

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 SUR-REPLY

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<i>Murray & Poole Enters. LTD v. Institut De Cardiologie De Montreal</i> , IPR2023-01064, Paper 49 (PTAB Jan. 14, 2025)	24
<i>On Demand Machine Corp. v. Ingram Industries, Inc.</i> , 442 F.3d 1331 (Fed. Cir. 2006)	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 via 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 overstates the prior art, leveraging an overly broad interpretation of the claimed "cross-over error" to paint it as a widely appreciated issue at the time of the invention. But under any interpretation, the problem from chimeric sequences formed in Illumina flow cell sequencing workflows was not realized until after the '852 Patent. Petitioner fails to establish a motivation to combine to address that unknown problem, or a reasonable expectation of success in doing so. Petitioner's shifting assertions regarding the claimed "discarding" step additionally fail. And Petitioner still has not proven that Gloor was prior art. The Challenged Claims should be found patentable.

II. Claim Construction

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

Petitioner argues for a broad interpretation of "cross-over error" but undermines its own construction. Petitioner first chides Patent Owner for adding context to the claim language, but Petitioner's construction adds incomplete context, improperly broadening the claim. Petitioner then argues that including "error" in the construction would be "repeating" a word, but Petitioner's proposed construction eliminates the idea of "error" entirely.

The main dispute is the source of the claimed cross-over error, and Petitioner argues four alleged contradictions in Patent Owner's constructions. Reply, 3-5. None of Petitioner's arguments has merit.

First, Petitioner argues that because the claimed method is for “reducing template cross-over error introduced during sequencing workflow,” the claim must cover error introduced at any point in the sequencing workflow. But the claim language is consistent with Patent Owner's construction, as the claims require combining nucleic acid templates into a single sample and then “amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters, at least one of which comprises a chimeric sequence.” EX1001, claim 1. Even Petitioner's proposed construction, requiring chimeric sequences to be formed from template nucleic acids from *different* samples, acknowledges that the claimed chimeric sequence could not be formed prior to the “combining” step (i.e., when different samples are combined). The claim further requires that the chimeric sequence be present in clusters on the flow cell. Thus, the *only* step during which chimeras could form between two nucleic acids from different samples is during the amplifying step,

and Petitioner's argument that the claim "does not require that chimeric sequences are 'created' during cluster amplification" is belied by the claim language itself.¹

Petitioner argues that the plain language of the claims does not require chimeras "created" during cluster amplification to imply that Patent Owner improperly limits the claims. Reply, 3-4. But even Petitioner's proposed construction shows that Patent Owner's proposed construction is **not** an improper narrowing exercise, but rather a clarification of scope consistent with claim language and specification. Acknowledging that the term "cross-over error" requires construction, Petitioner proposes clarifying that the cross-over error is from chimeras formed from two different nucleic acid templates. Petitioner reasons that

¹ Petitioner selectively cites deposition testimony out of context. Reply, 3-4. Dr. Adalsteinsson actually refutes Petitioner's argument. Dr. Adalsteinsson distinguished chimeras giving rise to claimed cross-over error from "other" chimeras. EX1055, 91:18-92:5 ("The claims of the '852 patent are very clearly directed at reducing template cross-over error, which is the error from the chimeric sequences that arise during cluster amplification on the surface of the flow-cell. It's possible that other chimeras can be formed and sequenced, too, ***but the method of the invention -- or, rather, the invention is directed at reducing template cross-over error, and that's what's reflected in the claims***").

“[t]he specification mentions the term ‘cross-over error’ four times, each describing the formation of a chimeric molecule made from template nucleic acids derived from different samples.” Petition, 22. Petitioner ignores that in each of the specification quotes Petitioner relies on for its construction, and in every figure in the patent, the chimeric molecule also forms *during cluster amplification*. EX1001, 5:41-6:6 (cross-over arises from “improper molecular overlap” during cluster amplification), 6:53-7:38 (cross-over arising when molecules are “hybridized, forming a bridge” during cluster amplification). Indeed, the ’852 Patent repeatedly explains that the “chimeric molecule [was] formed *in this amplification reaction*.” *Id.*, 7:20-23.

Second, Petitioner’s proposed construction improperly broadens the claim language by including only one of the two qualities defining every instance of cross-over error in the ’852 Patent. *On Demand Machine Corp. v. Ingram Industries, Inc.*, 442 F.3d 1331, 1340 (Fed. Cir. 2006) (reversing construction in view of specification’s consistent use of a term, finding “claims cannot be of broader scope than the invention that is set forth in the specification”). Patent Owner’s construction accurately includes the full context of those examples Petitioner cites. *See* Paper 18 (“POR”), 13; EX2001, ¶¶23-30.

Petitioner does not dispute that every example of cross-over error in the specification arises from chimeras formed during amplification. Petitioner ignores that fact (which under the logic it used for its own construction—that every example

of cross-over error forms from templates derived from two different samples—should prevail) and instead argues that the specification “identifies cluster amplification as merely one possible theory for chimera formation.” Reply, 4 (citing EX1001, 6:25-29). But Petitioner once again omits the context of its quote. Read in full, the nonlimiting statement refers to the biochemical method by which the chimeric molecule forms on a flow cell, not the stage of sequencing during which it forms. EX2001, ¶29; EX2002, 100:7-10 (Dr. Spellman describing the heteroduplex described in the specification is one way of biochemically getting to a chimeric sequence, among others). Indeed, the same example in the specification states in no uncertain terms that chimeric sequences “formed early *in the amplification process*.” EX1001, 6:25-52.

Third, Petitioner argues that requiring amplification “on the surface of a flow cell” during prosecution did not narrow the claims to errors arising from said amplifications. Reply, 4-5. Petitioner once again ignores the context. The Applicant amended the claims such that the entire method revolves around pooling samples and amplifying them on the surface of a flow cell. EX1007, 304 (distinguishing the claims from methods of pyrosequencing, with different amplification methods). The novelty relates to identifying and reducing specific problems that arise during the claimed amplification step *on the surface of the flow cell*, which is when clusters form. Accordingly, Patent Owner’s construction is consistent with the prosecution

history in that it too brings in the context of the error arising during cluster amplification. POR, 18; EX2001, ¶¶25-30, 42-48.

Fourth, Petitioner argues that general knowledge in the art was that “chimeric sequences made of template samples could arise at many stages during sequencing, not just cluster amplification on a flow cell.” Reply, 5. But Petitioner’s contention of what was known about chimeras generally does not govern what the claimed “cross-over error” is in the context of the claims and specification of the ’852 Patent. EX1003, ¶26 (Dr. Spellman stating it was well known that chimeric sequences, *including those resulting from cross-over errors*, were problematic); EX2002, 84:3-85:9 (Dr. Spellman explaining he would not consider certain types of chimeric molecules to cause the claimed cross-over error); EX1055, 91:18-92:5 (“It’s possible that other chimeras can be formed and sequenced, too, but the method of the invention -- or, rather, the invention is directed at reducing template cross-over error, and that’s what’s reflected in the claims.”). In line with Patent Owner’s construction, the ’852 Patent consistently describes cross-over error (1) arising from chimeric sequences created during cluster amplification and (2) made from template nucleic acids derived from different samples. POR, 13-20.

With regard to whether “cross-over error” should be a chimeric sequence itself or error from said chimeric sequence, Petitioner does not meaningfully respond to

Patent Owner's proposal. Petitioner only states that it "explains what the claimed 'error' constitutes," and that "repeating the term 'error' adds nothing." Reply, 5-6.

Petitioner is incorrect that its construction explains what the claimed "error" constitutes. The error to be reduced is not the formation of chimeric molecules, as Petitioner appears to assert, but rather the incorrect identification of sequences based on those chimeras. EX1001, 1:54–56, 5:41–6:6. Indeed, the entire premise of the '852 Patent is that chimeric sequences *will* form in pooled samples during amplification. *Id.*, 1:51–54, 5:41-58, 21:8-13. Rather than targeting a way to prevent those sequences from forming, the '852 Patent provides methods to identify the sequences and discard them to reduce error. *Id.*, 2:13–29, 20:65–21:19.

Petitioner also misapprehends the application of the claim construction to the claim language. Petitioner's construction removes the concept of "error" from the claim, which eviscerates the purpose of the claim. Patent Owner's construction, by contrast, leaves intact the concept of removing "error" as claimed while providing necessary context. Patent Owner's construction should be adopted.

III. Ground 1: 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 chimeric sequence issues were widely known in Illumina sequencing and addressed by Parameswaran. Reply, 7-13. While Petitioner faults Patent Owner for failing to address “numerous prior-art references disclosing the problem of chimeric sequences in Illumina sequencing” (Reply, 7), the references on which Petitioner now focuses were *never* discussed, cited, or submitted prior to Petitioner's Reply. Reply, 7-8 (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., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d

1359, 1369 (Fed. Cir. 2016). But even if considered, Petitioner’s new arguments fall far short of rebutting Patent Owner’s evidence showing a lack of such awareness. POR, 20-36.²

First, Petitioner relies heavily on Campbell (EX1057), which Petitioner incorrectly characterizes as describing widespread awareness of chimeric sequences in Illumina sequencing. Reply, 7 (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 giving rise to cross-over error as claimed. Reply, 7-8; EX1054, ¶¶40-44. Nor is there any explanation how any of the particular rearrangements listed in Campbell—

² Petitioner also takes a half-hearted shot at Dr. Adalsteinsson’s credentials. Reply, 7. 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, 20-22; EX1055, 11:19-12:11.

including “deletions,” “duplications,” and “inversions” (EX1057, 3 (Table 1))—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, 37-41. 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, 7-9. 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, 7-8. 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 detecting chimeras “in close proximity on the flow cell.” Reply, 7-8. But Maher detects *biological* chimeras (i.e., those naturally present in a sample when collected) as potential cancer markers. EX1061, 1. The “artifacts” occurring “in close proximity on the flow cell” 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, 8. 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 ’852 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, 8-10. 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 and claimed in the ’852 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 rehashes its assertions of older PCR references (Reply, 9 (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, 29-30; EX2001, ¶¶105-115.

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, 10 (citing EXS2003-2004)), but those references illuminate Petitioner’s mischaracterizations of the prior art. POR, 3-4, 7, 35. EX2004—published months after the ’852 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, 10 (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 ’852 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, 11-13. 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, 27-28, 30-32. 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, 13. Petitioner now makes that assertion based on certain reverse complement barcodes (Reply, 12-13), 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 claimed cross-over error. Reply, 12-13.

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, 16-21. 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, 16-18. 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, 32, 35. Petitioner’s string cite quotes from EX1056 and EX1068 (Reply, 17) 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, 18-19. 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, 22-23; *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, 19-20. 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, 23-24; EX2001, ¶¶95-97; *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, 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, 36-37.

Fifth, Petitioner defends its “obvious to try” rationale (Reply, 21), 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 III.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, 22-23. But Petitioner cannot avoid the evidence Patent Owner provided (POR, 24-27, 38-39), 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, 22. 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, 25; EX2001, ¶¶99-101. 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, 23-24. And Petitioner's claim that the heightened barcode synthesis requirements were "a suitable option" even if not "the best option" (Reply, 23) 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, 22-23), 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 III.A.2.

B. Petitioner's Failure To Establish A Reasonable Expectation Of Success Is A Significant Shortcoming And An Independent Reason For Upholding The Challenged Claims. (All Claims)

Petitioner incorrectly dismisses Patent Owner's arguments regarding reasonable expectation of success as " cursory." Reply, 23. The lack of reasonable expectation of success is a critical gap in Petitioner's assertions, and Patent Owner explained in detail why that gap destroys Petitioner's validity challenges. POR, 39-42. Petitioner cannot avoid its failure on reasonable expectation of success by claiming (incorrectly) that it is "not meaningfully dispute[d]."

The Challenged Claims require "discarding sequence reads ... *thereby to reduce cross-over error.*" EX1001, claim 1. Thus, the obviousness analysis requires that a POSA have a reasonable expectation of successfully reducing cross-over error, as Dr. Spellman admitted. EX2015,132:20-133:5. But the problem of cross-over error was not at all appreciated for flow cell sequencing in December 2010. *See* Section III.A.1.; POR, 29-36. That is particularly true under the proper construction of "cross-over error" that specifies it arises from chimeras formed during cluster amplification. *See* Section II; POR, 13-20. Having no awareness of cross-over error, there was no way for a POSA to have any reasonable expectation of success to

reduce it (i.e., expecting to reduce something that was not known to exist in the first place), and Petitioner's arguments fail for a lack of such expectation.

C. Petitioner Fails To Show That The Combination Renders Obvious The Claimed “Discarding” Step (Claim 1)

Petitioner shifts its theory for the “discarding” step in claim 1. In the Petition, Petitioner asserted that its prior art disclosed this step. Petition, 34 (“Parameswaran discloses claim 1[f] [the discarding step].”), 35 (“Gloor also identified and discarded chimeric sequences.”). Patent Owner pointed out the flaws in those arguments (POR, 42-45), and Petitioner now asserts that the discarding step might be obvious depending upon the “purpose” of a particular application (Reply, 13-16), apparently abandoning its prior assertions of disclosure in the prior art. Even if the Board considers the new “purpose” theory, Petitioner's new arguments fail to show the claimed discarding step was obvious.

Petitioner relies on new interpretations of Parameswaran that are unsupported. For example, Petitioner asserts that Parameswaran only used “expected” motifs in its analysis, and that this indicates the “mismatched” motifs in Parameswaran could be discarded. Reply, 14. But Parameswaran contains no such explanation, and Petitioner cites to none. By definition, a mismatch is one expected barcode paired with one unexpected barcode. Otherwise, the pair would be a perfect expected pair (two expected barcodes), which Parameswaran listed separately from its mismatched

barcodes, or would have no perfect matches (zero expected barcodes), which Parameswaran explicitly states were discarded. EX1004, 7 (Table 3), 8 (sequences with “no perfect matches” were “discarded”). Parameswaran explained instead that “all sequences with a match to *one of the two expected*” barcodes, which encompasses a “mismatch,” were used in the analysis. *Id.*, 8; POR, 10, 42-43; EX2001, ¶¶139-144.

Petitioner asserts that Parameswaran “greyed out” certain cells of data to indicate they were not used. Reply, 15. But Parameswaran contains no such explanation, and Petitioner cites to none. Instead, Parameswaran is explicit about what it discards: sequences having “no perfect matches” to any barcode. EX1004, 8; EX2001, ¶140. In the data tables Petitioner references, those discarded sequences correspond to the four blank cells in the bottom right of the table, *not to the greyed out cells*, as Dr. Spellman admitted. EX2015, 102:3-12, 102:20-103:9, 103:22-104:19. Neither Dr. Spellman nor Petitioner explain why the grayed boxes they highlight were not simply left blank if those sequences were actually discarded, like the four boxes in the data table corresponding to sequences Parameswaran actually states were discarded. Moreover, when explaining the “barsort” source code that analyzed Parameswaran’s data, Dr. Spellman admitted that only sequences containing no perfect matches were put in an “unassigned” bin, while *the rest of the*

sequences—which would include perfect mismatches—went into a “raw” data bin for analysis. *Id.*, 115:15-116:11, 117:22-118:2, 118:12-119:2.

D. 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, 24-28. The new evidence and arguments should be disregarded. *Intelligent Bio-Sys.*, 821 F.3d at 1369. 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, 12 (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, 24–28. 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, 25; 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 25–26 (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.³ *Align Tech.*, IPR2023-01369, Paper 42 at 31-32.

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, 26; 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, 12; Reply,

³ 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 suggests the PDF at that link was created in November 2018. EX1002, ¶¶16–17, 19; EX1046, ¶18.

24–25; 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 assertion that Gloor’s availability has been confirmed by arXiv.org database records (Reply, 26), 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 And Bentley.

Petitioner fails to show why a POSA would have applied Parameswaran’s unique dual indexing method with Bentley’s whole genome sequencing project. Petitioner explains that Bentley “demonstrated how Illumina sequencers work” and points to Parameswaran’s “speculat[ion] on the use of [its] pyrosequencer tailored barcodes with other sequencing platforms” as its motivation to combine. Reply, 28 (citing EX1004, 1-2). But Petitioner’s argument fails to identify any error in Bentley or otherwise demonstrate a need that Parameswaran’s unique dual indexing method would have been known to address.

Additionally, Petitioner's assertions for Ground 2 fail for the same reasons as explained above for the proposed combination for Ground 1, including that Petitioner fails to show a reasonable expectation of success and fails to show that the combination discloses or renders obvious the claimed "discarding" step. *See* Sections III.A-C; POR, 51-53.

V. Conclusion

For the foregoing reasons, the Challenged Claims are patentable.

Dated: August 11, 2025

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 5,598 words as counted by Microsoft Word 365, the word-processing system used to prepare this paper.

Dated: August 11, 2025

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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:

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