

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. IPR2024-00965
Patent No. 11,768,200

PETITIONER'S REPLY

TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
II. CLAIM CONSTRUCTION	2
Claim 1[a]: “Detecting the Presence of <i>Two or More Identifier Sequences That Are Uniquely Associated With the Nucleic Acid of Interest</i> ”	2
III. GROUND 1: CLAIMS 1-6 ARE ANTICIPATED BY <i>GLOOR</i>	4
A. <i>Gloor</i> Anticipates Claim 1	4
B. <i>Gloor</i> Anticipates Claim 5	5
IV. GROUND 2: CLAIMS 1-6 ARE ANTICIPATED BY <i>PARAMESWARAN</i>	5
A. <i>Parameswaran</i> Anticipates Claim 1	5
B. <i>Parameswaran</i> Anticipates Claims 5 and 6	7
V. GROUND 3: CLAIMS 1-6 WOULD HAVE BEEN OBVIOUS OVER <i>PARAMESWARAN</i> AND <i>GLOOR</i>	8
A. Patent Owner Fails to Rebut the Strong Motivation to Combine <i>Parameswaran</i> and <i>Gloor</i>	9
1. “Chimeric Sequences, Such as Cross-Over Error,” Were a Known Problem	9
2. <i>Parameswaran</i> Provided a Solution to the Known Problem of Chimeric Sequences	13
3. Multiple Reasons Support Applying <i>Parameswaran</i> ’s Method to <i>Gloor</i> ’s Illumina Sequencer	15
4. Patent Owner Fails to Identify Anything That Would Have Motivated a POSA to Avoid <i>Parameswaran</i> ’s Unique Dual-Indexing Methods	21

B.	Patent Owner’s Cursory Treatment of Reasonable Expectation of Success Confirms Obviousness	22
C.	Patent Owner’s Silence on Secondary Considerations Speaks Volumes.....	23
D.	<i>Parameswaran</i> and <i>Gloor</i> Render Claim 5 Obvious	23
VI.	GROUND 4: CLAIMS 1-6 WOULD HAVE BEEN OBVIOUS OVER <i>PARAMESWARAN</i> AND <i>BINLADEN</i>	24
A.	<i>Parameswaran</i> and <i>Binladen</i> Render Claim 1 Obvious	24
B.	<i>Parameswaran</i> and <i>Binladen</i> Render Claims 5 and 6 Obvious.....	25
VII.	GROUND 5: CLAIM 6 WOULD HAVE BEEN OBVIOUS OVER <i>PARAMESWARAN</i> , <i>BINLADEN</i> , AND <i>BENTLEY</i>	26
VIII.	GLOOR WAS PUBLICLY AVAILABLE	26
IX.	CONCLUSION.....	29

TABLE OF AUTHORITIES

	Page(s)
Federal Cases	
<i>Alcon Rsch., Ltd. v. Apotex Inc.</i> , 687 F.3d 1362 (Fed. Cir. 2012)	14
<i>Align Tech., Inc. v. Dental Monitoring SAS</i> , IPR2023-01369, Paper 42 (PTAB Mar. 3, 2025)	27
<i>Aventis Pharma Deutschland GmbH v. Lupin, Ltd.</i> , 499 F.3d 1293 (Fed. Cir. 2007)	9
<i>Bayer Healthcare Pharms., Inc. v. Watson Pharms., Inc.</i> , 713 F.3d 1369 (Fed. Cir. 2013)	20
<i>Bayer Pharma AG v. Watson Lab 'ys, Inc.</i> , 874 F.3d 1316 (Fed. Cir. 2017)	22
<i>Constant v. Advanced Micro-Devices, Inc.</i> , 848 F.2d 1560 (Fed. Cir. 1988)	26
<i>CQV Co. v. Merck Pat. GmbH</i> , 130 F.4th 1344 (Fed. Cir. 2025)	29
<i>Donner Tech., LLC v. Pro Stage Gear, LLC</i> , 979 F.3d 1353 (Fed. Cir. 2020)	11
<i>Elekta Ltd. v. ZAP Surgical Sys., Inc.</i> , 81 F.4th 1368 (Fed. Cir. 2023)	19
<i>GoPro, Inc. v. Contour IP Holding LLC</i> , 908 F.3d 690 (Fed. Cir. 2018)	28
<i>KSR Int'l Co. v. Teleflex Inc.</i> , 550 U.S. 398 (2007)	14, 15
<i>Leo Pharm. Prods., Ltd. v. Rea</i> , 726 F.3d 1346 (Fed. Cir. 2013)	15

<i>In re Applied Materials, Inc.</i> , 692 F.3d 1289 (Fed. Cir. 2012)	7
<i>UCB, Inc. v. Actavis Lab'ys UT, Inc.</i> , 65 F.4th 679 (Fed. Cir. 2023)	18

TABLE OF ABBREVIATIONS

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

The challenged claims are anticipated by *Gloor* and *Parameswaran* and obvious over those references, *Binladen*, and *Bentley*.

Patent Owner's response misconstrues the claims and misinterprets the prior art. For example, Patent Owner's contention that *Gloor* does not anticipate fails because the plain claim language permits using the same identifier sequence for different analytes. Indeed, the specification confirms that “***individual barcode sequences can be used with multiple templates*** as long as no pair is used across two different samples.” EX1001, 2:50-53.

Patent Owner's argument that the problem of chimeric sequences was unknown for Illumina sequencers likewise fails. Nothing in the claims limits sequence validation to chimera detection, and regardless, numerous prior-art references confirm that this problem was well known. *E.g.*, EX1057, 2, 7 (detecting and excluding nearly 1.2 million chimeric sequences during Illumina sequencing); EX1061, 8 (detecting candidate chimeras formed among templates in “close proximity on the [Illumina] flow cell”); EX1005, 8 (detecting chimeras arising “during PCR or because of overlapping spots” on an Illumina flow cell).

Patent Owner's additional arguments lack merit for the reasons set forth in the Petition, this Reply, and the declarations of Drs. Spellman and Gloor. Every challenged claim is unpatentable.

II. CLAIM CONSTRUCTION

Claim 1[a]: “Detecting the Presence of *Two or More Identifier Sequences That Are Uniquely Associated With the Nucleic Acid of Interest*”

Patent Owner does not expressly seek to construe any term in claim 1, but implicitly seeks to narrow the claims to avoid anticipation by *Gloor*. EX1054, ¶¶7-8. Patent Owner argues that the claims require that “any identifier sequence can be used with *only one* template nucleic acid of interest.” POR, 14-15. *Gloor*’s combinatorial dual-indexing strategy used *pairs* of identifier sequences uniquely associated with each analyte of interest, but individual identifier sequences were used for multiple analytes. EX1005, Abstract.

Patent Owner’s argument contradicts the plain claim language. EX1054, ¶9. Claim 1 requires “*two or more* identifier sequences that *are uniquely associated* with the nucleic acid analyte of interest.” EX1001, cl. 1. It does *not* state that *each* individual identifier sequence is unique to the analyte of interest. The specification supports this reading, stating: “[w]hile the pair of barcodes associated with any given template is unique, *individual barcode sequences can be used with multiple templates* as long as no pair is used across two different samples.” EX1001, 2:50-53; *see also id.*, 3:20-23.

Patent Owner nevertheless argues that one could not exclude sequences with an “incorrect pair of identifiers,” as recited in claim 1, if individual identifier

sequences were used for more than one analyte of interest. POR, 14-15. This is incorrect. EX1054, ¶¶10-16. *First*, as Patent Owner’s expert Dr. Adalsteinsson admitted, the last clause of claim 1 recites excluding sequences containing “only one identifier *or* an incorrect pair of identifiers.” EX1055, 64:21-65:3, 68:17-22. Regardless 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*. EX1005, 7; EX1055, 76:9-77:12; EX1054, ¶11.

Second, regardless of whether identifier sequences are used with multiple analytes of interest, sequences containing “an incorrect pair of identifiers” could also be excluded—for example, those containing an identifier sequence containing a truncation, insertion, or erroneous base. EX1054, ¶12. This was also done in *Gloor*. *Id.*; EX1005, 7-8; EX1055, 77:13-20.

Third, when identifier sequences are used with multiple analytes of interest, but the combinations of identifiers are not saturated (“sparse” non-unique dual-indexing), analytes of interest could be excluded if an incorrect pair of identifiers occurs that was not originally associated with an analyte of interest. EX1054, ¶¶13-14; EX2002, 14:20-16:1. Tellingly, the claims are silent on the specific number of identifiers or analytes used and the ratio between the two. EX1054, ¶15; EX1055, 62:5-64:1.

Patent Owner's effort to avoid *Gloor* should therefore be rejected. But even if the claims were construed as Patent Owner suggests, they are still anticipated by *Parameswaran* and obvious over the asserted combinations. EX1054, ¶¶16, 140-141; *see infra* §§ IV-VII.

III. GROUND 1: CLAIMS 1-6 ARE ANTICIPATED BY *GLOOR*

A. *Gloor* Anticipates Claim 1

Patent Owner's response to *Gloor* relies entirely on its erroneous claim construction. POR, 13-15; *see supra* § II. Properly construed, claim 1 permits using individual identifier sequences with multiple analytes, provided that no *pair* of identifier sequences is used for more than one analyte. *See supra* § II. *Gloor* disclosed this. EX1005, 2; EX1054, ¶¶17-19.

Gloor also disclosed identifying and excluding sequences with a “*complete lack* of the left or right end sequence tag”—i.e., sequences with “only one identifier,” which Dr. Adalsteinsson ignored. EX1005, 7; EX1054, ¶¶18-19; EX2001, ¶¶83-85; EX1055, 68:7-16. *Gloor* likewise excluded sequences with a “*truncation* of the sequence tag” or a “small numbers of *errors* including additional 5' bases” or “misincorporated bases”—i.e., sequences with “an incorrect pair of identifiers.” EX1005, 7-8; EX1054, ¶¶18-19.

Gloor therefore anticipates claim 1. Other than claim 5, Patent Owner did not separately contest anticipation by *Gloor* for the remaining dependent claims, which are also anticipated. EX1054, ¶20.

B. *Gloor* Anticipates Claim 5

Patent Owner and Dr. Adalsteinsson concede that *Gloor*'s identifier sequences "lack" "homopolymer regions." POR 15-16; EX1055, 79:18-80:2. But they wrongly allege that *Gloor* does not anticipate because it does not state that the identifiers were "*designed* to have no homopolymer regions." *Id.* *Gloor*'s identifier sequences were self-evidently designed to have no homopolymer regions, since they "lack" "homopolymer regions." EX1054, ¶¶21-24.

IV. GROUND 2: CLAIMS 1-6 ARE ANTICIPATED BY *PARAMESWARAN*

A. *Parameswaran* Anticipates Claim 1

Patent Owner contends that *Parameswaran* does not anticipate claim 1 because it purportedly "included," rather than "excluded," nucleic acid analytes containing "only one identifier or an incorrect pair of identifiers." POR, 23-25. But *Parameswaran* characterizes sequences with mismatched barcode motifs (i.e., sequences with "an incorrect pair of identifiers") as "*false-discover[ies]*." EX1004, 8; EX1054, ¶25. This contrasts with *Parameswaran*'s treatment of sequences containing "both 5' and 3' barcode motifs" (i.e., sequences with a correct pair of

identifiers), which were validated through placement “into the correct bin.” EX1004, 8; EX1054, ¶25.

To support its “inclusion” argument, Patent Owner points to the following 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.” POR, 23 (emphasis in original); EX1004, 8. But this sentence refers to ***expected*** sequence motifs—not what *Parameswaran* calls “perfect, ***unexpected*** occurrences,” where each of the two barcode motifs belong to different samples (i.e., mismatched barcoded motifs) and therefore “[t]here wouldn’t be a sample to assign.” EX1004, 7; EX1055, 52:15-53:5; EX1054, ¶¶26-27. Notably, *Parameswaran*’s data table (reproduced below) ***greyed out*** sequences with mismatched barcode motifs, signifying that they were excluded. EX1004, 23, 29; EX1054, ¶¶28-29, 33. 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, ¶¶30-32. *Parameswaran* therefore “excluded” sequences containing “an incorrect pair of identifiers.” EX1054, ¶34.

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	292	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)	349	167	210	122	237	154	317	172	268	280	208	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 (EX1004, 29; Shading in Original)

Claim 1 is therefore anticipated. Other than claims 5 and 6, Patent Owner did not separately contest anticipation by *Parameswaran* for the remaining challenged claims, which are also anticipated. EX1054, ¶35.

B. *Parameswaran* Anticipates Claims 5 and 6

Patent Owner contends that *Parameswaran* does not anticipate claim 5 because *Parameswaran's* specific barcodes, which contain two of the same bases in a row, contain a “homopolymer region” under the specification’s definition. POR, 25-26. But a “reference must be considered for everything that it teaches, not simply the described invention or a preferred embodiment.” *In re Applied Materials, Inc.*, 692 F.3d 1289, 1298 (Fed. Cir. 2012). *Parameswaran* expressly discloses designing

barcodes “with care taken to *avoid* homopolymeric runs.” EX1004, 4; EX1054, ¶36. Regardless of whether *Parameswaran*’s specific barcodes contain repeat bases, *Parameswaran*’s recommendation anticipates claim 5. Notably, the ’200 Patent *verbatim* copied the definition of “homopolymer regions,” and instruction not to use them, from a prior-art publication. *Compare* EX1001, 9:7-9 with EX1037, 5:2-5; EX1054, ¶37.

Patent Owner contends that *Parameswaran* does not anticipate claim 6, arguing that in Roche 454 pyrosequencing, the analyte of interest is not “on the surface of the flow cell,” as claimed, “but suspended *above* the flow cell.” POR, 26-27. Claim 6, however, does not require the analyte to physically contact the flow cell surface. EX1054, ¶¶38-39. Patent Owner’s pyrosequencing depiction (POR, 26) confirms that, whether suspended or not, the analyte in *Parameswaran* was located on the surface of the flow cell.

V. GROUND 3: CLAIMS 1-6 WOULD HAVE BEEN OBVIOUS OVER *PARAMESWARAN* AND *GLOOR*

Parameswaran and *Gloor* anticipate the challenged claims because each discloses every claim limitation. Their combination also renders the claims obvious, regardless of how the claims are construed.

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

1. “Chimeric Sequences, Such as Cross-Over Error,” Were a Known Problem

Relying on Dr. Adalsteinsson, Patent Owner argues the claims would not have been obvious because “chimeric sequences, such as cross-over error” were an unknown problem for NGS. POR, 36-42. Patent Owner refers to “chimeras” or “chimeric sequences” more than 30 times and “cross-over” 26 times in its Response. But these are not claim limitations. EX1054, ¶¶40-42.

Instead, the claims recite validating nucleic acid analytes of interest by excluding sequences “containing only one identifier or an incorrect pair of identifiers.” EX1001, cl. 1. A POSA would have been motivated, with a reasonable expectation of success, to exclude erroneous sequences containing only one identifier, as reported in *Gloor*. See Pet., 47-49; EX1054, ¶¶40-42. Patent Owner does not contend otherwise. This alone renders the challenged claims obvious. *Aventis Pharma Deutschland GmbH v. Lupin, Ltd.*, 499 F.3d 1293, 1300 (Fed. Cir. 2007) (obvious prior-art species renders later genus claim obvious).

Patent Owner and Dr. Adalsteinsson are also wrong that a POSA in December 2010 “would not have known that cross-over error was problematic for NGS, particularly for the pyrosequencer in *Parameswaran* or the Illumina sequencer in *Gloor*.” POR, 36. At that time, 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 chimeras arising during Illumina sequencing. EX1054, ¶¶43-73. *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 on Illumina’s flow cells. *Id.*, 2, 7; EX1054, ¶¶52-55. Other articles similarly detected and excluded erroneous chimeric sequences, including PCR-induced artifacts, during Illumina sequencing. EX1066, 10; *see also* EX1061, 8, 16 (reporting artificial chimeric sequences during Illumina sequencing); EX1005, 8; EX1054, ¶¶56-57, 48-51.¹

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

¹ Patent Owner argues that EX2005 shows that “cross-over error for NGS” was “not a problem.” POR, 37; EX2001, ¶124. As Dr. Adalsteinsson testified, however, “though [EX2005] uses the term ‘cross-over error,’ I don’t believe it is even about what the patent discloses.” EX1055, 25:22-26:15; EX1054, ¶¶65-69.

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 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, ¶¶48-51. This was a sequencer-agnostic problem for multiplex sequencing on any platform relying on PCR. EX1065, 5 (Illumina sequencers use “bridge PCR” to amplify templates as clusters); EX1067, 3 (“mixed clusters can be grown starting from more than one template” in Illumina sequencing); EX1005, 8, 22 (reporting chimeras formed during Illumina multiplex sequencing); EX1054, ¶¶45-47.

Patent Owner wrongly dismisses early references discussing chimera formation during PCR (EXS1017-20) because they published before NGS became available. POR, 36-37. 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). These references were understood as relevant in December 2010 because many NGS platforms used PCR during sequencing, including bridge amplification on a flow cell. EX1003, ¶¶62-64, 70; EX1054, ¶¶61-63; 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, ¶¶63-64.

Patent Owner also contends that these early references concerned chimeras formed “within a sample,” whereas the challenged claims concern those formed “between samples.” POR, 36-37. 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, ¶63; 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, ¶¶70-73. 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, ¶71. 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, EX2004's authors 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, ¶71. Thus, "chimeric sequences, such as cross-over error," were a recognized problem in December 2010. EX1054, ¶73.

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

A POSA would have been motivated to exclude erroneous sequences formed during multiplex sequencing—including inter-sample chimeric sequences—because such sequences were not present in the original samples and undermined sample identification. Pet., 48-49; EX1054, ¶¶74-75.

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., 49; EX1054, ¶¶76-80. Using unique dual-indexing, *Parameswaran* identified chimeric sequences based on 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 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 excluded inter-sample chimeric sequences. EX1054, ¶¶76-80.

Solely focusing on the “specific problem” allegedly identified by the inventors—“chimeric sequences, such as cross-over error”—Patent Owner argues that a POSA would not have adopted *Parameswaran*'s method because *Parameswaran* does not explicitly mention “cross-over error.” POR, 38-40; 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 during multiplex sequencing. *See supra* § V.A.1. A POSA would have been motivated to use *Parameswaran*'s method to exclude these erroneous sequences, regardless of how *Parameswaran* described that source of error. EX1054, ¶81.

Patent Owner also argues that “*Parameswaran*'s mismatched barcode motifs were *not* cross-over error.” POR, 38-39 (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, ¶¶82-93. 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, ¶¶86-88. 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.*, ¶85.

The challenged claims—which apply an obvious solution (offered by *Parameswaran*) to a known problem (chimeric sequences arising during multiplex sequencing)—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. 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, 28-29. But *Parameswaran*'s method would benefit *any* NGS platform, regardless of error rate or sequencing capacity. EX1054, ¶¶94-95. 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, ¶¶96-100. 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 '200 Patent acknowledged this in *Fuller*, a 2009 prior-art article. EX1059, 3; EX1054, ¶¶99. *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)).²

Alleged Reason #2: Patent Owner contends that *Parameswaran*’s approach applies only to pyrosequencing because its barcodes are “tailored” for pyrosequencing. POR, 29-30. 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, ¶¶101-102; EX1055, 104:13-105:11 (“So by and large, I’d say that was known in the art.”). Indeed, this had

² 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.

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 '200 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, 20:40-44; Pet., 20; EX1054, ¶102.

Nor does *Gloor* “warn[] against applying pyrosequencing approaches to Illumina sequencers.” POR, 29-30. *Gloor* commented on methods for analyzing 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, ¶103.

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, 30-31. 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). Instead, a POSA would have understood the respective benefits of unique

and non-unique dual-indexing and chosen the appropriate strategy accordingly. EX1054, ¶¶104-105. *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 these considerations were less important, non-unique dual-indexing could have been considered.³ EX1054, ¶105. 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,” but argues this would have led a POSA to “consider” Illumina sequencers, not “motivate” their use. EX1004, 1-2, 8; POR, 42-43. This semantic argument is a distinction without a difference. *See Bayer Healthcare Pharms., Inc. v. Watson*

³ 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, ¶¶90-91; EX1055, 77:13-78:8. This also would have motivated a POSA to use *Parameswaran*'s approach.

Pharms., Inc., 713 F.3d 1369, 1376 (Fed. Cir. 2013) (finding motivation based on “prior art’s direct recommendations”); EX1054, ¶¶106-107. 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, ¶204.

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, 43-44. On the contrary, Petitioner identified the known problem of chimeric sequences “such as cross-over error” in NGS platforms. Pet., 49; *see supra* § V.A.1. Petitioner explained that, to improve efficiency and accuracy, it would have been obvious to try *Parameswaran*’s method (which detects chimeras) on *Gloor*’s Illumina sequencer (which offered superior accuracy and cost). Pet., 49. 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; EX1054, ¶108.

4. 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*.⁴ POR, 32-35, 44-45. Patent Owner emphasizes the 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, ¶¶92-93, 111. 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 a '200 patent inventor

⁴ Of course, combining *Gloor* with *Parameswaran* does not require sequencing 272 samples. EX1054, ¶¶109-110.

who cited *Parameswaran* as disclosing “an effective technology” for “reduc[ing] the inefficiencies” of NGS platforms. EX1059, 3; EX1054, ¶111.

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, ¶¶111-112. A POSA would have been motivated to combine *Parameswaran* and *Gloor* to improve sequencing accuracy and facilitate sample assignment. Pet., 48-51.

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 chimera formation was even a problem for NGS, including “Illumina sequencers like Gloor’s,” and therefore would not know whether such molecules “give rise to validation issues.” POR, 45-46.

Not so. By December 2010, a POSA would have recognized chimeric sequences as a problem for multiplex sequencing, including for Illumina sequencers. *See supra* § IV.A.1. Multiple references disclosed chimeric sequence formation during Illumina sequencing. *Id.* Further, concerns about chimeric sequences applied

generally to PCR amplifications involving templates from multiple samples. *Id.*; EX1054, ¶¶113-115. Notably, Patent Owner did not dispute that the combination of *Parameswaran* and *Gloor* could in fact validate sequences of analytes of interest. *See* POR, 45-46.

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 prior art for improving sequencing accuracy. *See supra* § V.A.3.

D. *Parameswaran* and *Gloor* Render Claim 5 Obvious

Contrary to Patent Owner’s arguments (POR, 46-47), claim 5 would have been obvious in view of *Gloor*’s design of barcode sequences lacking homopolymer regions, as well as *Parameswaran*’s caution to “avoid homopolymeric runs.” *See supra* §§ III.B, IV.B; EX1054, ¶¶118-119.

Patent Owner makes no separate arguments regarding any other challenged claim, all of which would have been obvious over *Parameswaran* and *Gloor*. EX1054, ¶¶116-117.

**VI. GROUND 4: CLAIMS 1-6 WOULD HAVE BEEN OBVIOUS OVER
PARAMESWARAN AND BINLADEN**

A. *Parameswaran* and *Binladen* Render Claim 1 Obvious

Parameswaran discloses the last clause of claim 1, including “analyzing both identifiers.” *See supra* § IV.A. Patent Owner argues *Binladen* does not teach this limitation because it analyzes the two identifiers in two separate sequence reads. EX1029, 3; POR, 48. But the claim does not require sequencing the identifiers in a single sequence read. EX1054, ¶¶120-123. By separately analyzing sequence reads containing the 5'-end barcode and sequence reads containing the 3'-end barcode, *Binladen* teaches “analyzing both identifiers.” *Id.*, ¶124.

Contrary to Patent Owner’s characterization (POR, 48), *Binladen* also discloses excluding sequences with “only one identifier.” EX1054, ¶¶125-126. *Binladen* discloses “discard[ing] all sequence reads without an exact match to any of the primers used in the studies.” EX1029, 5. This includes sequences “with only one identifier,” which therefore were excluded.

Patent Owner also argues there was no motivation to combine these references because *Parameswaran* found *Binladen*’s barcodes too short for multiplexing and *Binladen* is purportedly a “step backwards” from *Parameswaran*. POR, 52-54. But this only **strengthens** the motivation to combine. EX1054, ¶¶127-128. To improve multiplex sequencing accuracy, a POSA would have been motivated to use

Parameswaran's unique dual-indexing method, including its longer barcodes. *Id.* To improve sequence fidelity, a POSA would have excluded sequences with one identifier (as in *Binladen*) or an incorrect pair of identifiers (as in *Parameswaran*). Implementing this “predictable variation” results in the claimed method. Pet., 56; EX1054, ¶¶127-130.

B. *Parameswaran* and *Binladen* Render Claims 5 and 6 Obvious

Patent Owner argues that *Binladen* does not disclose barcode sequences designed to have no homopolymer runs, even though its tetranucleotide barcodes had no homopolymer runs and it concluded that these barcodes without repeat bases improved sample assignment accuracy over those with repeat bases. POR, 49-51; EX1029, 7. Combined with *Parameswaran*'s recommendation to “avoid homopolymeric runs” (*see supra* § IV.B), *Binladen* renders claim 5 obvious. EX1054, ¶¶131-135.

Claim 6 merely specifies the location of the analyte as “on the surface of a flow cell.” *See supra* § IV.B. Patent Owner's depiction of pyrosequencing confirms that the analytes in *Parameswaran* and *Binladen* were located on the surface of a flow cell. *Id.* Claim 6 also would have been obvious. EX1054, ¶136.

**VII. GROUND 5: CLAIM 6 WOULD HAVE BEEN OBVIOUS OVER
*PARAMESWARAN, BINLADEN, AND BENTLEY***

Instead of specifically addressing this prior-art combination, Patent Owner refers back to its arguments regarding *Parameswaran, Gloor, and/or Binladen*. POR, 54. As detailed above, these arguments are flawed. Claim 6 would have been obvious. *See supra* §§ VI; EX1054, ¶¶137-139.

VIII. 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, 17-18. 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, 18-19. 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, 21. 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.

IX. CONCLUSION

The challenged claims should be found unpatentable.

Dated: July 3, 2025

By: /William B. Raich/
William B. Raich, Reg. No. 54,386
Lead Counsel

CERTIFICATE OF COMPLIANCE

The undersigned certifies that the foregoing **PETITIONER'S REPLY** contains 5,586 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.

Dated: July 3, 2025

By: /William B. Raich/
William B. Raich, Reg. No. 54,386
Lead Counsel

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:

Brian D. Matty
Ashley DaBiere
Michael Wueste
Kevin Goon
DESMARAIS LLP
230 Park Avenue
New York, NY 10169
bmatty@desmaraisllp.com
adabiere@desmaraisllp.com
mwueste@desmaraisllp.com
kgoon@desmaraisllp.com
MolecularLoopService@desmaraisllp.com

Bridget Belcher
DESMARAIS LLP
101 California Street
San Francisco, CA 94111
bbelcher@desmaraisllp.com

Patent Owner has consented to electronic service.

Dated: July 3, 2025

By: /William Esper/
William Esper
Legal Assistant and PTAB Coordinator
FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, LLP