

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA INC.,
Petitioner,

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner.

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

PETITIONER'S REPLY

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TABLE OF ABBREVIATIONS

'852 Patent	U.S. Patent No. 11,041,852
<i>Bold and italicized</i>	Emphasis added unless otherwise indicated
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
POSA	Person of ordinary skill in the art

I. INTRODUCTION

Patent Owner does not dispute that the combination of *Parameswaran* and *Gloor* (or *Bentley*) discloses a dual-indexing method that could be used to identify and reduce cross-over error. Instead, Patent Owner attempts to narrow the plain claim language to limit “cross-over error” solely to chimeric sequences “created during cluster amplification.” Patent Owner also argues a lack of motivation to combine, alleging that: (1) the problem of cross-over error purportedly had not been identified for Illumina sequencers; and (2) *Parameswaran*’s method, validated using a Roche 454 sequencer, was deemed incompatible with the Illumina sequencers in *Gloor* and *Bentley*.¹ These arguments fail.

First, Patent Owner’s construction contradicts the claim language and intrinsic evidence. The claims recite reducing cross-over error “**during** sequencing workflow,” without restricting how or when that error arises. Nothing in the intrinsic record limits the claims to reducing cross-over error arising at a particular stage in the sequencing workflow. Further, the claims would have been obvious even under Patent Owner’s erroneous construction. A POSA would have been motivated to remove **all** chimeric sequences formed between templates from different samples

¹ Patent Owner also disputes *Gloor*’s prior-art status, but the evidence overwhelmingly establishes its public availability.

created at *any* stage during sequencing workflow, because they could lead to sample misassignment. Applying *Parameswaran*'s method to the Illumina sequencers in *Gloor* or *Bentley* would remove all such erroneous sequences, thus reducing *all* cross-over error, including cross-over error "created during cluster amplification."

Second, Patent Owner's assertion that chimeric sequences "created during cluster amplification" was an "unrecognized" problem in December 2010 is incorrect. A POSA would have known from numerous prior-art references that chimeras created on flow cells during Illumina sequencing was a significant problem. *E.g.*, EX1057, 2, 7 (detecting nearly 1.2 million chimeric sequences during Illumina sequencing); EX1061, 8 (detecting candidate chimeras formed among templates in "close proximity *on the flow cell*" during Illumina sequencing); EX1005, 8 (detecting chimeras arising "during PCR or because of overlapping spots" on an Illumina flow cell). These chimeric sequences were routinely excluded, typically using bioinformatic analyses. *Parameswaran*'s unique dual-indexing method offered an alternative solution, allowing more precise and efficient identification of these erroneous sequences during multiplex sequencing. It would have been obvious to apply that method to Illumina sequencers, one of three main NGS platforms used in 2010.

Third, Patent Owner's incompatibility argument trivializes the POSA's understanding and contravenes the patent specification. It also contradicts numerous

prior-art statements to the contrary, including a statement from an '852 patent inventor praising *Parameswaran* as teaching an “effective technology” for addressing the challenge of tracking fragments pooled from multiple samples—using Illumina sequencers (citing *Bentley*) and other NGS platforms. EX1059, 3.

As explained in the Petition, this Reply, and the declarations of Drs. Spellman and Gloor, the challenged claims of the '852 patent are unpatentable as obvious.

II. CLAIM CONSTRUCTION

The terms “template cross-over error” and “cross-over error” should be construed as “sequences of chimeric molecules made from template nucleic acids derived from different samples.” Pet., 22-23. Patent Owner seeks to limit these terms to “error from sequences of chimeric molecules *created during cluster amplification* and made from template nucleic acids derived from different samples.” POR, 13. Patent Owner emphasizes that the patent is directed to “chimeric sequences arising after sample pooling *during amplification*, not at other points during sequencing workflow.” POR, 14 (emphasis in original). This is wrong. EX1054, ¶¶7-29.

First, it contradicts the plain claim language. Claim 1 recites a method for “reducing template cross-over error *introduced during sequencing workflow*.” EX1001, cl. 1. It does not require reducing cross-over error created at any specific point in that workflow, much less “during cluster amplification.” EX1054, ¶¶10-12.

Further, claim 1 recites only that at least one cluster on the flow cell “*comprises* a chimeric sequence.” EX1001, cl. 1; EX1055, 88:18-90:12, 91:14-92:5 (Patent Owner’s expert Dr. Adalsteinsson testifying that “other chimeras can be formed and sequenced”). It does not require that the chimeric sequences are “created” during cluster amplification. EX1054, ¶¶13-14; *Sisvel Int’l S.A. v. Sierra Wireless, Inc.*, 81 F.4th 1231, 1236-37 (Fed. Cir. 2023) (rejecting narrower construction when “the claim language itself is not so limited”).

Second, Patent Owner’s proposal contradicts the specification, which identifies cluster amplification as merely one possible theory for chimera formation. EX1001, 6:25-29 (“[w]ithout being limited by any particular theory or mechanism of action”), 6:64-7:1 (same); EX2002, 87:5-88:3; EX1054, ¶¶15-16. The specification does not support limiting the claims to reducing error arising at a specific stage in the sequencing workflow. *Hill-Rom Servs., Inc. v. Stryker Corp.*, 755 F.3d 1367, 1371 (Fed. Cir. 2014) (refusing to “read limitations from the embodiments in the specification into the claims”); *QuantifiCare S.A. v. Canfield Sci., Inc.*, No. 23-1917, 2025 WL 901279, at *4 (Fed. Cir. Mar. 25, 2025) (rejecting construction limiting claims to specific embodiments characterized as “possible” or “exemplary”).

Third, the prosecution history of the ’852 patent does not support Patent Owner’s construction. EX1054, ¶¶17-19. Patent Owner suggests that amending the

claims to recite amplifying nucleic acids “on a surface of a flow cell” to form clusters somehow narrowed the claims to cross-over errors “created” on the flow cell. POR, 18. But Applicant never suggested that the claimed chimeric sequence must “arise” during flow cell amplification. Instead, Applicant merely contrasted formation of clusters on “a flow cell” (Illumina) from “bead-bound templates” (Roche 454). EX1007, 304-306, 311; EX1054, ¶¶18-19.

Fourth, Patent Owner’s construction contradicts the general knowledge in the art. EX1054, ¶¶20-25. It was known that chimeric sequences made of templates from different samples could arise at many stages during sequencing, not just during cluster amplification on a flow cell. *Id.*, ¶¶21-22. The claimed method will detect these sequences regardless of when or how they arise. *Id.*, ¶¶23-25. Nothing in the claims or specification explains how to selectively detect and reduce cross-over error arising “during cluster amplification” versus other points during the sequencing workflow. *Id.*

Patent Owner separately argues that the construction should include “***error from*** sequences of chimeric molecules,” as opposed to “sequences of chimeric molecules,” as Petitioner proposed. POR, 13-14. But Petitioner’s construction explains what the claimed “error” constitutes—the presence of sequences of chimeric molecules made from template nucleic acids derived from different

samples. Pet., 22-23. Repeating the claim term “error” adds nothing. EX1054, ¶¶27-29.

Patent Owner’s erroneous construction should be rejected. That said, the challenged claims would have been obvious under either construction for the reasons set forth in the Petition and below. EX1054, ¶¶30, 119-127; *infra* §§ III-IV.

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

In December 2010, a POSA would have been motivated to use *Parameswaran*’s unique dual-indexing method on the Illumina sequencer in *Gloor*, and would have reasonably expected to successfully reduce cross-over error, because: (1) *Parameswaran*’s method directly addressed the known problem of chimeric sequences arising on Illumina sequencers; (2) *Parameswaran* expressly recommended trying its methods with other platforms; (3) Illumina sequencers were faster, more accurate, and more efficient than the Roche 454 sequencer in *Parameswaran*; and (4) it would have been obvious to apply *Parameswaran*’s methods on Illumina sequencers, one of only three main NGS platforms in 2010. Pet., 36-45. Any minor modifications required to transfer *Parameswaran*’s method to an Illumina sequencer would have been routine, as the ’852 patent admits. *Id.*, 42-44.

A. Patent Owner Fails to Rebut the Strong Motivation to Combine *Parameswaran* and *Gloor*

1. Chimeric Sequences, Including Those Arising “During Cluster Amplification,” Were a Known Problem

Relying on its expert Dr. Adalsteinsson, Patent Owner argues that chimeric sequences arising “during cluster amplification” were an “unrecognized” problem in December 2010. POR, 1-2, 29-36. At that time, however, Dr. Adalsteinsson had just graduated from college and was not a POSA, yet he did not consult anyone who was. EX1055, 11:11-18, 13:10-14, 24:20-25:5; EX1054, ¶¶5-6.

Patent Owner and Dr. Adalsteinsson failed to consider numerous prior-art references disclosing the problem of chimeric sequences in Illumina sequencing. EX1054, ¶¶31-48. *Campbell*, for example, used an Illumina sequencer to detect biologic chimeras existing before sequencing. EX1057, 1. *Campbell* detected and excluded nearly 1.2 million erroneous chimeric sequences—including PCR-induced artifacts formed “within the PCR colony” on Illumina’s flow cells. *Id.*, 2, 7; EX1054, ¶¶40-44. Other studies similarly detected and excluded erroneous chimeric sequences during Illumina sequencing, including PCR-induced artifacts formed “within the bridge amplified cluster.” EX1066, 10; *see also* EX1061, 8, 16

(detecting candidate chimeras formed among templates “in close proximity on the flow cell”); EX1005, 8; EX1054, ¶¶44-45, 36-39.²

POSAs understood that many chimeric sequences arose on Illumina flow cells. EX1054, ¶¶46-48. By 2010, it was known that chimeras form during PCR, that Illumina sequencers use a “PCR process known as bridge PCR to achieve clonal amplification of sequencing templates on the surface of a glass flow cell,” and that different templates can seed and amplify on the same spot on an Illumina flow cell. EX1065, 5; EX1018, 1 (chimeras are “composed of parts of two different sequences artificially combined during PCR”); EX1067, 3 (“mixed clusters can be grown starting from more than one template” in Illumina sequencing).

When multiple samples were sequenced using NGS platforms, chimeric sequences were particularly problematic because they created “false” sequences and decreased accuracy. EX1033, 8; EX1063, 1 (“The formation of chimeras by PCR leads to the genetic mixing of sequences which can generate new diversity that is not present in the initial sample.”); EX1060, 1 (“When PCR amplification is carried

² Patent Owner argues that EX2005 shows that “cross-over error for NGS” was “not a problem.” POR, 30; EX2001, ¶114. As Dr. Adalsteinsson testified, however, EX2005 was *not* about “template cross-over that occurs during cluster amplification on the surface of a flow-cell.” EX1055, 25:22-26:15; EX1054, ¶¶53-57.

out using a mixture of two or more non-identical but similar genotype target sequences as templates, this can lead potentially to the formation of artificial chimeras between the amplification products”); EX1054, ¶¶36-39. This was a sequencer-agnostic problem for multiplex sequencing on any platform relying on PCR. EX1005, 8, 22 (reporting chimeras formed during Illumina multiplex sequencing); EX1054, ¶¶33-35.

Patent Owner wrongly dismisses early references discussing chimera formation during PCR (EXS1017-20) because they published before NGS became available. POR, 29-30. But obviousness is determined “from the vantage point” of a POSA as of the priority date. *Donner Tech., LLC v. Pro Stage Gear, LLC*, 979 F.3d 1353, 1360 (Fed. Cir. 2020). By December 2010, these references were understood as relevant because many NGS platforms used PCR during sequencing, including bridge amplification on a flow cell. EX1003, ¶¶59-61, 67; EX1054, ¶¶49-51; EX2002, 115:6-24. Tellingly, contemporaneous publications cited these foundational references to explain the source of chimeras formed during Illumina sequencing. *E.g.*, EX1005, 8 (citing EX1017); EX2004, 2 (citing EXS1017-20); EX1054, ¶¶51-52.

Patent Owner also contends that these early references concerned chimeras formed “within a sample,” whereas the challenged claims concern those formed “between samples.” POR, 29-30. A POSA would have understood, however, that

chimera formation is problematic whenever multiple templates are amplified via PCR, whether from a single or multiple samples. EX1015, 9 (warning of “jumping PCR” between mixed samples); EX1054, ¶51; EX2002, 115:6-24 (“[A] POSA reading [EX1020] would understand that anytime one had done PCR with more than one sample present in the PCR, [chimera formation] was a possibility.”).

Patent Owner cites two post-priority references, EX2003 and EX2004, but neither undermines the recognition of chimera formation as problematic as of December 2010. POR, 3-4; EX1054, ¶¶58-61. EX2003 states that “[i]ndex misassignment between multiplexed libraries is a *known* issue that has impacted NGS technologies *from the time sample multiplexing was developed*.” EX2003, 1. *Parameswaran* and *Gloor* are two prior-art examples of “sample multiplexing.” EXS1004-05; EX1054, ¶59. EX2004 applied dual-indexing methods to an Illumina sequencer. Citing the same early references cited in the Petition (EXS1017-20), EX2004 explains that “PCR can produce chimeras by recombining different template molecules (a process often referred to as ‘jumping PCR’).” EX2004, 2. Notably, before December 2010, the authors of EX2004 expressly warned of “jumping PCR” between samples during Illumina sequencing—the phenomenon Dr. Adalsteinsson mischaracterizes as unknown at that time. EX1015, 9; *see* EX1055, 95:19-96:2; EX1054, ¶59. Thus, chimeras, including those arising “during cluster amplification,” were a recognized problem in December 2010. EX1054, ¶61.

2. *Parameswaran* Provided a Solution to the Known Problem of Chimeric Sequences

A POSA would have been motivated to discard *all* inter-sample chimeric sequences, whether or not “created during cluster amplification,” because such sequences were not present in the original samples and undermined sample identification. Pet., 37-38; EX1054, ¶¶62-63.

Parameswaran’s solution avoided bioinformatic analyses, which were not always suitable for or precise at identifying chimeras (e.g., where no reference sequences were available for comparison). Pet., 37-38; EX1054, ¶¶64-65, 119-125. Using unique dual-indexing, *Parameswaran* identified chimeric sequences based on barcode motifs that should not have been found together, i.e., mismatched barcode motifs corresponding to two different samples. EX1004, 7-8 (characterizing sequences with mismatched barcode motifs as “false-discover[ies]”). As Dr. Adalsteinsson admitted, unique dual-indexing is a “powerful approach” that can address many “weaknesses” in sequencing workflow, allowing for identifying erroneous inter-sample chimeric sequences formed at various sequencing stages using mismatched barcode motifs as a proxy. EX1055, 98:3-14, 95:9-97:10. A POSA using *Parameswaran*’s method on an Illumina sequencer would have readily identified and discarded inter-sample chimeric sequences arising at *any* stage during the sequencing workflow—including those “created” on the flow cell. EX1054,

¶¶64-65, 119-125. The claims thus would have been obvious even under Patent Owner’s erroneous construction.

Solely focusing on the “specific problem” allegedly identified by the inventors—“cross-over error” created on Illumina’s flow cells—Patent Owner argues that a POSA would not have adopted *Parameswaran*’s method because *Parameswaran* does not explicitly mention “cross-over error.” POR, 32-34; EX1055, 35:11-36:10, 38:16-39:2. But it is error to “look only to the problem the patentee was trying to solve.” *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 419-20 (2007); *Alcon Rsch., Ltd. v. Apotex Inc.*, 687 F.3d 1362, 1368 (Fed. Cir. 2012). In December 2010, chimeric sequences were known to arise at *any* stage during sequencing workflow, including amplification on the flow cell. *See supra* §§ II, III.A.1. A POSA would have been motivated to use *Parameswaran*’s method and discard these erroneous sequences, regardless of whether *Parameswaran* used the phrase “cross-over error” to describe that source of error. EX1054, ¶66.

Patent Owner also argues that “Parameswaran’s mismatched barcode motifs were *not* cross-over error.” POR, 31 (emphasis in original). According to Patent Owner, if those sequences were “a source of cross-over error,” the number of sequences with mismatched barcodes would “remain steady” even after adding bases from the adjoining cloning linkers to form “barcode motifs.” *Id.* This argument misapprehends *Parameswaran*. EX1054, ¶¶67-78. In *Parameswaran*, “six of the

forward barcodes [were] exact reverse complements of six reverse barcodes.” EX1004, 7. These barcodes became indistinguishable following PCR, creating ambiguity in assigning sequences to distinct samples. *Id.* Adding three bases from the adjoining cloning linkers generated “barcode motifs” unique to each sample (i.e., no forward and reverse barcodes were exact reverse complements anymore), decreasing the number of sequences with mismatched barcode motifs. *Id.* That the number of these sequences decreased as barcode motifs became truly unique indicates that “sequencing error” was not the likely source of error. EX1054, ¶¶71-73. Further, that *multiple* sequences with mismatched barcode motifs remained after removing barcode ambiguity indicates that *Parameswaran*’s method can indeed identify “cross-over error.” *Id.*, ¶70.

The challenged claims—which apply an obvious solution (offered by *Parameswaran*) to a known problem (chimeric sequences arising during sequencing workflow, including amplification on an Illumina flow cell)—would have been obvious. *KSR*, 550 U.S. at 419-20; *cf. Leo Pharm. Prods., Ltd. v. Rea*, 726 F.3d 1346 (Fed. Cir. 2013).

3. “Discarding” Chimeric Sequences Would Have Been Obvious to a POSA

The Petition explains that a POSA would have been motivated to combine *Parameswaran* and *Gloor* because doing so would facilitate identifying and

discarding chimeric sequences, thus reducing cross-over error. Pet., 37-38. In response, Patent Owner alleges that *Parameswaran* and *Gloor* “distinguished” chimeric sequences but did not “discard” them. POR, 34, 42-44. Patent Owner focuses on a single sentence in *Parameswaran*: “***For our purposes***, all sequences with a match to one of the two ***expected*** sequence motifs were considered as true members of the corresponding dataset.”³ EX1004, 8.

A POSA would have understood from *Parameswaran* that chimeric sequences could be kept or discarded, depending on the sequencing “***purpose***.” Pet., 35; *KSR*, 550 U.S. at 421 (a POSA is “a person of ordinary creativity, not an automaton”); EX1054, ¶¶79-82. For example, if sample tracking and sequence fidelity were important, discarding chimeras would have been obvious to avoid false positives. *Id.*, ¶¶81, 77-78; EX1055, 113:16-114:10 (Dr. Adalsteinsson agreeing that some applications “require highly accurate sequencing”).

Further, this sentence refers to sequences with “***expected*** sequence motifs”—where a sequence would be assigned to one sample based on the first barcode motif and the second barcode motif does not contradict that sample assignment. EX1004, 7. This sentence does ***not*** refer to “perfect, ***unexpected*** occurrences”—sequences where one barcode motif belongs to one sample and the other motif

³ Patent Owner omits the language “[f]or our purposes.” POR, 10.

belongs to a different sample (i.e., mismatched barcode motifs) and therefore “[t]here wouldn’t be a sample to assign.” *Id.*; EX1055, 52:15-53:5; EX1054, ¶83. Notably, *Parameswaran*’s data table (reproduced below) **greyed out** sequences with mismatched barcode motifs, signifying that they were discarded. EX1004, 23, 29; EX1054, ¶¶84-85, 89. By contrast, sequences containing expected sequence motifs were not grayed out and were included in the tallies labeled “(close) or (no match).” EX1004, 23, 29; EX1054, ¶¶86-88. These tables indicate that *Parameswaran* “discarded” chimeric sequences to reduce cross-over error. EX1054, ¶89. At a minimum, they reinforce that it would have been obvious to do so. *Id.*; *see also* EXS1057, 1061, 1066 (excluding erroneous chimeric sequences from analysis).

D. SEARCH MOTIF = Barcode (10 nt) + Cloning-Linker (3 nt)																											
	454-66	454-67	454-68	454-69	454-70	454-71	454-72	454-73	454-74	454-75	454-76	454-77	454-78	454-79	454-80	454-81	454-82	454-83	454-84	454-85	454-86	454-87	454-88	454-89	454-90	(close)	(no match)
454-66	13566	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	639	85
454-67	0	7459	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	221	33
454-68	0	0	13260	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	314	139
454-69	0	0	0	6518	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	108	115
454-70	0	0	0	0	9484	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	350	23
454-71	0	0	0	0	0	9397	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	297	55
454-72	0	0	0	0	0	0	12302	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	264	54
454-73	0	0	0	0	0	0	0	7273	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	145	32
454-74	0	0	0	0	0	0	0	0	13923	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	211	51
454-75	0	0	0	0	0	0	0	0	1	15060	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	418	97
454-76	0	0	0	0	0	0	0	0	1	0	10266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	308	50
454-77	0	0	0	0	0	0	0	0	0	0	0	9602	0	0	0	0	0	0	0	0	0	0	0	0	0	241	49
454-78	0	0	0	0	0	0	0	0	0	0	0	0	12082	0	0	0	0	0	0	0	0	0	0	0	0	226	34
454-79	0	0	0	0	0	0	0	0	0	0	0	0	0	10349	0	0	0	0	0	0	0	0	0	0	0	269	48
454-80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10019	0	0	0	0	0	0	0	0	0	0	294	153
454-81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6799	0	0	0	0	0	0	0	0	0	200	42
454-82	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	11674	0	0	0	0	0	0	0	0	357	28
454-83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12141	0	0	0	0	0	0	0	392	43
454-84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14049	0	0	0	0	0	0	405	77
454-85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7632	0	0	0	0	0	155	44
454-86	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8838	0	0	0	0	250	77
454-87	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	10431	0	0	0	390	57
454-88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10395	0	0	202	65
454-89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8148	1	201	56
454-90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5028	136	27
(close)	369	167	210	122	237	154	317	172	268	280	209	215	299	319	240	221	325	268	346	218	198	269	258	191	126		
(no match)	37	26	76	48	28	20	48	29	64	46	42	36	78	59	60	47	58	35	52	26	77	27	70	59	29		

Parameswaran’s Data Table 5D (EX1004, 29; Shading in Original)

Patent Owner's analysis of *Gloor* is similarly flawed. EX1054, ¶¶90-91. Focusing on the single sentence that "[t]he dataset was used without further regard to chimeric sequences" (EX1005, 8), Patent Owner ignores that *Gloor* **already** discarded many chimeric sequences through "the strict requirement for proper sequence tag and primer sequences on the left and right ends" and "a requirement for long overlapping segments of a defined length" (EX1005, 22).

4. Multiple Reasons Support Applying *Parameswaran's* Method to *Gloor's* Illumina Sequencer

Patent Owner proffers several reasons why a POSA allegedly would not have applied *Parameswaran's* method to *Gloor's* Illumina sequencer. None has merit.

Alleged Reason #1: Patent Owner contends that, given Illumina sequencers' higher capacity, they "did not have the same needs of pyrosequencing that guided the design of *Parameswaran*." POR, 21-22. But *Parameswaran's* method would benefit **any** NGS platform, regardless of its error rate or sequencing capacity. EX1054, ¶¶92-93. Indeed, notwithstanding the low error rate reported in *Gloor*, chimeras were still formed during Illumina multiplex sequencing. *Id.*; EX1005, 8, 22.

The applicability of *Parameswaran's* method to Illumina sequencers was widely appreciated by December 2010. EX1054, ¶¶94-98. For example, *Pfrender* discussed "Next-Generation Sequencing Strategies," including both Roche 454 and

Illumina sequencers. EX1062, 11. *Pfrender* cited *Parameswaran*, explaining that “[a]pplying a tagging strategy to uniquely identify samples would allow the combination of multiple whole community assays in a single run, thereby reducing the per sample cost.” *Id.*; *see also* EX1056, 18 (“Coupled with the use of barcoded amplicon primers to multiplex multiple patients in a single run [in *Parameswaran*], this approach may become one of the first applications of high-throughput sequencing to be adopted by clinical molecular laboratories.”); EX1068, 7 (“[I]t is not a problem to sequence many samples in a Solexa [Illumina] instrument run if using a barcoding technique. It is becoming a standard approach to increase the number of samples run on high-throughput instruments. [*Parameswaran*].”).

An inventor of the ’852 patent acknowledged this in *Fuller*, a 2009 prior-art article. EX1059, 3; EX1054, ¶97. *Fuller* cited *Parameswaran*, stating that a “streamlined, robust sample preparation” method would be “an effective technology to judiciously add sequence tags to the fragments of several different samples,” such that samples could be combined and processed together, with the resulting sequences separated based on their tags. EX1059, 3. *Fuller* explained that *Parameswaran*’s method would reduce the inefficiencies of “ensemble-based [sequencing-by-synthesis] architectures.” *Id.* Ensemble-based sequencers refer to platforms that

sequence multiple identical copies of a DNA (*id.*, 1), including Illumina sequencers (*id.*, Figure 1, citing *Bentley* (EX1006)).⁴

Alleged Reason #2: Patent Owner contends that *Parameswaran*'s approach applies only to pyrosequencing because its barcodes are “tailored” for pyrosequencing. POR, 22-23. But in December 2010, barcodes posed no barrier for switching from one NGS platform to another; a POSA understood how to design barcodes suitable for Illumina sequencers. EX1054, ¶¶99-100; EX1055, 104:13-105:11 (“So by and large, I’d say that was known in the art.”). Indeed, this had already been done. EX1038, Table 1 (performing unique dual-indexing on Roche 454 and Illumina sequencers using the same set of barcodes). Consistent with the routine nature of barcode design, the ’852 patent does not exemplify specific barcodes, instead trusting a POSA to design suitable barcodes for “any sequencing methodology” and copying nearly verbatim from others’ patent publications on barcode designs. EX1001, 8:65-9:3, 20:37-41; Pet., 19; EX1054, ¶100.

Nor does *Gloor* “warn[] against applying pyrosequencing approaches to Illumina sequencers.” POR, 22-23. *Gloor* commented on methods for analyzing

⁴ Patent Owner was aware of *Fuller* at least through co-pending litigation (*see* EX1064, ¶¶88-96), but did not cite *Fuller* in its Response or have Dr. Adalsteinsson address *Fuller*'s disclosures. *See generally* POR; EX2001.

microbiome data based on the comparative read lengths obtained by Roche 454 (unidirectional reads) and Illumina (bidirectional, paired-end reads) sequencers. EX1005, 2. *Gloor* then taught how to account for these read-length differences. *See id.*, 6-7. This would have further motivated a POSA to adopt *Parameswaran*'s method for Illumina sequencing. EX1054, ¶101.

Alleged Reason #3: Patent Owner contends that “a POSA would not have had a reason to use the [unique dual-indexing] methods disclosed in *Parameswaran*,” because *Parameswaran* and *Gloor* discuss nonunique, combinatorial dual-indexing. POR, 23-24. But the mere existence of an alternative (e.g., non-unique dual-indexing) would not dissuade a POSA from using *Parameswaran*'s unique dual-indexing method. *See UCB, Inc. v. Actavis Lab 'ys UT, Inc.*, 65 F.4th 679, 692 (Fed. Cir. 2023). Specifically, a POSA would have understood the respective benefits of unique and non-unique dual-indexing and chosen the appropriate strategy accordingly. EX1054, ¶¶102-103. *Parameswaran*'s unique dual-indexing conferred exceptional sequencing accuracy by efficiently reducing cross-over error, and therefore would have been appropriate where sample tracking and sequence fidelity were important, regardless of platform. *Id.*; EX1055, 113:16-114:10. Where sample tracking and error identification were less important, non-unique dual-indexing

could have been considered.⁵ EX1054, ¶103. Consequently, “common sense, common wisdom, and common knowledge” support using *Parameswaran*’s unique dual-indexing with Illumina sequencers. *Elekta Ltd. v. ZAP Surgical Sys., Inc.*, 81 F.4th 1368, 1374 (Fed. Cir. 2023).

Alleged Reason #4: Patent Owner acknowledges that *Parameswaran* expressly recommended applying its method to “other sequencing platforms.” EX1004, 1-2, 8; POR, 36-37. But Patent Owner argues this would have led a POSA to “consider” Illumina sequencers, not “motivate” their use. POR, 36-37. This semantic argument is a distinction without a difference. *See Bayer Healthcare Pharms., Inc. v. Watson Pharms., Inc.*, 713 F.3d 1369, 1376 (Fed. Cir. 2013) (finding motivation based on “prior art’s direct recommendations”); EX1054, ¶¶104-105. Patent Owner also downplays other express prior-art motivations to use Illumina sequencing, including *Gloor*’s statements that Illumina sequencers had “an exceedingly low error rate” and were “an approximate order of magnitude cheaper than the per-read cost of pyrosequencing.” EX1005, 2; EX1003, ¶¶158-159.

⁵ Dr. Adalsteinsson acknowledges that *Parameswaran*’s unique dual-indexing approach permits sample assignment with a single valid identifier, which could not be done with non-unique dual-indexing. EX2001, ¶140; EX1055, 77:13-78:8. This also would have motivated a POSA to use *Parameswaran*’s approach.

Alleged Reason #5: Patent Owner disputes Petitioner’s “obvious to try” rationale, alleging that Petitioner did not identify “a problem” to be solved, even if *Parameswaran*’s unique dual-indexing method and Illumina sequencing were well known. POR, 37-38. On the contrary, Petitioner identified the known problem of chimeras arising during sequencing, including “during amplification” in NGS platforms. Pet., 37-38; *see supra* § III.A.1. Petitioner explained that, to improve efficiency and accuracy, it would have been obvious to try *Parameswaran*’s method (which reduces cross-over error) on *Gloor*’s Illumina sequencer (which offered superior speed, accuracy, and cost). Pet., 36-37. Prior-art studies comparing results from Roche 454 and Illumina sequencers further confirm that Illumina sequencers were an obvious choice. *See* EX1015, 1 (adopting protocols developed for Roche 454 to Illumina); EX1038, Table 1 (performing unique dual-indexed, multiplex sequencing using both platforms). By December 2010, moving from a Roche 454 sequencer to an Illumina sequencer was a natural progression in the field. EX1058, 8 (“Many projects initiated with the 454 technology are being continued with Illumina or SOLiD platforms and will certainly use the latest methods for finishing.”); EX1054, ¶106. A POSA had a “good reason to pursue the known options within his or her technical grasp.” *KSR*, 550 U.S. at 421.

5. Patent Owner Fails to Identify Anything That Would Have Motivated a POSA to Avoid *Parameswaran*'s Unique Dual-Indexing Methods

Patent Owner identifies purported “downsides” in applying *Parameswaran*'s unique dual-indexing approach to *Gloor*, arguing it would have required more barcodes to sequence the 272 samples in *Gloor* versus the 48 samples in *Parameswaran*.⁶ POR, 24-27, 38-39. Patent Owner emphasizes increased cost and error associated with barcode synthesis. *Id.*

This criticism is unwarranted, and certainly does not render the claims nonobvious. *First*, other considerations affecting cost and efficiency outweighed alleged concerns resulting from using more barcodes. EX1054, ¶¶77-78, 109. For example, non-unique dual-indexing could result in sample misassignment, and a POSA would have understood this could incur substantial cost, substantial time, and even clinical misdiagnosis. *Id.*; EX1056, 18-19. Patent Owner disregards the significant value of the combination.

Second, Patent Owner's speculation that a POSA would have been driven away from *Parameswaran*'s method lacks literature support. If anything, researchers *praised* *Parameswaran*'s approach, including an '852 patent inventor

⁶ Of course, combining *Gloor* with *Parameswaran* does not require sequencing 272 samples. EX1054, ¶¶107-108.

who cited *Parameswaran* as disclosing “an effective technology” for “reduc[ing] the inefficiencies” of NGS platforms. EX1059, 3; EX1054, ¶109.

Third, even if barcode synthesis cost or error was relevant for some applications, a POSA would have recognized *Parameswaran*’s method as “a *suitable* option” for many situations, even if not the “*best* option” in every possible situation. *See Bayer Pharma AG v. Watson Lab ’ys, Inc.*, 874 F.3d 1316, 1328 (Fed. Cir. 2017); EX1054, ¶¶109-110. A POSA would have been motivated to combine *Parameswaran* and *Gloor* to improve sequencing accuracy and facilitate sample assignment. Pet. 36-41.

B. Patent Owner’s Cursory Treatment of Reasonable Expectation of Success Confirms Obviousness

Patent Owner does not meaningfully dispute expectation of success, instead repeating the flawed argument that a POSA had “no knowledge that cross-over error was even a problem for NGS, and particularly Illumina sequencers like *Gloor*’s.” POR, 39-41.

Not so. By December 2010, a POSA would have recognized “cross-over error” as a problem for multiplex sequencing, including for Illumina sequencers. *See supra* § III.A.1. Multiple references disclosed chimeric sequence formation, including PCR-induced artifacts, on Illumina flow cells. *Id.* Concerns about chimera formation applied generally to PCR amplifications involving multiple

templates, and there is no scientific basis for Patent Owner’s suggestion that flow cells were understood to change this. *Id.*; EX1054, ¶¶111-112.

It is also immaterial that *Parameswaran* did not attribute its sequences with mismatched barcode motifs to “cross-over error.” POR, 40-41. A POSA would have understood that those sequences would have included chimeras created during sequencing—and would have had a reasonable expectation of success in reducing cross-over error by discarding them. *See supra* §§ III.A.2-3; EX1054, ¶¶113-115. Notably, Patent Owner did not dispute that the combination of *Parameswaran* and *Gloor* could in fact reduce cross-over error, including chimeric sequences created during cluster amplification. *See* POR, 39-41.

C. Patent Owner’s Silence on Secondary Considerations Speaks Volumes

Patent Owner’s brief includes attorney statements about the purported significance of the challenged claims (POR, 1-2), but no objective evidence of nonobviousness. If anything, the field praised the asserted prior art for improving sequencing accuracy. *See supra* § III.A.4.

D. *Gloor* Was Publicly Available

Patent Owner never actually disputes that *Gloor* is prior art. Instead, Patent Owner argues that the evidence at most shows *Gloor* was submitted by July 28,

2010, not that it was publicly available one day later. POR, 46-47. Overwhelming evidence confirms *Gloor*'s public availability as of July 29, 2010:

EXS1047-48: Archived arXiv.org webpages from June and September 2010 (i.e., flanking *Gloor*'s July 2010 submission and publication dates) establish arXiv.org's routine practice of publishing articles within 1-2 business days of submission. EX1046, ¶¶1-6; EX1049 (declaration from Internet Archive employee attesting that these are "true and accurate copies of browser screenshots of the Internet Archive's records"); *Constant v. Advanced Micro-Devices, Inc.*, 848 F.2d 1560, 1569 (Fed. Cir. 1988) ("Evidence of routine business practice can be sufficient to prove that a reference was made accessible before a critical date.").

These publication policies exemplify when submitted articles would be made publicly available and announced to subscribers (typically persons interested in the paper's field). They state, for example, that a "submission made on Monday before 16:00" will be "publicly available on the web after 20:00" that same day and "appear in the Tuesday AM regular subject mailing." EX1047, 2; EX1048, 2.

This is consistent with Dr. Gloor's first-hand experience as an arXiv.org user and his recollection that *Gloor* was published shortly after submission, further establishing *Gloor*'s publication date of July 29, 2010. EX1002, ¶¶12-13; EX1046, ¶¶11-13. *Align Tech., Inc. v. Dental Monitoring SAS*, IPR2023-01369, Paper 42 at

32 (PTAB Mar. 3, 2025) (arXiv.org’s publication policy at time of submission sufficient to establish publication date).

EX1002, Exs. E-F, EXS1039, 1043, 1050: Multiple emails from arXiv.org state that *Gloor* was announced and made public as of July 29, 2010. See EX1002, ¶¶15-17; EX1046, ¶¶7, 13-15. For example, EX1050, an e-mail from arXiv.org to Dr. Gloor, states that *Gloor* appeared online after midnight or 1AM UTC on July 30 (i.e., after 8PM or 9PM ET on July 29) and that *Gloor*’s announcement and public availability “has been confirmed by [arXiv.org’s] database records.” EX1050; EX1046, ¶15; EX1039, 5 (*Gloor* “was made public on arxiv.org at 8PM Eastern on 7/29/2010”); EX1043.

EX1051: A search for *Gloor* on arXiv.org states that *Gloor* was submitted on July 28, 2010, and that its publication was “originally announced” in “July 2010.” EX1046, ¶¶8, 16.

This evidence negates Patent Owner’s argument that Petitioner has not adequately shown *Gloor* was “disseminated or made available in 2010,” as well as Patent Owner’s suggestion that only the abstract may have been made available. POR, 47-49. Indeed, Dr. Gloor confirms that, once articles were made publicly available by arXiv.org, the **full article text** could be located through simple keyword, author, or field-of-interest searches. EX1046, ¶¶14, 16; EXS1047-48.

Additional evidence indicates *Gloor*'s public dissemination before December 2010. For example, in October 2010, *Gloor* was discussed in a public forum (mothur.org; EX1053) used by professionals in the sequencing field. In a string that included Dr. Gloor, a third-party user wrote that Dr. Gloor's "paper" was available "at <http://arxiv.org/abs/1007.5075>"—the same URL arXiv.org identified in its July 29, 2010 email to Dr. Gloor, and where *Gloor* appears today. EX1046, ¶¶9-10, 16-18; EX1052; EX1002, ¶17. Before December 2010, *Gloor* was also cited in a publicly presented poster, allowing "interested members of the relevant public" to access *Gloor* by exercising reasonable diligence. EX1002, ¶18, Exs. G, H; *GoPro, Inc. v. Contour IP Holding LLC*, 908 F.3d 690, 693 (Fed. Cir. 2018).

Patent Owner challenges Dr. Gloor's familiarity with arXiv.org, noting he became a registered user on the submission date. POR, 50. But Dr. Gloor used and was familiar with arXiv.org before registering on July 28, 2010, consistent with arXiv.org's policy of providing free access to submitted manuscripts without registration. EXS1047-48; EX1046, ¶¶11-13. Indeed, speedy public availability and easy accessibility were precisely why Dr. Gloor submitted *Gloor* to arXiv.org. EX1046, ¶¶11, 18.

A reference's prior-art status must be determined based on the evidence "as a whole" without "overstat[ing] the required degree of certainty." *CQV Co. v. Merck Pat. GmbH*, 130 F.4th 1344, 1350 (Fed. Cir. 2025). Here, Patent Owner presents *no*

contrary evidence. The Board should find that Petitioner has proved *Gloor*'s prior-art status by a preponderance of the evidence.

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

In December 2010, a POSA would have been motivated to apply *Parameswaran*'s method to Illumina sequencers as taught in *Bentley*. Pet., 55-58. Patent Owner argues that the references cannot be combined because *Parameswaran* pooled and sequenced multiple samples while *Bentley* performed whole-genome sequencing without sample pooling. POR, 51-52.

Patent Owner again fails to read the prior art from “the vantage point” of a POSA. *Donner*, 979 F.3d at 1360. *Bentley*, an oft-cited 2008 article, demonstrated how Illumina sequencers work using whole-genome sequencing as a model system. See EX1006. Before December 2010, Illumina sequencers had been used for many other purposes, including multiplex sequencing. E.g., EX1005, 1; EX1055, 73:4-7; EX1054, ¶118. Given *Parameswaran*'s express recommendation and the many advantages Illumina sequencers offered, a POSA would have been motivated to combine *Parameswaran* and *Bentley*. EX1004, 1-2; EX1054, ¶¶116-118.

Patent Owner otherwise recycles the same flawed arguments made and addressed above for *Parameswaran* and *Gloor*. POR, 52; *supra* § III. *Parameswaran* and *Bentley* render the challenged claims obvious. EX1054, ¶116.

V. CONCLUSION

The challenged claims should be found unpatentable.

Dated: July 3, 2025

By: /William B. Raich/
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CERTIFICATE OF COMPLIANCE

The undersigned certifies that the foregoing **PETITIONER'S REPLY** contains 5,585 words, excluding those portions identified in 37 C.F.R. §42.24(c), as counted by the word-processing software used to prepare this paper.

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CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing **PETITIONER'S REPLY** was served on July 3, 2025, via e-mail directed to counsel of record for the Patent Owner at the following:

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