

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

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA INC.,
Petitioner

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner

Case IPR2024-00965

U.S. Patent No. 11,768,200

PATENT OWNER'S SUR-REPLY

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P.O. Box 1450
Alexandria, VA 22313-1450

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<i>Sierra Wireless, ULC v. Sisvel, S.P.A.</i> , 130 F. 4th 1019 (Fed. Cir. 2025)	4

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).

Ex. #	DESCRIPTION
2013	Declaration of Michael Wueste in Support of Motion for Admission <i>Pro Hac Vice</i>
2014	Declaration of Kevin Goon in Support of Motion for Admission <i>Pro Hac Vice</i>
2015	Deposition Transcript of Paul T. Spellman, Ph.D., August 1, 2025

All citations to Exhibits refer to stamped exhibit page numbers.

I. Introduction

Petitioner's Reply (Paper 25) fails to remedy the deficiencies of its Petition. Petitioner misinterprets the claims, overstates the disclosures of the prior art, and fails to show that the Challenged Claims are anticipated or obvious. For dependent Claims 5 and 6, Petitioner fails to show disclosure of the additional limitations of those claims in its prior art. And after a second attempt, Petitioner still has not proven that Gloor was prior art. The Challenged Claims should be found patentable.

II. Claim Construction

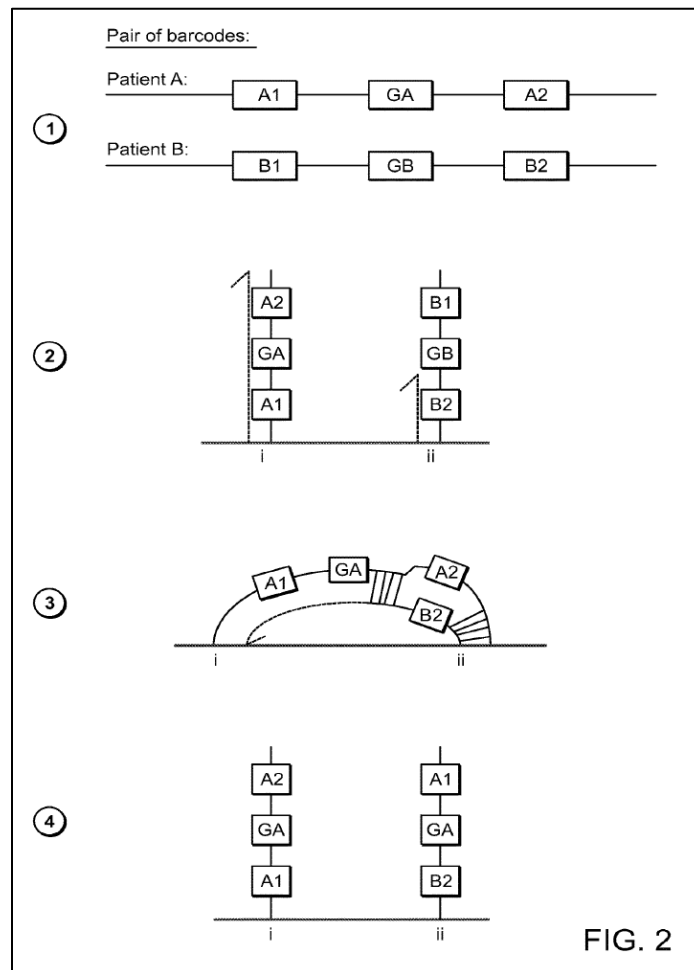
A. “Detecting the Presence Of Two Or More Identifier Sequences That Are Uniquely Associated With The Nucleic Acid Of Interest” (Claim 1).

The plain language of Claim 1 requires “[a] method for validating the sequence of a nucleic acid analyte of interest” that includes “detecting the presence of two or more *identifier sequences* that are *uniquely associated* with the nucleic acid analyte of interest.” EX1001, claim 1; POR, 13–15. Petitioner argues that claim 1 implies re-using the same identifier sequence with more than one sample, such that the identifier sequence is *not* uniquely associated with a sample. Reply, 2–4. Petitioner contradicts the plain language of the claims.

Petitioner's argument is that Claim 1 does not require the *identifier sequences* to be *uniquely associated* with the nucleic acid of interest, but instead requires that two or more sequences *together* be uniquely associated with the nucleic acid of

interest. That interpretation is contrary to the claimed method for “validating” nucleic acids. Under Petitioner’s construction, the claimed method would fail to validate sequences because the “validated” samples would include incorrectly identified samples that the claimed methods are aimed to eliminate.

As an illuminating example, Figure 2 of the ’200 Patent (reproduced below) depicts “how methods of the invention allow one to detect, during analysis, whether the integrity of a patient’s sample was maintained during a multiplex sequencing reaction.” EX1001, 4:64–5:1.



In Figure 2, the identifiers are uniquely associated with their respective sample, and the chimeric sequence formed due to improper molecular overlap is not validated because “A1 should not be seen together with barcode sequence B2,” as it is in the chimeric molecule. EX1001, 5:59-6:6 (“Thus during analysis, it is determined that the integrity of the patient’s sample was not maintained and the data can then be excluded from final analysis.”). But if nucleic acids from two samples (GA and GB) shared the same identifier sequence (A1) but had unique pairs of identifiers (for example, A1-A2 and A1-B2), then nucleic acids could be misassigned because the “A1-B2” chimeric molecule in Figure 2 would be assigned to a different sample. Thus, under Petitioner’s interpretation, sequences in a multiplex reaction would not be properly validated, and sequences could be misassigned to the wrong samples.

Petitioner’s justifications for its claim construction are not compelling. *First*, Petitioner argues that the specification states “[w]hile the pair of barcodes associated with any given template is unique, individual barcodes sequences can be used with multiple templates as long as no pair is used across two different samples.” Reply, 2; EX1001, 2:50-53. That language does not reflect the claimed configuration, in which the “*identifier sequences* [] are *uniquely associated* with the nucleic acid analyte of interest,” and thus does not reflect the proper interpretation of the claim. *Hill-Rom Servs., Inc. v. Stryker Corp.* 755 F.3d 1367, 1371–72 (Fed. Cir. 2014). Moreover, where the specification *does* track the claim language and describes

“identifiers [that] are unique to the sample,” it explains that incorrect pairs of those unique identifiers will be excluded so it is “possible to determine whether the integrity of the sample was maintained during a multiplex sequencing reaction, and prevent the assigning of sequence data to the wrong sample.” EX1001, 2:12-29.

Petitioner points to the “validating” step to argue that it excludes sequences containing only one identifier *or* an incorrect pair, and “[r]egardless of whether individual identifier sequences are used with multiple analytes, sequences ‘containing only one identifier’ can be identified and excluded, as was done in Gloor.” Reply, 3. The fact that Gloor excluded sequences with only one identifier does not change the interpretation of the claims, nor does it remove the claim’s requirement that incorrect pairs of identifier sequences also be excluded. *Sierra Wireless, ULC v. Sisvel, S.P.A.*, 130 F. 4th 1019, 1022 (Fed. Cir. 2025) (explaining two limitations are not mutually exclusive where “the premise describing the claim language” does not support such a conclusion). And the ’200 Patent explains that an “incorrect pair” of identifiers refers to a “pair or more of identifiers not associated with the same template.” EX1001, 2:23–24. If the claims were read with Petitioner’s understanding of the “detecting” limitation, then “incorrect pairs” would not appear in the validation step because nucleic acids with “incorrect pairs” of identifiers would instead be validated and incorrectly assigned to the wrong sample—the sample for which the pair of identifiers appear “correct.” Such an interpretation

effectively reads the phrase “or an incorrect pair of identifiers” out of the claim and cannot be correct.

Second, Petitioner asserts that the claimed excluding “incorrect pairs of identifiers” includes sequences where the “identifier sequence [itself] contain[s] a truncation, insertion, or erroneous base.” Reply, 3. That explanation is not based on anything in the ’200 Patent and is contradicted by the specification, which explains that incorrect pairs of identifier sequences are pairs of identifier sequences that are associated with the wrong sample, not instances where the identifier itself contains an error (e.g., a mutation). EX1001, 2:23-24 (“[T]emplate 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.”), 5:65-6:6 (identifying an “invalid barcode sequence pair” because “barcode sequence A1 should not be seen together with barcode sequence B2 and hence a crossover has occurred”), Figure 2.

Third, Petitioner offers a new theory that incorrect pairs of identifiers could be excluded when “combinations of identifiers are not saturated,” but that theory is again not based on the ’200 Patent. Indeed, Petitioner cites no support in the specification or claims for that description. Reply, 3. And Petitioner acknowledges that “the claims are silent on the specific number of identifier or analytes used and the ratio between the two,” yet relies on a strained interpretation that would require specific numbers or ratios to create “sparse” indexing to validate sequences. *Id.* That

interpretation finds no basis in the claim language. Instead, the plain language of claim 1 requires that the “identifier sequences”—not just their pairing—be “uniquely associated” with the nucleic acid of interest, consistent with Patent Owner’s interpretation.

III. Ground 1: The Challenged Claims Are Patentable Over Gloor.

A. Claim 1 Is Patentable Over Gloor.

Petitioner does not dispute that Gloor used the same individual identifier sequences across multiple different samples. Reply, 2, 4. Therefore, under the proper interpretation explained above in Section II, Gloor cannot anticipate claim 1 because it does not disclose “detecting the present of two or more *identifier sequences that are uniquely associated* with the nucleic acid analyte of interest.” POR, 13-15.

B. Claim 5 Is Patentable Over Gloor.

Gloor does not disclose “t[he] barcode sequences are *designed to* have no homopolymer regions,” as recited in Claim 5. Dr. Spellman attempts to fill the gaps in Gloor’s disclosure by calculating a chance of all barcodes having no homopolymer regions. See EX1054, ¶24. But that is not what the claim requires. And as Dr. Spellman admits, Gloor’s selection of barcodes was not random; Gloor “*chose sequence tags to ensure all 4 nucleotides were represented in each of the first four positions* of the primers.” EX1005, 5; EX1054, ¶22. Dr. Spellman’s

calculations do not account for the first four nucleotide positions representing all four available nucleotides.

The words “designed to” in claim 5 require a narrower read of the limitation than that imparted by Petitioner, under which the claim could be met by mere chance. *See, e.g., In re Giannelli*, 739 F.3d 1375, 1379-80 (Fed. Cir. 2014) (citing *Aspex Eyewear, Inc. v. Marchon Eyewear, Inc.*, 672 F.3d 1335, 1349 (Fed. Cir. 2012)) (holding that “the mere capability [of performing a function] is not the inquiry that the Board should have made”). Petitioner tacitly acknowledges as much with its later argument (which fails for other reasons discussed below) that Parameswaran meets this claim based on its alleged intended design “with care taken to avoid homopolymeric runs.” Reply, 7-8; EX1054, ¶36. There is no discussion of any consideration of homopolymers in Gloor for its barcode design, nor has Petitioner argued any such design was inherent. Accordingly, Gloor does not anticipate claim 5.

C. Petitioner Has Not Shown That Gloor Was Publicly Available (All Claims)

Petitioner attempts to backfill its Petition with new evidence regarding Gloor’s public availability—an issue on which Petitioner bears the burden—with no justification for not submitting the evidence with the Petition. Reply, 26-29. The new evidence and arguments should be disregarded. *Intelligent Bio-Sys., Inc. v. Illumina*

Cambridge Ltd., 821 F.3d 1359, 1369 (Fed. Cir. 2016). But even with the new evidence, Petitioner does not meet its burden to establish Gloor as prior art.

First, despite the Board twice telling Petitioner what evidence might establish public availability, Petitioner has twice failed to provide such evidence. Paper 7, 9 (explaining testimony from a “custodian with any ***personal knowledge*** of arXiv.org or its regular practices” might show publication); Paper 16, 6 (explaining the “alleged email arXiv.org sent to subscribers on July 29, 2010” might show publication). Petitioner has not provided such evidence.

Despite the Board’s express invitation, Petitioner still has not provided any email announcement of Gloor. Instead, Petitioner latches onto a suggestion that testimony from Archive.org could help for public accessibility. Paper 16, 5 (citing *Murray & Poole Enters. LTD v. Institut De Cardiologie De Montreal*, IPR2023-01064, Paper 49 (PTAB Jan. 14, 2025)). In *Murray*, Archive.org was used to retrieve webpages actually containing the publication in question. *Murray*, IPR2023-01064, Paper 49, 22–24. Here, Petitioner does not provide any archived webpages of the publication or webpages containing the alleged publicly available link. Reply, 26–29. Petitioner instead cites Archive.org webpages allegedly describing arXiv’s publication policy in 2010, but does nothing to establish that policy was actually followed. Reply, 26–27; EXS1047–1049.

Second, Petitioner’s reliance on *Align Tech.* is misplaced. Petitioner argues that “arXiv.org’s publication policy at [the] time of submission [is] sufficient to establish [the] publication date.” Reply at 27 (citing *Align Tech., Inc. v. Dental Monitoring SAS*, IPR2023-01369, Paper 42 at 32 (PTAB Mar. 3, 2025)). But in *Align Tech.*, the Board had additional evidence absent from the record here: (1) archived webpages containing links to the arXiv.org publication and (2) metadata associated with the arXiv.org publication corroborating the alleged priority date. *Align Tech.*, IPR2023-01369, Paper 42 at 31-32. As discussed above, Petitioner does not provide any such evidence for Gloor. Petitioner instead stretches with a claim that Gloor was publicly available based on a chat forum, but does not provide an archived webpage for the forum to authenticate or establish the availability of the forum beyond Dr. Gloor’s alleged recollection. EX1052; *Align Tech.*, IPR2023-01369, Paper 42 at 31; EX1046, ¶18; EX1049. Furthermore, unlike the declaration in *Align Tech.* that authenticated metadata for the publication on arXiv.org, Petitioner fails to provide any metadata here to corroborate the alleged publication date.¹ *Align Tech.*, IPR2023-01369, Paper 42 at 31-32.

¹ Review of the metadata associated with the PDF downloaded from the link (<https://arxiv.org/abs/1007.5075>) provided in Dr. Gloor’s declaration actually

Third, Petitioner submits a second declaration from Dr. Gloor and a present-day search for Gloor on arXiv.org as evidence that Gloor was publicly available by July 2010. Reply, 27-28; EX1046; EX1051. But that search does not confirm the actual date of the alleged announcement nor what the announcement contained. EX1051; EX1002, Ex. F. Importantly, Petitioner does not provide archived records of any arXiv.org search page containing Gloor at the time of the alleged publication date. EX1049; EX1051.

Fourth, conflicting exhibits highlight the need for corroborative evidence. Although Petitioner argues Gloor became publicly available on July 29, 2010, Petitioner at other times admits Gloor might not have been publicly available on July 29, 2010, with exhibits explaining Gloor was not publicly available on July 29 but suggesting a potential July 30 date. *Compare* EX1002 ¶¶16–18; Petition, 11; Reply, 26-27; EX1039, 6; *with* EX1050, 1. The uncertainty about Gloor’s public availability date, including from arXiv.org itself, shows that the records do not conclusively establish a public availability date and exemplifies the need for corroborating archived evidence (such as metadata, an email announcement, an archived webpage, or a declaration from an employee at arXiv.org). Despite Petitioner’s hearsay

suggests the PDF at that link was created in November 2018. EX1002, ¶¶16–17, 19; EX1046, ¶18.

assertion that Gloor’s availability has been confirmed by arXiv.org database records (Reply, 27), without the records themselves for corroboration, Petitioner cannot show that Gloor was publicly available prior to December 23, 2010.

IV. Ground 2: The Challenged Claims Are Patentable Over Parameswaran.

A. Claim 1 Is Patentable Over Parameswaran.

Petitioner argues that Parameswaran’s “characterization” of mismatched barcode motifs discloses excluding sequences with an incorrect pair of identifier sequences because Parameswaran “greyed out” sequences in its data tables and labeled them as “false discoveries.” Reply, 5–6. But Parameswaran never discloses excluding such sequences, as discussed in more detail below in Section V.A. Parameswaran only discarded sequences having no perfect matches to either barcode, which Dr. Spellman admits are not represented by the greyed out boxes. EX1004, 8 (discarding “sequences [that] had no perfect matches to *any of the sequence motifs*”); EX2015, 110:18–111:4. And Parameswaran explicitly includes “*all sequences with a match to one of the two expected sequence motifs*” in its analysis. EX1004, 8.

B. Claims 5 And 6 Are Patentable Over Parameswaran.

Parameswaran does not disclose “t[he] barcode sequences are *designed* to have no homopolymer regions,” as recited by Claim 5. Dr. Spellman admitted that *every* barcode in Parameswaran includes homopolymer regions (EX2002, 67:3-18),

and that they were specifically “designed” to include their repeated nucleotides, which are homopolymers (EX2015, 111:14-113:11). Petitioner now relies on a statement in Parameswaran about avoiding “homopolymeric runs” (Reply, 7-8) but that is different than the claimed “homopolymer regions,” which includes the dinucleotide homopolymers present in *every* barcode in Parameswaran. POR, 25.

Parameswaran also does not disclose “the nucleic acid analyte of interest is on the surface of a flow cell,” as recited in Claim 6. Petitioner argues that claim 6 “does not require the analyte to physically contact the flow cell surface,” but does not dispute that the analyte of interest is suspended above the surface of the flow cell in pyrosequencing. Reply, 8. Petitioner’s interpretation is contradictory to the plain claim language—a nucleic acid “suspended above” a surface is not “on” the surface. POR, 26-27. Accordingly, Parameswaran does not anticipate claim 6.

V. Ground 3: The Challenged Claims Are Patentable Over Parameswaran And Gloor.

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

1. Chimeric Sequences Arising In Illumina Sequencing Were Not A Known Problem, And Parameswaran’s Methods Did Not Address That Problem.

Petitioner continues to overstate the prior art, including a host of new references submitted with the Reply, to strain for support for the incorrect assertion that errors, including chimeric sequence issues, were widely known in Illumina sequencing and addressed by Parameswaran. Reply, 9-15. Petitioner notes that

Patent Owner's Response discussed "chimeras" and "cross-over," just as the '200 Patent does in describing its invention, but notes that these are not claim limitations. *Id.*, 9. Chimeras and errors they cause are referred to in the Petition as an allegedly known problem motivating prior art combinations (*e.g.*, Petition, 6-7, 19, 49), so Patent Owner's arguments addressed them in response. And while Petitioner faults Patent Owner for failing to address "numerous prior-art references disclosing the problem of chimeras arising during Illumina sequencing" (Reply, 10), the references on which Petitioner now focuses were *never* discussed, cited, or submitted prior to Petitioner's Reply. Reply, 10-11 (citing EX1057, EX1061, EX1066 and related opinions from Dr. Spellman (EX1054), all submitted with the Reply). It was Petitioner's burden to establish obviousness in its Petition, including establishing prior awareness of chimeric sequences in Illumina sequencing, which was a central component for Petitioner's obviousness rationale. Petitioner's late attempt to backfill the Petition on that issue is improper and deprives Patent Owner of the opportunity to address the new evidence with expert testimony. The Board should decline to even consider the new arguments and evidence regarding the alleged awareness of chimeras in Illumina sequencing. *Intelligent Bio-Sys.*, 821 F.3d at

1369. But even Petitioner’s new arguments fall far short of rebutting Patent Owner’s evidence showing a lack of such awareness. POR, 32-46.²

First, Petitioner relies heavily on Campbell (EX1057), which Petitioner incorrectly characterizes as describing widespread awareness of chimeric sequences in Illumina sequencing. Reply, 10 (claiming Campbell described “nearly 1.2 million erroneous chimeric sequences”). But Campbell contains no such description. Campbell never once mentions a chimera or a chimeric sequence. *See generally* EX1057. Instead, Campbell merely stated that it identified reads that “suggested a possible rearrangement.” EX1057, 2. Petitioner does not explain how a POSA would understand such discussion of “rearrangements” to refer to chimeric sequences or cross-over error that they can cause. Reply, 10-11; EX1054, ¶¶52-56. Nor is there any explanation how any of the particular rearrangements listed in Campbell—including “deletions,” “duplications,” and “inversions” (EX1057, 3 (Table 1))—

² Petitioner also takes a half-hearted shot at Dr. Adalsteinsson’s credentials. Reply, 9-10. Petitioner does not seriously dispute Dr. Adalsteinsson’s expertise for the technology and prior art at issue here. Dr. Adalsteinsson explained his intimate knowledge of NGS and Illumina sequencing from his years of experience, including his PhD work at MIT and his ten years as a researcher at the preeminent Broad Institute. EX2001, ¶¶5-9, 21-23; EX1055, 11:19-12:11.

could be a chimera. To the contrary, Dr. Spellman explained such rearrangements by giving examples of removed nucleotides (including as few as one nucleotide removed) and repetition of short sequences in a gene, which have no connection to chimeric sequences created from template nucleic acids derived from different samples. EX2015, 50:6-17, 51:11-53:8, 55:1-56:4.

Petitioner's read of Campbell as describing rampant chimera errors on Illumina platforms also conflicts with a linchpin of Petitioner's obviousness rationale: that Illumina sequencers were much better and more accurate, allegedly motivating their use in any sequencing application. Petition, 49-51. Specifically, even after applying various quality filters to obtain data for analysis, the Illumina sequence reads in Campbell (according to Petitioner's interpretation) included *an error caused by chimeric sequences in over 1-out-of-10 sequences*. As Dr. Spellman explained, Campbell obtained 36.2 million reads, removed 18.8 million reads in a quality screen, removed 7.2 million reads in a duplicate screen, and removed 1.2 million reads near telomeres or centromeres, leaving 10.4 million reads for analysis. EX1057, 2; EX2015, 88:6-89:18. Thus, if Petitioner's unsupported interpretation were correct it would mean *nearly 11.5% of the analyzed sequences after quality screening* (1.2 million / 10.4 million) on an Illumina platform in Campbell would be chimeric sequence errors created by the platform itself. EX2015, 93:3-94:2. That would be a staggering number of false molecules created by the

sequencer for a platform that supposedly increased accuracy dramatically to motivate its use for any sequencing application, and it highlights Petitioner's mischaracterization of Campbell. Campbell instead explained only that "incorrect mapping induced by sequencing errors"—*not* chimeric sequences—were responsible for its sequences that "suggested a possible rearrangement." EX1057, 2. That explanation comports with Dr. Spellman's understanding that the reference genome has evolved with increased scientific knowledge over time, and at the time of Campbell was limited because it was "just one haplotype," leading to mapping errors from the incomplete reference genome. EX2015, 76:22-78:13, 79:6-20.

Second, Petitioner cites new references discussing "artifacts" in Illumina sequencing and mischaracterizes them as evidencing awareness of erroneous chimeras created by the platform. Reply, 10-12. Petitioner fails to support its misreading.

Petitioner cites Varela (EX1066), which shares multiple authors with Campbell, as allegedly disclosing chimeras and artifacts formed within a cluster. Reply, 10. But Varela never mentions chimeras. *See generally* EX1066. And like Campbell, Varela never attributes its artifacts to chimera formation but instead posits they may be due to "*intramolecular* rearrangement" (which cannot be a chimera of two *different* molecules) or "mispriming" of the oligonucleotide used for sequence reading (which occurs *after* amplification on the flow cell). EX1066, 10.

Petitioner cites Maher (EX1061) as allegedly “reporting artificial chimeric sequences during Illumina sequencing,” noting they occur in “close proximity on the flow cell.” Reply, 1, 10. But Maher detects **biological** chimeras (i.e., those naturally present in a sample when collected) as potential cancer markers. EX1061, 1. The artifacts in Maher refer to artifacts arising from the optical detection in the sequencing, **not** to artifacts created by artificial chimeric molecules. Specifically, Maher explains that the artifacts are “identical mate pair reads.” *Id.*, 8. Those are not chimeric sequences, and Maher never says they are. Instead, the identical mate pair reads refer to “optical duplicates,” which occur when one cluster seeded by one initial nucleic acid is falsely detected as two separate clusters having identical sequences during the analysis pipeline. Indeed, Maher describes them as merely “**duplicates** [that] were likely an artifact of the **analysis pipeline**.” *Id.* And Dr. Spellman admitted that is “the likely reading of this section” in Maher. EX2015, 122:16-123:6; *see also id.*, 28:22-29:12, 30:16-20, 124:17-125:10.

Petitioner cites Whiteford (EX1067) to incorrectly suggest that its reference to “mixed clusters” describes chimeras on an Illumina flow cell. Reply, 11. But that reference again refers only to artifacts arising from the optical detection in the sequencing, **not** to artifacts created by artificial chimeric molecules. As Dr. Spellman admitted, a “mixed cluster” is a situation where two separate clusters—each having their own separate sequences, **not** a chimeric sequence—overlap and are potentially

detected as one cluster in the analysis pipeline. EX2015, 26:2-27:3, 30:21-31:5. Such mixed clusters were identified by optical filtering and are unrelated to chimeras or the cross-over error discussed in the '200 Patent. *Id.*, 46:6-48:11 (discussing “purity filters” in Bentley (EX1006) identifying mixed clusters).

Third, Petitioner cites new references for the unremarkable principle that chimeric sequences could form during standard PCR (not during a flow cell sequencing workflow), for example by “jumping PCR.” Reply, 10-12. Petitioner cites a single line from Gorzer (EX1060), which merely explains that chimeras can form within a single sample during “initial PCR” prior to pooling or sequencing, which is **not** the cross-over error discussed throughout the '200 Patent. EX1060, 1. The single line Petitioner cites from Smyth (EX1063) similarly repeats old notions of jumping PCR during initial PCR of a single sample. EX1063, 1. And Petitioner re-hashes its assertions of older PCR references (Reply, 11-12 (citing EXS1017-1020)), but provides no new color to address the dearth of evidence of awareness in the field—either from Illumina or anyone else—of cross-over error and chimera formation on flow-cell based sequencing platforms prior to December 2010. POR, 36-42; EX2001, ¶¶115-125.

Petitioner sidesteps strong evidence that cross-over error was not appreciated on Illumina sequencers until after December 2010 by calling them “post-priority references” (Reply, 12-13 (citing EXS2003-2004)), but those references illuminate

Petitioner’s mischaracterizations of the prior art. POR, 3-4, 7, 41-42. EX2004—published a month after the ’200 Patent—remains the earliest evidence of anyone other than the inventors recognizing the problem of cross-over error on Illumina sequencers. And while the authors of EX2004 acknowledged pre-NGS references discussing chimeras during “initial PCR” (e.g., EXS1017-1020), the authors explained that others—including Illumina—had not yet realized that chimeras created during flow cell amplification could lead to errors in the workflow, which the authors sought to reduce with double indexing. EX2004, 2 (characterizing their realization months after December 2010 as “a *new* double-indexing method”). EX2003 also remains the earliest evidence of *Illumina* finally acknowledging the potential for crossover error on its NGS machines. Petitioner notes Illumina’s claim that index hopping was a known issue “from the time multiplexing was developed” (Reply, 12 (citing EX2003, 1)), but tellingly the only citation provided for that claim *is to the 2011 Kircher publication (EX2004)*, further stressing that neither Illumina nor researchers were aware of the issue at the earlier time of the ’200 Patent invention. EX2003, 1 (citing reference 2 (EX2004)). Petitioner’s citation to EX1015 does not show otherwise, as it merely points to another example of discussing “jumping PCR” in initial amplification prior to loading onto a flow cell. EX1015, 9.

Fourth, Petitioner again stretches the disclosures of Parameswaran to pitch it as a solution to the unrecognized problem of cross-over error. Reply, 13-15.

Petitioner still fails to rebut Patent Owner's showing that Parameswaran's mismatched sequences merely reflect pyrosequencing-induced sequencing error that Parameswaran claimed to address, *not* cross-over error from chimeric sequences created during the sequencing workflow. POR, 32-35, 38-40. Indeed, Dr. Spellman previously agreed that "the large numbers of perfect unexpected barcode motifs that were found were the result of *sequencing errors, not barcode swapping*." EX2002, 168:6-17 (Dr. Spellman stating "that is a likely interpretation, yes"). Petitioner now takes the opposite position, claiming that "'sequencing error' was not the likely source of error" without addressing the contradiction between that assertion and Dr. Spellman's prior explanation. Reply, 15. Petitioner now makes that assertion based on certain reverse complement barcodes (Reply, 14-15), but Dr. Spellman admitted he did not consider those barcodes and how they might have contributed to errors in Parameswaran in forming his opinions. EX2002, 170:17-22. And even now, Petitioner does not explain how its new interpretation of the issues arising from those barcodes would be understood to be the cross-over error discussed throughout the '200 Patent. Reply, 14-15.

2. Petitioner's Rebuttals Fail To Fill The Gaps In The Proffered Motivation To Apply Parameswaran's Method To Gloor's Illumina Sequencer.

Petitioner offers scattershot rebuttals to five "alleged reasons" undermining motivation to combine. Reply, 15-20. Petitioner's responses fail to overcome Patent

Owner's showing that, on balance, the evidence shows a lack of motivation to combine. *Arctic Cat Inc. v. Bombardier Recreational Prods. Inc.*, 876 F.3d 1350, 1363 (Fed. Cir. 2017).

First, Petitioner attempts to dismiss the significant differences between pyrosequencing and Illumina sequencing. Reply, 16-17. Petitioner's reliance on Pfreder (EX1062) ignores that Pfreder's reference to "a tagging strategy" said nothing of incorporating unique dual indexing. EX1062, 11. Indeed, in addition to citing Parameswaran, Pfreder cites two Meyer papers that required only **one barcode** for assignment, just as Parameswaran did. *Id.* (citing Meyer 2007 (EX2010) and Meyer 2008 (EX1014)); POR, 23–25, 41–42. Petitioner's string cite quotes from EX1056 and EX1068 (Reply, 16) fare no better, as they merely discuss incorporating "barcoded amplicon primers" or "a barcoding technique," with no discussion of requiring dual barcodes or their significance. Petitioner similarly twists Fuller (EX1059), which discusses Parameswaran's sequence tags and ensemble-based sequencing generally, but says nothing about the applicability of Parameswaran's disclosures to Illumina sequencers or adapting Parameswaran's pyrosequencing-tailored approach for unique dual indexing on an Illumina machine. EX1059, 3.

Second, Petitioner downplays Gloor's warnings against pyrosequencing approaches by claiming that barcodes could be adapted for Illumina sequencing. Reply, 17-18. But even if true, that assertion does not show why a POSA would be

motivated to make such adaptation in view of the explicit statements in Gloor that pyrosequencing approaches like Parameswaran’s are “often unsuitable” for the Illumina sequencers in Gloor. POR, 29-30; *Arctic Cat*, 876 F.3d at 1363.

Third, Petitioner mischaracterizes the strong urging in both Parameswaran and Gloor to pursue **combinatorial** indexing as “mere existence of an alternative” or a “common sense” application. Reply, 18-19. Both references pitch CDI as much more than a mere alternative or choice. Instead, both conclude that CDI is the approach to use because of its lower cost and higher efficiency, along with their view that the problem of mismatched barcodes was so small (based on their data sets) that it was not an issue in their analysis, evidencing a complete lack of appreciation for the benefit of UDI approaches in Illumina sequencing. POR, 30-31; EX2001, ¶¶106-108; *Medtronic, Inc. v. Teleflex Innovations S.a.r.l.*, 69 F.4th 1341, 1351 (Fed. Cir. 2023).

Fourth, Petitioner rehashes its argument that Parameswaran’s mention of Illumina sequencers would have motivated their use. Reply, 19-20. Petitioner fails to address the point that Parameswaran referred only to its individual barcode designs—and not its unique dual indexing approach as a whole—as potentially applicable to other platforms. EX1004, 8. And Petitioner still fails to explain why mere consideration of what a POSA **could** do with different platforms shows what a POSA **would** have been motivated to do. POR, 42-44.

Fifth, Petitioner defends its “obvious to try” rationale (Reply, 20), but bases its assertions on the flawed premise that the problems of chimeric sequences and cross-over error were well known to a POSA. That premise is incorrect for the reasons discussed above in Section V.A.1. And Petitioner’s assertion that moving to an Illumina sequencer “was a natural progression” again fails to appreciate that Parameswaran explicitly designed its methods (at a time when Illumina sequencing already existed) to be *pyrosequencing-tailored* instead of simply using an Illumina platform. EX1004, 1.

3. Petitioner Does Not Overcome The Evidence Showing That A POSA Would Not Have Incorporated Parameswaran’s UDI Methods.

Petitioner downplays the significant downsides a POSA would appreciate when considering Parameswaran’s methods in view of Gloor. Reply, 21-22. But Petitioner cannot avoid the evidence Patent Owner provided (POR, 32-35, 44-45), including the supporting opinions of Dr. Adalsteinsson, to salvage a motivation to combine the prior art.

Petitioner misunderstands the point in comparing Gloor’s 272 samples to Parameswaran’s 48 samples. Reply, 21. This is not an issue of two references having different sample numbers; the point is *however many* samples are being studied (whether Gloor’s 272 or Parameswaran’s 48), implementing UDI as Petitioner urges would require designing and synthesizing significantly more barcodes. That is

exactly the point Patent Owner made, highlighting that Gloor achieved its CDI approach using 34 unique barcodes for its 272 samples, but would have been required to design and synthesize *16 times more* barcodes (544 total) to implement a UDI approach *for the same 272 samples*. POR, 33; EX2001, ¶¶110-112. Petitioner’s additional point that there are other considerations, for example “clinical misdiagnosis” again fails to appreciate that both Parameswaran and Gloor found that potential errors were so minimal and inconsequential that they urged *CDI approaches* over UDI approaches. POR, 30-34. And Petitioner’s claim that the heightened barcode synthesis requirements were “a suitable option” even if not “the best option” (Reply, 22) similarly fails to explain why Parameswaran would be seen as “suitable,” particularly in view of Parameswaran urging a move towards combinatorial indexing, like that already employed in Gloor.

Petitioner also incorrectly claims that researchers “praised” Parameswaran’s UDI approach (Reply, 21-22), but provides no evidence of praise. Instead, Petitioner merely repeats its mischaracterization of Fuller (EX1059) as lauding Parameswaran’s UDI, which it does not for the reasons explained above in Section V.A.2.

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

Petitioner incorrectly dismisses Patent Owner's arguments regarding reasonable expectation of success as " cursory." Reply, 22. Petitioner provides no further explanation how a POSA would have reasonably expected to successfully validate sequences as claimed, and its showing on reasonable expectation of success still fails. POR, 45-46.

C. Claim 5 Is Patentable Over Parameswaran And Gloor.

For the same reasons explained in Sections III.B and IV.B, claim 5 is not rendered obvious by Parameswaran and Gloor. POR, 46-47.

D. Petitioner Has Not Shown That Gloor Was Publicly Available.

Petitioner fails to establish Gloor as prior art for the reasons explained above in Section III.C, and Ground 3 fails for the same reasons.

VI. Ground 4: The Challenged Claims Are Patentable Over Parameswaran And Binladen.

A. Claim 1 Is Patentable Over Parameswaran And Binladen.

Petitioner's arguments (Reply, 24-25) fail to overcome Patent Owner's showing that (1) this combination does not disclose all claim limitations and (2) a POSA would not have been motivated to make the combination. POR, 47-49, 52-54. *First*, Petitioner points out that Binladen *sequences* both barcodes (Reply, 24), but that does not show "*analyzing* both identifiers and excluding..." as claimed.

Indeed, Binladen includes sequences based on only *one primer sequence*, not by analyzing both sequences. POR, 47-48; EX2001, ¶¶144-145. *Second*, Petitioner claims that Parameswaran's disparagement of Binladen somehow motivated a combination. Reply, 24-25. But Petitioner's new proposal to use Parameswaran's longer barcodes to improve Binladen now runs directly contrary to Binladen's reasons for using short barcodes in the first place in view of its limited read length capability. POR, 53-54; EX2001, ¶¶152-154.

B. Claims 5 And 6 Are Patentable Over Parameswaran And Binladen.

For the reasons explained in Section IV.B, Parameswaran does not disclose claim 5. Binladen cannot fill the gaps left by Parameswaran's disclosure because the lack of homopolymeric sequences in *some of* Binladen's barcodes does not overcome that other barcodes in Binladen were *designed to* include homopolymer regions. POR, 49-51.

Both Parameswaran and Binladen employed pyrosequencing, which does not meet Claim 6 for the reasons explained in Section IV.B. POR, 51-52. Claim 6 thus is not rendered obvious by Parameswaran and Binladen.

VII. Ground 5: Claim 6 Is Patentable Over Parameswaran, Binladen, And Bentley.

For the same reasons explained in Sections V and VI, there is no motivation to combine Parameswaran with Binladen and Bentley. POR, 54. And for the same

reasons explained in Section IV.B, claim 6 is not rendered obvious by Parameswaran, Binladen, and Bentley. POR, 54.

VIII. Conclusion

For the foregoing reasons, the Challenged Claims are patentable.

Dated: August 11, 2025

Respectfully submitted,

/Brian D. Matty/

Brian D. Matty (Reg. No. 70,358)

DESMARAIS LLP

230 Park Avenue

New York, NY 10169

Telephone: 212-351-3400

Facsimile: 212-351-3401

Email: bmatty@desmaraisllp.com

*Counsel for Patent Owner Molecular Loop
Innovations LLC*

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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 5,591 words as counted by Microsoft Word 365, the word-processing system used to prepare this paper.

Dated: August 11, 2025

Respectfully submitted,

/Brian D. Matty/

Brian D. Matty (Reg. No. 70,358)

DESMARAIS LLP

230 Park Avenue

New York, NY 10169

Telephone: 212-351-3400

Facsimile: 212-351-3401

Email: bmatty@desmaraisllp.com

*Counsel for Patent Owner Molecular Loop
Innovations LLC*

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), the undersigned certifies that on August 11, 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:

William B. Raich (Reg. No. 54,386)
Charles T. Collins-Chase (Reg. No. 78,019)
Finnegan, Henderson, Farabow, Garrett & Dunner, LLP
901 New York Avenue, NW
Washington, DC 20001-4413
Tel: 202-408-4210
Fax: 202-408-4400
william.raich@finnegan.com
charles.collins-chase@finnegan.com

Dated: August 11, 2025

Respectfully submitted,

/Brian D. Matty/
Brian D. Matty (Reg. No. 70,358)
DESMARAIS LLP
230 Park Avenue
New York, NY 10169
Telephone: 212-351-3400
Facsimile: 212-351-3401
Email: bmatty@desmaraisllp.com

*Counsel for Patent Owner Molecular Loop
Innovations LLC*