

Case IPR2024-01419
U.S. Patent No. 11,840,730

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA INC.,
Petitioner

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner

Case IPR2024-01419

U.S. Patent No. 11,840,730

PATENT OWNER'S RESPONSE

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Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

TABLE OF CONTENTS

	<u>Page</u>
I. Introduction.....	1
II. Background.....	3
A. DNA Sequencing.....	3
B. Overview Of The '730 Patent Technology.....	5
C. Overview Of The '730 File History.....	12
III. Overview Of The References Asserted By Petitioner	14
A. Chee (EX1010).....	14
B. Bignell (EX1013).....	17
C. McCloskey (EX1012).....	18
D. Gahan (EX1015).....	20
E. Bentley (EX1017).....	21
F. Chiang (EX1033).....	22
IV. The '730 Patent Priority Date	22
A. Claims 1-3 And 9 Of The '730 Patent Are Entitled To A Priority Date Of At Least April 29, 2009	22
1. The Inventors' March 6, 2009 Document Demonstrates Conception Of All Elements Of Claims 1-3 And 9.....	24
2. Dr. Porreca And The Prosecuting Attorneys Exercised Reasonable Diligence During The Critical Period From April 29, 2009 Through May 18, 2009.....	33
V. The Level Of Ordinary Skill In The Art	36
VI. Grounds 1-3: The Challenged Claims Are Patentable Over Chee Alone Or Over Chee In View Of Gahan.....	37

	<u>Page</u>
A. Chee Is Not Prior Art To Claims 1-3 And 9 Of The '730 Patent Because Those Claims Are Entitled To An Earlier Priority Date.....	37
B. Chee's April 30, 2009 Provisional Application Does Not Disclose All Elements Relied Upon By Petitioner. (All Claims)	37
C. Chee Does Not Disclose "Collapsing Target:Differentiator Tag Combinations Observed More Than Once Into A Single Count." (Claim 1)	44
1. The '730 Patent Claims "Collapsing" As A Separate Step From "Determining A Consensus Base Call."	44
2. Chee Does Not Disclose The Claimed Collapsing Step.....	47
D. Chee Does Not Disclose "Collapsing All Sequence Reads Comprising A Same Differentiator Tag Into A Single Read Before Determining A Consensus Base Call." (Claim 2).....	51
E. Petitioner Fails To Show A Motivation To Combine Chee In View Of Gahan. (Claims 5, 6, and 8)	52
VII. Ground 4: The Challenged Claims Are Patentable Over Bignell And McCloskey.	54
A. Bignell In View Of McCloskey Does Not Disclose The "Correcting" Step Of Claim 1.....	54
B. Bignell In View Of McCloskey Does Not Disclose All Limitations Of Claims 4, 5, and 6.....	58
C. Petitioner Fails To Show A Motivation To Combine Bignell and McCloskey. (All Claims)	59
VIII. Ground 5: The Challenged Claims Are Patentable Over Bignell, McCloskey, And Bentley.....	64
IX. Ground 6: The Challenged Claims Are Patentable Over Bignell, McCloskey, And Gahan.	65

	<u>Page</u>
X. Ground 7: The Challenged Claims Are Patentable Over Bignell, McCloskey, And Chiang.....	65
XI. Conclusion	65

TABLE OF AUTHORITIES

	Page(s)
<u>Cases</u>	
<i>Burroughs Wellcome Co. v. Barr Lab'ys, Inc.</i> , 40 F.3d 1223 (Fed. Cir. 1994).....	25
<i>Ex parte Koji Muramaya</i> , 2020 WL 1663636 *4-5 (P.T.A.B. April 1, 2020).....	35
<i>Ex parte Koji Muramaya</i> , 2020 WL 1663636 (P.T.A.B. April 1, 2020)	35
<i>In re Riggs</i> , 131 F.4th 1377 (Fed. Cir. 2025).....	38, 39
<i>Monsanto Co. v. Mycogen PlantSci., Inc.</i> , 261 F.3d 1356 (Fed. Cir. 2001).....	22
<i>Perfect Surgical Techniques, Inc. v. Olympus America, Inc.</i> , 841 F.3d 1004 (Fed. Cir. 2016).....	22, 24, 33
<i>Singh v. Brake</i> , 317 F.3d 1334 (Fed. Cir. 2003).....	24
<i>Spanion, Inc. v. Int'l Trade Comm'n</i> , 629 F.3d 1331 (Fed. Cir. 2010).....	25
<i>Unified Patents, LLC v. Flexiworld Techs.</i> , IPR2022-00775, Paper 41 (P.T.A.B. November 1, 2023)	35
<i>Unified Patents, LLC v. Flexiworld Techs.</i> , IPR2022-00775, Paper 41, 17–20 (P.T.A.B. November 1, 2023)	35

All citations to 35 U.S.C. §§102 and 103 in this paper refer to the pre-AIA statute.

All emphases are added unless otherwise noted.

PATENT OWNER’S EXHIBIT LIST

Ex. #	DESCRIPTION
2001	Declaration of Michael Wueste in Support of Motion for Admission <i>Pro Hac Vice</i>
2002	Declaration of Kevin Goon in Support of Motion for Admission <i>Pro Hac Vice</i>
2003	D. Endy, <i>Reconstruction of the Genomes</i> , SCIENCE (Feb. 28, 2009)
2004	July 17, 2025 Declaration of Gregory Porreca, Ph.D.
2005	July 18, 2025 Declaration of Patrick Waller, Ph.D., J.D.
2006	March 6, 2009 Draft Provisional Application
2007	March 6, 2009 Draft Provisional Application Figure 1
2008	March 6, 2009 Draft Provisional Application Figure 2
2009	Metadata Associated with March 6, 2009 Draft Provisional Application
2010	Metadata Associated with March 6, 2009 Draft Provisional Application Figure 1
2011	Metadata Associated with March 6, 2009 Draft Provisional Application Figure 2
2012	April 24, 2009 Email Thread between Gregory Porreca, Patrick Waller, and Daniel Young
2013	April 30, 2009 Email Thread between Gregory Porreca, Patrick Waller, and Eric Boutin
2014	May 6, 2009 Email Thread between Gregory Porreca, Patrick Waller, and Daniel Young
2015	May 14, 2009 Email Thread between Gregory Porreca, Patrick Waller, and Daniel Young
2016	May 15, 2009 Email Thread between Gregory Porreca, Patrick Waller, and Daniel Young
2017	<i>Invitae Acquiring Good Start Genetics and CombiMatrix, Adding Comprehensive Reproductive Health Capabilities to Serve Every Stage of Life</i> , INVITAE (Jul. 31, 2017)
2018	J.D. Keasling et al., <i>Production of the antimalarial drug precursor artemisinic acid in engineered yeast</i> , NATURE (Apr. 13, 2006) (“Keasling 2006”)
2019	J.D. Keasling, <i>Synthetic Biology for Synthetic Chemistry</i> , ACS CHEMICAL BIOLOGY (Jan. 18, 2008) (“Keasling 2008”)
2020	C.A. Hutchison, R.Y. Chuang, J.C. Venter (Mar. 16, 2016) (“Hutchison”)
2021	March 6, 2009 Email Thread between Gregory Porreca, Patrick Waller, Daniel Young, and Eric Boutin

Ex. #	DESCRIPTION
2022	M.P. Weiner, S.B. Gabriel, and J.C. Stephens, <i>Genetic Variation: A Laboratory Manual</i> , COLD SPRING HARBOR LABORATORY PRESS (2007) (excerpted)
2023	July 18, 2025 Declaration of Joseph Puglisi, Ph.D.
2024	July 9, 2025 Deposition Transcript of Stacey Gabriel, Ph.D.

All citations to Exhibits refer to stamped exhibit page numbers.

I. Introduction

U.S. Patent No. 11,840,730 (the “’730 Patent”) covers important discoveries for reducing errors in next-generation sequencing (“NGS”). NGS technologies can sequence nucleic acids and gather information about the genotype of a sample collected from a patient, and could be used as the basis for important decisions. But, prior to the ’730 Patent, errors introduced during sequencing workflows, including during library preparation, amplification, and sequencing, could hamper analysis of the sequencing data. For example, during amplification, certain nucleic acid molecules from the original sample could be over- or under-amplified (a phenomenon known as amplification bias), resulting in a skewed number of amplicons attributable to a single original copy of a sequence. These errors could lead to incorrect assumptions during the analysis of sequence reads, such as wrongly designating an individual as being homozygous for a particular allele of a gene instead of heterozygous. Thus, there existed a need for a scalable, cost-effective method to achieve more accurate results by reducing the influence of errors introduced during sequencing workflows in next-generation sequencing. *See, e.g.*, EX1001, 1:38-41.

The ’730 Patent addressed the need in the field by providing a method for associating a set of tags with target nucleic acid molecules prior to amplification, amplifying the tagged molecules such that each amplified molecule contains a unique sequence, and collapsing all amplicon sequence reads containing that unique

sequence into one read prior to subsequent analyses. *See, e.g.*, EX1001, 5:12-24, 28:25-48. For example, using the '730 Patent invention, the inventors were able to accurately track amplicons back to the correct original target nucleic acid molecule, before determining the number of times that certain sequences appeared in the sample, enhancing genotyping accuracy. Since their invention, the methods of the '730 Patent have manifested as an efficient, cost-effective solution to the problem recognized by its inventors.

Petitioner cannot prevail on any of its grounds for several reasons. ***First***, Petitioner applies Chee as prior art based on its provisional application filing on April 30, 2009. But the inventors of the '730 Patent conceived of many of its inventions before Chee's provisional application was filed. And the '730 Patent's inventors and prosecuting attorneys worked diligently to reduce the invention reflected in certain claims to practice beginning from at least April 29, 2009 (i.e., the day before Chee's provisional application was filed) through May 18, 2009, the day the prosecuting attorneys filed the provisional application for the '730 Patent and thereby constructively reduced its inventions to practice. Petitioner additionally fails to show that all portions of Chee that Petitioner relies on in its invalidity arguments were also included in Chee's provisional application. For at least these reasons, Petitioner's Grounds 1-3 fail because Chee is not prior art to the '730 Patent claims.

Second, Petitioner's Grounds 1-3 fail for additional substantive reasons. For independent claim 1, Petitioner relies on Chee alone, but fails to show that Chee

discloses the “collapsing” step of the claim. And for Ground 3, Petitioner fails to explain why a POSA would be motivated to combine Gahan’s and Chee’s disparate methods.

Third, Petitioner relies on the combination of McCloskey and Bignell for Grounds 4–7 but fails to show that the combination discloses all claim limitations. These grounds rely on McCloskey and Bignell for independent claim 1, but Petitioner fails to show that the combination discloses claim 1’s “collapsing” step. Furthermore, Petitioner fails to show that a POSA would have been motivated to combine McCloskey’s barcodes into Bignell’s method.

Accordingly, Petitioner cannot prevail on any of Grounds 1-7, and claims 1-9 (“Challenged Claims”) are patentable.

II. Background

A. DNA Sequencing

In 2009, there were multiple sequencing technologies available. EX2023, ¶¶25–39. Sanger sequencing was first developed in the 1970s and was the most common type of DNA sequencing platform until the mid-2000s. EX2023, ¶¶25–32; Pet., 4. While Sanger sequencing reported accurate reads of long DNA fragments, it suffered from major drawbacks that pushed sequencing costs “beyond the range of most individual laboratories.” EX1014, 1141; EX2023, ¶¶26–31. For example, Sanger sequencing techniques use time-intensive chemistry to sequence a limited

number of DNA fragments (on the order of hundreds) in a single run. EX2023, ¶¶30-31.

At the time of the '730 Patent invention, next-generation sequencing technologies were emerging as an alternative to Sanger sequencing and “democratiz[ed] the extent to which individual investigators can pursue projects at a scale.” EX1014, 1143; EX2023, ¶¶31–39. Next-generation sequencing provided researchers with hundreds of millions of short sequence reads in a single sequencing run that could then be further analyzed with downstream software. EX2023, ¶¶34–39. Next-generation sequencing improved upon traditional Sanger sequencing and allowed individual investigators to carry out large-scale genomic sequencing projects. EX2023, ¶¶31, 36–39; EX1014, 1143.

DNA sequencing technologies had been used for diagnostic genotyping applications. The '730 patent highlights the use of next-generation sequencing for such diagnostic purposes. *See e.g.*, EX1001, 1:28-53, 2:10-24; EX2023, ¶¶24, 44–49. For example, next-generation sequencing could be used to obtain genotype information relevant to a range of diseases and disorders that could be used for diagnoses. EX1001, 1:28-53, 3:4-20; EX2023, ¶¶44–49.

The field of synthetic biology had also begun utilizing DNA sequencing technologies. At the time of the '730 Patent invention, synthetic biology was “a promising new field” that focused on the artificial creation of materials, such as nucleic acid sequences, for further research and manipulation, including genome

reconstruction. EX1010, 1:11-31; EX2023, ¶¶40–43; EX2003, 1196. Synthetic biology “require[d] highly skilled experts and considerable resources,” particularly due to the errors inherent in the process of synthesizing DNA molecules. EX2003, 1997; EX2023, ¶42; EX1010, 1:38–43. Synthetic biology applications use DNA sequencing technologies, for example, to confirm the accuracy of synthetically created molecules and distinguish correctly synthesized molecules from molecules containing errors from chemical synthesis. EX1010, 1:38-52, 13:5-19.

B. Overview Of The ’730 Patent Technology

The ’730 Patent provides inventive methods “for evaluating genetic markers” and “determining genotypes in patient samples.” EX1001, 1:1-2, 1:23-24. As the ’730 Patent recognizes, “[f]or cost-effective and reliable medical and reproductive counseling on a large scale, it is important to be able [to] correctly and unambiguously identify the allelic status *for many different genetic loci in many subjects*.” EX1001, 1:38-41. However, at the time of the invention in 2009, prior approaches lacked a solution for effective quantitative error correction on next-generation sequencing (NGS) platforms. EX2023, ¶¶50-57.

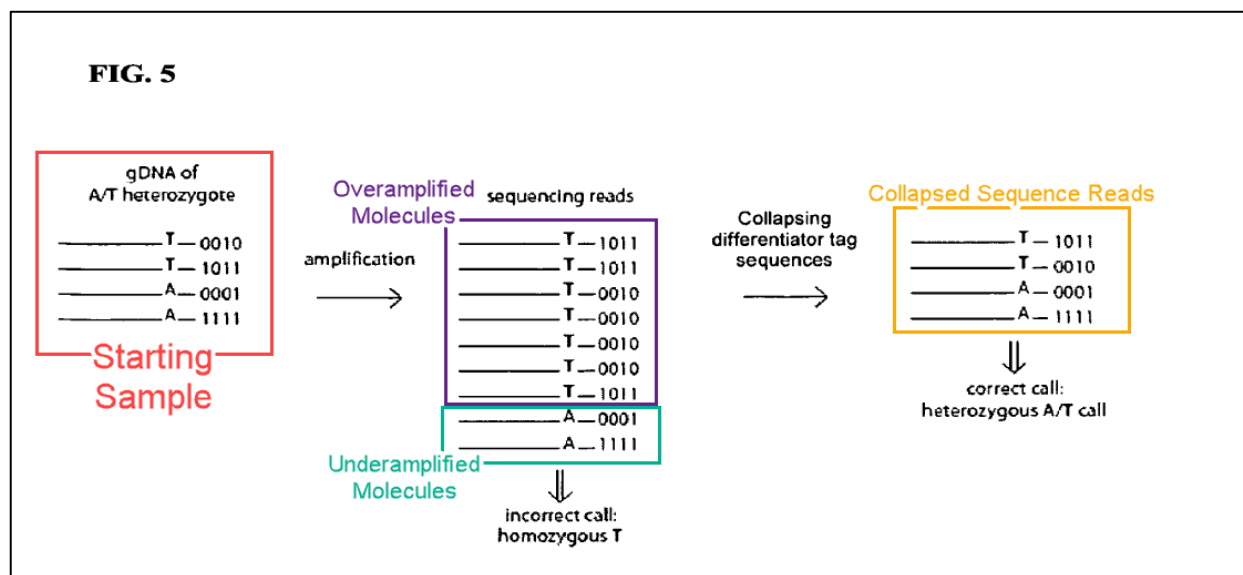
As the ’730 Patent explains, although technologies existed for genotyping patient samples, they were “not sufficiently robust and cost-effective to be scaled up for *reliable high throughput analysis of many genetic loci in large numbers of patients*” because of the expense and time needed to resolve the high rates of incorrect or ambiguous calls when handling large numbers of patient samples.

EX1001, 1:42-53. A high number of quantitative errors or ambiguities could be introduced at different stages in the sequencing workflow, including during “the preparative stages (e.g. capture amplification, etc.), determining stages (e.g. allele specific detection, sequencing etc.), data interpretation stages (e.g. determining whether the assay information is sufficient to identify a subject as homozygous or heterozygous), and/or other stages.” *Id.*, 1:62-2:9.

Previous methods failed to address these errors because they “waste[d] resources and increase[d] the expense required to perform such experiments” or “reduced statistical confidence in certain conclusions (e.g., diagnostic calls).” *Id.*, 27:57-67. For example, the ’730 Patent explains that because “most downstream sequencing library quantification techniques depend on stochastic counting processes, these problems have typically been addressed by sampling enough (oversampling) to obtain a minimum number of observations necessary to make statistically significant decisions.” *Id.*, 27:57-62. But oversampling wastes resources and increases expenses while reducing statistical confidence when making diagnostic calls. *Id.*, 27:62-67. Thus, the prior approaches did not provide a reliable, efficient, and accurate method for genotyping patient samples for diagnostic purposes using NGS platforms.

The ’730 Patent provides examples of particular types of quantitative errors that the inventors discovered. For example, the ’730 Patent notes that systematic and random errors “can be particularly problematic when it results in target sequences

from a genome being absent or undetectable in the sequencing libraries.” *Id.*, 27:45-53. Such errors can occur due to an “over- or under-representation of particular sequences from a source genome.” *Id.*, 27:47-50. The ’730 Patent illustrates an example of this issue in Figure 5, which depicts a particular target region of the sample’s genome as being heterozygous (i.e., having two different versions of that region), but being incorrectly designated as homozygous (i.e., having two of the same versions of that region).



Id., Figure 5 (annotated); *see also* EX2023, ¶¶51–57, 137–144.

As Figure 5 shows in the **red** box, the **original sample** collected contained two copies of a “T” allele at a certain target region and two copies of an “A” allele at the same target region. EX2023, ¶139. Because one allele of the target region was inherited from each parent, this means that the original sample had two copies of that target region and was heterozygous (i.e., had two different alleles of the same

target region). *Id.* However, to prepare the sample for sequencing to determine the sample's genotype, the '730 Patent discloses amplifying the original nucleic acid molecules. EX1001, 6:5-13; EX2023, ¶¶139–140. During amplification, certain original molecules are amplified more than others; for example, original molecules with the “T” allele were **over-amplified**, resulting in seven sequence reads as shown in the **purple** box, while original molecules with the “A” allele were **under-amplified**, resulting in only two sequence reads as shown in the **teal** box. EX2023, ¶¶140-142. This disproportionate amplification of original molecules with the “T” allele skews the population of sequence reads analyzed and results in incorrectly genotyping the sample as homozygous for the “T” allele, based on the assumption that the amplicons showing the “A” allele are actually just errors and not reflective of any actual sequences in the original sample. EX2023, ¶¶140-142. In other words, if one simply counts the total number of each amplicon (e.g., seven instances of the “T” allele amplicon, and two instances of the “A” allele amplicon), without first addressing the amplification bias error, the final genotype call will be incorrect.

The '730 Patent provided a novel solution for this problem by using tags to efficiently and more accurately identify sample genotypes collected from patients for medical diagnostic and treatment purposes. EX1001, 3:59-62, 4:57-5:11, 19:8-13, 21:7-10. The '730 Patent explains that “a ‘differentiator tag sequence’ is a sequence of a nucleic acid (a preparative nucleic acid), which in the context of a plurality of different isolated nucleic acids, identifies a unique independently

isolated nucleic acid.” *Id.*, 28:53-57; EX2023, ¶¶50–55, 88. Introducing “the differentiator tag sequence permits the detection of bias based on the frequency with which pairs of differentiator tag and target sequences are observed in a sequencing reaction.” *Id.*, 28:18-21; EX2023, ¶¶51–57. For example, differentiator tags “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). This information can be used to help analyze a threshold number of independently isolated target nucleic acids from a biological sample in order to obtain sequence information that is reliable and can be used to make a genotype conclusion (e.g., call) with a desired degree of confidence. This information also can be used to detect bias in one or more nucleic acid preparation steps.” *Id.*, 5:12-23; EX2023, ¶53. The ’730 Patent provides “non-limiting examples of methods for combining differentiator tag sequence and target sequences” in Figure 4. *Id.*, 19:32-34; EX2023, ¶57.

The ’730 Patent explains that “the differentiator tag sequence permits the detection of bias based on the occurrence of ***combinations of differentiator tag and target sequences*** observed in a sequencing reaction.” EX1001, 5:5-8. “[I]n the context of a multiplex nucleic acid amplification reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, primary amplicons of a target nucleic acid, for example. Thus, ***combinations of target***

nucleic acid and differentiator tag sequence (target:differentiator tag sequences) of an isolated nucleic acid of a preparative method provide a basis for identifying unique, independently isolated target nucleic acids.” *Id.*, 28:62-29:4; EX2023, ¶¶54–60, 177-183. And as shown in Figure 5 above in **gold**, redundant amplicons with the ***same unique differentiator tag:target combinations*** are removed to create **collapsed sequence reads**, which resolves issues such as over- and under-amplification and improves the accuracy of subsequent analyses, like copy number determination and genotyping. *Id.*, Figure 5, 73:3-5 (“FIG. 5 depicts a method for ***making diploid genotype calls*** in which ***repeat target:differentiator tag combination are collapsed.***”), 131:47-49; EX2023, ¶¶51–57, 142, 182.

The ’730 Patent explains that “collapsing” sequence reads is a process distinct from determining a consensus base call, which can involve reading the same position in amplicon sequences multiple times to determine the most probable nucleotide present at a particular position in the original sequence. EX2023, ¶¶131–144, 150. For example, the specification explains that collapsing corrects for quantitative errors such that, “[f]or any target:differentiator tag combination observed more than once, all such reads are ‘collapsed’ into a single read ***before*** consensus basecalls are determined,” which “will cancel the effect of bias on consensus basecall accuracy.” EX1001, 72:63-73:5. In other words, the ’730 Patent discloses using the ***collapsed*** sequence reads to determine a consensus base call, not reading multiple identical copies of the same original molecule, which could lead to incorrect calls. EX2023,

¶¶131–144, 150. The '730 Patent discusses multiple types of biological samples that are analyzed and primarily focuses its methods on solving problems related to analyzing naturally-occurring nucleic acids, such as those collected from patients for diagnostic purposes. EX2023, ¶165. The '730 Patent discloses “methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample) as aspects of the invention are not limited in this respect.” EX1001, 3:13-20. In this way, the '730 Patent explains that its methods can provide “a diagnosis, prognosis, or disease risk assessment [] to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject).” EX1001, 3:43-47. The '730 Patent further provides examples of patients who may want to know their genotype, such as a couple interested in having children or an individual interested in determining their cancer prognosis and appropriate treatment options. EX1001, 3:47-58.

C. Overview Of The '730 File History

The '730 Patent's application was filed on November 19, 2020, as Application No. 16/952,764, claiming priority to provisional applications filed in April and May 2009. EX1004, 113-115; EX1001, Cover. In particular, the '730 Patent claims priority to U.S. Provisional Application No. 61/178,358 (the “'358 provisional”),

filed on May 18, 2009. EX1001, Cover. Petitioner uses the May 18, 2009 filing date of the '358 provisional as the effective priority date of the '730 Patent for purposes of the Petition. Pet., 1, 15. But for the reasons described in Section IV.A below, certain claimed inventions of the '730 Patent are entitled to a priority date of at least April 29, 2009, because the claimed inventions were conceived of by March 6, 2009 and diligently reduced to practice from at least April 29, 2009 until May 18, 2009.

In an Office Action dated February 10, 2021, the Examiner rejected pending claims as obvious in view of Porreca (EX1031) and Wang. EX1004, 866-872. In response, Applicant submitted a declaration from inventor Dr. Greg Porreca on March 4, 2021. *Id.*, 964-967.

In his declaration, Dr. Porreca explained the '730 Patent's "application discloses tagging target individual nucleic acid molecules with differentiator tags before any amplification or sequencing steps" so that "each individual target nucleic acid molecule is tagged, and therefore associated, with a differentiator tag." *Id.*, 964. Dr. Porreca clarified that "each target:differentiator tag ***combination*** uniquely identifies a single nucleic acid molecule through subsequence [*sic*] amplification and sequencing steps." *Id.* Dr. Porreca explained that "[b]ecause each target:differentiator tag ***combination*** can be associated back to its original, individual nucleic molecule based on the ***unique combination of individual nucleic acid molecules and their respective differentiator tags***, the invention corrects for bias in subsequent steps." *Id.*, 965.

The Examiner issued a final rejection on April 9, 2021, which Applicant appealed. In its appeal, the Applicant highlighted that the differentiator tags in the '730 Patent's application are not designed to capture a specific exon of interest and thus can differentiate between separate single molecules. *Id.*, 1070-1073, 1142-1146. Applicant reiterated none of the cited references “disclose a method comprising tagging individual nucleic acid molecules with differentiator tags prior to amplification or sequencing steps.” *Id.*, 1071, 1144. The Board reversed the Examiner's rejection, and the claims were allowed on September 28, 2023. *Id.*, 1194-1196.

III. Overview Of The References Asserted By Petitioner

A. Chee (EX1010)

Chee discloses a method in “*synthetic biology*” for selecting “error-free synthesized oligonucleotides” *containing a desired sequence*, which can be used to reconstruct longer nucleic acid segments, including entire genomes. EX1010, 1:23-55; EX2023, ¶¶100–109. Chee notes that the “de novo chemical synthesis of the DNA building blocks is quite error prone” and recognizes that the high error rate during the chemical synthesis of nucleic acid molecules is a “major limitation in the cost effective assembly of larger DNA molecules.” EX1010, 1:38-43; EX2023, ¶¶101–102.

Chee's method allows for selecting “error-free synthesized oligonucleotides” by “providing constructs, sets of constructs, and methods for ensuring accuracy of

oligonucleotides having a desired sequence.” EX1010, 1:53-58; EX2023, ¶¶100–109. Specifically, Chee describes creating constructs comprising a region of interest (“ROI”) and a unique identifier. EX1010, 12:1-27. Chee explains that these constructs “*can be synthesized as a single unit* with all elements provided in a *single synthesis step*” and that “[i]t is a fundamental element of the invention that *the unique identifier is associated with a construct, not the ROI*.” EX1010, 2:21-28, 12:1-6; EX2023, ¶103. Chee further describes association of the unique identifier with the construct—instead of the ROI itself—as “a fundamental element of the invention” to ensure that “ROIs having the *correct sequence* can be differentiated from constructs having *substantially the same ROI but containing errors*.” EX1010, 2:23-28, *see also id.*, 2:34-38; EX2023, ¶104. In other words, if two constructs contain very similar ROIs, but one construct has sequencing errors while the other does not, associating the tag with the construct itself instead of the ROI allows identification of the error-free construct (differentiated from the error-containing construct) for subsequent analyses. EX1010, 2:21-28; EX2023, ¶¶104–106.

By ensuring each construct with an artificially synthesized ROI is associated with a unique identifier, Chee’s method can be used to identify a discrete population of validated (i.e., desired) sequences. EX1010, 2:5-9; EX2023, ¶¶101–109. For example, Chee explains that “the use of the uniquely identifiable constructs of the invention to ensure the determination, selection and/or isolation of error free regions

of interest (ROIs) are correct is extremely effective, and *allows specific sequences to be selected with high accuracy and high throughput.*” EX1010, 2:9-20; EX2023, ¶¶103–109. Chee also notes that “for example, if one lane of a sequencing experiment obtains 10M reads, and the average redundancy of the constructs comprising an ROI is specified to be 100 fold to *ensure a sufficiently high probability of obtaining at least one correct copy of an ROI*, then a set of constructs of the invention would comprise approximately 100K separate constructs with unique identifiers.” EX1010, 2:44-51, 2:53-64; EX2023, ¶¶106–108.

Chee explains that its method is particularly useful for selecting validated sequences for further synthetic biology uses, such as constructing an artificial genome from the selected constructs. For example, Chee discloses adding excision sites to constructs so that ROIs with a validated sequence can be isolated and excised from the construct. EX1010, 14:65-67, 23:36-39 (“[b]y obtaining long, highly pure oligos of desired sequence, it will be possible to assemble relatively long constructs by annealing the oligos together so that hybridization ensures that correct sequences are brought together”); EX2023, ¶¶103, 172.

To determine whether a construct contains a validated sequence, Chee analyzes multiple clonal copies of amplicons attributable to that construct, identified as such by having the same differentiator tag as the construct, and derives a single consensus sequence based on those sequence reads. For example, Chee explains that “[m]ultiple instances of the *same molecule* can be sequenced, and the ROI of an

individual parent construct can be identified by its unique identifier, as molecules with identical identifiers arise via amplification of the same original construct. Thus, a variation in the sequence of a single copy of a construct is most likely to be a synthesis or sequencing error if it differs from a consensus sequence derived from *multiple clonal copies* of the construct, which are identified as identical by descent via the shared unique identifier.” EX1010, 14:42-50, 9:34-39 (“Furthermore, because molecules that are identical by descent can be identified, *a consensus sequence can be determined using data from multiple molecules*, thereby achieving a much higher accuracy rate than most conventional high throughput methods.”); EX2023, ¶¶109, 161.

B. Bignell (EX1013)

Bignell sought to create “methods for *indexing samples* during the sequencing of polynucleotide templates.” EX1013, 1:24-28; EX2023, ¶121. Bignell recognizes that “separate serial analysis of *individual DNA samples*” was a costly barrier to large-scale genotyping with next-generation sequencing. EX1013, 1:51-55; EX2023, ¶122. Bignell acknowledges that pooling *multiple samples of DNA libraries* together prior to sequencing allows multiple DNA samples to be sequenced together in a single sequencing run. EX1013, 1:56-65; EX2023, ¶¶122–124. However, Bignell explains that the inability to determine which source or sample a given DNA sequence belongs to presents a major limitation to pooling DNA libraries from multiple samples together. EX1013, 1:66-2:8; EX2023, ¶122.

Bignell provides methods to analyze DNA in a single sequencing run by *tagging all the DNA fragments from a given sample with the same tag* before *pooling multiple samples* of DNA libraries together. EX1013, 1:24-3:59; EX2023, ¶123; EX2024, 77:15-21. Bignell explains that if each DNA fragment in a given sample was “tagged with different nucleic acid tags,” then “the source of the sample can be identified” with the sample-specific tag. EX1013, 1:36-38; EX2023, ¶¶123–125. Therefore, a POSA would understand that Bignell provides a cost-reducing method to sequence multiple DNA samples together in a single sequencing run. EX1013, 1:24-3:59, 6:36-48; EX2023, ¶¶122–125.

C. McCloskey (EX1012)

McCloskey was considered by the Examiner during prosecution of the '730 patent and is listed on the face of the '730 patent. EX1001, 2; EX1004, 919; EX2023, ¶114. McCloskey describes methods to “verify[] the authenticity of PCR products.” EX1012, [0004]; EX2023, ¶115. McCloskey explains that contamination and redundancy are two problems associated with PCR amplification. EX1012, [0003] (“The polymerase chain reaction (PCR) allows multiple copies of selected DNA sequences to be produced from limited amounts of a DNA template. Reactions with limited amounts of template, however, increase the risk of amplifying contaminant DNA and can result in a skewed yield of PCR products such that there is a high degree of redundancy for a small portion of the original genomic sequences.”); EX2023, ¶115–120. McCloskey provides methods that use barcodes and batch-

stamps to authenticate a nucleic acid sequence by identifying and distinguishing valid sequences from contaminant and redundant sequences. EX1012, [0005]; EX2023, ¶¶117–120.

McCloskey's methods require a "bar-coded oligonucleotide" that comprises a "first sequence" that is complementary to the target nucleic acid sequence, a "second sequence" containing a random barcode, and a "third sequence" that is not complementary to any sequencing in the sample. EX1012, [0008]. *First*, McCloskey anneals the bar-coded oligonucleotide to the target nucleic acid before extending the bar-coded oligonucleotide to create a bar-coded target nucleic acid. EX1012, [0008]; EX2023, ¶¶117–118. *Second*, McCloskey uses PCR amplification to produce an amplification product. EX1012, [0033]; EX2023, ¶110. *Third*, McCloskey authenticates the amplification product "by ***detecting the presence of the second sequence*** in the amplification product." EX1012, [0034]; EX2023, ¶120. McCloskey discloses additional methods that introduce a "fourth sequence" containing a "batch-stamp or experiment verification information" into the bar-coded oligonucleotide. EX1012, [0027]. In those methods, McCloskey discloses "authenticating the amplification product by ***detecting the presence of the second sequence and the fourth sequence***." EX1012, [0035]; EX2023, ¶¶115–120.

McCloskey suggests using Sanger sequencing to detect the barcode and batch-stamp sequences. For example, McCloskey explains that "[e]xemplary conditions for analysing bar-coded target nucleic acid molecules by gel electrophoresis,

cloning, and *sequencing* are described in” its examples and its own examples use Sanger sequencing to detect the barcode and batch-stamp sequences. EX1012, [0034], [0049] (“Verified reactions were then cleaned with Microclean (Gel Company) and *sequenced on an ABI 3100* with Big Dye.”); EX2023, ¶¶186–187. All of McCloskey’s examples were, therefore, limited to experiments that recovered fewer than 150 sequences. EX1012, [0053], [0063], [0076], [0104]; EX2023, ¶195.

D. Gahan (EX1015)

Gahan describes “circulating nucleic acids” in biological samples, such as blood and urine, collected from organisms. EX1015, 2; EX2023, ¶¶110-112. Gahan explains using different kits to isolate nucleic acids from biological samples and the various potential benefits of using isolated nucleic acids in diagnostic techniques. EX1015, 3-5; EX2023, ¶111. However, Gahan notes that “there is still a lack of clarity concerning the use of the broad range of kits and their applicability” and that, “the methodology is *far from consistent*, and if there are to be reliable protocols for diagnostic purposes, there needs to be a form of quality assurance for these procedures.” EX1015, 3; EX2023, ¶111. Gahan does not describe any particular sequencing method or sequencing workflow, nor does Gahan describe any method directed to synthetically created nucleic acid molecules. EX1015, 3; EX2023, ¶112.

E. Bentley (EX1017)

Bentley discloses then-nascent sequencing technologies, including sequencing by synthesis on arrays. EX1017, 546; EX2023, ¶¶128-130. This category

includes the Solexa sequencer, which was the early version of the contemporary Illumina sequencer. EX1017, 546; EX2023, ¶130. Bentley explains that many factors related to these new sequencing technologies were still actively under investigation by the scientific community, including trying to lower costs and improve throughput, accuracy, and completeness in sequencing workflows. EX1017, 548; EX2023, ¶¶129-130. Bentley also describes the intersection between these goals and the challenge of improving one factor at the expense of others. EX1017, 548; EX2023, ¶130.

In describing Solexa sequencers, Bentley explains that “new technologies work on a range of different principles that will require different ways to define good quality metrics.” EX1017, 549. Bentley goes on to say that “[m]ore work is required in this area to assess the accuracy of each sequencing method, to look for loss of accuracy in particular sequence contexts for each method, and to enable assembly of sequence data obtained used more than one technology.” EX1017, 549; EX2023, ¶130. Bentley does not disclose a method for improving accuracy, however, and instead notes that, once factors such as accuracy are improved, the “new challenge will be to analyze all the [sequencing] data” collected. EX1017, 549; EX2023, ¶130.

F. Chiang (EX1033)

Chiang describes an analysis of “issues involved in identifying copy-number alterations using massively parallel sequencing.” EX1033, 99; EX2023, ¶¶126-127. Chiang describes analyzing “statistical power to detect copy-number alterations”

and the development of SegSeq, an algorithm used for mapping these alterations. EX1033, 99; EX2023, ¶127. Chiang also describes testing this methodology on sequencing reads collected from an Illumina sequencer, which showed that “copy-number estimates from massively parallel sequencing achieved greater sensitivity, higher dynamic range and greater precision for mapping breakpoints than similar estimates based on microarray hybridization.” EX1033, 99, 101; EX2023, ¶127.

IV. The ’730 Patent Priority Date

A. Claims 1-3 And 9 Of The ’730 Patent Are Entitled To A Priority Date Of At Least April 29, 2009

A patent owner can “antedate a reference by proving earlier conception and reasonable diligence in reducing to practice.” *Perfect Surgical Techniques, Inc. v. Olympus America, Inc.*, 841 F.3d 1004, 1007 (Fed. Cir. 2016) (citing *Monsanto Co. v. Mycogen PlantSci., Inc.*, 261 F.3d 1356, 1362 (Fed. Cir. 2001)). A patent owner must show that there was “*reasonably* continuous diligence,” but “need not prove the inventor continuously exercised reasonable diligence throughout the critical period.” *Id.*, 1009. The critical period for showing diligence begins “one day prior to [the alleged prior art’s] publication date, and end[s] the date the invention was constructively reduced to practice by filing the application that resulted in the [patent] with the PTO.” *Id.*, 1007.

Here, the inventors conceived of the inventions in claims 1–3 and 9 of the ’730 Patent at least as early as March 6, 2009, as described further in Section IV.A.1,

infra. Specifically, all of the elements of claims 1–3 and 9 were documented in March 6, 2009, in an initial draft application created by the inventors and sent to their prosecuting attorney. EX2023, ¶¶64, 65-84; EX2004, ¶¶5, 7-9; *see also* Section IV.A.1, *infra*.

Further, the inventions were diligently reduced to practice during the critical period of April 29, 2009 (i.e., one day before the Chee provisional application was filed) through May 18, 2009 (when the '358 provisional was filed and the claims were constructively reduced to practice), as described further in Section IV.A.2, *infra*. EX2004, ¶¶10-23; EX2005, ¶¶9-23; *see also* EX2023, ¶¶85-99. During that time, the inventors communicated with the attorneys regularly to ensure the application was progressing, and the inventors provided the attorneys with multiple rounds of additional information and edits needed to prepare the patents, which the attorneys promptly implemented. EX2004, ¶¶8–23; EX2005, ¶¶7–23; *see also* Section IV.A.2.

The actions of the prosecuting attorneys further show the reasonable diligence exercised by all parties in preparing the application that became the '730 Patent. For example, the prosecuting attorney explained that Dr. Porreca took prosecution of the patent seriously and that together they put significant effort into ensuring the patent application was edited, finalized, and filed promptly. EX2005, ¶¶22–23; EX2004, ¶¶7, 23; Section IV.A.2. The prosecuting attorney also explained that he maintained

an active practice at the time, and that he spent on average 7 hours per day on his various active applications. EX2005, ¶¶6, 9; Section IV.A.2.

The activities of both the inventors and prosecuting attorneys all show more than a “reasonably continuous” diligence in reducing the inventions to practice. *Perfect Surgical Techniques*, 841 F.3d at 1009. For at least these reasons, claims 1-3 and 9 of the ’730 Patent are entitled to a priority date of at least April 29, 2009.

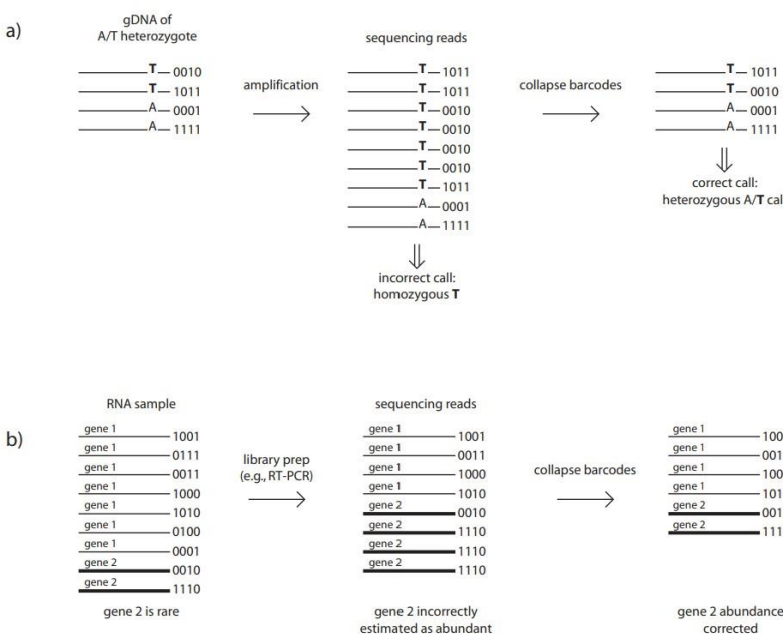
1. The Inventors’ March 6, 2009 Document Demonstrates Conception Of All Elements Of Claims 1-3 And 9

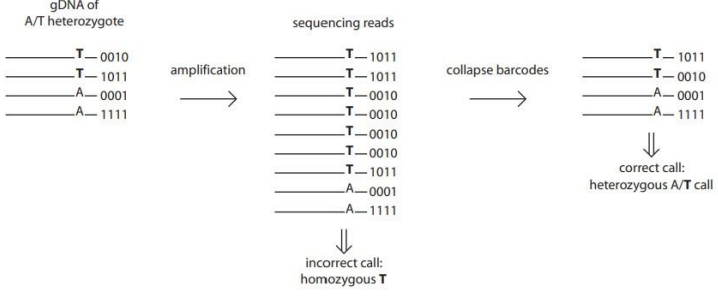
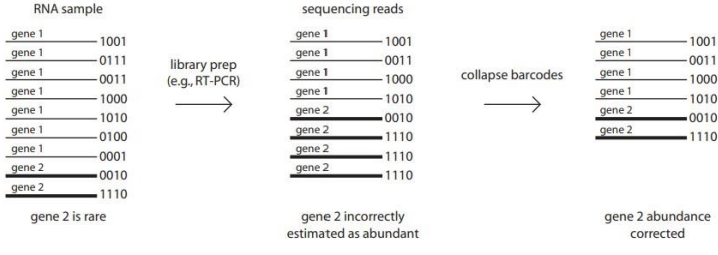
“Conception is the formation in the mind of the inventor of a definite and permanent idea of the complete and operative invention.” *Singh v. Brake*, 317 F.3d 1334, 1340 (Fed. Cir. 2003) (quotations and citations omitted). Conception “does not require perfection,” instead “conception is complete when ‘the idea is so clearly defined in the inventor’s mind that only ordinary skill would be necessary to reduce the invention to practice without extensive research or experimentation.’” *Spansion, Inc. v. Int’l Trade Comm’n*, 629 F.3d 1331, 1356 (Fed. Cir. 2010) (quoting *Burroughs Wellcome Co. v. Barr Lab’ys, Inc.*, 40 F.3d 1223, 1228 (Fed. Cir. 1994)).

The inventors of the ’730 Patent, including Dr. Gregory Porreca, conceived of claims 1–3 and 9 by March 6, 2009 and constructively reduced those claims to practice by May 18, 2009. EX2004, ¶¶7–9; EX2023, ¶¶61–99. For example, on March 6, 2009, Dr. Porreca sent the beginning of a draft patent application that the inventors had created (the “March 6 Application”), which eventually led to the ’730

patent disclosure (and its related figures), to the attorneys responsible for prosecuting the '730 patent. EX2004, ¶¶8–9; EX2005, ¶7; EX2006; EX2007; EX2008. On May 18, 2009, the attorneys filed the '358 provisional, constructively reducing the invention to practice. EX2023, ¶¶85–99. As shown in the chart below, and explained by Dr. Puglisi, each limitation in claims 1–3 and 9 of the '730 patent is described in the March 6 Application and therefore was conceived of by the inventors by at least March 6, 2009. EX2023, ¶¶61-84.

Claims	<i>March 6 Application</i>
Claim 1: A method for correcting error or bias introduced during nucleic acid analysis workflow, the method comprising the steps of:	“The invention relates to preparative steps in DNA sequencing-related technologies that reduce bias and increase the reliability and accuracy of downstream quantitative applications.” EX2006 at 1. EX2023, ¶¶67, 84; <i>see also</i> EX2023, ¶¶86, 99.
obtaining a biological sample comprising a plurality of target nucleic acid molecules from more than one locus of origin;	“One example of the use of the barcodes is in genome resequencing. ... Furthermore, since there are two alleles at every locus, it is important to sample enough to ensure that both alleles have been observed a sufficient number of times to confidently determine whether the sample is homozygous or heterozygous. Indeed, the sequencing is performed to sample the composition of molecules in the originating sample. However, after multiple reads have been collected for a given locus, it is possible that due to bias (e.g., caused by PCR amplification steps), a large fraction of the reads derive from a single originating molecule.” EX2006, 4-5. “[T]hese methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids.” EX2006, 1.

introducing a set of differentiator tags, wherein members of said set of differentiator tags are associated with members of said plurality, such that one or more of said loci of origin are associated with more than one differentiator tag;	EX2023, ¶¶68, 84; <i>see also</i> EX2023, ¶¶87, 99. “The invention is the composition and use of a large library of unique molecular barcodes in preparative steps for reducing the effects of the bias described above. During the library preparation process, each molecule is tagged with a unique [barcode] in such a configuration such that the barcode is sequenced along with the information of interest for which the library is being prepared.” EX2006, 2. “This allows the random barcodes to be used in any preparative process where the ultimate output is sequencing of many molecules in parallel.” EX2006, 4.  EX2008. EX2023, ¶¶69, 84; <i>see also</i> EX2023, ¶¶88, 99.
amplifying each of the plurality of tagged target nucleic acid molecules to	“One example of the use of the barcodes is in genome sequencing.... Indeed, the sequencing is performed to sample the composition of molecules in the originating sample. However, after multiple reads have been collected for a given locus, it is possible that due to bias (e.g., caused by PCR amplification

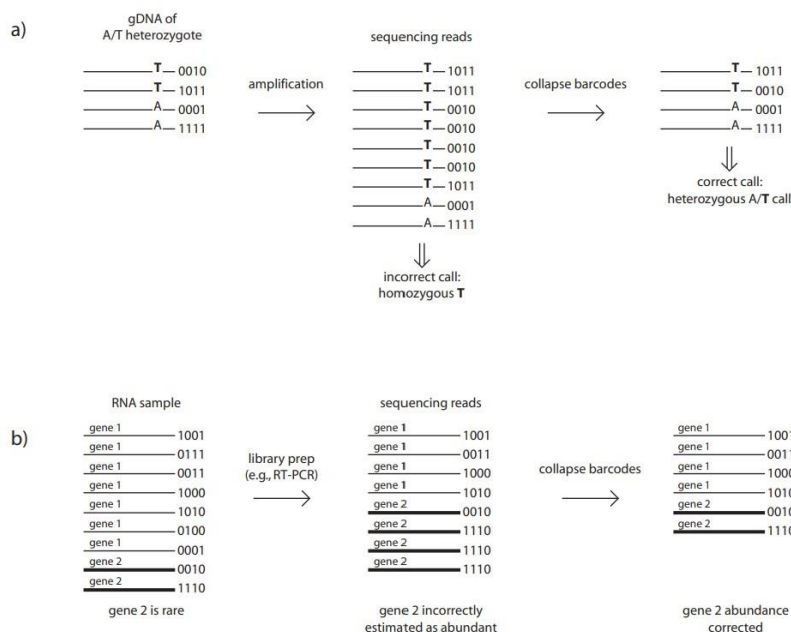
generate amplicons;	steps), a large fraction of the reads derive from a single originating molecule. EX2006, 4-5. “A common problem to overcome is that these techniques commonly introduce biases into the sequencing libraries, generally due to biases in enzyme function or to exponential and stochastic nature of amplification methods like PCR.” EX2006, 1. “This may include chemical or biochemical treatment of the DNA (e.g., reverse transcription, bisulphite treatment), capture of a specific subset of the genome (e.g., padlock probe capture, solution hybridization), and a variety of amplification techniques (e.g., polymerase chain reaction, whole genome amplification, rolling circle amplification).” EX2006, 1. a)  b)  EX2008. EX2023, ¶¶70, 84; <i>see also</i> EX2023, ¶¶89, 99.
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sequencing the amplicons obtained in said amplifying step to obtain sequence reads of each of the amplicons, wherein each of the sequence reads comprises a target nucleic acid molecule sequence and a differentiator tag sequence; and

“The sequencing primers must be incorporated afterwards such that they sequence both the barcode and the molecule of interest.” EX2006, 3.

“This allows the random barcodes to be used in any preparative process where the ultimate output is sequencing of many molecules in parallel.” EX2006, 3.

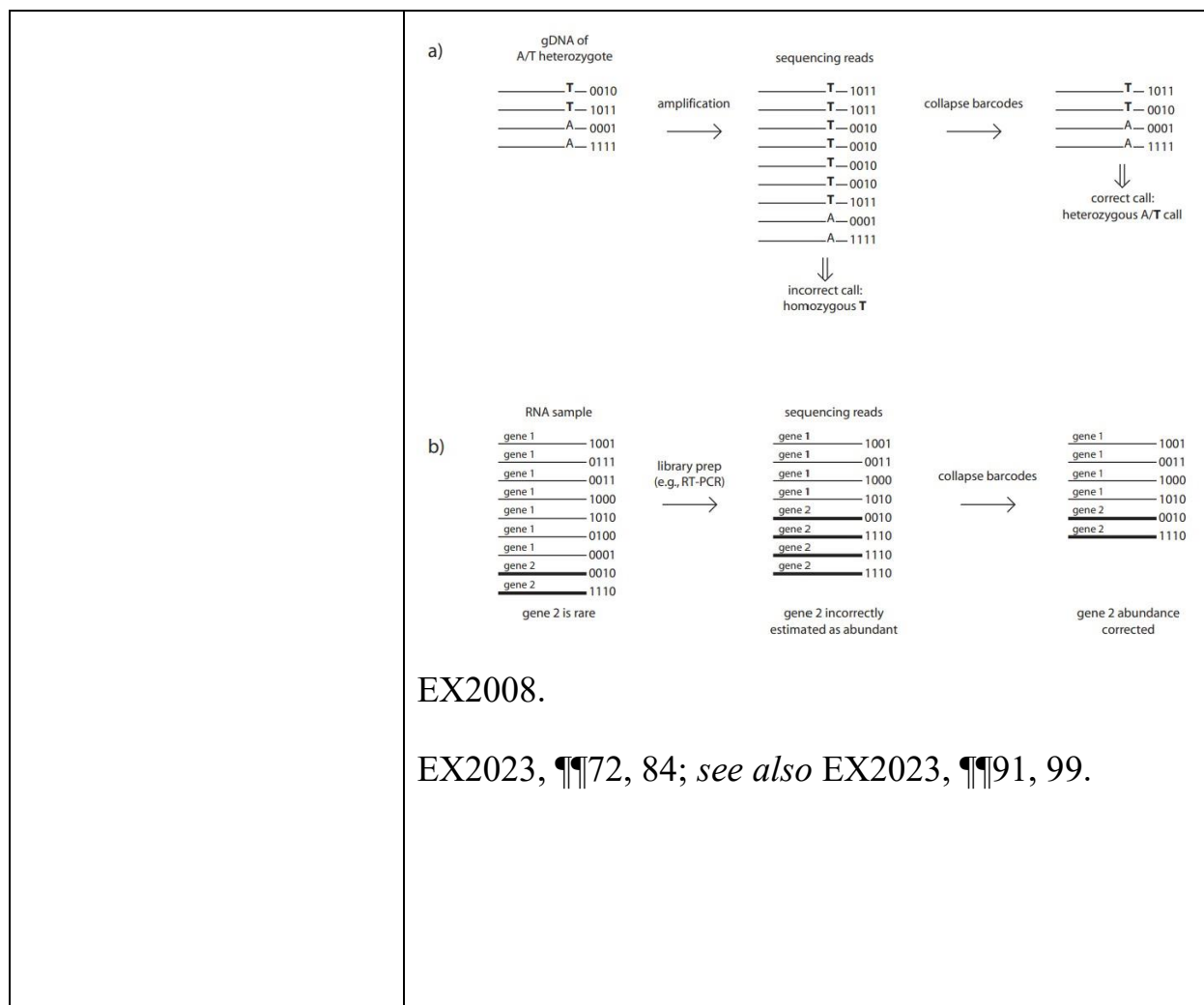
“The sequences of the barcodes will be read at the final stage of the sequencing.” EX2006, 2.



EX2008.

EX2023, ¶¶71, 84; *see also* EX2023, ¶¶90, 99.

correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.	“[B]y observing how the distribution of random barcodes changes after the sequencing is performed, we have collected the information that is necessary to correct the biases. By undoing the distribution changes that are measured for the random barcodes, the corresponding changes in the counts for the sequences of interest will provide a higher fidelity representation of the composition of the original complex pool.” EX2006, 3. “However, if we also have information on random barcodes, this situation could be averted, because the over-represented allele would be seen to also have an over-represented random barcode (i.e., the sequences with the overrepresented barcode all originated from the same single molecule). Therefore, the sequences and corresponding distribution of random barcodes can be used as an additional input to the genotype-calling algorithm to significantly improve the accuracy and confidence of the genotype calls.” EX2006, 5. “The incorporation of the barcode at a given step allows the detection and correction of biases in any of the following steps of the protocol.” EX2006, 2. “The observations of the random barcode sequences are used to detect and correct biases in the final read-out of the library.” EX2006, 2. “[I]f each RNA/cDNA molecule is coupled to a unique random barcode sequence, then any repeated barcodes in the sequencing reactions can simply be collapsed into each other. By collapsing similar clones in such a way that the sequencing data only includes a single representative for each barcode (or barcode-molecule combination), the biases that were introduced in preparation are essentially eliminated.” EX2006, 5.
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EX2008.

EX2023, ¶¶72, 84; *see also* EX2023, ¶¶91, 99.

Claim 9 is similar to claim 1 and contains many of the same limitations as claim 1. For at least the reasons in the chart above and in Dr. Puglisi’s declaration, the March 6 Application also demonstrates conception of claim 9. EX2023, ¶¶78–84. Furthermore, the March 6 Application discloses using unique barcodes that are “randomly incorporated” with individual molecules and “allows [for] the detection and correction of biases.” EX2006, 2 (“The incorporation of the barcode at a given step allows the *detection and correction of biases* in any of the following steps of the protocol.... [S]ince the barcodes are *randomly incorporated*, the probability that

two individual molecules receive the same barcode sequence is very small.”). The March 6 Application explains that “observing how the distribution of random barcodes changes” allows for the identification and correction of bias. EX2006, 3. Therefore, the March 6 Application also demonstrates conception of independent claim 9. EX2023, ¶¶78–84.

Claim 2 requires collapsing all target:differentiator tag combinations before determining a consensus base call. The figures accompanying the March 6 Application depict a method that collapses sequence reads before determining a consensus sequence. EX2008 (depicting a first step of “collapse barcodes” and a second step of “correct call: heterozygous A/T call”). Therefore, a POSA would have understood that the March 6 Application demonstrates conception of claim 2. EX2023, ¶¶74–75, 84.

Claim 3 requires “determining the presence of a sequencing or amplification error based upon the number said differentiator tags.” EX1001, 131:54-57. The March 6 Application discloses methods where “random barcode sequences are used to *detect* and correct biases in the final read-out of the library.” EX2006, 2. Therefore, a POSA would have understood that the March 6 Application demonstrates conception of claim 3. EX2023, ¶¶76–77, 84.

2. Dr. Porreca And The Prosecuting Attorneys Exercised Reasonable Diligence During The Critical Period From April 29, 2009 Through May 18, 2009

To show reasonable diligence, an “inventor is not required to work on reducing his invention to practice every day during the critical period” nor do “periods of inactivity within the critical period ... automatically vanquish a patent owner’s claim for reasonable diligence.” *Perfect Surgical*, 841 F.3d at 1009. Instead, the relevant inquiry involves whether there was “*reasonably continuous diligence*” through the critical period. *Id.* Where diligence is shown by preparing a patent application, “the absence of evidence that an inventor and his attorney revised or discussed the application on a daily basis is alone insufficient to determine that the invention was abandoned or unreasonably delayed.” *Id.*

Dr. Porreca and the prosecuting attorneys exercised reasonable diligence from at least April 29, 2009 through May 18, 2009. EX2004, ¶¶1–23; EX2005, ¶¶1–23. For example, on April 24 Dr. Porreca received an initial set of claims for the application that became the ’730 patent. EX2004, ¶10; EX2005, ¶10; EX2012, 1. On April 27, Dr. Porreca provided his edits the prosecuting attorneys and on April 28, Dr. Porreca received a revised set of claims for the application that became the ’730 patent. EX2004, ¶11; EX2005, ¶11; EX2012, 3. On April 29, Dr. Porreca spoke with the prosecuting attorneys regarding the inventions that became the ’730 patent. EX2004, ¶12; EX2005, ¶12; EX2012, 1–2.

On April 30, Dr. Porreca provided further edits to the draft patent application for the prosecuting attorneys to add. EX2004, ¶¶12–13 ; EX2005, ¶¶12–13; EX2012, 1–2; EX2013. On May 5, Dr. Porreca provided more materials to add to the application. EX2004, ¶14; EX2005, ¶14; EX2012, 1. On May 5, Dr. Porreca also met with the prosecuting attorneys to discuss the application that became the '730 patent. EX2004, ¶15; EX2005, ¶15; EX2014, 4–5. On May 8, Dr. Porreca set up a call with the prosecuting attorneys that took place on May 9. EX2004, ¶16; EX2005, ¶15; EX2014, 3. On May 11 and May 13, Dr. Porreca exchanged emails with the prosecuting attorneys regarding the application that became the '730 patent. EX2004, ¶¶17–18; EX2005, ¶¶16–17; EX2014, 1. On May 14, Dr. Porreca received an updated draft of the application that became the '730 patent from the prosecuting attorneys, and Dr. Porreca provided his edits on May 15. EX2004, ¶¶19–21; EX2005, ¶18–20; EX2015, 1; EX2016.

On May 18, 2009, the prosecuting attorneys filed the '358 provisional. EX2004, ¶¶22; EX2005, ¶21; EX1008. By filing the '358 provisional, Dr. Porreca and the inventors constructively reduced the '730 patent to practice on May 18, 2009. EX2004, ¶¶19–23; EX2005, ¶¶7–23; EX2015, 1; EX2016.

Inventor and attorney testimony accompanied by corroborating documentary evidence, like the evidence identified above, has consistently been found sufficient to demonstrate reasonable diligence. *Unified Patents, LLC v. Flexiworld Techs.*, IPR2022-00775, Paper 41, 17–20 (P.T.A.B. November 1, 2023); *Ex parte Koji*

Muramaya, 2020 WL 1663636 *4-5 (P.T.A.B. April 1, 2020).. For example, in *Koji* the Board found that an attorney’s statement that he “continuously and repeatedly communicated with the inventor” and that his “actions were reasonably continuously diligent based on the many hundreds of other cases [he] was handling at the time” demonstrated reasonable diligence. *Ex parte Koji*, 2020 WL 1663636 at *5. Particularly, the Board in *Koji* found that there was “sufficient evidence of reasonable diligence during the critical period” even where there was a lack of documentary evidence for the first 45 days of a 57-day critical period. *Id.* at *5. The Board specifically explained if an “attorney has a reasonable backlog of unrelated cases which he takes up in chronological order and carries out expeditiously, that is sufficient” evidence of reasonable continuous diligence. *Id.*

Here, Dr. Waller explains that he spent an average of 7 hours a day on his intellectual property cases, regularly communicated with the inventor regarding the application, and created multiple drafts of the patent application throughout the entire critical period. EX2005, ¶¶1–21; EX2013; EX2014; EX2015; EX2016. Furthermore, the actions of Dr. Porreca’s attorneys demonstrate far more consistent diligence than those found to be sufficient in *Koji*, as shown by documentary evidence corroborating the testimony of Dr. Waller’s diligence early and throughout the critical period. EX2005, ¶¶1–21; EX2013; EX2014; EX2015; EX2016. For the reasons discussed above, Dr. Porreca’s testimony and the corroboration from the documentation and the testimony of the prosecuting attorneys demonstrate

reasonable diligence for at least the period of April 29, 2009 through May 18, 2009. Claims 1–3 and 9 of the '730 patent are, therefore, entitled to a priority date of at least April 29, 2009.

V. The Level Of Ordinary Skill In The Art

Petitioner proposes a POSA would be a person having either “(i) a Ph.D. in molecular biology, genetics, bioinformatics, or a related field, and at least two years of experience in high-throughput sequencing technologies, or (ii) a Master’s degree in one of the same fields with at least four years of experience in high-throughput sequencing technologies.” Pet., 10. Patent Owner¹ adopts this narrow proposed definition for purposes of this proceeding. EX2023, ¶¶22-23.

However, Patent Owner disagrees with Petitioner’s additional requirement that a POSA must have “also commonly worked as part of an interdisciplinary team.” Pet., 10; EX2023, ¶22. Petitioner fails to explain what that vague requirement means, including what is required for the “interdisciplinary team,” such as the disciplines studied by the other team members. Petitioner also does not explain why a Ph.D. or Master’s degree in the relevant fields combined with an appropriate level of experience in high-throughput sequencing technologies would not suffice. For these reasons, Patent Owner disagrees with Petitioner’s vague addition to the

¹ As indicated in the Updated Mandatory Notices filed February 27, 2025 (Paper No. 8), the Patent Owner in this proceeding is Molecular Loop Innovations LLC.

definition of a POSA beyond what would have been required in the field at the time of the invention.

VI. Grounds 1-3: The Challenged Claims Are Patentable Over Chee Alone Or Over Chee In View Of Gahan.

A. Chee Is Not Prior Art To Claims 1-3 And 9 Of The '730 Patent Because Those Claims Are Entitled To An Earlier Priority Date.

As explained in Section IV, *supra*, claims 1-3 and 9 of the '730 Patent are entitled to a priority date of at least April 29, 2009. Accordingly, Chee is not prior art for claims 1-3 and 9, because Chee's provisional application was not filed until April 30, 2009. EX1010, cover. For at least this reason, claims 1-3 and 9 are patentable, and Petitioner's Grounds 1-3 fail for those claims.

B. Chee's April 30, 2009 Provisional Application Does Not Disclose All Elements Relied Upon By Petitioner. (All Claims)

Petitioner also has not shown that Chee is entitled to the April 30, 2009 filing date of Provisional Application No. 61/174,442 (EX1011, the "'442 Provisional"). Specifically, Petitioner cannot show that Chee's '442 Provisional supported statements in Chee that Petitioner relies upon to disclose the "obtaining a biological sample comprising a plurality of target nucleic acid molecules" claim limitation in independent claims 1 and 9 of the '730 Patent. The '442 Provisional also does not disclose performing Chee's method with blood, biopsy tissue, bacterial or viral nucleic acids, tumor nucleic acid molecules, maternally-circulating fetal or placental nucleic acid molecules, or any other sample collected from a patient as recited in

claims 4-8 of the '730 Patent. Therefore, those portions of Chee that Petitioner relies on in its invalidity analyses are not entitled to the '442 Provisional's filing date, and it is not prior art. In particular, Petitioner relies on disclosure in Chee that it may perform its technique on “‘naturally-occurring nucleic acids (e.g., DNA or RNA or fragments thereof...)' for clinical or diagnostic use.” EX1010, 11:51-54; Pet., 26. But Chee's description of “naturally-occurring nucleic acids” is not present *anywhere* in the '442 Provisional. Thus, Chee's provisional did not disclose the material that Petitioner relies on to allege that Chee taught using its method by “obtaining a biological sample,” as recited in independent claims 1 and 9, or using the particular sample types recited in claims 4-8, as of April 30, 2009. Chee thus is not prior art to the Challenged Claims.

To show that a patent was prior art as of its provisional filing date, “[e]ven if one demonstrates that a provisional application provides written description support for one claim of the non-provisional application or patent, the provisional application must *also* provide written description support *for the specific portions of the patent specification identified and relied on in the prior art rejection.*” *In re Riggs*, 131 F.4th 1377, 1384 (Fed. Cir. 2025); *see also id.*, 1384-85 (“It makes *no sense* to suggest that if a single claim is supported by the provisional application, then *everything* in the later filed application gets the benefit of the provisional date whether supported or not.”).

Petitioner relies on Chee’s description of “naturally-occurring nucleic acids” as allegedly disclosing the ’730 Patent’s claimed step of “obtaining a biological sample.” Pet., 26. For that portion of Chee to qualify as prior art, Petitioner must show that the ’442 Provisional provides written description support for that disclosure. It does not. EX2023, ¶¶164-169. Those lines are not present in the ’442 Provisional, and Petitioner does not attempt to show that they are. Accordingly, Petitioner does not show that the “specific portions of the patent specification identified and relied on in the prior art rejection” (*In re Riggs*, 131 F.4th at 1384)—e.g., Chee’s description of “naturally-occurring nucleic acids”—are present in the ’442 Provisional, and Chee is therefore not entitled to the ’442 Provisional’s April 30, 2009 priority date for any Challenged Claim.

Petitioner asserts that Chee is entitled to a priority date of April 30, 2009 based on the allegation that “at least one claim in the issued Chee patent has written description support in the provisional application” for ***claims 1 and 2*** of the issued Chee patent, but does not show disclosure for ***all*** of the portions of Chee actually relied upon in the prior art grounds in the Petition. Pet., 23-24; *see also* EX1005, ¶¶121-129. Indeed, Dr. Gabriel confirmed that she only analyzed whether or not claims 1 and 2 in the issued Chee patent were sufficiently described by the ’442 Provisional and did not provide “any other analysis beyond claims 1 and 2.” EX2024, 69:3-6; EX2023, ¶164. Thus, Petitioner does not show how the ’442

Provisional disclosed performing Chee's method on *naturally-occurring* nucleic acid samples.

Rather than showing the required support in the '442 Provisional, Petitioner and Dr. Gabriel cherry pick paragraphs that allegedly describe analyzing “nucleic acids *derived* from a biological sample.” Pet., 24, 26; EX1005, ¶¶121-129; *see also* EX2023, ¶¶166-167. Deriving a biological sample refers to synthetic biology—e.g., artificial creation of materials such as nucleic acid sequences—not to obtaining the naturally-occurring biological materials themselves. EX2023, ¶167; EX2024, 59:21-60:7 (“‘Synthetic biology,’ as Chee describes it, means constructing the genome as opposed to decoding or -- decoding a genome, correct? A. I think it refers to – to synthesizing nucleic acid fragments. Q. And referencing our discussion earlier, you know, *that would be an artificial nucleic acid sequence as opposed to a naturally occurring one, like the ones you're developing in your genomic profiles, for example, correct? A. That's fair.*”). Indeed, the '442 Provisional begins by stating that its invention “relates to the field of oligonucleotide selection and *synthetic biology*.” EX1011, [0001]; EX2023, ¶¶166-168. It continues, explaining that “*synthetic biology* is a promising new field at the interface of engineering and biology,” recognizing that “*de novo chemical synthesis of the DNA building blocks is quite error-prone*,” and noting the “need to select error-free synthesized oligonucleotides for use in *synthetic biology* and other analytical methods,” which it claims is met by its invention. EX1011, [0003]-[0005].

The '442 Provisional is entirely directed to performing its method on *synthetically-created* nucleic acids. EX2023, ¶¶166-168. The language later added to Chee and relied upon by Petitioner as allegedly disclosing “obtaining a biological sample” is telling. Petitioner cites a subsequently added portion of Chee that says its “technique can be used to analyze [] ‘naturally-occurring nucleic acids (e.g., DNA or RNA or fragments thereof . . .)’ for clinical or diagnostic use,” but this language *does not appear* in the '442 Provisional. Compare EX1010, 11:51-54 with EX1011, [0066]. Instead, this paragraph of the '442 Provisional noted that its methods were “*primarily described in the context of synthetic biology*,” language that was later *removed* from Chee’s specification. Compare EX1010, 11:51-54 with EX1011, [0066]; EX2023, ¶¶166-167. The '442 Provisional does not provide *any method* in this paragraph or elsewhere to provide sufficient written description support for the creation of constructs containing ROIs from a *naturally-occurring* sample. EX1011, [0066]; EX2023, ¶¶166-168. And Dr. Gabriel confirmed that she did not address whether the '442 Provisional also describes the subject matter from Chee she relies upon as allegedly disclosing the “obtaining a biological sample” limitation. EX2024, 70:7-16.

The remaining paragraphs of the '442 Provisional string-cited by Petitioner as allegedly providing support for “obtaining a biological sample” do nothing more than explain how to perform the disclosed method on *synthetically-created* nucleic acids. EX2023, ¶¶164, 166-168; Pet., 27. For example, Petitioner points to generic

language that merely says “another feature of the invention is that multiple oligos with different ROIs can be processed in a single reaction,” but this again says **nothing** about the source of the nucleic acids. EX1011, [0029]; EX2023, ¶¶164, 166-168. Petitioner then cites to two paragraphs suggesting that nucleic acids from “biological samples” may be analyzed, but those disclosures again say nothing about the “naturally-occurring” nucleic acids that Petitioner relies on for its analysis, and in fact highlight that the samples discussed are **synthetic**. EX1011, [0060] (addressing “error encountered in **conventional synthetic biology**”), [0069]; EX2023, ¶167. In sum, none of these paragraphs provide sufficient written description support for the portions of Chee relied upon by Petitioner to disclose “obtaining a biological sample” by discussing **naturally-occurring** nucleic acids. And notably, Dr. Gabriel does not point to **any** portion of the ’442 Provisional that allegedly discloses this limitation. EX1005, §VII.A.2.(a); *see also id.*, ¶¶137-143; EX2024, 69:21-70:16; EX2023, ¶164.

The ’442 Provisional further explains using its methods “to assemble oligos into larger constructs, and these can be used to assemble larger nucleic acids using the isolated ROIs,” another example of synthetic biology. EX1011, [0129]; *see also id.*, [0100], [0114]; EX2023, ¶¶166-168. It also describes the benefits of using its methods to create these longer sequences, noting that, “[b]y obtaining long, highly pure oligos of desired sequence, it will be possible to assemble relatively long constructs by annealing the oligos together so that hybridization ensures that correct

sequences are brought together.” EX1011, [0130]; EX2023, ¶¶166-168. Again, none of these disclosures suggest using Chee’s method by obtaining naturally-occurring nucleic acids, but instead point *solely* toward its use in the context of synthetic biology.

For at least these reasons, Chee is not entitled to an effective filing date of April 30, 2009 for any of its alleged disclosures related to “obtaining a biological sample” using naturally-occurring nucleic acids—i.e., the precise disclosure Petitioner relies on to argue that Chee discloses claim 1 and 9—or with blood, biopsy tissue, bacterial or viral nucleic acids, tumor nucleic acid molecules, maternally-circulating fetal or placental nucleic acid molecules, or any other sample collected from a patient as recited in claims 4-8. Thus, Petitioner did not establish Chee as prior art for any Challenged Claim, and Petitioner’s Grounds 1-3 fail for these additional reasons.

C. Chee Does Not Disclose “Collapsing Target:Differentiator Tag Combinations Observed More Than Once Into A Single Count.” (Claim 1)

Claim 1 recites “*collapsing target:differentiator tag combinations* observed more than once into a single count.” EX1001, 131:47-49. Petitioner argues that Chee discloses the claimed collapsing step because it generates a single consensus read by sequencing multiple clonal copies of all molecules with the same unique identifier. Pet., 31-32; *see also* EX1005, ¶¶175-178. But determining a single consensus read by looking at multiple clonal copies of the same original starting molecule is not

collapsing as claimed by the '730 Patent. Thus, Petitioner fails to point to any part of Chee that discloses the claimed collapsing.

1. The '730 Patent Claims “Collapsing” As A Separate Step From “Determining A Consensus Base Call.”

A POSA would understand “collapsing” and “determining” to be distinct steps in the Challenged Claims. EX2023, ¶¶131-147. Petitioner interprets a “consensus base call” as “using multiple sequencing reads of the same molecule to determine the nucleotide (base) at each position of a sequence.” Pet., 19. But even under that interpretation, as the '730 Patent explains, “collapsing” is directed toward fixing quantitative errors and is a different step distinct from determining a “consensus base call,” which is directed toward fixing sequence errors. EX2024, 46:10-25.

First, the plain language of the claims demonstrates that “collapsing” and “determining a consensus base call” are distinct steps. For example, the '730 Patent recites a “collapsing” step in independent claim 1. EX1001, 131:47-49. The '730 Patent then goes on to claim that the “collapsing” step is performed *before* “determining a consensus base call” in dependent claim 2. EX1001, 131:50-54. Thus, claim 2 describes determining a consensus base call from *collapsed* reads, *not* performing “collapsing” by determining a consensus base call. The use of different terms to describe different steps, performed sequentially, shows that “collapsing”

and “determining a consensus base call” are distinct steps. EX2023, ¶133; EX2024, 57:11-58:22.

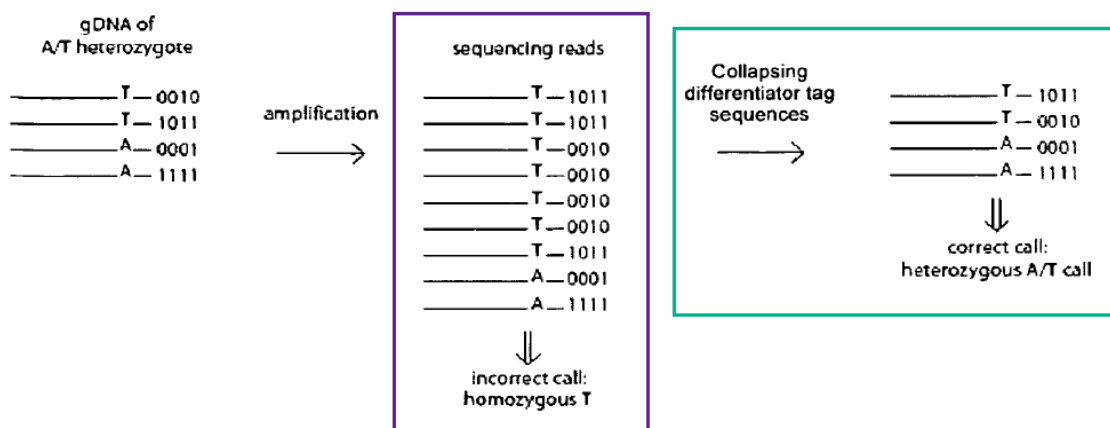
Second, the ’730 Patent’s specification consistently shows that “collapsing” is a different step than “determining a consensus base call.” *See, e.g.*, EX1001, 72:63-73:5, claim 2; EX2023, ¶¶134-144. For example, the specification explains that “[a]ll ‘count’ entries should be ‘1’, since any particular target:differentiator tag mapping will not occur more than once by chance, and therefore will only be observed if bias was present somewhere in the sample preparation process. **For any target:differentiator tag combination observed more than once**, all such reads are ‘collapsed’ into a single read **before consensus basecalls are determined. This will cancel the effect of bias on consensus basecall accuracy.** FIG. 5 depicts a method for making diploid genotype calls in which repeat target:differentiator tag combination are collapsed.” EX1001, 72:63-73:5; EX2023, ¶¶133, 138-142.

The specification of the ’730 Patent also explains that collapsing corrects for skewing, an issue related to over- or under-amplification (EX1001, 27:47-50, 32:29-34), which is a separate and distinct problem from the sequence errors that a consensus base call is designed to address, which Chee says arise from adding an incorrect nucleotide at a given position during sequencing or synthesis. EX1010, 9:34-39, 14:42-50; EX2023, ¶¶134, 136-137; EX1005, ¶¶76-78, ¶¶177-178; EX2024, 46:2-46:25. “Collapsing” is a different step, performed separately from determining any consensus base call, to provide even *greater* accuracy when

subsequently determining a consensus base call. EX2023, ¶¶134-137. In fact, if one were to collapse *by* determining the consensus base call, errors from amplification bias could still be represented in the consensus base call generated. EX2023, ¶¶134-137.

Figure 5 of the '730 Patent, *infra*, also shows that collapsing is a different step from determining a consensus base call. In **purple** below, Figure 5 shows that, when certain starting molecules are over- or under-amplified and redundant sequence reads are not removed, the skewed population of amplicons will result in generating a consensus base sequence using *all amplicons* and thus causing an **incorrect genotype call**. EX1001, Fig. 5; EX2023, ¶¶138-142. Instead, as shown in **teal** below, when identical sequence reads are collapsed prior to generating a consensus base call, errors arising during amplification bias will be eliminated from the calculations, resulting in a **correct genotype call**. EX1001, Fig. 5; EX2023, ¶¶138-142.

FIG. 5



EX1001, Figure 5 (annotated); *see also* EX2023, ¶138.

For at least these reasons, the plain language of the claims requires that collapsing is a distinct step from determining a consensus base call.

2. Chee Does Not Disclose The Claimed Collapsing Step.

Petitioner and Dr. Gabriel do not offer a construction for “collapsing,” but incorrectly conflate it with Chee’s alleged process of determining a consensus base sequence. For example, Dr. Gabriel appears to define “collapsing” as “counting the unique tags associated with each sequence (and ignoring duplicates) to determine the number of times a sequence appeared in the original sample” (EX1005, ¶175), but goes on to say that Chee inherently collapses by determining a consensus base sequence to arrive at the sequence of a single starting molecule. EX1005, ¶¶176-178. That interpretation is incorrect. As described in Section VI.C.1, *supra*, “collapsing” is a different step, separate and distinct from determining a consensus

base call, each being designed to solve a different problem. And Chee does not disclose a method for collapsing. EX2023, ¶157.

First, collapsing is different from the consensus base sequence in Chee’s method, and the quantitative errors corrected by collapsing are different from sequence errors corrected by determining a consensus base sequence. EX2024, 46:2-25. Chee’s method relies on reducing *sequence* errors. EX1010, 14:42-50; EX2023, ¶¶148-153. Chee’s primary goal is to select constructs containing a desired (i.e., “validated”) sequence by determining a consensus sequence through reading “*multiple clonal copies* of the construct, which are identified as identical by descent via the shared unique identifier,” and attributing “a variation in the *sequence* of a single copy of a construct” to an error occurring during sequencing or synthesis of the construct. EX1010, 14:39-56; EX2023, ¶¶152-153; EX2024, 12:17-13:1, 13:15-24. Petitioner recognizes that, by obtaining the consensus base sequence for a given construct, Chee corrects for *sequence* errors. Pet., 32.

But Petitioner fails to recognize that collapsing serves a different purpose: to remove redundant sequence reads prior to counting. EX2023, ¶¶149-153. Collapsing does not determine a consensus base call by analyzing multiple reads of the same construct, because collapsing does the opposite; it assumes that all sequence reads for a single count will be identical, so there is no need to analyze multiple reads. EX2023, ¶¶158-163. Thus, collapsing resolves quantification errors, allowing

identical sequence reads to be counted only once without considering the precise sequence. EX2023, ¶¶154-156.

Second, Petitioner cherry picks language from Chee noting the possibility of using its method to address issues related to amplification bias but does not provide any mechanism for actually doing so. For example, Petitioner points to generic language in Chee to suggest that its unique identifiers can be used to determine that two amplicons are “copies of the same construct from the original sample.” Pet., 31. But, beyond pointing to language that tags can be used to represent amplicons that are “identical by descent,” Petitioner does not, and cannot, point to any precise method of Chee that explains how a POSA would use this information to count the number of original starting molecules of a given sequence. *See* Pet., 31-32; EX2023, ¶¶158-162. Petitioner also selects sentences in Chee that merely state its method “can” be used to identify amplification bias by associating each construct with a unique identifier but fails to cite to any disclosure to explain what that method entails. Pet., 31-32; EX2023, ¶¶151-153; *see also* EX1010, 23:56-64 (noting bias could be identified “by determining the amounts of constructs with unique identifiers” and that “the quantification of the molecules can be corrected” but providing no way of doing so).

Tellingly, although Dr. Gabriel seemingly proposes defining collapsing as “counting the number of unique tags associated with each sequence (and ignoring duplicates),” she does not cite any disclosure in Chee of an actual method of counting

or ignoring duplicate sequence reads. EX1005, ¶¶173-175; *see also* EX2023, ¶¶153-156. Indeed, as described above, Chee does not “ignore duplicates” of the same sequence, but rather reads *multiple clonal copies* to determine a consensus sequence. EX1010, 14:39-56; EX2023, ¶158. And Dr. Gabriel states that generating a consensus call involves performing multiple sequence reads of the same molecule and counting the nucleotide that appears most frequently at a given position to determine the most probable sequence of the molecule. EX2024, 13:15-24. But still, Dr. Gabriel asserts the *same step* is both “collapsing” and determining a consensus base sequence (EX1005, ¶¶175-178, 185), despite the clear language of the claims that she agrees requires the two steps to be different. EX2024, 57:11-58:22.

For at least these reasons, Petitioner fails to show that Chee discloses the claimed collapsing step. Thus, Petitioner’s Grounds 1-3 fail.

D. Chee Does Not Disclose “Collapsing All Sequence Reads Comprising A Same Differentiator Tag Into A Single Read Before Determining A Consensus Base Call.” (Claim 2)

Claim 2 depends on claim 1 and “further comprises collapsing all sequence reads comprising a same differentiator tag into a single read before determining a consensus base call for the target nucleic acid molecule sequences.” EX1001, 131:50-54. Petitioner and Dr. Gabriel allege that Chee discloses this limitation because “Chee generates a single ‘consensus sequence derived from [those] multiple clonal copies of [a] construct’” and thus “discloses ‘collapsing’ all of the ROI reads of multiple copies of a construct into a single read or ‘consensus sequence.’” Pet.,

33; EX1005, ¶185. But as described in Section VI.C.1, collapsing is *not* the same step as determining a consensus base sequence. EX2023, ¶¶132-144. And Petitioner ignores the two separate steps in claim 2 and their temporal relation: “[1] *collapsing* all sequence reads comprising a same differentiator tag into a single read *before* [2] *determining a consensus base call.*” EX1001, 131:50-54. Petitioner fails to explain how the *same step* of Chee can perform *both* collapsing *and* determining a consensus base call, when they are two distinct steps performed sequentially. EX2023, ¶¶131-133.

For at least these reasons, Petitioner fails to point to any part of Chee to show determining a consensus base call after obtaining collapsed sequence reads. Thus, Petitioner’s Grounds 1-3 fail for claim 2 for this additional reason.

E. Petitioner Fails To Show A Motivation To Combine Chee In View Of Gahan. (Claims 5, 6, and 8)

Claims 5, 6, and 8 each depend on claim 1 and recite the plurality of target nucleic acid molecules is comprised of “bacterial or viral nucleic acids isolated from said biological sample” (claim 5), “circulating tumor nucleic acid molecules” (claim 6), or “maternally-circulating fetal or placental nucleic acid molecules” (claim 8). Petitioner argues that Chee in view of Gahan render claims 5, 6, and 8 obvious. Pet., 42-44. As described in Sections VI.B-C, *supra*, neither Chee nor Chee’s ’442 Provisional disclose performing its method on a biological sample, such as blood or biopsy tissue, from a patient or analyzing bacterial or viral nucleic acids, tumor

nucleic acids, or maternally-circulating fetal or placental nucleic acids. Nor does Petitioner show that a POSA would have been motivated to combine its method, directed to synthetic DNA, to performance on naturally occurring nucleic acids. EX2023, ¶¶170-176. Thus, Petitioner’s Ground 3 fails.

First, Chee and Gahan are in different fields of art. As described in Section VI.B, *supra*, Chee is directed to solving errors that arise when selecting *synthetically-created* nucleic acids with the correct sequence. *See, e.g.*, EX1010, 1:23-58. Petitioner asserts that “[a] POSA would have known that it was useful to sequence DNA circulating in blood for diagnostic purposes.” Pet., 43. But Petitioner fails to point to any part of Chee that explains why a POSA would be motivated to look to Gahan in the first place when performing Chee’s method specifically designed for correcting errors that arise in synthetic biology. Pet., 43-44. Instead, Petitioner and Dr. Gabriel ignore Chee’s clear teachings and again cite to generic language about “biological samples” without reading Chee’s method in context. EX1005, ¶¶224, 226-227, 229-230, 232; EX2023, ¶¶172-173. In fact, beyond picking a single textbook out of the eleven listed in Chee, Petitioner fails to point to even a single sentence about any type of “biological sample” in Chee. Pet., 43; EX1005, ¶222-223; EX2023, ¶175. Thus, Petitioner fails to explain why a POSA would look to two different fields of art.

Second, Petitioner fails to provide a motivation to combine Chee’s method, which it alleges solved issues in synthetic biology when a sufficient number of

constructs were present, when read with the uncertainties about cell-free DNA expressed in Gahan. For example, Chee explicitly states that “[t]he number of nucleic acid constructs in a set should be *large enough to ensure a high probability of obtaining at least one correct sequence or to confirm a correct sequence associated with a particular unique identifier.*” EX1010, 2:44-51; EX2023, ¶174. However, Gahan explains the methodology for isolating cell-free nucleic acids was “*far from consistent*, and if there are to be reliable protocols for diagnostic purposes, there needs to be a form of quality assurance for these procedures.” EX1015, 3; EX2023, ¶174. In describing the kits used for isolation, Gahan also explains that “there is still a lack of clarity concerning the user of the broad range of kits and their applicability,” despite “an attempt to determine the kit with the greatest yield” of nucleic acids. EX1015, 3; EX2023, ¶174. Thus, while a POSA would have been able to artificially construct a large enough set of constructs, Gahan would have created uncertainty about having a reliably sufficient amount to perform Chee’s method. Coupled with Chee’s teachings toward performing its method with synthetic biology, Petitioner fails to show why a POSA would even consider combining Chee and Gahan.

For at least these reasons, Petitioner’s Ground 3 fails.

VII. Ground 4: The Challenged Claims Are Patentable Over Bignell And McCloskey.

A. Bignell In View Of McCloskey Does Not Disclose The “Correcting” Step Of Claim 1.

Claim 1 requires “correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.” EX1001, 131:47-49. Petitioner relies solely on McCloskey for this limitation. Pet., 56–57. Petitioner’s analysis is wrong.

First, Petitioner begins with a flawed understanding of the claim limitation. Petitioner states “the ’730 patent shows performing the ‘collapsing’ step by counting only unique tags to determine the number of times a sequence appeared in the original sample.” Pet., 57 (citing EX1001, 30:48-51, 72:63-73:2). But the ’730 patent specifically claims and requires “collapsing *target:differentiator tag combinations*.” EX1001, 30:48-51, 72:63-73:5, 131:47-49. Claim 1 recites collapsing *combinations* of targets (*i.e.*, target nucleic acid molecules) *and* differentiator tags, not tags alone as Petitioner incorrectly states. EX1001, 131:47-49.

And the specification of the ’730 Patent describes why collapsing target:differentiator tag combinations (as claimed) provides advantages that counting tags alone (as in McCloskey) does not. For example, the specification of the ’730 patent explains that collapsing target:differentiator tag combinations provides methods to make diploid genotype calls. EX1001, 73:3-5 (“FIG. 5 depicts

a method for *making diploid genotype calls* in which repeat *target:differentiator tag combination[s] are collapsed.*”), Figure 5. The specification of the ’730 patent further explains that collapsing “any *target:differentiator tag combination* observed more than once ... into a single read before consensus basecalls are determined ... will cancel the effect of bias on consensus basecall accuracy.” *Id.*, 72:63-73:3. Thus, a POSA would have understood that the inclusion of the target nucleic acid molecule when collapsing target:differentiator tag combinations allows a POSA to make diploid genotype calls and lays the groundwork for more accurate consensus basecalls. Pet., 57.; EX2023, ¶¶57, 182. Unlike Petitioner’s assertion that claim 1 can be performed by “counting only unique tags” themselves, claim 1 requires “correcting for errors or bias ... by collapsing *target:differentiator tag combinations.*” Pet., 57; EX1001, 131:47–49. Accordingly, Petitioner’s characterization of the claims of the ’730 Patent is incorrect, and infects the rest of Petitioner’s analysis.

Second, McCloskey does not “correct for errors or bias ... by collapsing target:differentiator tag combinations,” because McCloskey does not disclose using “target:differentiator tag combinations” to correct for errors or bias. Instead, McCloskey only discloses identifying *tags* alone without any analysis of the *target* to correct for errors or bias. Pet., 56 (“McCloskey corrects the skewing by *identifying duplicate barcodes* in an amplified sample.”); EX1012, [0032] (“authenticating the amplification product by *detecting the presence of the second*

[barcode] sequence in the amplification product”), [0035] (“authenticating the amplification product by *detecting the presence of the second [barcode] sequence and the fourth [batch-stamp] sequence*”), [0036] (“authenticating the amplification product of step (c) by *detecting the presence of the second [barcode] sequence*”). McCloskey does **not** disclose using the target nucleic acid in **combination** with the tag to correct for errors or bias. A significant advantage of collapsing target:differentiator tag combinations instead of detecting the presence of a barcode alone is the ability to “mak[e] diploid genotype calls” without any further steps. EX1001, 73:3–5. The ’730 patent explains that utilizing target:differentiator tag **combinations** prior to determining consensus basecalls allows for ways to determine basecalls that “cancel the effect of bias on consensus basecall accuracy.” EX1001, 72:63-73:3. And Dr. Gabriel does not cite to any disclosure in McCloskey where its barcodes in combination with the target nucleic acid are used to correct for errors or bias, nor does Dr. Gabriel otherwise opine that a POSA would use the target nucleic acid in combination with McCloskey’s barcodes to correct for errors or bias. EX1005, ¶¶291–295.

Third, McCloskey does not disclose “collapsing” reads together. McCloskey teaches detecting or “identifying” a barcode to “count” amplification products with distinct tags. Pet., 56–57; EX1012, [0009]. Tellingly, Petitioner does not argue that McCloskey collapses sequences together. Pet., 56–57. Instead, Petitioner argue that “identifying duplicate barcodes” to “count[] only distinctly tagged sequences”

satisfies the collapsing limitation because “the ’730 patent shows performing the ‘collapsing’ step by counting only unique tags to determine the number of times a sequence appeared in the original sample.” Pet., 56–57. But a POSA would have understood that “identifying duplicate barcodes in an amplified sample” and “counting only distinctly tagged sequences,” is different and distinct from “collapsing target:differentiator tag combinations.” EX2023, ¶¶177–183.

While Petitioner argues that the combination of Bignell and McCloskey discloses claim 1 of the ’730, Petitioner relies solely on McCloskey to disclose the “collapsing” limitation. Pet., 56-57. Petitioner cannot rely on Bignell to disclose the collapsing limitation because Bignell discloses a sample indexing method that tags different target nucleic acids in a given sample with the *same tag* before pooling and sequencing multiple samples together. Section III.B *supra*; EX1013, 3:32-35; EX1005, ¶84; EX2024, 77:15-21. Unlike the ’730 patent, which requires collapsing target:differentiator tag combinations into a single count, Bignell discloses using sequences with the duplicate tags for analysis. EX1013, 6:36-48; EX1005, ¶¶84 (citing Bignell), 233–238; EX2023, ¶¶183–185. A POSA would have understood that Bignell is directed to a different problem than the ’730 patent, namely methods to sequence multiple samples together. EX2023, ¶¶183–185; EX2024, 77:2–4. Petitioner thus fails to show that the combination of McCloskey and Bignell teach “correcting for errors or bias introduced during [a nucleic acid analysis] workflow

by *collapsing target:differentiator tag combinations* observed more than once into a single count.” EX1001, 131:47–49.

B. Bignell In View Of McCloskey Does Not Disclose All Limitations Of Claims 4, 5, and 6.

Claims 4, 5, and 6, of the '730 patent depend on claim 1. For at least the reasons stated above, Bignell and McCloskey do not disclose all the limitations for claims 4, 5, and 6. Section VII.A *supra*.

C. Petitioner Fails To Show A Motivation To Combine Bignell and McCloskey. (All Claims)

Petitioner argues that a POSA would have been motivated to combine “McCloskey’s unique barcodes [with] Bignell’s method,” but its argument ignores the stated goals of both references and fails to address the disadvantages (including increased costs) associated with the combination. Pet., 46-51.

First, a POSA reading Bignell in view of McCloskey would have understood that the stated goals for McCloskey’s molecule-specific sequence tags conflict with the stated goals for Bignell’s sample-specific sequence tags. McCloskey is directed to solving issues with the authenticity of PCR products. EX1012, [0004]. Specifically, McCloskey contemplates using its methods on Sanger sequencing platforms. EX1012, [0034] (“*Exemplary conditions* for analyzing bar-coded target nucleic acid molecules by gel electrophoresis, cloning, and sequencing are *described in EXAMPLES 1-4, and 6*”), [0049] (“Verified SPCR reactions were then cleaned with Microclean (Gel Company) and sequenced on an *ABI 3100* with Big Dye.”). A

POSA would have known that Sanger sequencing platforms were costly and limited in total read capabilities. EX1014, 1135, 1141–43. To authenticate PCR products, McCloskey describes use of *random barcodes* that *are attached to every target molecule* in a sample and sequenced on Sanger sequencing platforms to produce a *limited amount of sequence reads*. EX2023, ¶¶113–120, 186, 195–196; EX1012, [0043]–[0104]. Accordingly, McCloskey requires use of different tags for each molecule within a sample in order to verify the authenticity of PCR amplification products. EX2023, ¶¶113–120, 184–187.

Unlike McCloskey, Bignell is directed to pooling multiple different samples, all containing DNA libraries, together in a single sequencing run. Specifically, Bignell contemplates pooling samples in NGS sequencing to reduce the costs of sequencing large populations of samples but explains that “[o]ne disadvantage of pooling samples prior to analysis is that information pertaining to individual DNA samples is lost.” EX1013, 1:66-2:8. To solve that problem of sample identification across pooled sample runs, Bignell describes *tagging all the nucleic acid fragments* in a given sample with the *same sequence tag* before pooling multiple samples (each with a different sample-specific tag) of DNA fragments together for a multiplex sequencing reaction on a next-generation sequencing platform that produces hundreds of millions of reads. EX2023, ¶¶121–125, 184–186; EX1005, ¶55. Accordingly, Bignell requires use of the *same* tag for each molecule within a sample

in order to distinguish *all* molecules originating from that sample from all molecules originating from a different sample.

McCloskey's goal of verifying the authenticity of PCR products requires a solution—tagging every molecule within a limited sample pool with a different tag—that is at odds with Bignell's goal of pooling multiple samples together to reduce costs, and Bignell's proposed solution of tagging every molecule within a given sample with the *same* tag. And Bignell not only recognizes this conflict, but distinguishes approaches like McCloskey's from Bignell's claimed goal. For example, Bignell considers a similar approach to McCloskey that envisions 16,777,216 different sequence tags within a single sample and concludes that “whilst [it] is ideal for treating one sample such that each individual molecule in the sample carries a different tag, *it does not permit attachment of the same tag to every molecule in the sample.*” EX1013, 3:32-35. Neither Petitioner nor Dr. Gabriel address this conflict at all. In fact, Dr. Gabriel agrees that a POSA “wouldn't want to have different tags within a sample”—e.g., using McCloskey's proposed solution—when using Bignell's method—e.g., using the same tags for every molecule within a sample—for its stated goal. EX2024, 76:7–14 (“Q. It wouldn't make sense to apply different tags to every molecule in the sample if ... Bignell's goal was identifying the source of the sample, right? ... A. If the goal is being able to tell one sample from another sample, *you wouldn't want to have different tags*

applied within a sample.”). Petitioner’s and Dr. Gabriel’s failure to address this conflict fails to prove a motivation to combine Bignell and McCloskey.

Second, even if Petitioner overcame the conflict between Bignell’s method and McCloskey’s method—which it has not—Petitioner has failed to show that a POSA would have a reasonable expectation of success in combining McCloskey and Bignell because Petitioner entirely ignores the costs of such a combination. Specifically, Petitioner fails to consider the outsized costs involved with using McCloskey’s molecule-specific different tags with Bignell’s massive, pooled sample runs. A POSA at the time of the invention would have understood that “[c]ost and throughput are the key limitations of sequencing.” EX1017, 545; EX2023, ¶190. Indeed, a major draw of NGS technology—like Bignell (*see e.g.*, EX1013, 45:48-50)—was its significant cost savings over first-generation technologies like the Sanger sequencing contemplated by McCloskey. Section II, *supra*. For example, a POSA at the time of invention of the ’730 Patent would have recognized that first-generation technologies, like Sanger sequencing, was costly and capable of roughly hundreds of reads in a single run. EX1005, ¶¶55-57; EX2024, 12:9-16, 80:18-81:2. In fact, a POSA would have recognized that a typical Sanger sequencing project ranged from \$300,000 to \$1,000,000 dollars. EX1014, 1141; EX2024, 83:7-84:1; EX2023, ¶¶36, 188–189. And adding McCloskey’s unique tags to each molecule within such a Sanger sequencing run would only increase those costs. EX2023, ¶¶191–197.

Conversely, a POSA would also recognize that the NGS technology contemplated by Bignell was cheaper, more efficient, and capable of far more sequencing reads in a single run than McCloskey's Sanger sequencing technology. For example, a POSA would have recognized that NGS technologies regularly processed millions of sequencing reads in a single run, as opposed to the hundreds contemplated by first-generation technologies. EX1005, ¶55; EX2024, 12:9-16. Bignell acknowledges that its sample-specific tags were to be applied across multiple samples in runs comprising entire DNA libraries. EX1013, 26:10-13. And a POSA would have recognized that NGS technologies reduced costs of sequencing by at least two orders of magnitude over first generation technologies, like McCloskey's Sanger sequencing. EX1005, ¶55; EX2024, 80:22-81:2. Indeed, Bignell specifically contemplates using pooled samples instead of non-pooled samples because of throughput and cost considerations. EX1013, 2:3-8 ("An ability to genotype large populations in a small number of reactions, while retaining the information relating to the source of the individual samples, would yield the information content of a full non-pooled population screen *in the time and at the cost of a pooled reaction.*"). Because of these major improvements in cost reduction and run throughput, NGS technology "democratiz[ed] the field by putting the sequencing capacity of a major genome center in the hands of individual investigators." EX1014, 1135, 1143; EX2023, ¶191. Accordingly, and as Dr. Gabriel admits, a POSA would have had to carefully consider the cost implications of

modifying Bignell's NGS techniques, so as not to impair the efficiency and affordability of Bignell's NGS technology. EX1005, ¶250.

But neither Petitioner nor Dr. Gabriel ever contemplate the cost considerations of applying McCloskey's different, molecule-specific tags to the millions of nucleic acids analyzed in the multiplexed sequencing reactions contemplated by Bignell. As described above, a typical Sanger-sequencing run, as contemplated by McCloskey, comprised mere hundreds of sequencing reads. Adding additional, molecule-specific pre-amplification tags to *millions* of DNA fragments in Bignell's NGS technology—the combination contemplated by Petitioner—would run counter to Bignell's stated cost advantage of pooling samples. EX1013, 2:3-8. Because neither Petitioner nor Dr. Gabriel ever even consider—let alone address—the high cost considerations of combining McCloskey and Bignell, they have failed to show either a motivation to combine or a reasonable expectation of success.

VIII. Ground 5: The Challenged Claims Are Patentable Over Bignell, McCloskey, And Bentley.

Petitioner argues that Bignell, McCloskey, and Bentley together disclose claims 2 and 3. Pet., 61–63. Claims 2 and 3 depend on claim 1, and Petitioner does not argue that the combination of Bignell, McCloskey, and Bentley provides any additional support for disclosing the elements of claim 1. *Id.* For the reasons explained above in Section VII, the combination of Bignell, McCloskey, and Bentley do not disclose claims 2 and 3 because the combination does not disclose claim 1.

IX. Ground 6: The Challenged Claims Are Patentable Over Bignell, McCloskey, And Gahan.

Petitioner argues that Bignell, McCloskey, and Gahan together disclose claims 6 and 8. Pet., 63–65. Claims 6 and 8 depend on claim 1, and Petitioner does not argue that the combination of Bignell, McCloskey, and Gahan provides any additional support for disclosing the elements of claim 1. *Id.* For the reasons explained above in Section VII, the combination of Bignell, McCloskey, and Gahan do not disclose claims 6 and 8 because the combination does not disclose claim 1.

X. Ground 7: The Challenged Claims Are Patentable Over Bignell, McCloskey, And Chiang.

Petitioner argues that Bignell, McCloskey, and Chiang together discloses claim 7. Pet., 65–66. Claim 7 depends on claim 1, and Petitioner does not argue that the combination of Bignell, McCloskey, and Chiang provides any additional support for disclosing the elements of claim 1. *Id.* For the reasons explained above in Section VII, the combination of Bignell, McCloskey, and Chiang does not disclose claim 7 because the combination does not disclose claim 1.

XI. Conclusion

For the foregoing reasons, the Challenged Claims are patentable.

Case IPR2024-01419
U.S. Patent No. 11,840,730

Dated: July 18, 2025

Respectfully submitted,

/Brian D. Matty/

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

DESMARAIS LLP

230 Park Avenue

New York, NY 10169

Telephone: 212-351-3400

Facsimile: 212-351-3401

Email: bmatty@desmaraisllp.com

*Counsel for Patent Owner Molecular Loop
Innovations LLC*

Case IPR2024-01419
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CERTIFICATION UNDER 37 C.F.R. § 42.24(d)

I hereby certify that this paper, excluding the portions exempted under 37 C.F.R. § 42.24(a), has 13,975 as counted by Microsoft Word 365, the word-processing system used to prepare this paper.

Dated: July 18, 2025

Respectfully submitted,

/Brian D. Matty/

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

DESMARAIS LLP

230 Park Avenue

New York, NY 10169

Telephone: 212-351-3400

Facsimile: 212-351-3401

Email: bmatty@desmaraisllp.com

*Counsel for Patent Owner Molecular Loop
Innovations LLC*

Case IPR2024-01419
U.S. Patent No. 11,840,730

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), the undersigned certifies that on July 18, 2025, a complete copy of Patent Owner's Response and corresponding exhibits were served on counsel of record for Petitioner by filing this document through P-TACTS and by sending this document via electronic mail to the following addresses:

SidleyIlluminaMLTeam@sidley.com

Thomas A. Broughan III
tbroughan@sidley.com

Ching-Lee Fukuda
clfukuda@sidley.com

Sharon Lee
sharon.lee@sidley.com

Dated: July 18, 2025

Respectfully submitted,

/Brian D. Matty/

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

DESMARAIS LLP

230 Park Avenue

New York, NY 10169

Telephone: 212-351-3400

Facsimile: 212-351-3401

Email: bmatty@desmaraisllp.com

*Counsel for Patent Owner Molecular Loop
Innovations LLC*