

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

QIAGEN Sciences, LLC,
Petitioner,

v.

Tecan Genomics, Inc.,
Patent Owner.

Case IPR2025-00026

U.S. Patent No. 11,098,357

**EXPERT DECLARATION OF MICHAEL L. METZKER, Ph.D.
IN SUPPORT OF THE PETITIONER'S REPLY REGARDING
THE '357 PATENT**

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I. INTRODUCTION

1. I, Michael L. Metzker, prepared a previous Declaration¹ in connection with QIAGEN Sciences, LLC's ("QIAGEN" or "Petitioner") Petition for Inter Partes Review of U.S. Patent No. 11,098,357 (the "'357 Patent").

2. After I submitted my previous Declaration, the Board issued an Institution Decision ("ID") granting review of the '357 Patent. Subsequently, Tecan Genomics, Inc. ("Tecan" or "Patent Owner") submitted its Patent Owner Response ("POR"), along with a declaration from Dr. Carlos D. Bustamante.² I have been asked by QIAGEN's counsel to opine on the claim construction and anticipation arguments set forth in Tecan's POR.

II. MATERIALS REVIEWED

3. I have reviewed the materials cited herein, including the Patent Owner's Response, the Board's Institution Decision, and the following exhibits.

Ex.	Description
1001	<i>Amorese et al., Compositions and methods for identification of a duplicate sequencing read</i> , U.S. Patent No. 11,725,241 (the "'241 Patent").
1002	<i>Amorese et al., Compositions and methods for identification of a duplicate sequencing read</i> , U.S. Patent No. 11,098,357 (the "'357 Patent").
1003	Expert Declaration of Michael L. Metzker, Ph.D.
1004	Miner et al, <i>Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR</i> , NUCLEIC ACID RESEARCH 32:e135 pp. 1-4 (2004)

¹ See Ex. 1003, Declaration of Michael L. Metzker, Ph.D.

² See Ex. 2107, Declaration of Carlos D. Bustamante, Ph.D.

Ex.	Description
	(“Miner 2004”)
1005	McCloskey <i>et al.</i> , <i>Molecular encoding of nucleic acid templates for PCR and other forms of sequence analysis</i> , U.S. Patent Publication No. 2007/0020640 (2007) (the “’640 Patent Pub. (McCloskey)”).
1006	Porreca <i>et al.</i> , <i>Methods and compositions for evaluating genetic markers</i> , U.S. Patent Publication No. 2012/0165202 (2012) (the “’202 Patent Pub. (Porreca)”).
1007	Schmitt <i>et al.</i> <i>Methods of lowering the error rate of massively parallel DNA sequencing using duplex consensus sequencing</i> , WO 2013/142378 (2013) (the “’389 PCT Appl. (Schmitt)”).
1008	Illumina Publication No. 770-2008-011, <i>Multiplexed sequencing with the Illumina Genome Analyzer system</i> (2008) (“Illumina (2008)”), available at https://web.archive.org/web/20101226144356/https://www.illumina.com/documents/products/datasheets/datasheet_sequencing_multiplex.pdf .
1009	Authenticating affidavit of Nathaniel Frank-White from the Internet Archive, including the archived copy of <i>Illumina 2008</i> .
1010	Metzker, <i>Sequencing technologies – the next generation</i> , NATURE REVIEWS GENETICS 11:31-46 (2010) (“Metzker (2010)”).
1011	Li <i>et al.</i> , <i>The Sequence Alignment/Map format and SAMtools</i> , BIOINFORMATICS 25:2078-2079 (2009) (“Li (2009)”).
1012	Prosecution History of U.S. Patent No. 11,725,241
1013	Prosecution History of U.S. Patent No. 11,098,357
1014	Authenticating declaration of Sylvia D. Hall-Ellis, Ph.D.
1015	McCloskey <i>et al.</i> , <i>Encoding PCR products with batch-stamps and barcodes</i> , BIOCHEMICAL GENETICS 45:761-767 (2007) (“McCloskey (2007)”).
1016	Curriculum Vitae of Michael L. Metzker, Ph.D.
1017	Metzker & Caskey, <i>Polymerase chain reaction</i> , In <i>ENCYCLOPEDIA OF MEDICAL DEVICES AND INSTRUMENTATION</i> , Second Edition, Volume 5, pp. 380-387 (2006) (“Metzker (2006)”).
1018	Saiki <i>et al.</i> , <i>Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia</i> , SCIENCE

Ex.	Description
	230:1350-1354 (1985) (“Saiki (1985)”).
1019	Saiki <i>et al.</i> , <i>Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase</i> , SCIENCE 239:487-491 (1988) (“Saiki (1988)”).
1020	Gibbs <i>et al.</i> , <i>Detection of single DNA base differences by competitive oligonucleotide priming</i> , NUCLEIC ACIDS RESEARCH 17:2437-2448 (1989) (“Gibbs (1989)”).
1021	Newton <i>et al.</i> , <i>Analysis of any point mutation in DNA: The amplification refractory mutation system (ARMS)</i> , NUCLEIC ACIDS RESEARCH 17:2503-2516 (1989) (“Newton (1989)”).
1022	Chamberlain <i>et al.</i> , <i>Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification</i> , NUCLEIC ACIDS RESEARCH 16:11141-11156 (1988) (“Chamberlain (1989)”).
1023	Gibbs <i>et al.</i> , <i>Multiplex DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families</i> , GENOMICS 7:235-244 (1990) (“Gibbs (1990)”).
1024	Grompe, <i>The rapid detection of unknown mutations in nucleic acids</i> , NATURE GENETICS 5:111-117 (1993) (“Grompe (1993)”).
1025	Syvänen, <i>Accessing genetic variation: genotyping single nucleotide polymorphisms</i> , NATURE GENETICS 2:930-942 (2011) (“Syvänen (2001)”).
1026	Metzker, <i>Emerging technologies in DNA sequencing</i> , GENOME RESEARCH 15:1767-1776 (2005) (“Metzker (2005)”).
1027	Rothberg <i>et al.</i> , <i>An integrated semiconductor device enabling non-optical genome sequencing</i> , NATURE 475:348-352 (2011) (“Rothberg (2011)”).
1028	Jain <i>et al.</i> , <i>Improved data analysis for the MinION nanopore sequencer</i> , NATURE METHODS 12:351-356 (2015) (“Jain (2015)”).
1029	Illumina, <i>Multiplexing, Sample preparation guide</i> (2010) (“Illumina Manual (2010)”).
1030	Illumina, <i>TruSeq™ RNA and DNA sample preparation kits</i> (2010) (“Illumina TruSeq Data Sheet (2010)”).
1031	Craig <i>et al.</i> , <i>Identification of genetic variants using barcoded multiplexed sequencing</i> , NATURE METHODS 5:887-893 (2008) (“Craig (2008)”).

Ex.	Description
1032	Li & Durbin, <i>Fast and accurate short read alignment with Burrows–Wheeler transform</i> , BIOINFORMATICS 25:1754-1760 (2009) (“Li & Durbin (2009)”).
1033	Quail <i>et al.</i> , <i>Improved protocols for the Illumina Genome Analyzer sequencing system</i> , CURRENT PROTOCOLS IN HUMAN GENETICS 18.2.1-18.2.27 (2009) (“Quail (2009)”).
1034	October 8, 2025, Carlos D. Bustamante, Ph.D Deposition Tr.
1035	Expert Declaration of Michael L. Metzker, Ph.D. in Support of the Petitioner’s Reply Regarding the ’357 Patent
2107	Declaration of Carlos D. Bustamante, Ph.D.

III. EXPERIENCE AND QUALIFICATIONS

4. My experience and qualifications are set out in my previous declaration and have not changed.³

IV. APPLICABLE STANDARDS

5. I am not a lawyer and offer no legal opinions. For the purposes of this Declaration, I have been informed by counsel about certain aspects of the law that are relevant to my analysis.

A. Claim Construction

6. I have been informed by counsel and understand that during an *inter partes* review proceeding, claims are to be construed in light of the specification and file history as would be read by one of ordinary skill in the art in the relevant art at

³ See Ex. 1003, Declaration of Michael L. Metzker, Ph.D. at § IV.

the time of invention. I have assumed that the relevant time for my analysis is on or before November 13, 2013, the earliest possible priority date for the '357 Patent. My opinions described *infra* would not change even if other priority dates, such as November 13, 2014, the filing date of the first nonprovisional application, were assumed.

7. I have been informed by counsel and understand that patent claim terms are generally given the plain and ordinary meaning that they would have had to one of ordinary skill in the art as of the relevant date. I also understand from counsel that the plain and ordinary meaning of claim terms must be considered in the context of the entire patent claims in which they appear, as well as in the context of the other claims, the specification, and the prosecution or file history of the patent. I further understand that an applicant may depart from the plain and ordinary meaning of a claim term in two instances. *First*, an applicant may provide a definition in the specification that differs from the plain and ordinary meaning, in which case the inventor's definition governs. However, I understand that any such definition must be clearly stated. *Second*, an applicant may disclaim the full scope of a term, such that the invention must include or not include a particular feature. I understand, however, that any such disclaimer must be clear and unmistakable in the specification and/or the prosecution history.

B. Anticipation

8. I have been informed by counsel and understand that a patent claim is invalid as anticipated if a single piece of prior art discloses each and every limitation of that claim.

9. I have been informed by counsel and understand that a single piece of prior art may anticipate without disclosing a particular limitation if that feature is necessarily present, *i.e.*, inherent, in that piece of art. When an anticipating reference is silent about an asserted inherent characteristic, such gap in the reference may be filled with recourse to extrinsic evidence. Such evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by one of ordinary skill in the art. I also understand that a failure of the skilled artisan to contemporaneously recognize an inherent property, function or ingredient of a prior art reference does not preclude a finding of anticipation.

10. I have been informed by counsel and understand that to anticipate a claim, a prior art reference must not only disclose all elements of the claim, but also must disclose those elements arranged as in the claim. A reference can anticipate even if it does not expressly spell out all the limitations arranged or combined as in the claim, if one of ordinary skill in the art reading the reference, would at once have envisaged the claimed arrangement or combination. I also understand that a prior

art reference need not always include an express discussion of the actual combination to anticipate. Instead, a reference may still anticipate if that reference teaches that the disclosed components or functionalities may be combined and one of ordinary skill in the art would have been able to implement the combination.

V. CLAIM CONSTRUCTION

A. “identifying sequence reads with identical identifier and target sequences as duplicates” (Claim 1 of the ’357 Patent)

Tecan’s Construction	QIAGEN’s Construction
“identifying sequencing reads as duplicates through a step that uses both the identifier and at least a portion of the target sequence”	Plain and ordinary meaning, which is identifying sequence reads with identical identifier and at least a portion of the target sequences as duplicates

11. I understand that Tecan proposed in its POR⁴ to construe the claim term “identifying sequence reads with identical identifier and target sequences as duplicates,” which appears in Claim 1 of the ’357 Patent. I understand that Tecan proposes that this claim term means “identifying sequence reads as duplicates through a step that uses both the identifier and at least a portion of the target sequence.” I further understand that Tecan interprets its construction as requiring that the “identifying” is done “in a single step.”⁵ In other words, I understand

⁴ See POR at pp. 14-20.

⁵ *Id.* at p. 29.

Tecan's construction to require that the comparison of the target and identifier sequences to occur in a single step.

12. I understand that QIAGEN contends that this term should be construed in accordance with its plain and ordinary meaning, which is "identifying sequence reads with identical identifier and at least a portion of the target sequences as duplicates." I further understand that QIAGEN's position is that the claim term does not limit the "identifying" to a single step, rather, the claim term allows for the "identifying" to occur in multiple steps.

13. I understand that QIAGEN agrees with Tecan that both the identifier sequence and a portion of the target sequence are compared to identify duplicates. As the parties agree on this point, I will not address it in this declaration. My opinions will instead focus on the portion of the construction that the parties dispute, namely whether the "identifying" must occur in a single step.

14. I disagree with Tecan's proposed construction. Specifically, I disagree that the claim term requires that the comparison of target and identifier sequences must occur in a single step. In my opinion, one of ordinary skill in the art would not have understood the claim term to require that the "identifying" be performed in a single step. Rather, one of ordinary skill in the art would have understood that the claim term requires a comparison of the target and identifier sequences without any restriction regarding how many steps the comparison takes. In my opinion, the claim

term covers a scenario where the target sequences are compared first, and the identifier sequences are compared in a separate, second step. The claim term would also cover a reverse scenario, where the identifier sequences are compared first, and the target sequences are compared in a separate, second step. I thus agree with QIAGEN's construction.

15. My opinions are based on my review of the intrinsic evidence. As an initial matter, I reviewed the claim language and confirmed that there is nothing in the claims to require comparing the identifier and target sequence "in a single step." Moreover, the specification of the '357 Patent discloses in Example 1 an embodiment that identifies duplicates in at least two steps. In my opinion, this example shows that the disputed term does not require a single step analysis.

16. Example 1 is titled, *Identification of Duplicate Sequencing Reads with NuGEN Ovation Target Enrichment Library System*.⁶ In this example, the DNA was fragmented to about 500 base pairs in length, after which it was treated with an end repair enzyme mix to produce blunt ended DNA fragments.⁷ To produce the library, an oligonucleotide containing an Illumina indexing read priming site, an eight base pair indexing site, a random six base pair identifier site, and a Illumina sequencing primer site, was annealed to a second oligonucleotide to form a partially double

⁶ See Ex. 1002, '357 Patent at 16:47-49.

⁷ *Id.* at 16:51-58.

stranded DNA adapter.⁸ These adapters were ligated onto the blunt ended DNA, and the adapter-ligated fragments were purified with magnetic beads. The beads were washed, and the ligated DNA fragments were collected and annealed to targeting probes.⁹ The adapter-ligated fragments were denatured by heat and then cooled slowly to allow targeting probes to anneal to their complementary sequences. The annealed probes were then extended with *Taq* DNA polymerase, and the extension products were purified using magnetic beads according to manufacturer's recommendations.¹⁰ The primer extension products were then enriched by 30 cycles of PCR using NuGEN universal primers that contain Illumina flow cell sequences.¹¹

17. The resulting libraries were quantitated using qPCR and then sequenced using an Illumina MiSeq sequencer.¹² There were three sequencing runs: "36 base first read, 14 base second read, and a 24 base third read."¹³ The 14 base pair second read (*i.e.*, the indexing read) was split into two files, with the first

⁸ *Id.* at 16:60-17:1.

⁹ *Id.* at 17:1-12.

¹⁰ *Id.* at 17:12-21.

¹¹ *Id.* at 17:21-26.

¹² *Id.* at 17:27-30.

¹³ *Id.* at 17:30-31.

containing the eight base indexing read and the second file containing the six random base identifier read that was “set aside for further sequence parsing.”¹⁴

18. Example 1 identified duplicate sequencing reads in at least a two-step process. First, the target sequence reads were aligned to the human reference genome using the “bowtie aligner” to determine their genomic start positions.¹⁵ One of ordinary skill in the art would have understood that this alignment process involves the comparison of at least a portion of the target sequence to the human reference genome. Target read sequences having the same genomic positions were identified as duplicates: “duplicate reads were identified by their genomic start positions.”¹⁶ Example 1 states “Fig. 5 provides the analysis results demonstrating the identification of the duplicative reads.”¹⁷ Figure 5 is a table, whereby its legend states that “all reads with the same start and end genomic coordinates were counted as duplicates.”¹⁸

19. Second, if duplicate sequences were identified having the same genomic positions, their identifier sequences were then check against the random identifier file. Those reads having the same identifier sequence were determined to

¹⁴ *Id.* at 17:32-37.

¹⁵ *Id.* at 17:39-43.

¹⁶ *Id.* at 17:42-43

¹⁷ *Id.* at 17:59-61.

¹⁸ *Id.* at Figure 5.

be “true duplicates.” “At this point, sequencing reads that started at the same genomic position were checked against the random bases file to see if they have the same or different set of random bases that were ligated to them.”¹⁹ The legend of Figure 5 describes “**True’ duplicates**” as follows: An identifier sequence is introduced through ligation to differentiate between fragments of DNA that randomly have the same start and end mapping coordinates,”²⁰ The ’357 Patent states that “[t]he use of an identifier site allowed for the determination of the number of true duplications versus the number of apparent or perceived duplicates.”²¹

20. As described *supra*, Example 1’s duplicate identifying process is a two-step process. Step (1) is a comparison of target sequences via the bowtie aligner to the human reference genome to determine genomic position, which is used to identify duplicates or apparent duplicates, and then Step (2) is a comparison of identifier sites to the random sequence identifier file to identify “true duplicates.” Example 1’s description further confirms that both the target and identifier sites are compared in this two-step process. “If the sequences of two libraries are identical, their duplication status is unknown since this could occur by chance in any library.

¹⁹ *Id.* at 17:43-47.

²⁰ *Id.* at Figure 5.

²¹ *Id.* at 17:61-63.

If an identifier sequence is used in combination with the library reads, the status can be determined (duplicates if identical, distinct if different).”²²

21. In my opinion, one of ordinary skill in the art would have understood from Example 1’s multi-step process for identifying duplicates that the claimed “identifying” limitation is not limited to a single step. It is thus my opinion that Tecan’s construction, which requires a single step, is erroneous and not in accordance with the plain and ordinary meaning of the term. QIAGEN’s construction, on the other hand, does not require any number of “identifying” steps and comports with the plain meaning as well as the intrinsic evidence.

VI. ANTICIPATION

A. The ’202 Patent Pub. (Porreca) discloses the adaptor recited in Claim 1 of the ’357 Patent

22. I understand that Tecan argues in its POR that the ’202 Patent Pub. (Porreca) fails to disclose the adaptor recited in Claim 1 of the ’357 Patent.²³ I understand that Tecan specifically argues that QIAGEN relies on separate, incompatible embodiments for limitations 1.a and 1.c.²⁴ According to Tecan, the ’202 Patent Pub. (Porreca)’s disclosure of a barcode (the claimed indexing site) is limited to only molecular inversion probe (“MIP”) embodiments, which are

²² *Id.* at 17:64-18:1.

²³ *See* POR at 22.

²⁴ *Id.* at § V.A.

allegedly incompatible with the other adaptor disclosures that QIAGEN relies on.²⁵

I disagree with Tecan's arguments. In my opinion, the '202 Patent Pub. (Porreca) teaches an adaptor that can include many different types of sequences, including the identifier and indexing site sequences recited in Claim 1 of the '357 Patent. In my opinion, one of ordinary skill in the art would have immediately envisaged the adaptor recited in Claim 1 of the '357 Patent after reviewing the '202 Patent Pub. (Porreca)'s disclosures that QIAGEN relies on in its Petition.²⁶

23. The '202 Patent Pub. (Porreca) teaches three different preparative methods for use in conjunction in the methods and compositions taught by the '202 Patent Pub. (Porreca). First, the '202 Patent Pub. (Porreca) teaches "a circularization selection-based preparative method, e.g., molecular inversion probe capture products."²⁷ The '202 Patent Pub. (Porreca) states that such methods "convert regions of interest (target nucleic acids) into a covalently-closed circular molecule which is then isolated typically by removal (usually enzymatic, e.g. with exonuclease) of any non-circularized linear nucleic acid."²⁸ The '202 Patent Pub. (Porreca) explains that this preparative method employs probes such as MIPs that

²⁵ *Id.*

²⁶ *See* Petition at 17-27.

²⁷ *See* Ex. 1006, '202 Patent Pub. (Porreca) at [0027].

²⁸ *Id.* at [0028].

have ends that flank the region of interest, which hybridize to the genomic target.²⁹ Enzymes are then used to fill in any gap between the probe ends. The '202 Patent Pub. (Porreca) further states that “[f]ollowing circularization, any remaining (non-target) linear nucleic acid is typically removed, resulting in isolation (capture) of target nucleic acid.”³⁰

24. Second, the '202 Patent Pub. (Porreca) teaches “an amplification-based [] preparative method.”³¹ In this method, “genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using one or more oligonucleotide primers.”³² The '202 Patent Pub. (Porreca) explains that primers may be employed that contain additional sequences “that facilitate downstream applications” such as “differentiator tag sequences.”³³ The '202 Patent Pub. (Porreca) further states that these methods “include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel).”³⁴

²⁹ *Id.*

³⁰ *Id.*

³¹ *Id.* at [0029].

³² *Id.*.

³³ *Id.*

³⁴ *Id.*

25. Third, the '202 Patent Pub. (Porreca) teaches “hybridization-based preparative methods.”³⁵ These methods involve “selectively immobilizing target nucleic acids for further manipulation.”³⁶ The '202 Patent Pub. (Porreca) explains that “one or more oligonucleotides (immobilization oligonucleotides), which comprise differentiator tag sequences, and which may be from 15 to 170 nucleotides in length, are used which hybridize along the length of a target region of a genetic locus to immobilize it.”³⁷

26. The '202 Patent Pub. (Porreca) also teaches that any of the three preparative techniques may be used with several disclosed techniques, including tagging. “It should be appreciated that any of the circularization, amplification, and/or hybridization based methods described herein may be used in connection with one or more of the tiling/staggering, tagging, size-detection, and/or sensitivity enhancing algorithms described herein.”³⁸ In my opinion, one of ordinary skill in the art reviewing paragraph 31 of the '202 Patent Pub. (Porreca) would have understood that it discloses that any of the three preparative methods may be used with the tagging techniques disclosed in the '202 Patent Pub. (Porreca).

³⁵ *Id.* at [0030].

³⁶ *Id.*

³⁷ *Id.*

³⁸ *Id.* at [0031].

27. The '202 Patent Pub. (Porreca) discloses several types of tags. For example, it discloses “differentiator tags,” which are “useful to determine whether target nucleic acid sequences identified in an assay are from independently isolated target nucleic acids or from multiple copies of the same target nucleic acid molecule (e.g., due to bias in a preparative step, for example, amplification).”³⁹ The '202 Patent Pub. (Porreca) also discloses tags that are useful in multiplex analysis involving multiple patient samples. The '202 Patent Pub. (Porreca) explains that “[i]n some embodiments, samples from different subjects are tagged with subject-specific (e.g., patient-specific) tags (e.g., unique sequence tags) so that the information from each product can be assigned to an identified subject.”⁴⁰ In my opinion, these “subject-specific” or “patient-specific” tags are synonymous with the “barcode” disclosed in paragraph 149 of the '202 Patent Pub. (Porreca). And because the “subject-specific” tag / “patient specific” tag / barcode are used to identify the source of a read in a multiplex analysis, they each satisfy the claimed “indexing site.”⁴¹

28. In my opinion, one of ordinary skill in the art reviewing the above disclosures would have understood that, because the '202 Patent Pub. (Porreca)

³⁹ *Id.* at [0024].

⁴⁰ *Id.* at [0102].

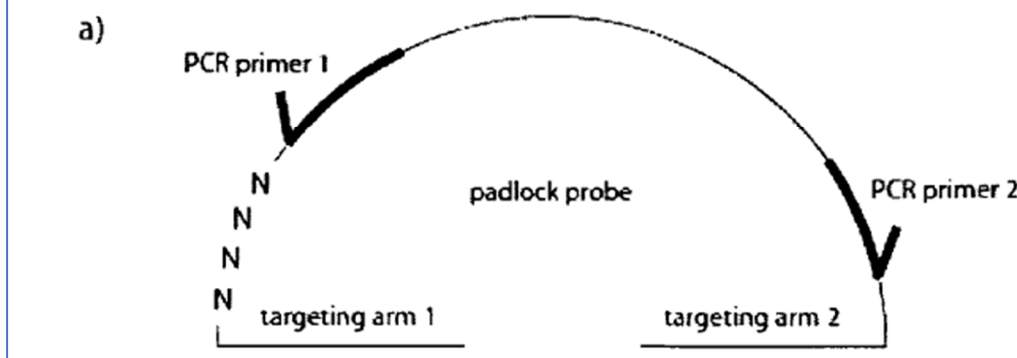
⁴¹ *See also* Ex. 1003, Metzker Declaration at § X.A.1.d: Limitation 1.c.

teaches that “tagging” may be used in connection with “any of the circularization, amplification, and/or hybridization based methods described herein,” the ’202 Patent Pub. (Porreca) teaches that the “subject-specific” tag / “patient specific” tag / barcode (the claimed indexing site) may be employed in an adaptor suitable to any of the three disclosed preparative methods. One of ordinary skill in the art would not have understood the “subject-specific” tag / “patient specific” tag / barcode to be limited only to MIP embodiments.

29. The disclosures of paragraph 149 of the ’202 Patent Pub. (Porreca) further support my opinion that the ’202 Patent Pub. (Porreca)’s barcode is not limited to only MIP embodiments. Paragraph 149 of the ’202 Patent Pub. (Porreca) states that “[a]dapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites.” Based on the use “and/or,” one of ordinary skill in the art would have understood that this paragraph discloses, among others, an adaptor embodiment containing both a PCR priming site *and* a sequencing priming site. In my opinion, one of ordinary skill in the art would have understood that such an adaptor would not be used with a MIP embodiment. Figure 4(a) of the ’202 Patent Pub. (Porreca) discloses a typical MIP padlock probe.⁴²

⁴² *Id.* at Figure 4(a).

FIGURE 4



After the targeting arms hybridize to the region flanking the target sequence and the gap between the arms is filled, a ligase reaction is performed to create the circular nucleic acid target. Thereafter, the PCR primer sites on the padlock probe are used to amplify the resulting circular nucleic acid. Adaptors containing the sequencing primers can then be subsequently ligated to the amplicons. I note that Tecan⁴³ and Dr. Bustamante⁴⁴ both agree that MIP adaptors are ligated after circularization and amplification via the PCR primers on the padlock. Thus, one of ordinary skill in the art would have known that adaptors used with MIPs typically do not contain an additional PCR priming site for additional amplification prior to sequencing on an NGS platform. In contrast, one of ordinary skill in the art would have known that with other preparative methods, the adaptor typically contains both a PCR priming site and a sequencing primer site. Paragraph 149 of the '202 Patent Pub. (Porreca)'s

⁴³ See POR at pp. 25-26.

⁴⁴ See Ex. 2107, Bustamante Declaration, at ¶¶ 117-19.

disclosure of an adaptor containing a sequencing primer and a PCR primer thus shows that one of ordinary skill in the art would have known that this paragraph is not specific to MIPs.

30. Paragraph 149 of the '202 Patent Pub. (Porreca)'s citation to '640 Patent Pub. (McCloskey) further shows that this paragraph is not limited to MIP embodiments.⁴⁵ Paragraph 149 of the '202 Patent Pub. (Porreca) states "barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007 /0020640A1 (McCloskey et al.)." I have reviewed the entirety of the '640 Patent Pub. (McCloskey).⁴⁶ There is no disclosure of any MIP or padlock probe embodiments in the reference. Rather, that reference discloses two types of embodiments containing multiplexing barcodes: a hairpin linker adaptor embodiment and an oligonucleotide primer embodiment.⁴⁷ The '640 Patent Pub. (McCloskey) does not disclose MIPs, let alone adaptors for use with MIPs. In my opinion, one of ordinary skill in the art reviewing paragraph 149 of the '202 Patent Pub. (Porreca) would not have considered its disclosures to be limited to only MIPs

⁴⁵ See Ex. 1006, '202 Patent Pub. (Porreca) at [0149].

⁴⁶ See Ex. 1005, '640 Patent Pub. (McCloskey).

⁴⁷ See Ex. 1003, Metzker Declaration at ¶¶ 65-66.

and would consider that its disclosures are generally applicable to all disclosed preparative methods.

31. The disclosures in paragraph 150 of the '202 Patent Pub. (Porreca) similarly show that the teachings of paragraph 149 are applicable to all disclosed preparative methods and not just to MIPs. That paragraph discloses an adaptor containing a common adaptor sequence, a differentiator tag sequence, and a PCR priming sequence “such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5’ to 3’ order).”⁴⁸ As discussed *supra*, the presence of a PCR priming sequence indicates that the adaptor is not intended for use in a MIP, as MIP embodiments have already undergone amplification by the time the adaptors are attached.

32. In my opinion, one of ordinary skill in the art reviewing paragraphs 149 and 150 of the '202 Patent Pub. (Porreca) would at once have envisaged an adaptor containing both a barcode (the claimed indexing site) and a random differentiator tag (the claimed unique identifier).

33. In my opinion, the disclosures of paragraph 102 of the '202 Patent Pub. (Porreca) additionally bolster my opinion that the disclosed barcodes are not limited in use to only MIPs. The first sentence of that paragraph states “[i]t should be

⁴⁸ See Ex. 1006, '202 Patent Pub. (Porreca) at [0150].

appreciated that aspects of the invention may be useful for high throughput, cost-effective, yet reliable, genotyping of multiple patient samples (e.g., in parallel, for example in multiplex reactions).”⁴⁹ There is no mention of MIP embodiments in this sentence. Another sentence in the same paragraph states “[i]n some embodiments, samples from different subjects are tagged with subject-specific (e.g., patient-specific) tags (e.g., unique sequence tags) so that the information from each product can be assigned to an identified subject.”⁵⁰ There is also no mention of MIPs in this sentence. Rather, this sentence mentions that the disclosed tags may be used “in some embodiments.” In view of the ’202 Patent Pub. (Porreca)’s earlier disclosure that “tagging” may be used with any of the three preparative methods,⁵¹ it is my opinion that one of ordinary skill in the art would have understood that paragraph 102 of the ’202 Patent Pub. (Porreca)’s disclosure of subject-specific and patient specific tags is not limited to only MIPs, and that these tags may be used with any of the disclosed preparative methods.

34. It is my opinion that one of ordinary skill in the art reviewing the disclosures described *supra* would have understood that the ’202 Patent Pub. (Porreca) teaches an adaptor having all the elements recited in Claim 1 of the ’357

⁴⁹ *Id.* at [0102].

⁵⁰ *Id.*

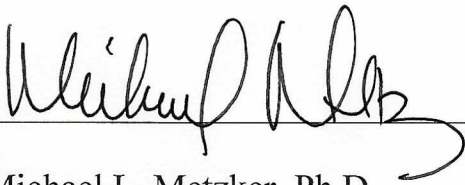
⁵¹ *Id.* at [0031].

Patent, including the indexing and identifier sites. In my opinion, the '202 Patent Pub. (Porreca) anticipates Claim 1 of the '357 Patent for the reasons stated *supra*, which are made in response to Tecan's POR arguments and which supplement the reasons stated in my previous declaration.⁵²

⁵² See Ex. 1003, Metzker Declaration at ¶¶ 70-88.

I declare under penalty of perjury that the foregoing is true and correct. I declare that all statements made herein of my knowledge are true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: November 12, 2025

By: 
Michael L. Metzker, Ph.D.