

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner.

Patent No. 11,840,730

Inter Partes Review No. IPR2024-01419

**Petition for *Inter Partes* Review of
U.S. Patent No. 11,840,730**

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1001	U.S. 11,840,730 (“’730 patent)
1002	’862 Application FH
1003	’687 Application FH
1004	’764 Application FH
1005	Declaration of Stacey Gabriel, Ph. D.
1006	Provisional Appl. No. 61/174,470
1007	Provisional Appl. No. 61/178,923
1008	Provisional Appl. No. 61/179,358
1009	Provisional Appl. No. 61/182,089
1010	US 9,085,798 to Chee
1011	Provisional Appl. No. 61/174,442
1012	US Pub. US2007/0020640 to McCloskey
1013	US 8,053,192 to Bignell
1014	J. Shendure & H. Ji, Next-Generation DNA Sequencing, Nature Biotechnology, Vol. 26, No. 10 (Oct. 2008)
1015	Gahan, Circulating Nucleic Acids in Plasma and Serum, Ann. N.Y. Acad. Sci. (2008)
1016	Weiner, Gabriel, Stephens, Eds. (2007), Genetic Variation: A Laboratory Manual (excerpts)
1017	D. Bentley, Whole-Genome Re-Sequencing, Current Opinion in Genetics & Development, 16:545-552 (2006)
1018	US Pub. 2008/0261204 to Lexow
1019	US 6,172,214 to Brenner
1020	Brenner et al., <i>In vitro</i> Cloning of Complex Mixtures of DNA on Microbeads: Physical Separation of Differentially Expressed cDNAs, PNAS, Vol. 97, No. 4 (Feb. 2000)

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1022	Annals of the New York Academy of Sciences ordering page for the <i>Circulating Nucleic Acids in Plasma and Serum</i> article by Gahan (Archive.org: Sept. 12, 2008), available at: http://web.archive.org/web/20080912061421/http://www.nyas.org/annals/detail.asp?annalID=934
1023	J. Shendure et al., <i>Next-generation DNA sequencing</i> , Nature Biotechnology, Nature Biotechnology, Vol. 26, No. 10 (Oct. 2008) (Archive.org: Jan. 24, 2009), available at: https://web.archive.org/web/20090124152730/http://nature.com/nbt/journal/v26/n10/full/nbt1486.html
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1026	Nickerson, US Pub. 2009/0163366
1027	Thiyagarajan et al., PathogenMIPer: A Tool for the Design of Molecular Inversion Probes to Detect Multiple Pathogens, BMC Bioinformatics, Vol. 7 (Nov. 2006) (“Pourmand(2006)”)
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1029	Ronaghi, US Pub. 2006/0008824
1030	Shoemaker, US Pub. 2008/0090239
1031	Porreca et al., Multiplex Amplification of Large Sets of Human Exons, Nature Methods, Vol. 4, No. 11 (Nov. 2007)
1032	Voelkerding et al., Next-Generation Sequencing: From Basic Research to Diagnostics, Clinical Chemistry, Vol 55, No. 4 (April 2009)
1033	D. Chiang et al., High-resolution mapping of copy-number alterations with massively parallel sequencing, Nature Methods Vol. 6 No. 1 (Jan. 2009)

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1034	Print out of Polymerase Chain Reaction (PCR), Genome.gov, available at: https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction
1035	Commings et al., Computational Biology Methods and Their Application to the Comparative Genomics of Endocellular Symbiotic Bacteria of Insects, Biological Procedures Online, Vol. 11, No. 1 (March 2009)
1036	Molecular Loop's Complaint, Case 1:24-cv-00680-UNA, Filed June 10, 2024
1037	Declaration of Nathaniel E. Frank-White, Internet Archive (September 26, 2024)
1038	Current Opinion in Genetics & Development, Vol. 16, No. 6 (Dec. 2006) (on file with the A. R. Mann Library at Cornell Univ. in Ithaca, NY)
1039	D. Chiang et al., Abstract, High-resolution mapping of copy-number alterations with massively parallel sequencing, Nature Methods Vol. 6 No. 1 (Jan. 2009) (Archive.org: Feb. 13, 2009), available at: https://web.archive.org/web/20090213081827/http://www.nature.com/nmeth/journal/v6/n1/abs/nmeth.1276.html
1040	Nature Methods Table of Contents for the January 2009 Issue (Vol. 6, No. 1) (Archive.org: Feb. 25, 2009), available at: https://web.archive.org/web/20090225213837/http://nature.com/nmeth/journal/v6/n1/index.html
1041	Declaration of Thomas A. Broughan III

Petitioner's Mandatory Notices**A. Real Party in Interest (§42.8(b)(1))**

The real party in interest of this petition pursuant to § 42.8(b)(1) is Illumina, Inc. (Petitioner or Illumina) located at 5200 Illumina Way, San Diego, CA 92122.

B. Other Proceedings (§42.8(b)(2))**1. Patents and Applications**

U.S. Patent No. 11,840,730 (“’730 patent,” EX1001) is assigned to Molecular Loop Biosciences, Inc. (Patent Owner). The ’730 patent is related to the following pending applications:

- U.S. Application No. 13/266,862
- U.S. Application No. 18/483,878

2. Related Litigation

On June 10, 2024, Petitioner Illumina along with Verinata Health, Inc. (“Verinata”) filed a declaratory judgment Complaint in the U.S. District Court for the District of Massachusetts against Patent Owner asserting non-infringement of five patents, including the ’730 patent (“Massachusetts Action”). *See Illumina, Inc. and Verinata Health, Inc. v. Molecular Loop Biosciences, Inc.*, Case No. 1:24-cv-11501, D.I. 1 (D. Mass.). That same day, Patent Owner filed a Complaint against Petitioner and Verinata in the U.S. District Court for the District of Delaware asserting infringement of three patents, including the ’730 patent

(“Delaware Action”). *See Molecular Loop Biosciences, Inc. v. Illumina, Inc. and Verinata Health, Inc.*, Case No. 1:24-cv-00680, D.I. 1 (D. Del.).

The parties subsequently agreed on some terms and filed a joint motion to transfer the Massachusetts Action to the District of Delaware where the related Delaware Action is pending. The joint motion was granted on August 2, 2024 and the Massachusetts Action was transferred to the District of Delaware on August 8, 2024. After the parties agreed on additional terms, including that Patent Owner grants Illumina a covenant not to sue under the two non-overlapping patents in the two Actions, they filed on August 29, 2024 a joint motion to dismiss the transferred Massachusetts Action.

3. Patent Office Proceedings

The '730 patent is not currently subject to any other proceedings before the Office. During prosecution of the '730 patent, the Board reversed the Examiner's final rejection in Appeal 2022-002597. The '730 patent's parents have not been subject to any other proceeding before the Board.

C. Lead and Backup Lead Counsel (§42.8(b)(3))

Lead Counsel is: Thomas A. Broughan III (Reg. No. 66,001), tbroughan@sidley.com, (202) 736-8314. Back-Up Lead Counsel are: Ching-Lee Fukuda (Reg. No. 44,334), clfukuda@sidley.com, (212) 839-7364; and Sharon Lee (*pro hac vice* to be requested), sharon.lee@sidley.com, (212) 839-7305.

D. Service Information (§42.8(b)(4))

Service on Petitioner may be made by e-mail
(SidleyIlluminaMLTeam@sidley.com), mail or hand delivery to: Sidley Austin
LLP, 1501 K Street, N.W., Washington, D.C. 20005. The fax number for lead and
backup lead counsel is (202) 736-8711.

I. Introduction

Petitioner Illumina respectfully requests *Inter Partes* Review (“IPR”) of the ’730 patent and cancellation of claims 1-9 (the “Challenged Claims”). The ’730 patent is directed to methods for reducing bias and errors when sequencing nucleic acids (*e.g.*, DNA) by attaching “tags” that include identifiers (or barcodes) unique to each individual nucleic acid molecule. The Examiner for the ’730 patent recognized that techniques for attaching tags containing various identifiers to nucleic acid molecules during a sequencing process had been known in the art prior to the ’730 patent’s May 2009 priority date.¹ To obtain allowance of the ’730 patent claims, Applicant and the first-named inventor, Dr. Gregory Porreca, argued to the Examiner and Board that “none of the [prior art] references report ***tagging individual nucleic acid molecules each with a unique barcode sequence***,” which is a “distinguishing feature” of the claims.² EX1004, 967, 965 (Porreca decl. ¶13, ¶6); *also id.*, 1071.

Applicant’s and Dr. Porreca’s statements to the PTO during prosecution were wrong. Multiple prior art references taught attaching unique tags to each

¹ As explained below (§IV.D), the Challenged Claims’ earliest possible priority date is May 18, 2009.

² All emphases added unless otherwise noted.

nucleic acid molecule to correct for error or bias during a sequencing workflow. EX1005, ¶85. For instance, Chee, a patent filed in April 2009, teaches tagging individual nucleic acid molecules with unique identifiers and using the identifiers to correct for bias and errors caused by the amplification and sequencing process. EX1010(Chee), 9:18-21, 10:41-46. McCloskey, a patent application published in January 2007, teaches adding a “barcode” that can “uniquely identify a target nucleic acid in an experiment,” EX1012(McCloskey), [0021], which allows it to “correct[] for skewing that can arise from PCR amplification,” *id.*, [0106]. Neither of these references was substantively considered during examination, and Chee was not cited to the Examiner.

This petition is support by the testimony of Dr. Stacey Gabriel, Ph.D. (EX1005), an expert in the DNA sequencing field and the director of the largest DNA sequencing center in the United States, who explains that, in May 2009, there was nothing inventive about using tags to uniquely identify each nucleic acid molecule during a sequencing workflow.

Accordingly, the Board should institute trial and find all Challenged Claims unpatentable.

II. Grounds

Claims 1-9 are unpatentable based on the following prior art and grounds.

Ground	Challenged Claims	Basis	References
1	1-4, 9	§102	Chee
2	1-4, 7, 9	§103	Chee
3	5-6, 8	§103	Chee + Gahan
4	1, 4-6, 9	§103	Bignell + McCloskey
5	2-3	§103	Bignell + McCloskey + Bentley
6	6, 8	§103	Bignell + McCloskey + Gahan
7	7	§103	Bignell + McCloskey + Chiang

III. The '730 Patent and Background Technology

A. DNA

DNA (deoxyribonucleic acid) is a molecule that carries genetic information. EX1005, ¶41. DNA is made up of two complementary strands that wind around each other in a double helix. EX1005, ¶43. Each strand has a backbone to which four building blocks (called bases or nucleotides)—adenine (A), thymine (T), cytosine (C), and guanine (G)—are attached. EX1005, ¶42. The two strands are connected by bonds between complementary pairs of these bases: A bonds with T, and C bonds with G. EX1005, ¶¶43-44. DNA sequencing is the process by which scientists determine the sequence of the bases in a strand of DNA. EX1005, ¶47. By reading the sequence of portions of an organism's DNA, researchers can identify genetic traits or abnormalities that may be associated with disease. *Id.*; see *id.*, ¶¶45-54.

B. DNA Sequencing Technologies

Until the early 2000s, the most common DNA sequencing techniques were based on Sanger sequencing, a method first developed in the 1970s. EX1014, 1135, 1144. Although it generated accurate sequence reads, the traditional Sanger-based approach was labor intensive, could only be partially automated, and was slow and expensive. *Id.*, 1135.

In the mid-2000s, several new techniques for sequencing DNA were developed that enabled parallel, simultaneous preparation and sequencing of DNA (“Next-Generation” or “Next-Gen” sequencing). EX1014, 1136. Unlike Sanger-based sequencing, these techniques could simultaneously sequence hundreds of thousands or even millions of strands of DNA. *Id.*, 1136. Using Next-Gen techniques, multiple DNA samples (*e.g.*, from multiple patients) could be sequenced in a single run, a process called “multiplex sequencing.” EX1005, ¶55. Next-Gen sequencing quickly gained popularity by being both highly efficient and cost-effective, providing more scientists with access to sequencing technology. EX1005, ¶¶55-56; *see* EX1031, 931.

Petitioner Illumina was at the forefront of these developments. For over 25 years, Illumina’s pioneering efforts in genomics and Next-Gen sequencing have made sequencing more affordable and accessible to scientists worldwide. In May 2009, Illumina was one of just several companies providing a Next-Gen platform.

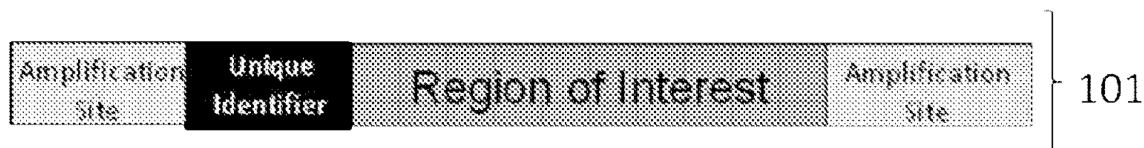
EX1014, 1136; EX1010(Chee), 10:2-7; EX1005, ¶¶56, 65. Illumina's platform used a technique called sequencing-by-synthesis, which allowed it to read the sequence of single-stranded template nucleic acids (*e.g.*, single strands of DNA) by synthesizing a nucleic acid strand complementary to each template. EX1005, ¶¶66-67; EX1029, [0027]-[0029]. Nucleotides are added to the complementary strand one-by-one, and methods are used to detect each nucleotide as it is incorporated (A, C, G, and T), revealing the sequence of the complementary strand, and hence, of the template strand as well. EX1005, ¶¶68-69; EX1014, 1139; EX1029, [0027]-[0028].

Next-Gen techniques generate reads on short DNA segments (*e.g.*, 150 nucleotides or less). EX1005, ¶59. Therefore, to prepare a DNA sample (*e.g.*, DNA collected from one person) for sequencing, longer DNA segments (*e.g.*, thousands of nucleotides in length) were commonly fragmented into shorter pieces. EX1005, ¶¶58-59; EX1029, [0017]. In many cases, to ensure the availability of large enough quantity of DNA for analysis, multiple copies of each fragment were made by amplifying the sample, for example through polymerase chain reaction ("PCR"). EX1005, ¶62; EX1010(Chee), 2:67-3:3. PCR works by denaturing double-stranded DNA into single strands, and building a complementary strand, thereby creating a copy. EX1005, ¶¶63-64. The process can be repeated for multiple cycles. *Id.*

The amplified nucleic acids (often called amplicons) are then sequenced, *e.g.*, on Illumina's Genome Analyzer sequencing machine. EX1014, 1136; EX1013(Bignell), 46:45-52; EX1005, ¶¶65-69. A computer takes the sequencing reads of the fragments and uses algorithms to align those reads, potentially correct errors, and re-assemble the sequence of the longer DNA segments. EX1005, ¶¶70-74.

C. Use of Tags/Barcodes Was Known

By the early 2000s, it was also well-known that “tags” (also called “barcodes” or “identifiers”) could be attached to nucleic acid molecules (*e.g.*, DNA fragments) before sequencing. EX1005, ¶¶79-86. Tags are artificially synthesized DNA molecules (commonly called oligonucleotides or oligos), and they often comprise sequences of nucleotides that encode information about the attached nucleic acid molecule. EX1018, [0028]; EX1012(McCloskey), [0021]; EX1029, [0014]. It was known, for example, that a tag could include a sequence that could identify the sample the molecule was part of, the gene the molecule came from, or the individual molecule in the sample. EX1018, [0001], [0028]-[0029]; EX1012(McCloskey), [0017], [0027]; EX1005, ¶¶81-85. An example of a nucleic acid molecule (labeled “region of interest”) with a unique identifier is shown below.



EX1010(Chee), Fig. 1. The amplification sites are adaptors with sequences to which primers can attach to so that a complementary strand can be synthesized. *Id.*, 13:33-37, 13:40-41; EX1005, ¶80. A primer is a short oligonucleotide to “prime” the DNA strand for the synthesis process. EX1005, ¶64.

One known use of tags was to attach the same identifier to every nucleic acid molecule in a sample to allow identification of the sample it originated from, such as during multiplex sequencing. *E.g.*, EX1005, ¶¶81-84; EX1018, [0029], Figs. 1-2; EX1013(Bignell), 1:24-28, 9:53-58; EX1012(McCloskey), [0005], [0027], Fig. 3; EX1029, [0007], [0014].

By 2005, it was also known that a unique tag could be attached to each original nucleic acid molecule in a sample prior to amplification. EX1005, ¶¶85-86. Such unique tags allowed each molecule, and any copies thereof, to be tracked throughout an analysis process. *Id.* This was taught by multiple references:

- Chee teaches that “‘unique’... identifiers are associated with individual molecules in the starting sample[,] [and] [t]herefore, any amplification products of these initial individual molecules bearing the unique identifier are assumed to be ‘identical by descent.’” EX1010(Chee), 5:31-36.

- McCloskey teaches that adding a “barcode” to a nucleic acid “can uniquely identify a target nucleic acid” and it provides “a unique identity to each target nucleic acid in the sample prior to amplification.” EX1012(McCloskey), [0021].
- Lexow teaches that “individual molecules... can be tracked throughout an analysis procedure by using a molecular tag that is unique to each specific molecule.” EX1018, [0005].

Not only were the many uses of unique tags in DNA sequencing processes well-known, but the process of making tags with unique sequences had been known for over a decade. *See* EX1005, ¶86; EX1019, Abstract; EX1020, 1665-68.

D. Errors and Bias in Typical Sequencing Workflows

It was known that error or bias could be introduced during different stages of the sequencing workflow, from sample preparation to sequencing. EX1010(Chee), 10:21-46; EX1001, 27:45-50, 2:1-9.

For example, sample preparation generally involved fragmenting DNA and amplifying the fragments to increase the number of molecules in the sample. EX1005, ¶¶58-59; EX1010(Chee), 10:50-54; EX1013(Bignell), 7:27-29; EX1001, 5:53-6:37. Amplification processes (*e.g.*, PCR) are random, and it was known they create more copies of some nucleic acid fragments, and fewer copies of others. EX1025, 8966 (noting the presence of “skewing” in studies “due to unequal amplification (PCR bias)”; EX1018, [0002], [0089]; EX1010(Chee), 10:11-14; EX1012(McCloskey), [0005] (“skewing [] can arise from PCR amplification”),

[0003]; EX1001, 27:45-50. Thus, the number of copies of each molecule in an amplified sample can be biased, making it difficult to determine whether a sequence appears multiple times because multiple copies of that sequence are in the genome or because the amplification process made more copies of it. EX1005, ¶77; EX1018, [0002].

Errors can also be introduced during the amplification and sequencing process. EX1005, ¶¶75-76. For example, when synthesizing a strand of DNA complementary to a template strand, from time-to-time an incorrect nucleotide may be added to the copy. *Id.*; EX1010(Chee), 10:54-57. Various known techniques had been developed to address those errors. EX1005, ¶78; EX1014, 1141.

IV. The '730 Patent

A. Overview

The '730 patent's claims are directed to methods for correcting errors or bias during a workflow for analyzing nucleic acids. EX1001, 1:62-67, Example 2. Such a workflow may include preparation of a sample and the sequencing of the nucleic acid molecules in that sample. The '730 patent discloses well-known sample preparation methods in which target nucleic acid molecules are amplified to generate a large enough library for sequencing. *Id.*, 5:53-6:37. The '730 patent also discloses well-known sequencing methods, including the sequencing method developed by Illumina. *Id.*, 72:45-47.

The Challenged Claims are directed to well-known processes for adding “tags” to the target nucleic acid molecules to be sequenced, amplifying the target nucleic acid molecules and tags, sequencing the target nucleic acid molecules and tags, and then using the tags to determine information about the original source of the sequenced target nucleic acid molecules. The ’730 patent includes two independent claims (claims 1 and 9) and 7 dependent claims (claims 2-8). All nine claims are reproduced in the Claim Appendix.

B. Person of Ordinary Skill in the Art

A person of ordinary skill in the art (“POSA” or “skilled person”) would have had (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. This description is approximate; varying combinations of education and practical experience also would be sufficient. The person also commonly worked as part of an interdisciplinary team.

Illumina’s positions regarding the ’730 patent claims and the prior art are supported by the testimony of Dr. Gabriel, an expert in genomics and DNA sequencing with over 25 years of experience.

C. File History

The '730 patent is the third application in a chain of continuations. During prosecution, the Applicant made multiple statements to the Office about the meaning of the claimed “*differentiator tags*” and how to use them to “*correct for bias or errors.*”

The earliest non-provisional application is U.S. Application No. 13/266,862 (the “’862 Application”), filed in March 2012.³ Prosecution of that application has focused on claims directed to a molecular inversion probe (MIP) for isolating a target region from a larger nucleic acid, which are not relevant to the ’730 claims. *See, e.g.*, EX1002, 122-28, 643-49, 1042-49, 1654-55, 1704-05, 1922-23, 2041-42. The ’862 Application is still pending today.⁴

In August 2016, Applicant filed U.S. Application No. 15/231,687 (the “’687 Application”) as a continuation, and included claims directed to using unique differentiator tags when sequencing nucleic acid molecules. EX1003, 33-34. The Examiner rejected the claims on several bases. He found the term “*unique differentiator tag*” indefinite because it was unclear what the tag had to be unique

³ The ’862 Application is the U.S. stage of a PCT application filed April 30, 2010.

⁴ In June 2024, Applicant submitted amendments requiring each MIP to include “*a unique differentiator tag sequence.*” EX1002, 2152.

relative to. *Id.*, 428-30; *also id.*, 516-18, 1124-26. He also found that the claims were unpatentable based on prior art directed to various techniques for tagging nucleic acids (*e.g.*, using sample identifiers or gene-specific identifiers). *Id.*, 430-45; *also id.*, 518-37, 859-77, 985-1005, 1127-53, 1219-48. Those references included Pourmand, Ronaghi, Shoemaker, and others.

There were several rounds of amendments and rejections that focused on the meaning of “*differentiator tags*.” Applicant argued that the claims “require *a unique barcode sequence per each individual target nucleic acid molecule*” while the prior art disclosed applying the same differentiator tag to multiple target molecules. *Id.*, 1177; *see, e.g., id.*, 1177-78 (Applicant arguing art taught associating “the same barcode” with multiple target molecules); *id.*, 1165-68 (first Porreca declaration).

In October 2020, the Applicant amended the pending claims to the language that ultimately issued in the '730 patent (i) to recite “*introducing a set of differentiator tags, wherein members of said set of differentiator tags are associated with members of said plurality [of target nucleic acid molecules from more than one locus of origin], such that one or more of said loci of origin are associated with more than one differentiator tag;*” and (ii) to further require the method to include the step of “*correcting for errors or bias. . . by collapsing target: differentiator tag combinations observed more than once into a single*

count.” EX1003, 1258. The Examiner rejected those claims finding they were directed to a different invention than the originally-filed claims. *Id.*, 1274.

Applicant filed a continuation (discussed below) and then abandoned the ’687 Application.

On November 19, 2020, Applicant filed U.S. Application No. 16/952,764 (the “’764 Application”) with the same claims the Examiner had rejected as improper.⁵ EX1004, 356-58. On February 10, 2021, the Examiner again rejected the claims, finding there was no written description teaching how to “*collapse*” reads as part of the “*correcting*” step. *Id.*, 861-62. The Examiner also found the claims obvious again in view of different references where the same identifier would be applied to every copy of the same gene in a sample, including Porreca(2007) and Wang(2007). *Id.*, 866-72.

On March 4, 2021, Applicant submitted a second declaration from Dr. Porreca. EX1004, 964-67. Dr. Porreca argued that none of the prior art disclosed that “*each individual nucleic acid molecule is tagged with a unique differentiator tag,*” and that such tags were a “*distinguishing feature of [his] claims.*” *Id.*, 965. The Examiner was not persuaded by Applicant’s arguments and issued a final rejection. *Id.*, 982-1003.

⁵ These claims issued as claims 1-9 in the ’730 patent.

The Applicant appealed to the Board. In its appeal brief, Applicant again distinguished its claims from the prior art by arguing that none of the art disclosed adding unique tags to each individual nucleic acid molecule prior to amplification. EX1004, 1071 (citing Porreca Decl. ¶¶6-12 (EX1004, 965-66)). In its reply brief, the Applicant made clear that the pending claims “*require a different tag on every single molecule*” and that they do not cover techniques where the same tag is attached to more than one nucleic acid. EX1004, 1146.

On September 6, 2023, the Board reversed the Examiner’s rejection, finding the specification provided sufficient written description support for the “*correcting*” step. EX1004, 1180-81; *also id.*, 1175-82. The Board also concluded the prior art of record did not render the claims obvious, finding the art did not teach “*collapsing*” the same target:differentiator tag combinations into a single count. *Id.*, 1191; *also id.*, 1189-92. After remand, the Examiner allowed the claims “in view of the PTAB decision.” EX1004, 1195.

D. The ’730 Patent Is Not Entitled to its Earliest Claimed Priority Date

The ’730 patent claims priority back to four provisional applications: one filed April 30, 2009 and three filed in May 2009 (May 15, 18, and 28). *See* EX1006, EX1007, EX1008, EX1009.

The two earliest provisional applications do not provide written description support for the claims of the ’730 patent. EX1005, ¶39. Every ’730 claim recites

“*differentiator tags*” and “*correcting for error or bias*.” Provisional Application No. 61/174,470 (the “’470 provisional”) filed on April 30, 2009, however, does not disclose differentiator tags, unique identifiers, or correcting for errors or bias. *Id.* Provisional Application No. 61/178,923 (the “’923 provisional”) filed on May 15, 2009, also does not disclose differentiator tags or unique identifiers. *Id.* Nor does it disclose correcting for error or bias by “*collapsing target:differentiator tag combinations... into a single count*,” as required by claims 1-8, or “*recognizing target/differentiator tags having the same sequence as being derived from the same input molecule*,” as required by claim 9. *Id.* Therefore, the ’730 patent is not entitled to claim priority to either the ’470 provisional or ’923 provisional.

Accordingly, the earliest date to which the ’730 patent could potentially claim priority is May 18, 2009, the date the ’358 provisional was filed.

V. Claim Construction

To construe the claims in a patent, the words are given their plain and ordinary meaning as they would have been understood by a person of ordinary skill in the art at the time of the alleged invention after reading the entire patent.

Phillips v. AWH Corp., 415 F.3d 1303, 1312-13 (Fed. Cir. 2005); 37 C.F.R.

§ 42.100. A term needs to be construed only if its meaning is disputed, and it needs to be construed only to the extent necessary to resolve the dispute. *Vivid Techs., Inc. v. Am. Sci. & Eng’g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999).

The related litigation is in its infancy, and the parties have neither identified claim terms to be construed nor proposed any constructions. Below, Petitioner proposes constructions for three groups of claim terms. If Patent Owner alleges any additional terms need to be construed, Petitioner reserves the right to respond.

A. “Set of [Unique] Differentiator Tags”

Claim 1 recites “*set of differentiator tags*” and claim 9 recites “*set of unique differentiator tags*.” Both terms mean a set of prepared tags where each tag includes a unique sequence as compared to the other tags. EX1005, ¶89. The specification defines the sequence of each differentiator tag as inherently unique. EX1001, 28:25-29 (“unique sequence tags (referred to as differentiator tag sequences)”), 28:53-57 (“a ‘*differentiator* tag sequence’ is a sequence of a nucleic acid..., which in the context of a plurality of different isolated nucleic acids, identifies a *unique*, independently isolated nucleic acid”). These unique differentiator tags are associated with target nucleic acids so that each nucleic acid is uniquely identifiable. EX1005, ¶90.

During prosecution, the Applicant distinguished the claims from cited prior art that disclosed use of tags to identify samples or to identify genes. EX1003, 1176-78, *also id.*, 1262-66, 1258; EX1005, ¶¶91-94.

For example, first-named inventor Dr. Porreca submitted a declaration in which he stated: “A distinguishing feature of the claims in my patent application is

that *each* individual nucleic acid molecule is tagged with a *unique* differentiator tag prior to any amplification or sequencing steps.” EX1004, 965. He also stated that “[e]ach differentiator tag has its own unique sequence so that each individual target nucleic acid molecule is tagged, and therefore associated, with a differentiator tag.” *Id.*, 964.

On appeal to the Board, Applicant made it explicit that each differentiator tag was unique and that repeats were not permitted:

The *Examiner’s Answer... states that the claims do not require a different tag on every single molecule* and therefore Appellant’s brief and declaration of Dr. Porreca are not arguing limitations of the claim. *The Examiner is simply wrong* and is not reading either the claim or the Porreca declaration correctly.”

EX1004, 1146. The inventor’s and Applicant’s statements about “*differentiator tags*” are consistent with the ’730 patent’s definition of “differentiator tag sequence” and inform “the meaning of the claim language.” *Phillips*, 415 F.3d at 1317. An interpretation of “*differentiator tags*” to include non-unique tags is not possible, and if it were, Applicant disclaimed any interpretation that would allow non-unique tags.

B. “Locus of Origin” Terms

Claims 1 and 9 use the terms “*locus of origin*.” That term means the location in a genome where a nucleic acid came from. EX1005, ¶96. In the field

of genetics, a “locus” refers to a fixed location on a chromosome where a gene can be located. *Id.* This is consistent with the ’730 patent specification, which describes a genetic loci as a location on the genome that can correspond to heritable disorders. EX1001, 3:2-20, 3:43-47, 66:3-11; *id.*, 31:50-42:12 (Table 1, listing exemplary genetic loci); EX1005, ¶¶96-98.

Claim 1 specifies that “*one or more of said loci of origin are associated with more than one differentiator tag.*” This means that, while the sample comprises a plurality of target nucleic acid molecules from more than one locus of origin, the sample includes multiple nucleic acids from at least one locus, and each of those nucleic acids is associated with a different unique tag. EX1005, ¶99.

The Applicant added the locus of origin terms in response to the Examiner’s finding that “the claims encompass the same unique tag on every sequence on a sample.” EX1003, 1263; *see id.*, 1258 (amendments). The Applicant’s amendment specified that the target nucleic acids come from more than one locus of origin, and that “one or more of said loci of origin are associated with more than one differentiator tag.” *Id.*, 1258. The limitation was added “to make clear that target nucleic acids (loci) may be associated with more than one tag.” *Id.*, 1263. Applicant explained the loci of origin language clarified that each molecule in the starting sample was associated with a different unique differentiator tag, even if

multiple molecules were from the same genetic locus of origin. *Id.*; EX1005, ¶¶100-01.

Claim 9 specifies that “*unique differentiator tags are randomly associated with members of said plurality of nucleic acids, such that any given tag is uniquely associated with one locus of origin.*” This means that each differentiator tag is associated with just one target nucleic acid from one locus of origin. EX1005, ¶102.

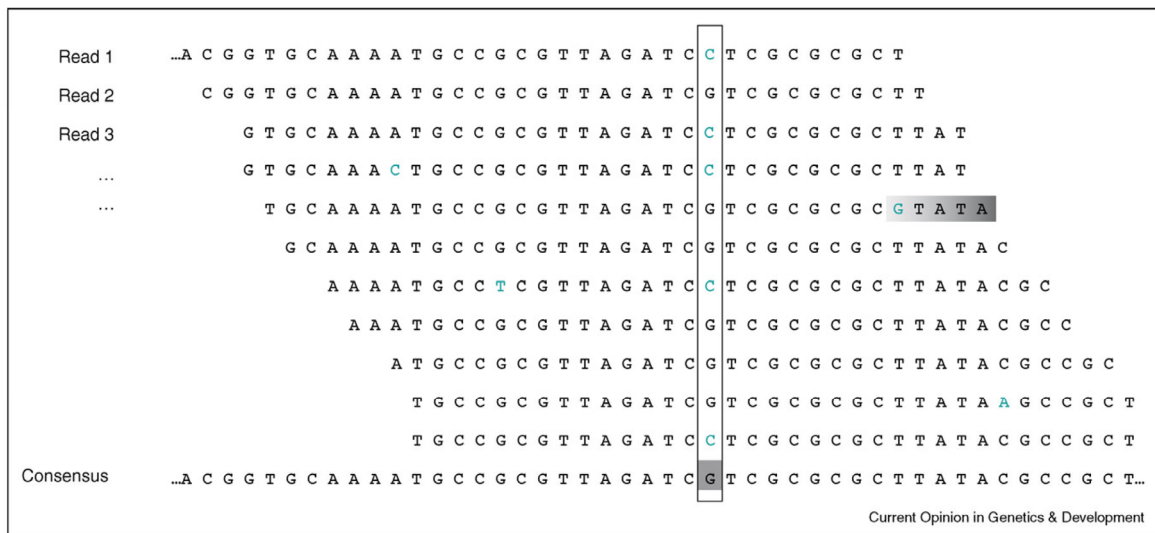
C. “Consensus Base Call”

Claim 2 recites “*determining a consensus base call for the target nucleic acid molecule sequences.*” The ’730 patent does not define or describe what a “*consensus base call*” is. Thus, a POSA would have given the term its ordinary meaning in the art, which is using multiple sequencing reads of the same molecule to determine the nucleotide (base) at each position of a sequence. EX1005, ¶¶103-06.

DNA sequencing commonly makes multiple sequence reads of the same DNA segment (*e.g.*, due to amplification or because the original sample included multiple copies). EX1005, ¶105. Those multiple reads are aligned to identify nucleotides (or bases) that are different than others at that position. *Id.* Scientists resolve those inconsistencies to generate a single “consensus” base call which comprises the nucleotide that appears most frequently at each position. *Id.*;

EX1017(Bentley), 549 (“multiple reads from the same sample can be aligned and used to derive a consensus base-call at each position”); EX1014, 1142. For example, a consensus sequence can be seen at the bottom of the figure below, with an example of a consensus base call (“G”) in the vertical rectangle.

Figure 2



EX1017(Bentley), 550; *also* EX1005, ¶¶105-06.

A POSA would have understood the term “*consensus base call*” to have its ordinary meaning in the field. EX1005, ¶103.

VI. Detailed Explanation Why the '730 Patent Claims Are Unpatentable

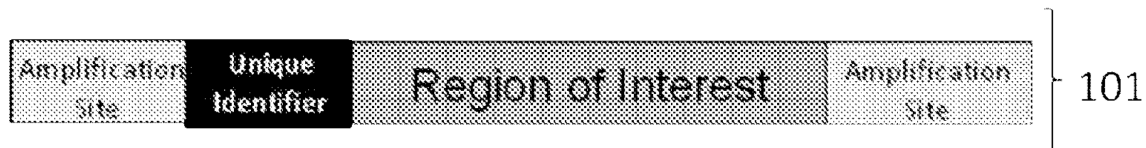
A. Ground 1: Chee Anticipates Claims 1-4 and 9

1. Overview of Chee

Chee teaches techniques for detecting and correcting errors or bias when sequencing nucleic acid molecules. EX1010(Chee), 1:44-58, 2:14-20. Chee’s techniques can be used to analyze both biological and synthetic nucleic acid

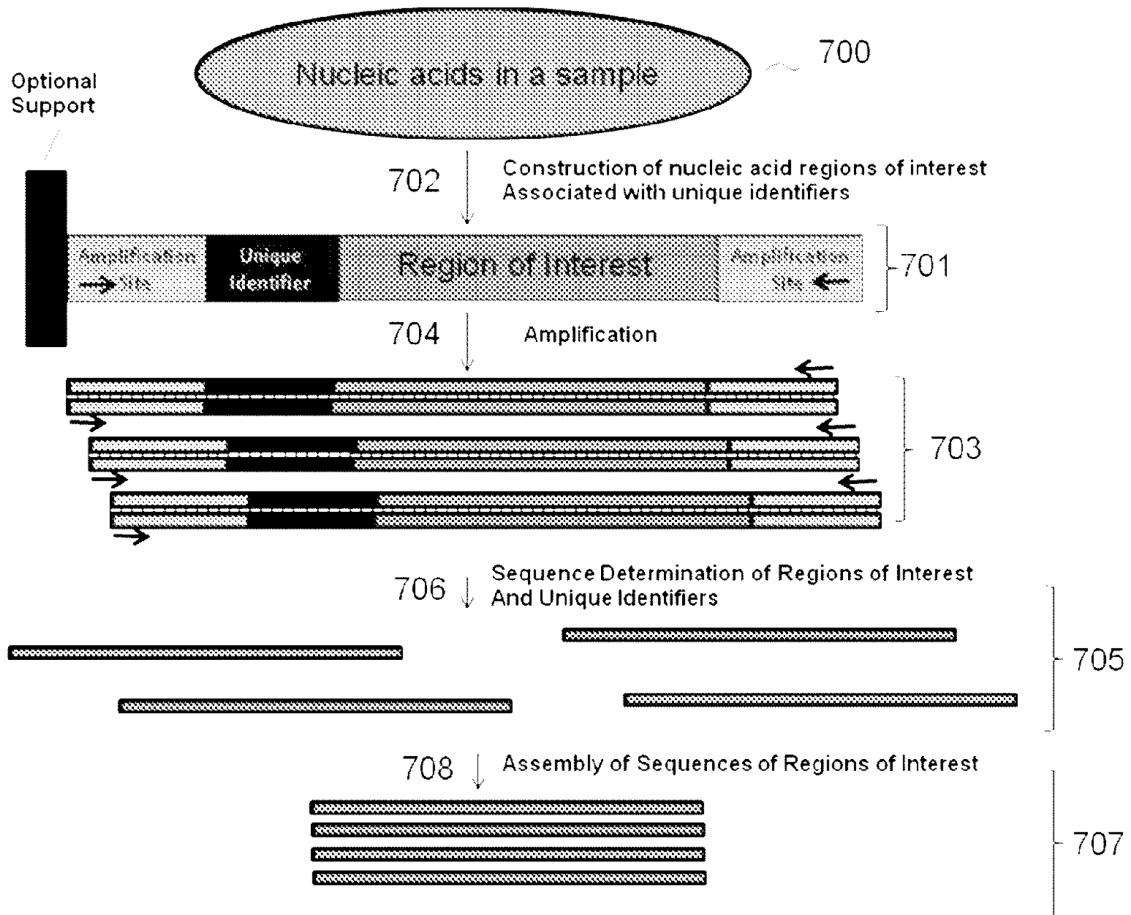
samples. *Id.*, 5:3-5, 10:21-28, 12:17-22, 8:6-11, 1:11-12. For example, Chee’s techniques can be used when sequencing naturally-occurring DNA. *Id.*, 11:51-54.

Chee observes that preparing a sample for sequencing could introduce errors or bias into the sample (*e.g.*, during amplification). EX1010(Chee), 1:43-47, 10:21-28. As a solution, Chee teaches creating a “construct” that includes a unique identifier, an amplification site, and a nucleic acid region of interest (“ROI”). *Id.*, 10:41-46, 2:34-38, 3:11-16, 4:1-3, 5:27-36. An exemplary construct is shown below. *Id.*, Fig. 1.



The ROIs “can be isolated from a natural... source” and can be “fragments of a larger nucleic acid.” EX1010(Chee), 3:51-55, 4:1-3. The constructs are created by adding the unique identifiers and amplification sites to the nucleic acids (ROIs) in a sample prior to amplification. *Id.*, 5:27-36, 21:61-64, Fig. 7.

The constructs allow each nucleic acid in a sample to be uniquely identified such that, following amplification, copies originating from the same nucleic acid can be identified. EX1010(Chee), 10:41-46, 5:27-36; EX1005, ¶¶117-19, 155, 167-68. An example of Chee’s process is shown in Figure 7 (below).



Chee discloses methods for determining the sequence of nucleotides that can be used for diagnostic or clinical use. EX1010(Chee), 9:42-52 (“genomic sequencing” for identifying “mutations” or a “sequence variant”), 11:60-63 (sequencing for “discovery research, diagnostic use, clinical use or other development techniques”); EX1005, ¶¶140-43. Chee is directed to the same field of endeavor as the ’730 patent.

Chee states its methods may include “conventional techniques” of, *e.g.*, molecular biology and sequencing technology. EX1010(Chee), 8:6-11. Chee states that such conventional techniques are described “in standard laboratory

manuals such as... Weiner, Gabriel, Stephens, Eds. (2007), *Genetic Variation: A Laboratory Manual*,” which is “incorporated in [its] entirety by reference.”

EX1010(Chee), 8:18-37; *see* EX1037. The *Genetic Variation Manual* (EX1016)⁶ describes, *inter alia*, techniques for DNA extraction (*e.g.*, from blood) and sample preparation, and different analyses that can be done on the sequenced DNA (*e.g.*, determining copy number variation). EX1016, 103-06, 120-28, 221-53; EX1005, ¶120.

2. Chee Is Entitled to Its Provisional Date and Is Prior Art

Chee issued from U.S. Appl. No. 13/266,568, filed on April 29, 2010. It claims priority to Provisional Application No. 61/174,442 (“’442 provisional”), filed on April 30, 2009. Chee is entitled to its provisional date, and thus, is prior art to the ’730 patent.⁷

As shown in the chart below, at least one claim in the issued Chee patent has written description support in the provisional application. *See Dynamic*

⁶ By November 18, 2007, the *Genetic Variation Manual* was available for purchase on Amazon.com. EX1021, 1. The *Genetic Variation Manual* (EX1016) is prior art under § 102(a).

⁷ As explained in §IV.D, above, the ’730 patent’s earliest possible priority date is May 18, 2009.

Drinkware, LLC v. Nat'l Graphics, Inc., 800 F.3d 1375, 1381 (Fed. Cir. 2015);

also EX1005, §VII.A.2.

Chee Claim Elements	Provisional Disclosure
1. A method for determining the sequences of a set of nucleic acid targets, comprising:	[00080] (“complete constructs are sequenced to confirm both the ROI and the unique identifier”); <i>see</i> [00048], [00061], [00081]
tagging each individual nucleic acid target in a set of nucleic acid targets to create a set of tagged constructs, with: (a) at least one unique identifier oligonucleotide that distinguishes the tagged construct from other tagged constructs in the set, wherein the at least one unique identifier oligonucleotide is not part of the nucleic acid target; and (b) at least one amplification site;	[00069] (“the nucleic acids containing the ROI are isolated, and... the constructs are separately created and added to the sample”), [0009] (“the constructs of the set comprise an ROI, a unique identifier, an amplification site, and an excision site”), [0008] (“the unique identifier is associated with a construct, not the ROI”), [00017] (“oligonucleotide constructs”), [00027] (“the different constructs have different unique identifiers”). <i>See</i> [00012], [00022], [00024], [00027], [00055]-[00057], [00060], [00070], [00076].
amplifying the set of tagged constructs; and	[00012] (“constructs allow the unique identifier and the ROI to be selectively amplified”); <i>see also</i> [00021]-[00022], [00059]-[00060], [00076]-[00079]
determining the sequences of the amplified set of tagged constructs comprising the unique identifier oligonucleotides and the nucleic acid targets.	[00080] (“complete constructs are sequenced to confirm both the ROI and the unique identifier”); <i>see also</i> [00048], [00059], [00061], [00081]
2. The method of claim 1, wherein the nucleic acid targets are isolated from a biological sample.	[00069]

In addition, the subject matter in Chee that Petitioner relies on to show the Challenged Claims are obvious was included in the provisional application (see citations in the claim mapping sections below).

3. Chee Discloses Claims 1-4 and 9

a) Claim 1

(1) A method for correcting for errors or bias introduced during nucleic acid analysis workflow

To the extent the preamble is limiting, Chee discloses it.

Chee teaches that there can be multiple sources of errors or bias in the process of sequencing biological samples (“*nucleic acid analysis workflow*”).

EX1010(Chee), 1:43-47, 10:11-14 (discussing “bias in amplification”), 10:21-28.

For example, errors or bias can be introduced when amplifying a sample, *id.*, 9:18-21, 10:11-14, 10:25-26, and by the sequencing process itself, *id.*, 10:54-59

(“sequencing error”), 10:21-28. EX1005, ¶¶132-33.

Chee teaches that its techniques can be used to “*correct[] for... bias.*”

EX1010(Chee), 9:29-39, 10:42-46, 23:53-64 (“quantification... can be **corrected** to reflect this manner of **bias**”). Chee also teaches that its techniques can be used to

“*correct[] for errors.*” *Id.*, 10:54-59 (“sequencing error... can be overcome”),

14:39-50; EX1005, ¶¶134-35.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00025], [00060], [00056], [000131].

(2) obtaining a biological sample comprising a plurality of target nucleic acid molecules from more than one locus of origin;

Chee discloses “*obtaining a biological sample comprising a plurality of target nucleic acid[s].*” EX1005, ¶¶137-38. Chee’s technique can be used to analyze “nucleic acids derived from a biological sample,” EX1010(Chee), 12:17-22, such as “naturally-occurring nucleic acids (e.g., DNA or RNA or fragments thereof...)” for clinical or diagnostic use, *id.*, 11:51-54.

Chee’s target nucleic acids come from “*more than one locus of origin.*” See §V.B, above; *also* EX1005, ¶¶139-43. A sample can include nucleic acids from “different regions” of a large nucleic acid, EX1010(Chee), 5:24-26, and the starting sample can be “quite large, e.g., a genome or a complex mixture of nucleic acids,” *id.*, 11:56-60. Chee’s technique can be used for “genomic sequencing” and identifying “polymorphisms[,] mutations,” or “a rare sequence variant.” EX1010(Chee), 9:42-52. Chee describes sequencing for “discovery research, diagnostic use, clinical use or other development techniques.” *Id.*, 11:60-63. A genome includes multiple loci of origin, and a POSA would have understood that diagnostic or clinical use, *e.g.*, detecting polymorphisms, mutations, and sequence variants, would have involved obtaining and analyzing nucleic acids from multiple regions of the genome. EX1005, ¶¶142-43; *see, e.g.*, EX1032, 654.

Chee's '442 provisional discloses this subject matter. *E.g.*, EX1011, [00029], [00060], [00066], [00069].

(3) 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;

Each construct comprises an ROI, a unique identifier, and one or more amplification sites. EX1010(Chee), 2:34-38, 2:65-3:9. Chee's constructs are created by adding unique identifiers ("*introducing a set of differentiator tags*") to a sample and attaching them to the individual ROI nucleic acids ("*members of said plurality*"). *Id.*, 4:1-3 ("the unique identifier[s]... are added to the individual fragments"), 3:61-63, 12:17-22; EX1005, ¶¶144-46.

Chee's '442 provisional discloses this subject matter. *E.g.*, EX1011, [00007]-[00009], [00011], [00017], [00068].

Chee discloses that "*one or more of said loci of origin are associated with more than one differentiator tag.*" As explained in § V.A, above, this limitation requires a unique tag per nucleic acid molecule. *See* EX1003, 1263.

Each unique identifier is associated with one individual construct (and thus one ROI), such that each construct is "uniquely identifiable." EX1010(Chee), 2:10, 2:21-28; EX1005, ¶¶153-54. As Chee explains, "the 'unique' (i.e. unique for

a construct comprising a particular ROI) identifiers are associated with individual molecules in the starting sample.” EX1010(Chee), 5:31-33; *see id.*, 9:13-18.

Chee shows that an initial sample may include multiple nucleic acids from the same region on the genome (a “*locus of origin*”). EX1005, ¶155. This would result in creating “two constructs with substantially the same ROI and different unique identifiers,” EX1010(Chee), 2:34-38, or even five or ten such constructs, *id.*, 2:38-43. Because each nucleic acid in the initial sample is associated with its own unique identifier, each sequence read can be traced back to a nucleic acid in the sample. *Id.*, 10:41-46, 24:1-4; EX1005, ¶155. As Chee explains:

It is a feature of the invention that the ***different constructs have unique identifiers***, including constructs that may have the same ROI, so the specific, correct ROIs can be ***distinctly identified*** and distinguished from the other constructs in the set.... Therefore, any amplification products of these initial individual molecules bearing the unique identifier are assumed to be ‘identical by descent’.

EX1010(Chee), 5:27-36.

Thus, Chee discloses that multiple nucleic acids in a sample can correspond to the same region of the genome (“*one or more of said loci of origin*”) and each such nucleic acid is associated with a different unique identifier (“*more than one differentiator tag*”).

Chee's '442 provisional discloses this subject matter. *E.g.*, EX1011, [00007]-[00009], [00011], [00027], [00029], [00055]-[00056], [00060]-[00061].

As explained in § V.A, above, a POSA would have understood that each “*differentiator tag*” has its own unique sequence.

Chee discloses several methods for generating the unique identifiers. In one, the sequence of each identifier can be “pre-defined.” EX1010(Chee), 11:18-20. Where the identifiers are predefined, each one is guaranteed to be unique. EX1005, ¶148. In another method, the sequence of each identifier is generated by randomly adding bases. EX1010(Chee), 11:14-18. For the random method, Chee generates “a large excess of possible identifiers relative to the ROIs in a given set,” which makes the chances of having one unique identifier associated with more than one nucleic acid “extremely low.” *Id.*, 9:54-56; *see id.*, 11:36-38; EX1005, ¶149.

Chee states that “the term ‘unique’ as used herein does not *necessarily* mean absolutely unique but rather having an extremely high probability of being unique.” EX1010(Chee), 9:60-63. Thus, although Chee does not *require* absolute uniqueness, Chee discloses that identifiers *can be* absolutely unique. For example, pre-defined identifiers are guaranteed to be absolutely unique. EX1005, ¶150. Where the identifiers have a high probability of being absolutely unique, Chee recognizes that scientists implementing its technique can choose the appropriate

number of identifiers, explaining that in practice, the “numbers of constructs in sets of the invention will depend on the current technology and the ultimate purpose of the set analysis.” EX1010(Chee), 2:62-64; EX1005, ¶¶150-51. Thus, a POSA would have understood Chee to disclose a method that uses identifiers that are absolutely unique. EX1005, ¶150.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00007]-[00009], [00011], [00055], [00057], [00064], [00066], [00074].

(4) amplifying each of the plurality of tagged target nucleic acid molecules to generate amplicons;

Chee discloses amplifying the constructs containing unique identifiers and nucleic acid ROIs. EX1010(Chee), 2:65-3:3 (“constructs allow the unique identifier and the ROI to be selectively amplified,” *e.g.*, by “PCR”), 3:10-17 (describing “amplification of the ROI and the unique identifier”), 13:20-61; EX1005, ¶¶161-63.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00012]-[00013], [00076]-[00078].

(5) 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

Chee discloses sequencing the amplified constructs (“*amplicons*”). EX1010(Chee), 14:42-63, 21:67-22:1. Chee sequences both the unique identifier

(“*differentiator tag*”) and ROI (“*target nucleic acid molecule*”). *Id.*, 14:3-10 (“complete constructs are sequenced to confirm both the ROI and the unique identifier”), 9:25-39 (discussing analyzing the sequence of the unique identifiers and ROIs), 14:42-50 (same), 2:53-64; EX1005, ¶¶165-68.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00055], [00080], [00083].

(6) correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

Chee corrects for both errors and bias. EX1010(Chee), 10:41-57, 23:51-59. First, Chee discloses that “technical bias” can be introduced when amplifying the original sample, which can “skew” the final sample to have more copies of a particular construct as compared to others. EX1010(Chee), 9:18-21; *see id.*, 10:36-46. By using the unique identifiers, “the quantification of the molecules can be *corrected* to reflect this manner of *bias*.” *Id.*, 23:59-64; *id.*, 10:41-46; EX1005, ¶¶172-74.

Chee corrects bias by “*collapsing target:differentiator tag combinations... into a single count*.” EX1005, ¶¶173-75. The unique identifiers allow Chee to determine that two sequenced constructs are “identical by descent,” *i.e.*, copies of the same construct from the original sample. EX1010(Chee), 5:27-36, 14:42-50. Chee’s unique identifiers allow it to accurately determine “the number of starting

molecules... by association of each initial ROI present in a sample with its unique identifier.” *Id.*, 10:36-46; *see id.*, 9:5-9, 24:1-8. Thus, Chee corrects for amplification bias by counting each unique construct once. *Id.*, 23:59-64. This is the same process for the “*collapsing*” step that is described in the ’730 patent: counting the unique tags associated with each sequence to determine the number of times a sequence appeared in the original sample. EX1001, 30:48-51, 72:63-73:5; EX1005, ¶¶173-75.

Second, Chee corrects for “sequencing errors” by sequencing multiple copies of the same original molecule (those with the same unique identifier) and comparing the ROI reads. EX1010(Chee), 9:34-39, 10:54-57, 14:39-50. Multiple reads of the same molecule are used to generate a single consensus read:

[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(Chee), 14:46-50; *see* EX1005, ¶¶176-78; EX1017(Bentley), 550. Thus, Chee corrects for error by “*collapsing target:differentiator tag combinations observed more than once into a single count.*” EX1005, ¶170.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00027], [00055], [00058]-[00060], [00083].

b) Claim 2

Claim 2 depends from claim 1 and specifies “*said correcting step 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.*” As explained in § V.C, a “*consensus base call*” refers to using multiple reads of the same molecule to determine the nucleotide at each position of a sequence.

As explained for the “*correcting*” step of claim 1, Chee generates a single “*consensus sequence* derived from [those] multiple clonal copies of [a] construct, which are identified as identical... via the shared unique identifier.”

EX1010(Chee), 14:46-50; *see id.*, 10:54-57. Thus, Chee discloses “*collapsing*” all of the ROI reads of multiple copies of a construct into a single read or “*consensus sequence.*” A POSA would have understood that to generate a consensus sequence, Chee determines a consensus base call at each position. EX1005, ¶¶184-85.

Chee’s ’442 provisional also discloses this subject matter. *E.g.*, EX1011, [00061], [00083].

c) Claim 3

Claim 3 depends from claim 2 and specifies the method “*further comprising the step of determining the presence of a sequencing or amplification error based upon a number of said differentiator tag sequences.*”

As explained for claim 2, Chee determines the presence of a “sequencing error” by comparing reads from “multiple clonal copies of [a] construct,” which are identified as copies “via the shared unique identifier.” EX1010(Chee), 14:46-50; *id.*, 9:29-34 (“sequence differences between molecules with the same ROI can be identified as technical errors”), 14:57-59. Thus, Chee identifies the presence of a sequencing error based upon multiple constructs with the same identifier (“*a number of said differentiator tag sequences*”). EX1005, ¶188.

Chee also can determine the presence of “technical errors” arising from “limitations of the amplification” technology. EX1010(Chee), 9:25-34; *see id.*, 10:21-28, 10:36-46. Chee discloses comparing amplification rates across molecules with different unique identifiers (“*a number of said differentiator tag sequences*”) to identify amplification errors. EX1010(Chee), 23:59-24:16, 10:42-46; EX1005, ¶¶189-90.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00058], [00060], [00083].

d) Claim 4

Claim 4 depends from claim 1 and specifies “*said biological sample is selected from the group consisting of blood and biopsy tissue.*”

Chee states that the practice of its methods may employ “conventional techniques” such as those described in “standard laboratory manuals” such as the *Genetic Variation Manual* (EX1016), which Chee incorporates by reference. EX1010(Chee), 8:6-37. The *Genetic Variation Manual* discloses techniques for extracting DNA from human tissue and blood. EX1016, 103-04, 110-13 (tissue), 120-28 (blood). A POSA would have understood Chee to disclose that these techniques can be used to acquire “nucleic acids in biological samples.” EX1010(Chee), 10:24; EX1005, ¶¶192-94. Thus, Chee discloses the additional elements of claim 4.⁸

The ’442 provisional incorporates by reference the *Genetic Variation Manual*. See EX1011, [00050].

e) Claim 9

Most of the steps of claim 9 are identical to steps of claim 1, which Chee discloses for the same reasons it does for claim 1. For the “*introducing...*” and

⁸ To the extent not anticipated, this element is obvious as explained in §VI.B.3, below.

“*correcting...*” steps, claim 9 uses slightly different words than claim 1, and Chee teaches these steps for the reasons provided below.

(1) introducing a set of unique differentiator tags, wherein members of said set of unique differentiator tags are randomly associated with members of said plurality of target nucleic acid molecules, such that any given tag is uniquely associated with said locus of origin;

This step is similar to claim 1’s “*introducing*” step, except it specifies “*unique*” differentiator tags that are “*randomly associated*” with targets such that any tag is “*uniquely associated*” with a locus of origin. As explained above in §V.A, Applicant made clear in prosecution that “*differentiator tags*” must be unique. Therefore, claim 9’s recitation of “*unique*” does not change the claim’s scope from claim 1. Chee’s unique identifiers are “*unique differentiator tags*” for the same reasons they are “*differentiator tags*.”

Chee discloses that its unique identifiers are “*randomly associated*” with nucleic acids. EX1010(Chee), 4:1-3, 11:12-18. The unique identifiers and amplification sites are separately synthesized and then added to a sample of nucleic acids. *Id.*, 4:1-3, 12:17-22, Fig. 7 (702). A POSA would have understood that each unique identifier would be randomly associated with a nucleic acid in the sample. EX1005, ¶158. One reason is that Chee’s identifiers do not have a region that is complementary to any the ROIs, and therefore the tags are not destined for any particular ROI. *Id.*

Chee discloses that each unique identifier is associated with one nucleic acid ROI in the original sample. EX1010(Chee), 5:27-36, Fig. 1. Thus, the unique identifier is uniquely associated with a “*locus of origin.*” EX1005, ¶159.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00017], [00027].

(2) correcting for error or bias introduced during said workflow by recognizing target/differentiator tags having the same sequence as being derived from the same input molecule.

Chee discloses “*correcting for error or bias*” for the same reasons as claim 1. As explained for claim 1, Chee creates a consensus sequence for each construct, and the “consensus sequence [is] derived from multiple clonal copies of the construct, which are identified as identical by descent via the shared unique identifier.” EX1010(Chee), 14:46-50; *see id.*, 5:27-36 (“any amplification products of these initial individual molecules bearing the unique identifier are assumed to be ‘identical by descent’”), 9:29-34. Thus Chee discloses “*recognizing*” constructs with the same unique identifier and ROI “*as being derived from the same input molecule.*” EX1005, ¶¶180-82.

Chee’s ’442 provisional discloses this subject matter. *E.g.*, EX1011, [00027], [00059]-[00061], [00083].

B. Ground 2: Chee Renders Claims 1-4, 7, and 9 Obvious**1. Claims 1 and 9**

Chee discloses every element of claims 1 and 9. Patent Owner may argue that Chee does not disclose the claimed “*differentiator tags*” because Chee states that its unique identifiers “need not be absolutely unique.” EX1010(Chee), 11:22-25; *also id.*, 9:60-63. Even if that distinction were accepted, configuring Chee’s technique to use identifiers that were absolutely unique would have been obvious in view of Chee’s own teachings. *See Arendi S.A.R.L. v. Apple Inc.*, 832 F.3d 1355, 1361 (Fed. Cir. 2016) (“a patent can be obvious in light of a single prior art reference”).

Chee’s statement that its identifiers “need not be absolutely unique” at the very least suggests that its method can be implemented with identifiers that are absolutely unique. EX1005, ¶¶196-97. Chee states that it is within the skill of a POSA to select appropriate parameters to be used to achieve the purpose of the intended analysis. EX1010(Chee), 2:62-64, 8:17-18, 8:67-9:3, 13:5-13, 19:51-56. A POSA would have been motivated to use identifiers that are absolutely unique to ensure that no two nucleic acid ROIs in a starting sample were associated with the same identifier. EX1005, ¶198. Chee groups constructs by unique identifier to correct for bias and to determine a consensus sequence because Chee assumes that constructs with the same unique identifier are copies of the same molecule in the

starting sample. EX1010(Chee), 10:41-57, 14:42-52. Configuring Chee to use identifiers that are absolutely unique would avoid any errors when grouping constructs. EX1005, ¶198. A POSA would have found configuring Chee to use absolutely unique identifiers obvious, or at least obvious to try. EX1005, ¶¶198-201.

2. Claims 2-3

Chee renders claims 2 and 3 obvious for the same reasons it anticipates them, explained for Ground 1 above.

3. Claim 4

Claim 4 depends from claim 1 and specifies “*said biological sample is selected from the group consisting of blood and biopsy tissue.*”

Chee’s methods may employ “conventional techniques” such as those described in “standard laboratory manuals” such as the *Genetic Variation Manual*, which Chee incorporates by reference. EX1010(Chee), 8:6-37. The *Genetic Variation Manual* describes, *inter alia*, conventional techniques for extracting DNA from human tissue and blood. EX1016, 103-04, 110-13 (tissue), 120-28 (blood). Prior to May 2009, it was routine to extract DNA from patient blood samples, and doing so was well within the skill of a POSA. EX1005, ¶207. Thus,

a POSA would have at least found it obvious to implement Chee's method using DNA isolated from a patient's blood or tissue. EX1005, ¶¶206-07.⁹

4. Claim 7

Claim 7 depends from claim 1 and specifies “*the step of determining the copy number of a genomic region or transcript in a patient from whom the sample is obtained.*”

The '730 patent does not explain what a “copy number” is nor how to determine a copy number of a genomic region or transcript. It simply notes that assays for analyzing “copy number” existed, and states “such techniques are not useful in a high throughput multiplex analysis.” EX1001, 56:54-63; *also id.*, 55:49-51. The '730 patent offers no guidance on how to use its methods to determine a copy number. EX1005, ¶¶209-10. Instead, the '730 patent relies on the knowledge of a POSA to supply that information. *Id.*

Prior to May 2009, it was well-known that variations in the number of copies of a particular segment of a gene (the “copy number”) are associated with certain disorders and that testing for such variation could be useful in identifying

⁹ If Patent Owner contends Chee does not disclose using its method with a “biological sample” as recited in claims 1 and 9, it would have been obvious to do for the same reasons as claim 4.

disease. EX1005, ¶211; EX1015(Gahan), 4. It was also known that massively parallel sequencing could be used to determine copy number, and that when doing so, any biases in the sequencing process should and could be addressed.

EX1033(Chiang), 99, 102; EX1026, [0074].

Chee teaches that its methods can be used for clinical and diagnostic purposes, which can include identifying sequence variants, polymorphisms and mutations. EX1010(Chee), 11:60-63, 9:22-52. The incorporated-by-reference *Genetic Variation Manual*, which discloses conventional techniques, states that “[t]he ability to discover and measure changes in chromosome copy number is becoming critical to our understanding of the differences between normal and disease states.” EX1016, xvi; EX1010(Chee), 8:6-37. The manual includes chapters on how to use assays and other available techniques to determine copy number. EX1016, ix, 221-53. Other publications from that time also recognized the importance of copy number. *See* EX1014, 1143 (noting that an “[i]mportant application[]” of next-generation sequencing was identifying “copy number variation”).

Chee suggests using its method to determine copy number in a level of detail that is commensurate with or exceeds the level of disclosure of copy number in the ’730 patent. Therefore, Chee’s teaching is sufficient to meet claim 7.

A POSA would have recognized that analyzing the DNA sequences generated from Chee's method to determine a copy number would have been beneficial and important for detecting and diagnosing certain diseases. EX1005, ¶¶212-14. A POSA would have also known that copy number analysis may be influenced by bias and would have been motivated to use Chee's methods to identify and correct for bias. *Id.*; EX1033(Chiang), 101. Thus, a POSA would have been motivated and found it obvious to use Chee's method when sequencing DNA to determine the copy number of a genomic region of a patient. EX1005, ¶¶214-15. Therefore, Chee renders claim 7 obvious.

C. Ground 3: Chee and Gahan Render Claims 5-6 and 8 Obvious

1. Claims 5-6 and 8 Recite Conventional Samples

Claims 5, 6, and 8 specify that the sample includes nucleic acids from bacteria or a virus, from a tumor, or from a fetus or placenta. Nothing about these limitation is novel, as they all were known sources of nucleic acids prior to May 2009. EX1005, ¶¶216-17. Aside from stating that its method can be used when analyzing DNA from the listed sources, the '730 patent does not describe these elements as having any impact on its methods. *Id.*

2. Overview of Gahan

Gahan is a scientific article published in September 2008 that describes different types of "circulating nucleic acids in blood." EX1015(Gahan), 1. By September 12, 2008, a summary of Gahan appeared on the NYAS website along

with a link to purchase the issue of the journal it was in. EX1022, 1; EX1037; EX1041, ¶4. Gahan is prior art under § 102(a).

Gahan reports on recent developments in the analysis of circulating nucleic acids as were discussed at the fifth international conference on circulating nucleic acids in plasma and serum. EX1015(Gahan), 1. Gahan states that “[t]he ability to isolate, quantify, and analyze these molecules has led to the identification of specific nucleic acid fragments related to particular disorders such as... cancer.” *Id.* Gahan states that DNA circulates in and can be isolated from the whole blood, serum, plasma. *Id.*

3. A Skilled Person Would Have Practiced Chee’s Methods Using Conventional Techniques

As explained for claim 4 above, a POSA would have found it obvious to obtain Chee’s biological samples from a patient’s blood, consistent with the incorporated-by-reference *Genetic Variation Manual*’s teaching of conventional methods for doing so. EX1005, ¶222; EX1010(Chee), 8:6-37.

A POSA would have known that different types of nucleic acids circulate in blood, and that these types of nucleic acids can be specifically studied. EX1005, ¶223. That conventional knowledge is reflected in Gahan, which discusses various types of DNA and RNA in blood or other plasmas. A POSA would have known that it was useful to sequence DNA circulating in blood for diagnostic purposes. EX1005, ¶224. For example, Gahan states such analysis allows for the

identification of “nucleic acids fragments related to particular disorders such as diabetes, cancer, myocardial infarction, and stroke, thereby permitting their early diagnosis.” EX1015(Gahan), 1. A POSA would have been motivated to isolate the different types of nucleic acids circulating in blood and analyze them using Chee’s method. EX1005, ¶224.

4. Claims 5, 6, and 8

Claims 5, 6, and 8 each depend from claim 1 and specify the type of nucleic acids analyzed.

Claim 5 specifies “*said plurality of target nucleic acid molecules comprises bacterial or viral nucleic acids isolated from said biological sample.*” A POSA would have understood that a biological sample (e.g., blood) can include nucleic acids from bacteria and viruses. EX1005, ¶¶226-27. In addition, Gahan states that nucleic acids can be extracted from blood using an Ultrasens Virus Kit. EX1015(Gahan), 3. A POSA would have understood that sequencing viral nucleic acids from blood could identify certain illnesses, and would have been motivated to use those with Chee’s methods. EX1005, ¶¶226-27.

Claim 6 specifies “*said plurality of target nucleic acid molecules are circulating tumor nucleic acid molecules.*” Gahan states that circulating DNA can be isolated from blood, EX1015(Gahan), 1, or can include “cancer-related DNA” from “tumors,” *id.*, 2. Gahan also states that cancer-related DNA can aid in the

diagnosis of certain cancers, such as breast and lung cancer. EX1015(Gahan), 4.

A POSA would have found it obvious to use Chee's method to screen for DNA associated with those cancers, as Gahan states that doing so is useful for diagnostic purposes. EX1005, ¶¶229-30.

Claim 8 specifies “*said plurality of target nucleic acid molecules are maternally-circulating fetal or placental nucleic acid molecules.*” Gahan states that circulating nucleic acids can include fetal and maternal DNA.

EX1015(Gahan), 3-4; *id.*, 2 (“fetal DNA”). Fetal DNA can be analyzed to identify certain disorders, and to determine a fetus's sex. EX1015(Gahan), 3-4. Maternal DNA can be analyzed to identify certain material complications. EX1015(Gahan), 3. A POSA would have found it obvious to use Chee's method to screen for maternal or fetal DNA, as Gahan states that doing so is useful for diagnostic purposes. EX1005, ¶232.

D. Ground 4: Bignell and McCloskey Render Claims 1, 4-6, and 9 Obvious

1. Overview of Bignell

Bignell teaches methods for sequencing nucleic acids (*e.g.*, DNA) that are particularly useful with Next-Gen sequencing technology. EX1013(Bignell), 8:21-23, 45:48-50, 43:48-50; EX1005, ¶¶233-35. Bignell uses tags to distinguish samples, and describes several methods for adding tags with sample-specific identifiers to target nucleic acid fragments. EX1013(Bignell), 1:24-28, 6:36-40,

9:48-52. That allows for multiplex sequencing, where the resulting template nucleic acids are amplified, pooled with other samples, and then sequenced together. *Id.*, 4:10-29; EX1005, ¶¶233-38. The sequence of each nucleic acid can be assigned to the correct sample using the sample-specific tag. EX1013(Bignell), 3:56-59, 6:40-43, 48:11-13.

2. Overview of McCloskey

McCloskey teaches techniques for adding a molecular barcode to each nucleic acid (*e.g.*, DNA fragments) in a sample to address problems with errors and bias caused by amplification. EX1012(McCloskey), [0003], [0106]; EX1005, ¶¶239, 246. McCloskey describes labeling nucleic acids with a tag that includes a barcode that uniquely identifies the fragment and a batch-stamp that identifies the sample (*e.g.*, patient). EX1012(McCloskey), [0005], [0021], [0027]. McCloskey provides that the unique barcodes are used to identify duplicate nucleic acids in the amplified sample, and to correct for any “skewing” caused by amplification. *Id.*, [0005], [0021], [0093], [0106]. The unique barcodes also allow for identifying “PCR errors,” *id.*, [0063], such as “mutations arising during PCR amplification,” *id.*, [0106].

3. A Skilled Person Would Have Modified Bignell to Incorporate Elements Shown in McCloskey

A POSA would have had reason to combine the teachings of McCloskey with those of Bignell. McCloskey teaches methods for preparing a sample so that

individual nucleic acids can be tracked during a sequencing analysis.

EX1012(McCloskey), [0002], [0106]. McCloskey does not require a specific sequencing technology, but notes that methods for “sequencing are standard in the art.” *Id.*, [0034]. To implement McCloskey’s method, a POSA would have looked to other references for details on known, standard sequencing technologies.

EX1005, ¶248. One such reference is Bignell.

Bignell teaches methods for preparing and then sequencing samples of DNA on next-generation sequencing machines. EX1013(Bignell), 17:12-27, 41:51-61, 46:45-47:3, 6:36-43. By early 2009, several recent developments in the DNA sequencing field, including the release of Next-Gen sequencing technologies, “undeniably reinvigorat[ed] this field” and increased the “incentive for developing entirely new strategies for DNA sequencing.” EX1014, 1135. A POSA would have been motivated to combine McCloskey specifically with a Next-Gen sequencing technique such as the ones taught in Bignell. EX1005, ¶¶249-50.

Similarly, a POSA reading Bignell would have understood that amplification processes were imperfect and introduced bias by making more copies of some nucleic acids than others, as that was well-known by May 2009. *See* EX1012(McCloskey), [0003]; EX1025, 8966; EX1018, [0002]; EX1005, ¶¶251, 77. McCloskey teaches that using unique barcodes for each molecule is a solution to the bias problem. EX1012(McCloskey), [0005]. A POSA would have

recognized that adding McCloskey's unique barcodes to Bignell's method, which includes several amplification steps, could improve it. EX1005, ¶251.

A POSA would have recognized that McCloskey's tagging technique was analogous to, and compatible with, Bignell's tagging techniques. EX1005, ¶252. Both Bignell and McCloskey teach that it is advantageous to tag nucleic acids with a sample identifier. EX1013(Bignell), 1:24-28, 3:54-59; EX1012(McCloskey) [0027]. Both describe attaching tags as well as adaptors for use in subsequent amplification steps. EX1013(Bignell), 7:45-49, 9:59-10:4, 21:18-22, Fig. 3; McCloskey, [0005], [0009] ("third sequence"). Both describe techniques that can be used on DNA fragments. EX1013(Bignell), 7:25-26, 8:4-5; EX1012(McCloskey), [0005].

Both Bignell and McCloskey describe methods for determining the sequence of nucleotides that can be used for diagnostic or clinical use. EX1013(Bignell), 1:24-32; EX1012(McCloskey), [0002] ("sequence analysis"), [0019], [0034]. They are directed to the same field of endeavor as the '730 patent.

A POSA would have had a reasonable expectation of success in combining McCloskey and Bignell. EX1005, ¶¶253-57.

Bignell discloses that each molecule (*e.g.*, DNA fragment) can have multiple adaptors attached to it. EX1013(Bignell), 11:65-12:9. Bignell's adaptor sequences may be selected by the user and could include additional sequence elements such

as tags. EX1013(Bignell), 9:62-67, 11:65-12:9. Bignell also shows examples where a nucleic acid includes two tags. *Id.*, Fig. 3. McCloskey shows that a tag can include both a unique barcode and a sample identifier. EX1012(McCloskey), [0046], [0027]. A POSA would have recognized that Bignell could have been readily adapted to include a tag containing McCloskey's unique, molecule-specific barcodes. EX1005, ¶¶253-54. It would have been within a POSA's skill to modify Bignell to use tags that included sample identifiers (as taught by both Bignell and McCloskey) and also McCloskey's unique barcode. EX1005, ¶255.

Patent Owner may argue that a POSA would not have added additional tags to Bignell because doing so would reduce how many nucleotides of the target nucleic acid could be sequenced. Such an argument would be wrong because Bignell's methods allow a sequencer to make multiple reads of each amplified nucleic acid. EX1013(Bignell), 17:36-39, 6:61-7:3. Thus, Bignell's sequencer can read the sequence of the nucleic acid fragment and then separately read the tag sequences. *Id.*, 6:61-7:3. This allows for use of longer tag sequences without compromising the read length for the nucleic acids. EX1005, ¶253.

In addition, by May 2009, it was known that making multiple reads allowed use of multiple identifiers without reducing the read length of the target nucleic acid. *See* EX1026, [0005], [0008]-[0016], [0068] (performing multiple reads on multiple tags on one template using a Next-Gen sequencing system).

Thus, a POSA would have reasonably expected the combination of McCloskey and Bignell to work. EX1005, ¶257.

Moreover, a POSA would have had a reasonable expectation of success in combining the teachings of Bignell and McCloskey as evidenced by the simultaneous inventions of the Chee and '730 patents. Simultaneous inventions are “evidence of the level of skill in the art” and “evidence that persons of ordinary skill in the art understood the problem and a solution to that problem.” *Univ. Cal. v. Broad Inst. Inc.*, 903 F.3d 1286, 1295 (Fed. Cir. 2018). Thus, the existence of simultaneous inventions is “evidence of the motivation to combine the prior art references.” *Id.* at 1296.

In the mid-2000s, methods of tagging, sequencing, and analyzing sequencing results were well-known in the art. *See* §§III.B- C. It was also well-known that a sequencing workflow could result in errors and/or bias. *See* §III.D. Around that time, multiple research teams were pursuing similar approaches to overcome these problems. The techniques described in the Chee and '730 patents, for instance, show independent development of the same improvement to the prior art to solve a known problem. Chee invented techniques for using unique identifiers to correct for errors and bias at least a few weeks or months before the '730 patent's provisional applications were filed. Therefore, these simultaneous inventions demonstrate that a POSA would have been motivated to combine

McCloskey's tags that include unique barcodes with the tagging methods described in Bignell. EX1005, ¶256.

4. Bignell and McCloskey Render Claim 1 Obvious

a) A method for correcting for errors or bias introduced during nucleic acid analysis workflow

To the extent the preamble is limiting, Bignell and McCloskey together teach it.

Bignell describes methods for sequencing nucleic acids (*e.g.*, DNA) and for preparing a sample for sequencing ("*nucleic acid analysis workflow*").

EX1013(Bignell), 8:21-23, 1:24-32, 6:36-40.

McCloskey teaches a method for "*correcting for errors or bias*" when amplifying a sample. EX1012(McCloskey), [0002]; EX1005, ¶261. McCloskey teaches that amplifying a DNA sample can result in "a skewed yield of PCR products" creating more copies of some molecules than others.

EX1012(McCloskey), [0003]; *see id.*, [0093] (noting "the challenges of PCR redundancy"). Labeling each nucleic acid with a distinct barcode prior to amplification allows its method to "correct[] for [the] skewing that can arise from PCR amplification." *Id.*, [0005]; *see id.*, [0106] (noting it can "correct[] for skewing"); EX1005, ¶262. The method also allows for the identification and analysis of "PCR errors," EX1012(McCloskey), [0063], such as "mutations arising during PCR amplification," *id.*, [0106].

b) obtaining a biological sample comprising a plurality of target nucleic acid molecules from more than one locus of origin;

Bignell and McCloskey each teach this element.

Bignell discloses obtaining a biological sample, such as “human genomic DNA molecules.” EX1013(Bignell), 8:4-16; *id.*, 26:10-13 (“human samples”). Bignell explains that samples can include DNA “molecules [that] represent the entire genetic complement of an organism, and are genomic DNA molecules.” *Id.*, 8:5-10. Bignell’s methods can be used to analyze the entire genome or subsets thereof (*e.g.*, particular chromosomes). *Id.*, 8:5-13. Thus, Bignell teaches that its method works with samples that include nucleic acids from “*more than one locus of origin.*” EX1005, ¶¶264-65.

McCloskey’s method can be used with “naturally-occurring” nucleic acids such as DNA. EX1012(McCloskey), [0018]. It describes examples where the sample is human DNA isolated from blood (“*obtaining a biological sample*”). *Id.*, [0043]-[0045]. McCloskey teaches using its method with “genomic DNA fragments,” which refers to DNA from anywhere on the genome. *Id.*, [0005]; EX1005, ¶267. McCloskey provides examples where the sample is taken from “the FMR1 locus.” EX1012(McCloskey), [0043]. Its method can be part of an analysis done for biomedical or forensic purposes, or for clinical purposes such as diagnosing cancers or other human diseases. *Id.*, [0006]-[0007], [0103] (“fragile X

syndrome”), [0019] (“clinical samples”), [0027] (“patient”). A POSA would have understood McCloskey’s reference to analyzing “genomic DNA fragments” for biomedical, clinical, or forensic purposes to refer to analyzing DNA fragments from “*more than one locus of origin*” on the genome. EX1005, ¶267.

- c) 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;**

Bignell discloses adding a tag to each nucleic acid in a sample.

EX1013(Bignell), 7:59-64, 9:41-52, 9:59-10:4. *See also id.*, 13:16-22, 13:32-35, 21:18-22. Bignell’s tags include a sample identifier that is the same for every nucleic acid in a sample. *Id.*, 9:46-52. The tags can be incorporated into an adaptor that is ligated to the ends of each nucleic acid in the sample and used to amplify them. *Id.*, 9:59-67; *see id.*, 10:23-26; EX1005, ¶¶269-71. Bignell explains the precise sequence of the adaptors can be selected by the user. EX1013(Bignell), 11:65-12:9.

As explained in §VI.D.3, above, a POSA would have been motivated and found it obvious to incorporate McCloskey’s molecule-specific unique barcodes into Bignell. *See* EX1005, ¶¶272-75.

McCloskey teaches associating a unique barcode with each nucleic acid in a sample. EX1012(McCloskey), [0005], [0009]. The “barcoded oligonucleotide[s]”

are synthesized and then added (“*introduced*”) to the sample of nucleic acids. *Id.*, [0046]; *see id.*, [0047]. McCloskey’s barcodes can contain multiple (*e.g.*, four) parts, including two identifiers:¹⁰

- a “random barcode” that will “uniquely identify a target nucleic acid in an experiment,” [0021] (“second sequence”); and
- a “batch-stamp” that identifies a sample or a patient, [0027] (“fourth sequence”).

The length of the random barcode should be “sufficient to provide, with high probability, a unique identity to each target nucleic acid molecule in the sample prior to amplification.” EX1012(McCloskey), [0021]; *see id.*, [0101]. McCloskey teaches that the random barcode can be between 3 and 30 nucleotides long. *Id.*, [0021], [0101]. A barcode 30 nucleotides in length yields over 1×10^{18} possible unique identifiers. EX1005, ¶¶276-77. Although McCloskey states the barcodes have a “high probability” of being unique, McCloskey includes examples where every molecule in the original sample has its own unique barcode. *E.g.*, McCloskey, [0053], [0063], [0043]. Thus, McCloskey discloses that every molecule in a sample has a different barcode. EX1005, ¶¶276-77.

¹⁰ The “first sequence” and “third sequence” are adaptors or primers.

EX1012(McCloskey), [0020], [0022].

McCloskey discloses that “*one or more of said loci of origin are associated with more than one differentiator tag.*” As explained in §V.A, above, the Applicant added this limitation to make clear that the claims require a unique tag per molecule. *See* EX1003, 1263, 1258.

McCloskey’s random barcode “can uniquely identify a target nucleic acid,” and the barcode provides “a unique identity to each target nucleic acid molecule in the sample prior to amplification.” EX1012(McCloskey), [0021]. In McCloskey’s examples, multiple nucleic acids correspond to the same genetic locus, but each one has its own unique barcode. *Id.*, [0043], [0053]. Thus, each nucleic acid associated with a loci of origin will have a different random barcode (“*more than one differentiator tag*”). EX1005, ¶278. As explained for the “*obtaining*” step above, McCloskey teaches that a sample can have multiple loci of origin.

d) amplifying each of the plurality of tagged target nucleic acid molecules to generate amplicons;

Both Bignell and McCloskey teach amplifying tagged nucleic acids. EX1013(Bignell), 4:51-53, 5:17-20; *see id.*, 10:16-19, 12:65-13:15, 15:5-11, 15:14-15; EX1012(McCloskey), [0032] (McCloskey teaches “amplifying the bar-coded target nucleic acid molecule... to produce an amplification product.”); *see id.*, [0031], [0033], [0035]; EX1005, ¶¶282-84.

e) 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

Bignell states that each “amplified strand” is sequenced, and both the target and tag sequences are read. EX1013(Bignell), 6:61-7:3; *id.*, 4:56-61, 5:25-30, 17:4-11, 17:36-39; EX1005, ¶286.

McCloskey similarly states that the “amplified products” may be sequenced, and that sequencing methods were standard in the art. EX1012(McCloskey), [0034]. McCloskey shows that both the barcodes and nucleic acids are sequenced. *Id.*, [0051]-[0052], [0063]; EX1005, ¶¶287-89.

Thus, in the combined method, both the nucleic acids and the tags are sequenced. EX1005, ¶290.

f) correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

As explained with respect to the claim preamble, McCloskey teaches “*correcting for errors or bias.*” EX1012(McCloskey), [0002].

For example, McCloskey’s unique barcodes allow it to “correct[] for [the] skewing that can arise from PCR amplification.” *Id.*, [0005]. McCloskey corrects the skewing by identifying duplicate barcodes in an amplified sample. *Id.*, [0053]; EX1005, ¶293. This allows McCloskey to determine that there are “multiple amplified sequences derived from a single original molecule.”

EX1012(McCloskey), [0093]. The '730 patent uses differentiator tags the same way. EX1001, 28:32-44; EX1005, ¶295. McCloskey explains that the “ability to distinguish **redundant** from valid data is an immensely powerful tool.”

EX1012(McCloskey), [0053].

McCloskey's barcodes allow it “to identify... redundant sequences and to quantify accurately the proportion of cells carrying a particular sequence variant by **counting only distinctly tagged sequences.**” EX1012(McCloskey), [0093]; *see id.*, [0106] (can “quantif[y] the relative abundance” of molecules). Similarly, 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.

EX1001, 30:48-51, 72:63-73:2. In other words, duplicates of a tag are identified but not included in the count. Thus, McCloskey teaches “*collapsing*” duplicate “*target:differentiator tag combinations... into a single count.*” EX1005, ¶¶294-95.

5. Claim 4

Claim 4 depends from claim 1 and specifies “*said biological sample is selected from the group consisting of blood and biopsy tissue.*”

McCloskey discloses using its technique on “[h]uman genomic DNA... isolated from blood.” EX1012(McCloskey), [0045]. Thus, McCloskey teaches claim 4. EX1005, ¶¶300-01.

6. Claim 5

Claim 5 depends from claim 1 and specifies “*said plurality of target nucleic acid molecules comprises bacterial or viral nucleic acids isolated from said biological sample.*”

Bignell teaches that its technique can be used to analyze the genomes of bacteria or viruses. EX1013(Bignell), 1:29-32, 8:47-53. Thus, it teaches claim 5. EX1005, ¶¶302-03.

7. Claim 6

Claim 6 depends from claim 1 and specifies “*said plurality of target nucleic acid molecules are circulating tumor nucleic acid molecules.*”

McCloskey discloses using its method “to assess the variations of methylation, and their quantification, in a heterogeneous population of cancer cells and normal cells.” EX1012(McCloskey), [0007]. McCloskey also shows that samples are taken from blood. *Id.*, [0045]. A POSA would have understood that such method would have involved analyzing nucleic acids released from tumors that was circulating in blood. EX1005, ¶305. Thus, McCloskey teaches claim 6. EX1005, ¶¶304-06.

8. Claim 9

Most of the steps of claim 9 are identical to elements of claim 1, which Bignell and McCloskey disclose for the same reasons as claim 1. For the “*introducing...*” and “*correcting...*” steps, claim 9 uses slightly different words

than claim 1. Bignell and McCloskey teach these elements for the reasons provided below.

- a) **introducing a set of unique differentiator tags, wherein members of said set of unique differentiator tags are randomly associated with members of said plurality of target nucleic acid molecules, such that any given tag is uniquely associated with said locus of origin;**

This step specifies the use of “*unique*” differentiator tags that are “*randomly associated*” with targets such that any tag is “*uniquely associated*” with a locus of origin. As explained above in §V.A, Applicant made clear in prosecution that “*differentiator tags*” must be unique. Therefore, claim 9’s recitation of “*unique*” does not change the claim’s scope from claim 1. McCloskey’s unique barcodes are “*unique differentiator tags*” for the same reasons they are “*differentiator tags*.”

McCloskey’s “random barcodes” are “*randomly associated*” with target nucleic acids. EX1012(McCloskey), [0017]. The barcodes are separately synthesized oligonucleotides, *id.*, [0021], that are added to the sample and attached to the target nucleic acids, *id.*, [0017], [0046]-[0047]. A POSA would have understood that the unique barcodes would have been randomly associated with the nucleic acids in the sample. EX1005, ¶280. One reason is that McCloskey’s barcodes do not have a sequence that is complementary to any the target nucleic acids, and therefore the barcodes are not destined for any particular molecule. *Id.*

McCloskey teaches that each barcode is associated with one target nucleic acid in the original sample. EX1012(McCloskey), [0021]. Thus, the barcode is uniquely associated with a “*locus of origin.*” EX1005, ¶281.

- b) correcting for error or bias introduced during said workflow by recognizing target/differentiator tags having the same sequence as being derived from the same input molecule.**

Bignell and McCloskey teach “*correcting for error or bias*” for the same reasons as claim 1.

McCloskey identifies “redundant sequences” where multiple nucleic acids have the same barcode. EX1012(McCloskey), [0093], [0053]. It explains that the “ability to distinguish redundant from valid data is an immensely powerful tool.” *Id.*, [0053]. McCloskey determines that amplified nucleic acids that have the same barcode are “derived from a single original molecule.” *Id.*, [0093]; *id.*, [0106] (noting it can detect when “multiple amplified sequences are derived from a single original molecule”). Thus, McCloskey teaches “*recognizing*” nucleic acids with the same unique barcode “*as being derived from the same input molecule.*” EX1005, ¶¶297-99.

E. Ground 5: Bignell, McCloskey, and Bentley Render Claims 2 and 3 Obvious

1. Overview of Bentley

Bentley is an academic article that was published in Science Direct's Current Opinion in Genetics & Development in October 2006. It was received in Cornell University's library on December 8, 2006. EX1038. It is prior art under § 102(b).

Bentley describes then-emerging techniques for resequencing genomes, such as Next-Gen sequencers like Illumina's. EX1017(Bentley), 546, 548. It describes how those emerging techniques measure and improve the accuracy of sequencing. *Id.* It explains that one such techniques used by Illumina's system was the generation of a consensus sequence. *Id.*, 549-50; EX1005, ¶¶307-08.

2. Claim 2

Claim 2 depends from claim 1 and specifies "*said correcting step 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.*"

McCloskey's unique barcodes allow for the identification and analysis of "PCR errors," EX1012(McCloskey), [0063], such as "mutations arising during PCR amplification," *id.*, [0106]. McCloskey discloses that its method can sequence duplicate copies of the same target nucleic acid. *Id.*, [0053], [0106].

McCloskey notes that for the exemplary experiments it reported data for, “errant base calling was corrected manually.” *Id.*, [0098]

A POSA would have been motivated to configure the method suggested by Bignell and McCloskey to correct errors by using duplicate copies of the same nucleic acid to generate a consensus sequence. EX1005, ¶¶323, 309-320. Such techniques were well-known in the art at the time. *Id.*, ¶¶105-06, 322-23. For example, Bentley explains that Illumina’s system was able generate a consensus sequence to improve read accuracy. EX1017(Bentley), 549 (“multiple reads from the same sample can be aligned and used to derive a consensus base-call at each position”), 550 (Fig. 2).

A POSA would have understood that McCloskey identifies and analyzes PCR mutation errors by using reads of multiple copies of the same nucleic acid. EX1005, ¶315. When combining McCloskey with Bignell, which describes techniques that can be used with Illumina’s sequencer, a POSA would have been motivated to configure the system to generate consensus sequences when the amplified products contained duplicates (amplified nucleic acids with the same barcode). EX1005, ¶¶309-320, 324. Doing so was a feature of Illumina’s system and improved accuracy as taught by Bentley.

3. Claim 3

Claim 3 depends from claim 2 and specifies the method “*further comprising the step of determining the presence of a sequencing or amplification error based upon a number of said differentiator tag sequences.*”

McCloskey’s use of unique barcodes allows it to determine the presence of “PCR errors,” EX1012(McCloskey), [0063], such as “mutations [in the DNA] arising during PCR amplification,” *id.*, [0106]. McCloskey sequences duplicate copies of the same target nucleic acids. *Id.*, [0106]. A POSA would have understood that McCloskey identifies “PCR errors” by comparing sequence reads of target nucleic acids with the same barcode. EX1005, ¶326. A POSA also would have understood that generating a consensus sequence would have involved comparing sequence reads of target nucleic acids with the same barcode. EX1005, ¶327. Thus, McCloskey and Bentley teach this element.

F. Ground 6: Bignell, McCloskey, and Gahan Render Claims 6 and 8 Obvious

McCloskey’s method can be used on nucleic acids collected from blood. EX1012(McCloskey), [0045] (“[h]uman genomic DNA... isolated from blood.”). A POSA would have known that different types of nucleic acids circulate in blood, which can be specifically studied. EX1005, ¶328. That conventional knowledge is reflected in Gahan, summarized in §VI.C, above. A POSA would have known that it was useful to sequence nucleic acids circulating in blood for diagnostic purposes,

which as Gahan explains, can allow for early diagnosis of certain diseases.

EX1005, ¶¶328-29; EX1015(Gahan), 1. A POSA would have been motivated to isolate the different types of nucleic acids circulating in blood and analyze them using Bignell's and McCloskey's method. EX1005, ¶329.

Claim 6 depends from claim 1 and specifies “*said plurality of target nucleic acid molecules are circulating tumor nucleic acid molecules.*”

Claim 8 depends from claim 1 and specifies “*said plurality of target nucleic acid molecules are maternally-circulating fetal or placental nucleic acid molecules.*” As explained in §VI.C.1, these claim elements do not recite inventive subject matter.

As explained in §VI.C.4, above, Gahan explains that “cancer-related DNA” from “tumors” circulates in human blood. EX1015(Gahan), 2. Identifying such cancer-related DNA can aid in the diagnosis of certain cancers, such as breast and lung cancer. EX1015(Gahan), 4. A POSA would have been motivated to use Bignell's and McCloskey's method to screen for DNA associated with those cancers for diagnostic purposes. EX1005, ¶332. Thus, Bignell, McCloskey, and Gahan render claim 6 obvious.

As explained in §VI.C.4, above, Gahan states that both fetal and maternal DNA circulate in human blood. EX1015(Gahan), 3. Fetal DNA can be analyzed to identify certain disorders, as well as to determine the sex of the fetus. *Id.*, 3-4.

Maternal DNA can be analyzed to identify certain maternal complications, such as fetal disorders. *Id.* A POSA would have been motivated to use Bignell's and McCloskey's method to screen for maternal or fetal DNA for diagnostic purposes. EX1005, ¶334. Thus, Bignell, McCloskey, and Gahan render the additional limitations of claim 8 obvious.

G. Ground 7: Bignell, McCloskey, and Chiang Render Claim 7 Obvious

Chiang is scientific article that describes techniques for determining copy number using Next-Generation Sequencing technology, such as Illumina's Genome Analyzer. EX1033(Chiang), 99. Chiang was published in Nature Methods in January 2009 and was published online November 30, 2008. EX1039, 1; EX1040, 7. Chiang is prior art under § 102(a).

Claim 7 depends from claim 1 and specifies “*the step of determining the copy number of a genomic region or transcript in a patient from whom the sample is obtained.*”

Chiang teaches that copy number variations “have been linked to numerous diseases” and that detecting such variations is a “powerful way to find cancer genes.” EX1033(Chiang), 99; EX1005, ¶¶335-36, 344. Chiang explains that “copy number estimates from massively parallel sequencing achieved greater sensitivity... and greater precision” than prior techniques. EX1033(Chiang), 99.

Chiang also shows algorithms for determining copy number using Next-Gen sequencers. *Id.*, 100-101; EX1005, ¶343.

A POSA also would have understood that determining copy number was an “important application[]” of next-generation sequencing. EX1014, 1143. Thus, a POSA would have been motivated to use in Bignell’s and McCloskey’s method to determine copy number and detect the presence of cancer genes. EX1005, ¶¶337-40. Therefore, a POSA would have found it obvious to use Bignell’s and McCloskey’s method to determine the copy number of a gene. EX1005, ¶¶343-44.

H. No Secondary Considerations of Non-obviousness

The Grounds 2-7 render the Challenged Claims obvious. No secondary indicia of non-obviousness exist having a nexus to the putative “invention” of these claims. Petitioner reserves its right to respond to any assertion of secondary indicia advanced by Patent Owner.

VII. The Board Should Not Deny Institution under 35 U.S.C. § 325(d) or § 314(a)

The Board should not deny institution under § 325(d). *See Advanced Bionics, LLC v. MED-EL Elektromedizinische Gerate GmbH*, IPR2019-01469, Paper 6 at 6-11 (PTAB 2020) (precedential); *Becton, Dickinson & Co. v. B. Braun Melsungen AG*, IPR2017-01586, Paper 8 at 17-18 (PTAB 2017) (setting forth a six factor test).

Grounds 1, 2, and 3, which together apply to every Challenged Claim, are based primarily on Chee, which was neither cited nor discussed during prosecution and is materially different than the art the Examiner considered. To overcome a rejection, Applicant argued that the prior art failed to disclose a method of tagging where “each individual nucleic acid molecule is tagged with a unique differentiator tag,” and that such unique tagging was a “distinguishing feature of the claims.” EX1004, 964-65. As explained in §VI.A.3.a, Chee discloses exactly this feature: “the ‘unique’ ... identifiers are associated with individual molecules in the starting sample” which allows the molecules to be “distinctly identified.” EX1010(Chee), 5:27-33. Thus, Chee is materially different from the art the Examiner considered during prosecution and is not cumulative (*Becton, Dickinson* factors (a) and (b)). Because it includes grounds containing new, materially different art, this Petition should not be denied.

Ground 4 is based on the combination of Bignell and McCloskey, to which Ground 5 adds Bentley, Ground 6 adds Gahan, and Ground 7 adds Chiang. Bignell, Bentley, Gahan, and Chiang were not cited or discussed during prosecution. Although McCloskey was cited in an IDS (and in the ’730 patent specification), it was not discussed by the Examiner.¹¹ Thus, neither the substance

¹¹ Over 600 references were disclosed to the Examiner in IDSs.

of McCloskey nor the combination of Bignell and McCloskey was considered during prosecution (*Becton, Dickinson* factor (c)).

McCloskey's teachings are materially different from the references discussed during examination. The Examiner's references were directed to tags with sample-identifiers or with gene-specific identifiers such that multiple nucleic acids would have the same identifier. *See, e.g.*, EX1004, 866-72, 994-1002; *also id.*, 1059-60, 1072; EX1003, 430-45, 518-37, 859-77, 985-1005, 1127-53, 1219-48. As noted above, Applicant distinguished those references by arguing they did not disclose that "each individual nucleic acid molecule is tagged with a unique differentiator tag." EX1004, 965; *also id.*, 977-78. McCloskey discloses that exact feature: tagging each nucleic acid with a "random barcode" that provides "a unique identity to each target nucleic acid molecule in the sample prior to amplification." EX1012(McCloskey), [0021]; *see id.*, [00053] (example where every nucleic acid was tagged with a different identifier). Thus, the Examiner erred by not considering the substance of the McCloskey reference (*Becton, Dickinson* factor (e)).

This petition further is supported by the declaration of Dr. Gabriel, which was not considered during prosecution (*Becton, Dickinson* factor (f)). *See* EX1004, 1060 (Applicant criticizing the lack of an expert affidavit to support the

Examiner's findings). Accordingly, the Board should not deny this petition under § 325(d).

The Board should not deny this petition under § 314(a). Illumina was served with a complaint alleging infringement of the '730 patent in the Delaware Action on June 10, 2024. Illumina filed its answer on September 13, 2024. No scheduling conference has occurred and no trial date has been set. The average time to trial in Delaware is 32.9 months, which is more than one year after this IPR would conclude, if instituted.¹²

VIII. Certifications

The Director is authorized to charge the fee specified by 37 C.F.R. § 42.15(a) to Deposit Account No. 50-1597.

Per CFR 42.104(a), Illumina certifies that the '730 patent is available for IPR as this Petition is being filed more than nine months after the patent issued. Illumina certifies it is not barred or estopped from requesting IPR of the '730 patent. Illumina has not filed a civil action challenging the validity of the '730 patent, nor has Illumina previously filed an IPR against the '730 patent.

¹² U.S. District Courts, National Judicial Caseload Profile, https://www.uscourts.gov/sites/default/files/data_tables/fcms_na_distprofile0630.2024.pdf

Per CFR 42.105, Petitioner certifies it served this petition on Molecular Loop Biosciences, Inc., the patent owner of record in the assignment records for the '730 patent.

IX. Conclusion

For the reasons set for above, Illumina respectfully requests that trial be instituted.

Dated: September 30, 2024

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Claim Appendix

The Challenged Claims are shown below:

1. A method for correcting for errors or bias introduced during nucleic acid analysis workflow, the method comprising the steps of:

obtaining a biological sample comprising a plurality of target nucleic acid molecules from more than one locus of origin;

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;

amplifying each of the plurality of tagged target nucleic acid molecules to generate amplicons;

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

correcting for error or bias introduced during said workflow by collapsing target: differentiator tag combinations observed more than once into a single count.

2. The method of claim 1, wherein said correcting step 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.

3. The method of claim 2, further comprising the step of determining the presence of a sequencing or amplification error based upon a number of said differentiator tag sequences.

4. The method of claim 1, wherein said biological sample is selected from the group consisting of blood and biopsy tissue.

5. The method of claim 1, wherein said plurality of target nucleic acid molecules comprises bacterial or viral nucleic acids isolated from said biological sample.

6. The method of claim 1, wherein said plurality of target nucleic acid molecules are circulating tumor nucleic acid molecules.

7. The method of claim 1, further comprising the step of determining the copy number of a genomic region or transcript in a patient from whom the sample is obtained.

8. The method of claim 1, wherein said plurality of target nucleic acid molecules are maternally-circulating fetal or placental nucleic acid molecules.

9. A method for correcting for errors or bias introduced during nucleic acid analysis workflow, the method comprising the steps of:

obtaining a biological sample comprising a plurality of target nucleic acid molecules from more than one locus of origin;

introducing a set of unique differentiator tags, wherein members of said set of unique differentiator tags are randomly associated with members of said plurality of target nucleic acid molecules, such that any given tag is uniquely associated with said locus of origin;

amplifying each of the plurality of tagged target nucleic acid molecules to generate amplicons;

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

correcting for error or bias introduced during said workflow by recognizing target/differentiator tags having the same sequence as being derived from the same input molecule.

CERTIFICATE OF COMPLIANCE

I hereby certify that this brief complies with the type-volume limitations of 37 C.F.R. §42.24, because it contains 13,925 words (as determined by the Microsoft Word word-processing system used to prepare the brief), excluding the parts of the brief exempted by 37 C.F.R. §42.24.

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CERTIFICATE OF SERVICE

I hereby certify that on this 30th day of September, 2024, copies of this Petition for *Inter Partes* Review, Attachments and Exhibits have been served in its entirety by Federal Express on the following counsel of record for Molecular Loop in Patent Center and on counsel of record in the Delaware Litigation:

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