of this Preliminary Response, Patent Owner does not set forth an alternative level of skill for a POSA because Petitioner fails to meet its burden even under its own proposal. Patent Owner's silence regarding the appropriate level of skill of a POSA is not an agreement with Petitioner's proposal. Patent Owner reserves the right to challenge Petitioner's proposal, and to propose an alternative or additional level of ordinary skill, in future proceedings.

V. Claim Construction

Petitioner construes "template cross-over error" to mean "sequences of chimeric molecules made from template nucleic acids derived from different samples." Pet., 23. For the purposes of this Preliminary Response, Patent Owner does not set forth an alternative construction because Petitioner fails to meet its burden even under its own proposed construction. Patent Owner's silence regarding the appropriate construction of any term is not an agreement with Petitioner's apparent construction of any such term. Patent Owner reserves the right to challenge Petitioner's proposed construction, and to propose alternative or additional claim constructions, in future proceedings.

VI. Institution Should Be Denied Because The Petition Does Not Establish A Reasonable Likelihood Of Prevailing On Any Ground.

A. Ground 1: Petitioner Does Not Establish A Reasonable Likelihood Of Prevailing On Its Ground Based On Parameswaran In View Of Gloor.²

Petitioner’s Ground 1 based on Parameswaran and Gloor is deficient because Petitioner fails to show the combination discloses all claimed steps and because Petitioner did not meet its burden to show that Gloor is prior art. *First*, Petitioner divides the “amplifying” step and alleges disclosures in Gloor and Parameswaran combine to meet the limitation, but Petitioner’s arguments leave gaps that fail to show how the combination meets the full claimed step. *Second*, Petitioner relies on only Parameswaran for the “discarding” step but fails to show that Parameswaran meets that step. *Third*, Petitioner fails to provide sufficient evidence to establish Gloor is prior art to the ’852 Patent. For at least those reasons, Petitioner failed to establish a reasonable likelihood of prevailing on Ground 1 in the Petition.

² For the purposes of this Preliminary Response, Patent Owner addresses certain issues with Petitioner’s ground. Patent Owner’s silence regarding any additional issue is not an agreement with Petitioner’s positions. Patent Owner reserves the right to address additional issues with this ground—including issues relating to additional claim limitations, motivation to combine, and reasonable expectation of success—in future proceedings.

1. Petitioner Does Not Show That The Combination Discloses Or Renders Obvious “Amplifying Said Nucleic Acid Templates On A Surface Of A Flow Cell 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.”

Petitioner alleges that a combination of Parameswaran and Gloor discloses the “amplifying” step of Claim 1. Pet., 31-33. Petitioner first relies on Gloor for “amplifying said nucleic acid templates on a surface of a flow cell to form clusters,” (Pet., 31-32), then relies on Parameswaran for “at least one of which comprises a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs,” (Pet., 32-33). As an initial matter, Petitioner fails to establish a reasonable likelihood that Gloor is prior art for the reasons explained in Section VI.A.3, and Petitioner’s assertions for this limitation fail for that reason alone. But even if the Board considers the disclosures of Gloor, by dividing the claimed step in this manner, Petitioner fails to show that the combination discloses or renders obvious *clusters* formed on a surface of a flow cell, with at least one *cluster* comprising a chimeric sequence, as the claim requires.

Petitioner relies on Parameswaran as allegedly disclosing clusters comprising chimeric sequences, equating the claimed chimeric sequences with “mismatched” or “misassigned” sequences containing an “unexpected” pair of forward and reverse

barcode motifs identified in Parameswaran.³ Pet., 32-33. In particular, Petitioner argues that “sequences with mismatched barcode motifs” in Parameswaran “include chimeric sequences resulting from cross-over events.” Pet., 32. However, Petitioner does not point to any disclosure in Parameswaran identifying these “mismatched” motifs as *chimeric* sequences—as opposed to mere sequencing or sample handling error—nor does Petitioner cite any disclosure in Parameswaran regarding such sequences resulting from cross-over events, and Petitioner does not explain how Parameswaran could disclose or render obvious *clusters* formed during amplification that comprise such chimeric sequences.

Parameswaran notes possible sources of error for sequences that contain “perfect but unexpected” motifs. Parameswaran posits that these errors can stem from “[1] rare coincidental sequence matches (due to multiple fortuitous errors during barcode synthesis or sequencing), [2] to contamination in PCR primer stockers, or [3] to cross-contamination of template DNAs in the second round of PCR.” Parameswaran, 7. Petitioner does not allege that either category [1] or [2] of

³ Petitioner admits that, because Gloor’s method involved *combinatorial* pairing of barcodes where certain identifiers were not unique across samples, a sequence with an incorrect pair of identifiers could “nevertheless match one of the pairs used for other nucleic acid templates.” Pet., 33.

the potential errors noted in Parameswaran produce the claimed chimeric sequences.

Pet., 32. Instead, Petitioner relies on only the third category (“cross-contamination of template DNAs in the second round of PCR”) and alleges that this category of errors could result in the generation of a chimeric sequence due to a cross-over event.

Id. However, Petitioner does not cite any disclosure identifying or inferring that this category of potential error included chimeric sequences resulting from a cross-over event. And Petitioner fails to explain how a POSA could have understood this category of error as including chimeric sequences resulting from a cross-over event in view of Parameswaran’s workflow.

Parameswaran specifically identifies the “second round of PCR” as the point of potential cross-contamination, but that second round of PCR in Parameswaran occurred *before* sample pooling (*i.e.*, *before* template nucleic acids derived from different samples were combined). Parameswaran, 7. After generating cDNA libraries containing cloning linkers, the cDNA pool from *each individual sample* was amplified for twenty cycles in a first round of PCR in Parameswaran. *Id.*, 3. Then “a *second round* of amplification with temperatures and cycle lengths identical to the initial round, but with variable number of total cycles (six, eight and ten), was performed using 1/25th of the first PCR product.” *Id.* Only *after* this second round of PCR, “PCR products from 25 samples were pooled together in roughly equimolar ratios” for running through the 454/Roche sequencing process. *Id.*, 4. Thus,

Parameswaran’s workflow shows that any of the “perfect but unexpected reads” arising from what Parameswaran calls “cross-contamination of template DNAs in the second round of PCR” occurs prior to sample pooling, not during any amplification and sequencing during the multiplex pyrosequencing. Petitioner fails to address that distinction and thus cannot show how the “mismatched” or “misassigned” sequences it relies on in Parameswaran could meet the claimed chimeric sequences.

Petitioner similarly fails to assert that Parameswaran discloses a cluster comprising a chimeric sequence, as claimed. Petitioner argues that “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.’” Pet., 32. However, as Petitioner admits, because Parameswaran used pyrosequencing on the surface of beads in a Roche 454 sequencer, it does not disclose the formation of *clusters*. Pet., 31. The plain language of the claim requires that at least one of the *clusters* formed during amplification comprises a chimeric sequence, not that the nucleic acid template includes such sequences. Petitioner’s mapping fails to address this requirement of the plain language of the claim, and its arguments for the “amplifying” step are deficient for this additional reason.

2. **Petitioner Does Not 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 requires “discarding sequences reads” that contain a “combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce cross-over error.” EX1001, 21:16-19. Petitioner asserts that Parameswaran discloses this “discarding” step. Pet., 34-35. However, Petitioner does not identify any disclosure in Parameswaran that “discards” the claimed sequence reads. Instead, Petitioner argues that 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.” Pet., 34. But nowhere does Petitioner argue that Parameswaran discloses *discarding* those sequences that were allegedly “distinguished.” Pet., 34-35. And Parameswaran discloses the opposite, explicitly stating that such sequences (containing “perfect but unexpected” barcode motifs) were not discarded, but instead were *kept and included in the analysis* along with “perfect and expected” barcode motifs. Parameswaran, 8 (“For our purposes, *all sequences with a match to one of the two expected sequence motifs were considered as true members of the corresponding data set.*”); Pet., 35. Thus, the Petition fails to identify any disclosure in Parameswaran of discarding

sequence reads that contain a “combination of identifier sequences that are different than any of said distinct pairs,” as required by Claim 1.

Tacitly acknowledging Parmeswaran’s lack of disclosure, Petitioner argues that Parmeswaran “disclosed a method in which these chimeric sequences *could be discarded*, and thus, reduce cross-over error.” Pet., 35. Petitioner contends that Parmeswaran’s suggestion that “any sequence with both 5’ and 3’ barcode motifs will be placed with reasonable accuracy into the correct bin” would inform a POSA that Parmeswaran disclosed the claimed “discarding” step. *Id.* But Petitioner fails to address that Parmeswaran’s reference to “any sequence with both 5’ and 3’ barcode motifs” refers to any sequence with “perfect” barcode motifs—both expected *and unexpected* pairings of such motifs—both of which Parmeswaran includes in its analysis, giving no reason to exclude the “unexpected” pairings that Parmeswaran explicitly considers and includes in its analysis. In particular, Parmeswaran defines “false discovery rate” as the “percentage of ‘perfect and unexpected’ occurrences” or as Petitioner suggests, “mismatched” 5’ and 3’ motifs. Parmeswaran then states, “[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.*” Parmeswaran, 8. Parmeswaran thus explains that the low incidence of “perfect and unexpected occurrences” shows that any sequence containing a 5’ and 3’ motif will be placed

with reasonable accuracy and provides that as the reason why Parameswaran *includes* the “perfect and unexpected occurrences” in its analysis. Petitioner provides no explanation why a POSA understanding they *could* exclude these sequences *would* do so in view of Parameswaran’s explanation of why the same sequences are *included* in its analysis to achieve reasonable accuracy.

Petitioner also cites to Gloor but fails to show how Gloor includes the claimed “discarding” step. Pet., 35-36. Petitioner acknowledges that Gloor does not disclose the discarding step at least because “Gloor disclosed non-unique dual-indexing.” Pet., 36. And Petitioner acknowledges the alleged chimeric sequences in Gloor “may contain pairs of identifier sequences assigned to template nucleic acids from another sample” and are thus not discarded. Pet., 36. Instead, Gloor explains “[t]he *dataset was used without further regard to chimeric sequences* because putative chimeric sequences composed a minuscule fraction of reads.” Gloor, 8.

3. Petitioner Fails To Establish A Reasonable Likelihood That Gloor Is Prior Art

Petitioner uses December 23, 2010, as the priority date of the ’852 Patent for purposes of the Petition (Pet., 17) but fails to show a reasonable likelihood that Gloor (EX1005) was publicly accessible as of that date. “[T]he burden is on the petitioner to identify with particularity evidence sufficient to establish a reasonable likelihood that the reference was publicly accessible before the critical date of the challenged patent, and therefore that there is a reasonable likelihood that it qualifies as a printed

publication.” *Hulu, LLC v. Sound View Innovations, LLC*, IPR2018-01039, Paper 29, at 15 (PTAB Dec. 20, 2019) (precedential).

The Petition dedicates only two sentences to Gloor’s public accessibility and does not mention or cite any evidence other than Dr. Gloor’s purported recollection in an attempt to establish a reasonable likelihood that Gloor was publicly accessible before the ’852 Patent. Petitioner argues that Gloor was publicly accessible by July 29, 2010, through “arXiv.org,” and cites as purported support to a declaration by Dr. Gloor, arguing that he “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.” Pet., 12 (citing EX1002, ¶¶12-19). That bare-bones assertion is insufficient to establish a reasonable likelihood that Gloor was publicly available under the Board’s precedent. *Hulu, LLC*, IPR2018-01039, Paper 29 at 13 (“***[T]he petition must identify, with particularity, evidence sufficient to establish a reasonable likelihood that the reference was publicly accessible*** before the critical date of the challenged patent and therefore that there is a reasonable likelihood that it qualifies as a printed publication.”). And to the extent Petitioner invites the Board to sift through the paragraphs of Dr. Gloor’s declaration and additional exhibits attached to the declaration—exhibits that are never cited or discussed anywhere in the Petition—

the Board should decline to do so because Petitioner improperly attempts to incorporate by reference arguments and evidence without explanation, or even citation, in violation of the Board’s regulations. *See* 37 C.F.R. § 42.6(a)(3); *Parus Holdings, Inc. v. Google LLC*, 70 F.4th 1365, 1371-73 (Fed. Cir. 2023) (affirming Board decision declining to consider evidence and argument alleged to antedate prior art that was improperly incorporated by reference); *General Access Solutions, Ltd. v. Sprint Spectrum L.P.*, 811 F. App’x 654, 657 (Fed. Cir. 2020) (The “prohibition against incorporation of arguments from other documents serves various policy goals, including to ‘minimize the chance that an argument may be overlooked’ and to ‘eliminate[] abuses that arise from incorporation and combination.’”) (quoting Rules of Practice for Trials Before the Patent Trial and Appeal Board and Judicial Review of Patent Trial and Appeal Board Decisions, 77 Fed. Reg. 48,612, 48,617 (Aug. 14, 2012)).

Even if the Board considers the additional evidence attached to Dr. Gloor’s declaration—evidence that Petitioner does not cite, explain, or even reference—it is still insufficient to show public availability. Dr. Gloor purports to support his testimony and recollection of events from fourteen years ago with documentation that at best indicates his article was ***submitted***, but does not show if and when it was actually ***published***, much less published in July 2010. Dr. Gloor’s Exhibits A-C and E-F show at best that the article was submitted in July 2010, but say nothing about

its date of publication or public availability. Dr. Gloor’s Exhibits D and I are websites that he claims show arXiv’s announcement practices, but the websites are from 2024 and say nothing about arXiv’s practices in July 2010, when Petitioner claims Gloor was publicly available. And Dr. Gloor’s Exhibits G and H are abstracts and a poster that he claims relate to a conference from 2010, but neither Petitioner nor Dr. Gloor even contend that the abstracts or poster actually include any of the details of Dr. Gloor’s article that are relevant to Petitioner’s challenge here. That evidence is wholly inadequate, particularly when compared to at least one prior case where the Board accepted an arXiv publication, relying on testimony from a Cornell University Library employee who was actually responsible for arXiv.org and knowledgeable regarding its practices. *Great West Casualty Company v. Intellectual Ventures II LLC*, IPR2015-01706, Paper 10 at 11 (PTAB Feb. 9, 2016) (“Mr. Warner testifies that he is an employee of the Cornell University Library (‘CUL’), and the Directory of Repository Architecture/Services within CUL Information Technology, which is responsible for arXiv.org, and explains that the data logs of arXiv.org indicate that MyLibrary was available, and arXiv.org users were notified of its availability via e-mail, as of February 3, 1999.”). Petitioner provides no testimony from any Cornell employee or any other custodian with any personal knowledge of arXiv.org or its regular practices. The Petition thus fails to provide even the minimum evidence previously found by the Board to be sufficient for showing

public availability at the institution stage. *Hulu*, IPR2018-01039, Paper 29 at 13; *see also Louisiana Pac. Group v. Huber Engineered Woods LLC*, IPR2020-00600, Paper 14 at 15-16 (PTAB Aug. 20, 2020).

Accordingly, Petitioner has failed to meet its burden to show that there was a reasonable likelihood that Gloor is prior art.

B. Ground 2: Petitioner Does Not Establish A Reasonable Likelihood Of Prevailing On Its Ground Based On Parameswaran In View Of Bentley⁴

Petitioner's Ground 2 based on Parameswaran and Bentley is deficient because Petitioner fails to show the combination discloses all claimed steps for reasons similar to those discussed above for Ground 1. *First*, Petitioner divides the “amplifying” step and alleges disclosures in Bentley and Parameswaran combine to meet the limitation, but Petitioner's arguments leave gaps that fail to show how the combination meets the full claimed step. *Second*, Petitioner relies on only

⁴ For the purposes of this Preliminary Response, Patent Owner addresses certain issues with Petitioner's ground. Patent Owner's silence regarding any additional issue is not an agreement with Petitioner's positions. Patent Owner reserves the right to address additional issues with this ground—including issues relating to additional claim limitations, motivation to combine, and reasonable expectation of success—in future proceedings.

Parameswaran for the “discarding” step but fails to show that Parameswaran meets that step. For at least those reasons, Petitioner failed to establish a reasonable likelihood of prevailing on Ground 2 in the Petition.

For the “amplifying” step, Petitioner first relies on Bentley for “amplifying said nucleic acid templates on a surface of a flow cell to form clusters,” (Pet., 53-54), then relies on Parameswaran for “at least one of which comprises a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs,” citing back to its corresponding argument in Ground 1 (Pet., 54). But as discussed above in Section VI.A.1, by dividing the claimed step in this manner, Petitioner fails to show that the combination discloses or renders obvious *clusters* formed on a surface of a flow cell, with at least one *cluster* comprising a chimeric sequence, as the claim requires. Specifically, Petitioner relies on Parameswaran but does not point to any disclosure in Parameswaran identifying its “mismatched” motifs as *chimeric* sequences or point to any disclosure in Parameswaran regarding such sequences being generated during sequences resulting from cross-over events, nor does Petitioner explain how Parameswaran could disclose or render obvious *clusters* formed during amplification that comprise such chimeric sequences. *See* Section VI.A.1; Pet., 54 (citing back to Ground 1 arguments).

For the “discarding” step, Petitioner relies on only Parameswaran, citing back to its corresponding argument in Ground 1 (Pet., 55). But as discussed above in Section VI.A.2, Petitioner fails to show how Parameswaran discloses or renders obvious this step.

Thus, Petitioner fails to establish a reasonable likelihood of prevailing on its grounds based on Parameswaran in view of Bentley.

VII. Conclusion

For the foregoing reasons, Patent Owner respectfully requests that the Board deny institution of the Petition.

Dated: October 31, 2024

Respectfully submitted,

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Case IPR2024-00964
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 6,117 words as counted by Microsoft Word 365, the word-processing system used to prepare this paper.

Dated: October 31, 2024

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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 October 31, 2024, 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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