

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PARSE BIOSCIENCES, INC.

Petitioner,

v.

10X GENOMICS, INC.

Patent Owner.

Case No. IPR2023-_____

U.S. Patent No. 10,619,207

**PETITION FOR INTER PARTES REVIEW
OF
U.S. PATENT NO. 10,619,207**

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PETITIONER'S EXHIBIT LIST

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EX1001	U.S. Patent No. 10,150,207 (the “’207 Patent”)
EX1002	Declaration of Gregory Cooper, Ph.D.
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EX1004	Lingyun Song and Gregory E. Crawford, <i>DNase-seq: A High-Resolution Technique for Mapping Active Gene Regulatory Elements across the Genome from Mammalian Cells</i> , 2010 Cold Spring Harbor Protocols, 1-11 (2010) (“Song”)
EX1005	Henriette O’Geen et al., <i>Using ChIP-Seq Technology to Generate High-Resolution Profiles of Histone Modifications</i> , 791 <i>Methods in Molecular Biology</i> , 265-286 (2011) (“O’Geen”).
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EX1008	Fraz Syed, 19 <i>Second-Generation Sequencing Library Preparation: In Vitro Tagmentation via Transposome Insertion, Tag-based Next Generation Sequencing</i> 311-321 (1st Ed. 2012) (“Syed”).
EX1009	Andrew Adey et al., <i>Rapid, Low-input, Low-bias Construction of Shotgun Fragment Libraries by High-density in vitro Transposition</i> , <i>Genome Biology</i> 1-17 (2010) (“Adey”).
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EX1012	Gerald Schochetman et al, <i>Polymerase Chain Reaction</i> , 158 <i>J. of Infectious Diseases</i> 1154-1157 (1988) (“Schochetman”).
EX1013	Peter E. Brodelius, Christoph Funk, and Raymond D. Shillito, <i>Permeabilization of Cultivated Plant Cells by Electroporation for Release of Intracellularly Stored Secondary Products</i> , 7 <i>Plant Cell Reports</i> 186-188 (1988) (“Brodelius”).

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EX1018	Terez Márián et al., <i>Hypo-osmotic Shock Induces an Osmolality-dependent Permeabilization and Structural Changes in the Membrane of Carp Sperm</i> , 41.2 <i>Journal of Histochemistry & Cytochemistry</i> 291-297 (1993) (“Márián”).
EX1019	Melissa A. Melan, <i>Overview of Cell Fixation and Permeabilization</i> , 34 <i>Immunocytochemical Methods and Protocols</i> 55-66 (1995) (“Melan”).
EX1020	Defendant Parse Biosciences Inc.’s Supplemental Brief In Support of Motion to Dismiss, <i>10X Genomics, Inc. and The Board of Trustees of the Leland Stanford Junior University v. Parse Biosciences, Inc.</i> , C.A. No. 22-1117-JHS (Dec 15, 2022 D. Del.)(No.33)
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EX1022	David S. Johnson et al., Genome-Wide Mapping of in Vivo Protein-DNA Interactions, <i>Science Express</i> 1-70 (2007) (“Johnson”).
EX1023	Curriculum Vitae of Gregory Cooper
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EX1025	Bruce Alberts, et al., “Chapter 6 How Cells read the Genome: From DNA To Protein”, <i>Molecular Biology of the Cell</i> , Garland Science 299-335 (4th ed. 2002).

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EX1028	Shigeyuki Esumi et al., <i>Method for single-cell microarray analysis and application to gene-expression profiling of GABAergic neuron progenitors</i> , 60 Neuroscience Research 439-451 (2008) (“Esumi”).
EX1029	Tweet by Jason Buenrostro, co-inventor of the 207 Patent (August 26, 2022) at https://twitter.com/JD_Buenrostro/status/1563327469293551618
EX1030	Clyde A. Hutchinson, <i>DNA sequencing: bench to bedside and beyond</i> , 35(18) Nucleic Acids Research 6227-6237 (2007) (“Hutchinson”).
EX1031	Elaine R. Mardis, <i>Next-Generation DNA Sequencing Methods</i> , 9 Annual Review Genomics and Human Genetics 387-402 (2008) (“Mardis”).
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EX1036	Lumen, <i>DNA Packaging in Eukaryotes and Prokaryotes</i> (March 15, 2023) at https://courses.lumenlearning.com/wm-biology1/chapter/reading-dna-packaging-in-eukaryotes-and-prokaryotes/
EX1037	Epigentek, <i>Antibodies for Histone Modifications</i> (March 15, 2023) at https://www.epigentek.com/catalog/antibodies-for-histone-modifications-lp-49.html

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EX1047	Federal Judicial Caseload Statistics (2022), at https://www.uscourts.gov/statistics-reports/analysis-reports/federal-judicial-caseload-statistics

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EX1049	Docket Navigator, <i>Motion Success for Stay Pending IPR in Delaware District</i> (March 31, 2023).
EX1050	Beáta G Vértessy and Judit Tóth, <i>Keeping Uracil Out of DNA: Physiological Role, Structure and Catalytic Mechanism of dUTPases</i> , Acc Chem Res. 1-20 (2009) (“Vértessy”).
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EX1055	J. Sambrook, E.F. Fritsch, T. Maniatis, <i>Molecular Cloning: A Laboratory Manual</i> (Ford et al, eds., 2d ed. 1989)
EX1056	<i>Recipe, RSB Buffer</i> , 2010 Cold Spring Harbor Protocols, available at https://cshprotocols.cshlp.org/content/2010/2/pdb.rec12151.full
EX1057	Sanzhen Liu, Cheng-Ting Yeh, Tieming Ji, Kai Ying, Hayan Wu, Ho Man Tang, Yan Fu, Dan Nettleton, Patrick S. Schnable, Mu <i>Transposon Insertion Sites and Meiotic Recombination Events Co-Localize with Epigenetic Marks for Open Chromatin across the Maize Genome</i> , 5 PLoS Genetics e100733 (2009)

I. INTRODUCTION

Parse Biosciences, Inc. (“Petitioner”) requests review of Claims 1-29 of U.S. Patent No. 10,619,207 (the “’207 Patent”), which issued on April 14, 2020 and is assigned to the Board of Trustees of the Leland Stanford Junior University.

The ’207 Patent pertains to a molecular biology technique known as “tagmentation.” Briefly, tagmentation entails using an enzyme, called a transposase, to fragment a DNA molecule into smaller chunks that are suitable for sequencing. Concurrent with DNA fragmentation, the transposase enzyme also adds “tags” to the DNA molecules that are necessary for the DNA to be sequenced with modern instrumentation. The term “tagmentation” is thus a combination of the words “tagging” and “fragmentation,” reflecting the simultaneous execution of the tagging and fragmentation processes.

Claim 1 of the ’207 Patent is directed to applying this tagmentation method to cell nuclei directly, specifically, by lysing cells and then subjecting the resulting nuclei of the cells to a tagmentation reaction. The instant petition is one of three that are directed to patents in the same family, the first of which Petitioner filed on April 20, 2023 and that is directed to U.S. Patent No. 10,150,995 (“the ’995 Patent”). *See* Paper No. 1, IPR2023-00878. The tagmentation claims of the ’207 Patent are little different from those of the ’995 Patent, except that the claims of the ’207 Patent expressly call for the use of cell lysis.

Importantly, however, neither tagmentation nor cell lysing are novel or inventive. Both are nothing more than routine, well-established prior art processes. The '207 Patent acknowledges, for instance, that tagmentation had not only been published, but was so well-developed that it had already been the subject of commercial product offerings that could be “readily adapted” for the alleged invention. *See* EX1001 at 14:35-42. Likewise, the '207 Patent does not remotely suggest that cell lysis was anything new. And, indeed, the accompanying declaration of Petitioner’s expert, Dr. Cooper, establishes that cell lysis was a routine method to access the interior of the cell. *See* EX1002 ¶ 90.

What, then, is the alleged invention of the '207 Patent? Nothing more than the trivial idea of using the prior art tagmentation process to cell nuclei so as to fragment and tag DNA as it exists in cells in its naturally-occurring form, known as “chromatin.” For instance, in parallel district court litigation, Patent Owner acknowledged that tagmentation has long been known, but that it was supposedly new to apply tagmentation to a cell. As Patent Owner argued, “tagmentation was being used as a way of doing that fragmenting process. And it was being used on DNA that had already been purified, it wasn't being used on a cell. Right?” EX1024 at 106:6-9.

Yet, there is nothing inventive or novel about applying tagmentation to a cell nucleus. Any such contention that this is inventive is negated by the earliest patent

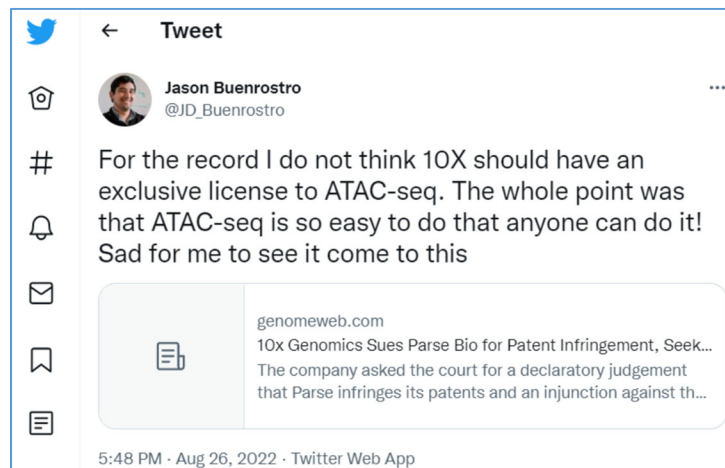
filings on the tagmentation process, such as the Grunenwald reference. Grunenwald unambiguously teaches applying tagmentation to living cells *in vivo* by permeabilizing a cell so the machinery of tagmentation can be introduced into the cell:

In some embodiments, the insertion of a transposon end into target DNA according to the present invention can also be carried out in vivo. If transposition is carried out in vivo, transposition into the target DNA is preferably achieved by electroporating a synaptic complex of a transposase and a suitable transposon end composition *into the host cell* as described in U.S. Pat. No. 6,159,736 (herein incorporated by reference).

EX1003 at [0157].

As proven herein and in the accompanying declaration of Dr. Gregory Cooper, the application of tagmentation to cells, and thus the cell nucleus, will unavoidably involve chromatin and Grunenwald thus anticipates and/or renders obvious every claim of the '207 Patent. In allowing the claims of the '207 Patent, however, the Examiner performed no analysis of Grunenwald, and certainly did not consider the disclosure of the cell-based embodiment taught in Grunenwald. See EX1007.152-156; 209-210. The Board should now take this opportunity to correct this oversight.

Indeed, any suggestion that the claimed invention of the '207 Patent is novel or inventive is put to rest by the primary inventor, Dr. Jason Buenrostro. He and the other inventors termed the application of prior art tagmentation to chromatin “ATAC-seq,” which is short for “Assay for Transposase Accessible Chromatin followed by high-throughput sequencing.” EX1001 at 29:63-64. In a tweet shortly after Patent Owner filed a lawsuit against Petitioner based on *inter alia* the '207 Patent, he lamented the litigation and confirmed that the application of “tagmentation” to chromatin was “so easy that anyone can do it!”



EX1029. Because the alleged inventions in the '207 Patent are not new and reflect nothing more than the previously disclosed application of tagmentation to cell nuclei, the claims of the '207 Patent should be cancelled.

II. MANDATORY NOTICES

A. Real Party-in-Interest

The real party-in-interest is Parse Biosciences, Inc.

B. Related Matters

As of the filing date of this Petition, the '207 Patent is involved in litigation in *10x Genomics v. Parse Biosciences, Inc.*, 22-cv-01117 (D. Del. filed Aug. 8, 2022).

C. Grounds for Standing

Petitioner certifies that the '207 Patent is available for *inter partes* review and that Petitioner is not barred or estopped from requesting *inter partes* review challenging the patent claims on the grounds identified in this Petition.

D. Lead and Back-Up Counsel and Service Information

Petitioner provides the following designation of Lead and Back-Up Counsel.

Lead Counsel	Back-Up Counsel
Derek C. Walter Registration No. 74,656 (derek.walter@weil.com)	Adrian C. Percer Registration No. 46,986 (adrian.percer@weil.com)
Postal & Hand-Delivery Address: Weil, Gotshal & Manges LLP 201 Redwood Shores Parkway Redwood Shores, CA 94065 T: 650-802-3934 F: 650-802-3100	Postal & Hand-Delivery Address: Weil, Gotshal & Manges LLP 201 Redwood Shores Parkway Redwood Shores, CA 94065 T: 650-802-3124 F: 650-802-3100
	William Sutton Ansley Registration No. 67,828 (sutton.ansley@weil.com)
	Postal & Hand-Delivery Address: Weil, Gotshal & Manges LLP 2001 M Street NW, Suite 600 Washington, DC 20036 T: 202-682-7018 F: 202-857-0940

III. RELATIONSHIP BETWEEN THIS IPR AND RELATED PROCEEDINGS

In addition to seeking review of the '207 Patent in the instant petition, Petitioner is also seeking review of U.S. Patent Nos. 10,150,995 (the "'995 Patent") and 10,738,357 (the "'357 Patent) in co-pending *inter partes* reviews. Petitioner requested review of the '995 Patent on April 20, 2023. *See* Paper No. 1, IPR2023-00878.

Petitioner notes that the claims of the '207 Patent at issue here are highly similar to those of the '995 and '357 Patents. Accordingly, the technical background material appearing in the Petition and supporting expert declaration in IPR2023-00878 is directly applicable to not only the instant Petition but also the co-pending petition directed to the '995 Patent. For the convenience of the Board, Petitioner notes that it should be sufficient for the Board to review only the technical background sections in IPR2023-00878 to gain an understanding of the necessary technical subject matter relevant to all three petitions.

IV. IDENTIFICATION OF CHALLENGE

Petitioner presents the following challenges to the claims of the '207 Patent:

- **Ground 1:** Claims 1-16 and 22-24 of the '207 Patent are unpatentable under 35 U.S.C. § 103 over Grunenwald (US 2010/0120098 (EX1003)) and Song (Cold Spring Harb Protoc;

2010; doi:10.1101/pdb.prot5384 (EX1004)) in view of the knowledge of a POSA.

- **Ground 2:** Claims 17-21 and 26-29 of the '207 Patent are unpatentable under 35 U.S.C. § 103 over O'Geen ("Using ChIP-Seq Technology to Generate High-Resolution Profiles of Histone Modifications" (EX1005)) in view of Grunenwald and Song and the knowledge of a POSA.
- **Ground 3:** Claims 13-16 Are Unpatentable Under 35 U.S.C. § 103 over Grunenwald and Song In View Of Adey ("Rapid, Low-input, Low-bias Construction of Shotgun Fragment Libraries by High-density *in vitro* Transposition" (EX1009)) and the knowledge of a POSA.

V. TECHNOLOGICAL BACKGROUND

A. DNA and RNA

Deoxyribonucleic acid, or DNA, is a double-stranded molecule having a chain of bases or "nucleotides." EX1002 ¶¶ 41-42. Each nucleotide comprises of four types of bases: adenine ("A"), cytosine ("C"), guanine ("G"), and thymine ("T"). *Id.* ¶ [[42]]. The order of these bases constitute genetic information, which can be copied to a ribonucleic acid ("RNA") strand, by a process called transcription. *Id.* ¶ [[47]].

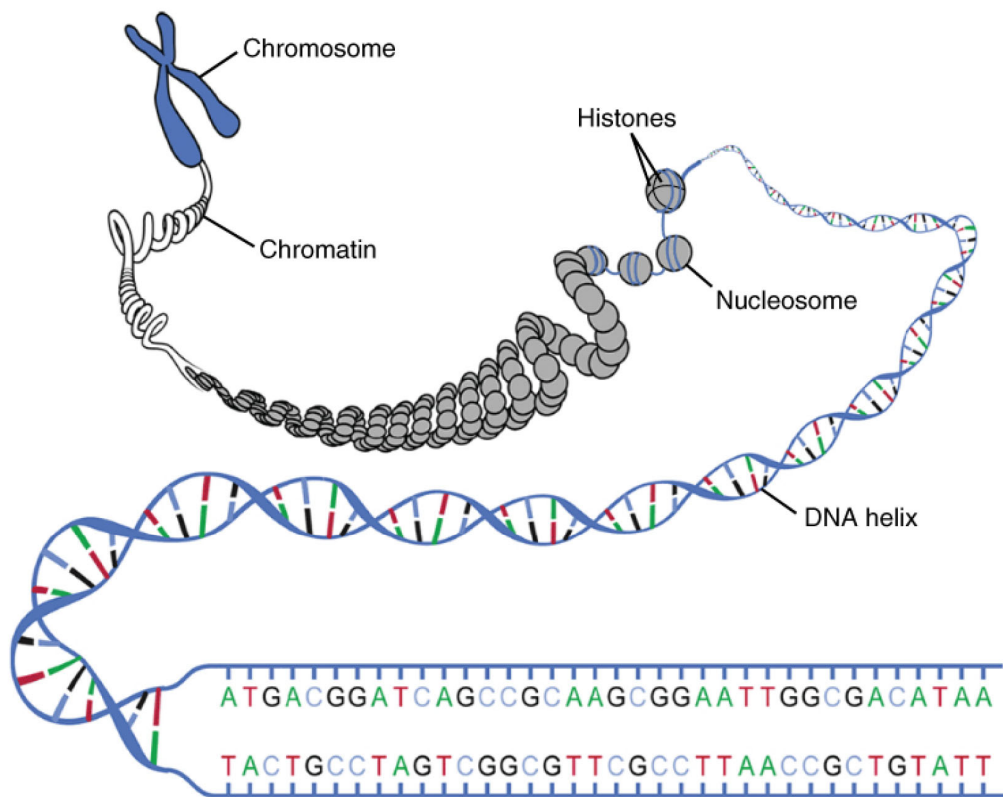
RNA is a molecule similar to DNA, where the nucleotides in RNA are adenine, cytosine, guanine, and uracil. Uracil and thymine are functionally

equivalent including for both information storage and transmission. *Id.* ¶¶ 44-45; EX1044.001. A specific type of RNA—messenger RNA (“mRNA”)—is read by ribosomes to make proteins. EX1002 ¶ 47. mRNA is critical to the study of epigenetics, or gene expression, because it shows the portions of DNA being copied for the production of proteins—*i.e.*, which genes are expressed. *Id.* ¶ 50.

B. DNA Packaging

For all of the DNA in a cell to fit inside of the nucleus of a cell, it must be very tightly packaged. EX1002 ¶ 52. This packaging is achieved with the aid of proteins called “histones,” which act as scaffolding around which the DNA is coiled. A histone protein with DNA coiled around it is a “nucleosome.” The collection of nucleosomes together is known as “chromatin.” *Id.* ¶¶ 55-56.

Chromatin is further packaged and condensed into chromosomes. *Id.* ¶ 53. This format for packaging DNA is shown in the figure below, which shows that an individual chromosome is made up of chromatin, which, in turn, consists of DNA wrapped around histones:



The DNA packaging depicted above is not static, but is rather dynamic, changing in the cell depending on the phase of the cell's life cycle. EX1002 ¶ 60. For example, at one point in time a portion of chromatin may be considered “closed” when the DNA in that portion is tightly coiled around a histone, but at another point in time the same chromatin portion may be considered “open,” when the DNA is not coiled, or not as tightly coiled, around the histone. *Id.* ¶ 60-61.

This accessibility of chromatin is directly associated with gene expression. *Id.* ¶ 61. While open chromatin regions are loosely packaged and hence more accessible to the cellular machinery for transcription, closed chromatin regions are tightly packaged and less accessible. *Id.*

C. Sequencing and Tagmentation

Nucleic acid sequencing, loosely speaking, is the process of determining the sequence of nucleotides in a polynucleotide such as DNA. EX1002 ¶ 62. Amongst the nucleic acid sequencing technologies, Next Generation Sequencing (“NGS”) emerged in the early 2000s. EX1033.002. NGS is a type of high-throughput sequencing technology that uses parallel sequencing of many fragments of DNA, enabling processing and detection of millions of sequence reads in a single run. EX1033.003. NGS platforms have a common workflow including the steps of “library preparation” (*i.e.*, making a collection of nucleic acid fragments), amplification, and sequencing. EX1002 ¶ 63.

Importantly, many NGS platforms are so-called “short read” sequencing platforms. In such platforms, one can generate only a short string of DNA sequence information from a nucleic acid molecule, typically only a couple of hundred nucleotides in length. The NGS platforms that are by far most commonly used in industry and academia, such as those sold by Illumina, fall into this “short read” category. For this reason, prior to sequencing, it is commonly essential for DNA to be broken up (*i.e.*, fragmented) into small chunks so that it is suitable for the sequencing instrument. EX1002 ¶ 64.

Historically, this was done through a process known as chromatin immunoprecipitation. *See, e.g.*, EX1022. In this process, chromatin is treated

with antibodies to pull DNA out of solution, at which point it could be fragmented by either digestion with restriction enzymes or by sonication. EX1005.005.

Library preparation, however, including the necessary fragmentation, can also be, and often is, accomplished by “tagmentation,” which was developed by researchers as early as 2010 to reduce the time and cost of preparing DNA libraries for NGS. EX1002 ¶ 65; EX1009.002; EX1008.003. Tagmentation is a one-step reaction where accessible DNA is fragmented and tagged for analysis. EX1008.003. A specific type of enzyme—transposase—can carry out this single-step reaction. EX1008.004. Oligonucleotide tags can be discrete segments of DNA with pre-defined lengths and sequences such that each tag is identifiable during sequencing, but tags may serve a variety of other purposes. EX1002 ¶ 65; EX1009.002; EX1010.002.

VI. OVERVIEW OF THE '207 PATENT

A. Priority Date of the '207 Patent

The '207 Patent, which was filed on May 21, 2019, identifies a provisional application, which was filed on May 23, 2013. EX1001 at (60). Thus, the priority date of the '207 Patent is no earlier than the date of this provisional application—May 23, 2013.

B. Overview of the Challenged Claims and Specification

The '207 Patent pertains to applying the prior art tagmentation technique to open chromatin, and sequencing the resulting tagged chromatin fragments.

Exemplary Claim of the '207 Patent is as follows.

1. A method for generating a sequencing library from a plurality of cells, comprising:

(a) lysing a plurality of cells to provide a plurality of cell nuclei, wherein the plurality of cell nuclei comprises chromatin;

(b) contacting a cell nucleus of the plurality of cell nuclei with a transposase complex such that polynucleotides of the cell nucleus are tagmented at regions of open chromatin to produce a plurality of tagged fragments; and

(c) performing one or more nucleic acid reactions on the tagged fragment to produce a sequencing library.

Step (a) of Claim 1 of the '207 Patent recites lysing cells to generate cell nuclei comprising chromatin. Lysing cells for this purpose had long been understood and widely adapted in genomics experiments. EX1002 ¶¶ 89,90,141.

Step (b) of Claim 1 of the '207 Patent recites using a “transposase complex”, an example of an insertional enzyme complex, on open chromatin to generate fragmented and tagged genomic nucleic acid molecules. EX1001 at Claim 1. The '207 Patent defines the term “insertional enzyme complex” to mean a complex

comprising an insertional enzyme (e.g., a transposase) and a payload of DNA tag sequences. EX1001 at 13:23-30; *see id.* at 14:32-34 (explaining that the insertional enzyme can be a transposase).

But as explained above, tagmentation—using a transposase to tag a target polynucleotide sequence with tag sequences—had been known and widely used in the art as of the filing of the '207 Patent. *See* Section IV.C, *supra* (explaining tagmentation has been known since 2010).

Indeed, the specification of the '207 Patent readily admits that tagmentation had long since been published and was even the subject of commercial product offerings that could be “readily adapted” for the alleged invention of the '207 Patents:

Methods for tagmenting isolated genomic DNA are known in the art (see, e.g., Caruccio *Methods Mol. Biol.* 2011 733: 241-55; Kaper et al, *Proc. Natl. Acad. Sci.* 2013 110: 5552-7; Marine et al, *Appl. Environ. Microbiol.* 2011 77: 8071-9 and US20100120098) and are commercially available from Illumina (San Diego, Calif.) and other vendors. Such systems may be readily adapted for use herein.

EX1001 at 14:35-42.

Independent claim 1 additionally recites in step (c) the conventional process of producing a sequencing library of tagged nucleic acid molecules. EX1001 at

Claim 1. As explained above, methods for sequencing nucleic acid molecules, including next-generation sequencing, had also been known and widely practiced in the art before the earliest priority date of the '207 Patent. Section IV.C, *supra*. The '207 Patent thus admits that sequencing techniques were well-known in the prior art and commercialized. EX1001 at 15:4-11. Similarly, producing sequencing libraries was well-known and widely practiced in the art before the earliest priority date of the '207 Patent. EX1002 ¶ 62.

As further documented herein *infra*, the '207 Patent's dependent claims further recite nothing more than aspects of genetic analysis that had also long been well-known in the art.

C. Prosecution History Overview

During prosecution, the Applicant submitted an Information Disclosure Statement identifying Grunenwald. EX1007.011. In the List of References cited by the applicant and considered by examiner, the examiner indicated that Grunenwald and Song were acknowledged and considered. EX1007.182. The examiner, however, neither cited nor discussed either Grunenwald or Song. O'Geen was never cited in the '207 Patent application, included in the IDS, or, by any indication, considered by the examiner.

The examiner issued one non-final rejection during the prosecution of the patent. EX1007.151-157. In the non-final rejection, the Examiner rejected a

number of claims as being indefinite. The Examiner also cited Boles as teaching a reagent system and method for generating DNA sequencing libraries and Fernandez *et al.* as teaching a method for tagging chromatin. EX1007.154-155. The Examiner then stated that the prior art considered does not teach “contacting the cell nucleus of the plurality of cell nuclei with a transposase complex such that polynucleotides of the cell nucleus are tagmented at regions of open chromatin to produce a plurality of tag fragments in combination with the additional steps.” EX1007.155.

As documented herein, however, the Examiner’s conclusion that the prior does not teach the application of a transposase complex to a cell nucleus was an error that stemmed from the Examiner’s failure to recognize express teachings to this effect in Grunenwald. The ’207 Patent then issued on April 14, 2020. EX1007.249. The Notice of Allowance did not provide any reasoning or explanation as to how the claims of the ’207 Patent are novel or inventive over the prior art. *See* EX1007.209-221.

VII. LEVEL OF ORDINARY SKILL IN THE ART OF THE ’207 PATENT

A person of ordinary skill in the art (“POSA”) relating to the ’207 Patent would have had (1) a Masters and/or Ph.D. in molecular biology, genetics, chemistry, engineering, or a related discipline and at least two years of post-Masters, postdoctoral, or industry experience or (2) a Bachelor of Science in such disciplines

and at least five years of academic or industry experience (including any experimental work toward a graduate degree), relating to DNA sample preparation techniques (including electroporation and permeabilization), tagmentation, amplification methods, and DNA sequencing technologies (including next generation sequencing).

As documented above, all aspects of the claims of the '207 Patent reflect techniques and competencies that would have been part of the toolkit of the ordinary skilled artisan. *See supra* Part V. In particular, the ordinary skilled artisan in 2013 would have been familiar with and would have fully understood the following concepts and techniques:

- The format of DNA packaging in living cells.
- The dynamic nature of DNA packaging in living cells.
- Next generation sequencing techniques, and methods of preparing nucleic acid for sequencing.
- The tagmentation process, and commercially available products for carrying out tagmentation.
- Methods of permeabilizing cells for nucleic acid analysis.
- Methods of lysing cells for nucleic acid experiments.
- Epigenetic analysis and techniques therefore.

Id.; *see also* EX1002 ¶¶ 93-94.

VIII. THE CITED PRIOR ART

A. Grunenwald

Grunenwald, entitled “Transposon End Compositions And Methods For Modifying Nucleic Acids,” was published on May 13, 2010. EX1003.001. Grunenwald is prior art under 35 U.S.C. § 102(a).

Grunenwald discloses methods of using transposase enzymes to generate libraries of tagged DNA fragments for sequencing and analysis. *See, e.g.*, EX1003 at [0017]. According to one method, Grunenwald teaches incubating the DNA with transposase and what it calls “transposon end compositions.” *Id.* at [0162]; *see also id.* at [0093]-[0094].

Grunenwald explains that a “transposon end composition” comprises tags, which may further comprise a “tag domain,” such as a “restriction site tag domain, a capture tag domain, a sequencing tag domain, an amplification tag domain, a detection tag domain, an address tag domain, and a transcription promoter domain.” *Id.* at [0103]. Grunenwald explains that as a result of this incubation, the transposase enzyme can fragment the DNA while simultaneously inserting tags into the DNA fragment. *Id.* at [0093].

Grunenwald teaches that the target DNA for fragmenting and tagging may be derived from any *in vivo* or *in vitro* source, such as “one or multiple cells, tissues, organs or organisms, whether living or dead, or from any biological or

environmental source (e.g., water, air, soil).” *Id.* at [0146]. According to Grunenwald, the DNA derived from these sources may be selected from a variety of types of DNA. *Id.*

Critically, Grunenwald further teaches that its methods of transposition fragmentation and tagging may be carried directly on target DNA found in a host cell, by “electroporating a synaptic complex of a transposase and a suitable transposon end composition into the host cell.” *Id.* at [0157]. A POSA reading these disclosures would have understood that performing transposition fragmentation and tagging on chromosomal target DNA within a host cell would mean that Grunenwald’s methods were applicable to chromatin in the nucleus of the cell. EX1002 ¶ 101.

B. Song

Song is a 2010 publication in Cold Springs Harbor Protocols entitled “DNase-seq: A High-Resolution Technique for Mapping Active Gene Regulatory Elements across the Genome from Mammalian Cells.” *See* EX1004. Song is prior art under 35 U.S.C. § 102(a).

Song teaches a technique for digesting regions of DNA in a cell that are not “tightly wrapped in nucleosome and higher-order structures” so that they can be subject to DNA sequencing. EX1004.001. This allows researchers to identify the “most active regulator regions from potentially any cell type.” *Id.* In connection

with this approach, Song teaches a technique for isolating cell nuclei by applying a lysis buffer containing the IGEPAL detergent to cells followed by centrifugation. *See id.* at .003.

C. O’Geen

O’Geen is a 2011 publication entitled “Using ChIP-Seq Technology to Generate High-Resolution Profiles of Histone Modifications.” EX1005.003. O’Geen is prior art under 35 U.S.C. § 102(a).

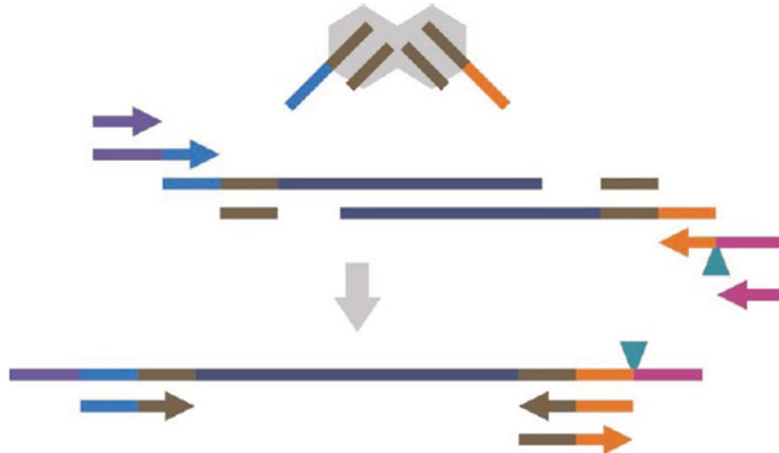
O’Geen teaches a prior art method of fragmenting and tagging DNA in preparation for sequencing and then using sequence information to determine epigenetic features of the DNA, known as chromatin immunoprecipitation and sequencing (ChIP-Seq). EX1005.005.

D. Adey

Adey is a 2018 publication entitled “Rapid, Low-input, Low-bias Construction of Shotgun Fragment Libraries by High-density *in vitro* Transposition” and was published in Genome Biology in 2010. EX1009.001. Adey is prior art under 35 U.S.C. § 102(a).

Adey describes a prior art method of tagmentation using Epicentre’s Nextera™ Technology—a transposase-based process for preparing DNA libraries by simultaneously fragmenting and tagging genomic DNA. Specifically, Adey discloses using a transposase to insert distinct adapter sequences. EX1009.002.

As depicted in Figure 1 (b), the transposome complex comprises the transposase enzyme (two grey hexagons), a sequence to assist the transposase to fragment DNA (brown), and two different adaptors (mild blue/orange):



EX1009.002 at Figure 1.

IX. CLAIM CONSTRUCTION

This Petition analyzes the claims using the ordinary and customary meaning in light of the specification and prosecution history. 37 C.F.R. § 42.100(b).

A. “Insertional Enzyme Complex”

The '207 Patent defines the term “insertional enzyme complex” as follows: “The term ‘insertional enzyme complex,’ as used herein, refers to a complex comprising an insertional enzyme and two adaptor molecules (the ‘transposon tags’) that are combined with polynucleotides to fragment and add adaptors to the

polynucleotides.” EX1001 at 13:23-30. This definition is consistent with the plain and ordinary meaning of the term as understood by a POSA. EX1002 ¶ 122.

B. “Nucleic Acid Insert Element”

The plain and ordinary meaning of a “nucleic acid insert element” in the context of the ’207 Patent is a nucleic acid for insertion in a polynucleotide by an insertional enzyme, such as transposase. EX1002 ¶ 123. This meaning is consistent with plain language of the claim term. EX1001 at claims 9, 13. Moreover, the ’207 Patent specification describes that an “insert element” may comprise a nucleic acid sequence and can be part of a composition also including a polynucleotide and an insertional enzyme. *See* EX1001 at 3:62-4:3, 4:22-29, 22:31-36, 22:50-54. The insert element can also be “bound to the insertional enzyme and the insertional enzyme is bound to the polynucleotide.” EX1001 at 4:10-11.

C. “Adapter Sequence”

The plain and ordinary meaning of an “adapter sequence” is the sequence of an adapter, which in the ’207 Patent refers to a tag. EX1002 ¶ 124. Indeed, the ’207 Patent uses the term “adaptor” interchangeably with “tag.”

For example, when defining an “insertional enzyme complex,” the ’207 Patent states that the complex comprises “an insertional enzyme”—*e.g.*, a transposase—“and two *adaptor molecules* (the ‘transposon *tags*’).” EX1001 at 13:23-27. This

description continues by explaining that the insertional enzyme complex is “combined with polynucleotides to fragment and *add adaptors* to the polynucleotides.” *Id.*

A POSA would have understood that this disclosure of the insertional enzyme complex fragmenting and adding adaptors is equivalent to the insertional enzyme complex fragmenting and tagging, as described throughout the '207 Patent. *See, e.g.,* EX1001 at Abstract (“...treating chromatin...with an insertional enzyme complex to produce *tagged* fragments....”) (emphasis added); *id.* at 14:57-60 (“...combining the isolated nuclei with the transposase and adaptors, wherein the combining results in...production of the adaptor-*tagged* fragments of genomic DNA....”); *id.* at 15:28-33 (“...inserting a plurality of molecular *tags* with an insertional enzyme into the polynucleotide...”) (emphasis added); *id.* at 14:27-31 (“...treating chromatin isolated from a population of cells with an insertional enzyme complex to produced *tagged* fragments of genomic DNA.”) (emphasis added); EX1002 ¶ 126.

Moreover, the use of the term “adaptor” in the '207 Patent specification is consistent with it being a tag. For example, the '207 Patent explains one process by using tagging and adding adaptors interchangeably: “chromatin is tagmented (*i.e.,* cleaved and *tagged* in the same reaction) using an insertional enzyme such as Tn5 or MuA that cleaves the genomic DNA in open regions in the chromatin and *adds*

adaptors to both ends of the fragments.” EX1001 at 14:31-35. The ’207 Patent only uses the term “adaptor” to refer to an element tagged to a polynucleotide.

Although the ’207 Patent also refers to a “sequencing adaptor,” this is just one type of tag or adaptor. See EX1001 at 16:30-34 (“The molecular tags can comprise sequencing adaptors....”).

D. “Barcode Sequence”

The ’207 Patent defines the term “barcode sequence” as follows:

The term “barcode sequence” or “molecular barcode,” as used herein, refers to a unique sequence of nucleotides used to a) identify and/or track the source of a polynucleotide in a reaction and/or b) count how many times an initial molecule is sequenced (e.g., in cases where substantially every molecule in a sample is tagged with a different sequence, and then the sample is amplified).

EX1001 at 12:30-36. This definition is consistent with the meaning of the term as understood by a POSA. EX1002 ¶ 125.

E. Tagged Fragments

The ’207 Patent defines the term “tagged fragments” as “polynucleotide fragments that are attached to tags.” EX1001 at 13:31-32. This definition is consistent with the meaning of the term as understood by a POSA. EX1002 ¶ 126.

X. IDENTIFICATION OF HOW THE CHALLENGED CLAIMS ARE UNPATENTABLE

A. Ground 1: Claims 1-16 and 22-24 of the '207 Patent Are Unpatentable Under 35 U.S.C. § 103 Over Grunenwald in View Of Song And The Knowledge Of A POSA

1. Claim 1:

a. [Preamble] A method for generating a sequencing library from a plurality of cells, comprising:

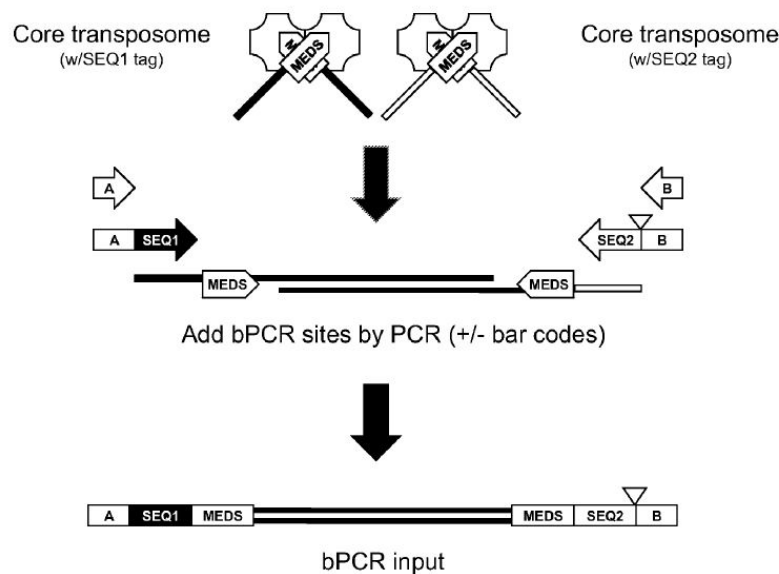
Grunenwald discloses methods for generating a sequencing library from a plurality of cells—specifically, a library of tagged DNA fragments. Grunenwald discloses “methods, compositions and kits for using transposase and a transposon end compositions for generating a library of tagged DNA fragments from target DNA.” EX1003 at [0002]. Grunenwald points out that the “[t]arget DNA’ can be derived from any in vivo or in vitro source, including *from one or multiple cells.*” EX1003 at [0146].

Grunenwald describes “incubating the target DNA with a transposase and a transposon end or transposon end composition” under such conditions that allow for a transposition reaction to occur, wherein the “target DNA is fragmented to generate a plurality of target DNA fragments” *Id.* at [0018]. Furthermore, Grunenwald explains that in this process, the “transferred strand of the transposon end or transposon end composition is joined to the 5' ends of each of a plurality of the target DNA fragments” *Id.*

Grunenwald describes the DNA fragment library as the population of tagged DNA fragments from target DNA, “wherein the combination of the tagged DNA fragments in the collection or population exhibits sequences that are qualitatively and/or quantitatively representative of the sequence of the target DNA from which the tagged DNA fragments were generated.” *Id.* at [0121].

Grunenwald further discloses that performing “fragmentation and tagging of target DNA” to produce a sequencing library “by insertion of tagged transposon ends in a transposition reaction, followed by tag-specific PCR+/- barcodes” that are compatible with Solexa/Illumina systems. *Id.* at [0071]. This process is depicted in Figure 13:

FIG. 13 Preparation of Bar Coded Illumina GAI-
Compatible Sequencing Libraries



- b. [1A] lysing a plurality of cells to provide a plurality of cell nuclei, wherein the plurality of cell nuclei comprises chromatin;**

Song teaches a method for lysing cells to provide a plurality of cell nuclei, wherein the plurality of cell nuclei comprises chromatin. And it would have been obvious to incorporate the method of Song into the tagmentation approach set forth in Grunenwald.

First, as documented above, Song teaches a routine method for nuclei isolation based on cell lysis using the IGEPAL detergent and that includes subsequent centrifugation. See EX1004.003; *supra* Section VIII.B. The lysis buffer used in Song comprises 10 mM Tris-Cl, pH7.4, 10mM NaCl, 3 mM MgCl₂, and 0.1% IGEPAL CA-630. The '207 Patent uses the exact same protocol based on the same detergent. See EX1001 at 25:4-15. Thus, the '207 Patent confirms that its approach for isolating nuclei via cell lysis was not new or special, but was rather merely a prior art approach taken verbatim from the Song reference, a publication from the 2010 volume of the Cold Spring Harbor Protocols, which, as Dr. Cooper explains, is a common source for routine laboratory protocols. EX1002 ¶ 139.

Second, it would have been obvious to include the Song protocol for isolating nuclei via cell lysis into the Grunenwald tagmentation technique and a person of ordinary skill would have been motivated to do so. As an initial matter,

Grunenwald and Song are analogous art. Both references are in the field of genetics and molecular biology. EX1002 ¶¶ 143-144. Further, both references cover the study of DNA modification and subsequent sequencing. *See, e.g.*, EX1003 at Abstract; EX1004.001. Indeed, Song is directed to a technique for analyzing DNA regions from cellular nuclei that are not “tightly wrapped in nucleosome and higher-order structures” and capturing such DNA so that it can be sequenced “by high-throughput, next-generation sequencing.” EX1004.001. Thus, the person of ordinary skill in the art with Grunenwald in hand would have naturally been inclined to consider Song.

In this regard, Grunenwald broadly teaches that its tagging method can be applied to any source of DNA. As Grunenwald explains, for instance, the target DNA “comprises or consists of genomic DNA, subgenomic DNA, *chromosomal DNA* (e.g., from an isolated chromosome or a portion of a chromosome, e.g., from one or more genes or loci from a chromosome), mitochondrial DNA, chloroplast DNA, plasmid or other episomal-derived DNA (or recombinant DNA contained therein), or double-stranded cDNA made by reverse transcription of RNA using an RNA dependent DNA polymerase or reverse transcriptase to generate first-strand cDNA and then extending a primer annealed to the first-strand cDNA to generate dsDNA.” EX1003 at [146].

Consistent with Grunenwald's teaching that its tagmentation method can be applied to just about any source of DNA—including chromosomal DNA—Grunenwald also discloses that its methods can be applied to DNA *in vivo*, which, as Dr. Cooper explains, would necessarily entail application of the method to chromosomal DNA in nuclei because chromosomal DNA exists in nuclei in cells *in vivo*. See EX1003 at [0146], [0157]; EX1002 ¶¶ 134-135. This *in vivo* embodiment in Grunenwald is accomplished by electroporating the cell to allow the transposome to enter the cell:

In some embodiments, the insertion of a transposon end into target DNA according to the present invention can also be carried out *in vivo*. If transposition is carried out *in vivo*, transposition into the target DNA is preferably achieved by electroporating a synaptic complex of a transposase and a suitable transposon end composition into the host cell as described in U.S. Pat. No. 6,159,736 (herein incorporated by reference).

EX1003 at [0157]. As Dr. Cooper explains, chromosomal DNA in a cell is packaged in nuclei in the form of chromatin. See EX1002 ¶¶ 52-59. By teaching a method whereby tagging is performed using an insertional enzyme complex *in vivo*, Grunenwald necessarily and inherently discloses performing its method on a plurality of cell nuclei wherein the cell nuclei comprises chromatin.

While Grunenwald teaches the use of electroporation so that the insertional enzyme complex can be introduced to the nuclear chromatin, a POSA interested in studying the DNA that is tagmented in Grunenwald's *in vivo* embodiment would be generally aware of the need to make the cellular DNA in the nuclei accessible for the tagmentation process. EX1002 ¶ 135. Rather than using the permeabilization method set forth in Grunenwald, it would have been a simple design choice to instead simply isolate the nuclei from the cells. The '207 Patent even admits that "the chromatin used in the method may be made by any suitable method." EX1001 at 14:45-47. The '207 Patent itself thus makes clear that the technique for making DNA accessible is not a special aspect of the invention.

Lysing the cell so as to isolate the nuclei for these purposes was a well-known, routine and commonly-used method and would have provided direct access to the genetic material in the nuclei. EX1002 ¶¶ 136-137. As Dr. Cooper explains, several methods of lysis were well-known and published long before 2013 for uses in a variety of cell types. EX1002 ¶ 136. For example, lysis may be done by application of a detergent, which is by far the most common method, but can also be accomplished mechanically or enzymatically. *Id.* Consistent with this, the '207 Patent does not contemplate any method of lysis that is novel or unique to the alleged invention nor does it characterize lysis for nuclei isolation as an inventive part of the protocol. Instead, it simply uses the prior art Song protocol.

Third, a POSA would have had a reasonable expectation of success in combining Song with Grunenwald. Grunenwald teaches a method for the modification of chromatin through tagmentation, analogous to the methods of chromatin modification taught in Song. *See supra* Sections X.A.1.b; *see also* EX1002 ¶¶ 143-146. A POSA combining the two would have a reasonable expectation of success on the basis that the references provide compatible methods of accomplishing analogous goals. EX1002 ¶ 146.

Additionally, cell lysis had long been used as a technique to break open a cell and access the interior; indeed, in the context of other assays requiring access to the organelles and proteins present in a cell, lysis has been described as “perhaps the most crucial step.” EX1002 ¶ 147; *see also* EX1055.004. A POSA would also have a reasonable expectation of success because Song teaches the combination of lysing a cell to provide nuclei and subsequently contacting the nuclei with an enzyme to modify the chromatin present. EX1002 ¶ 148. A POSA performing Grunenwald’s *in vivo* tagmentation method, combined with the nuclei isolation procedure based on application of a lysis buffer that is taught in Song, would have had a reasonable expectation that they would be successful in isolating and modifying DNA in preparation for sequencing. *Id.*

- c. **[1B] contacting a cell nucleus of the plurality of cell nuclei with transposase complex such that polynucleotides of the cell nucleus are tagged at regions of open chromatin to produce a plurality of tagged fragments; and**

Grunenwald discloses this claim element.

First, Grunenwald discloses a transposase complex. As explained in Section VIII.A, *supra*, a transposase complex is an example of an “insertional enzyme complex,” which the ’207 Patent defines as “a complex comprising an insertional enzyme and two adaptor molecules (the ‘transposon tags’) that are combined with polynucleotides to fragment and add adaptors to the polynucleotides.” EX1001 at 13:24-27. The ’207 Patent states that this “insertional enzyme can be a transposase.” *Id.* at 3:44-45.

Grunenwald discloses this transposase complex, which has a transposase and two transposon tags: “a single *transposome composition* comprising a stable complex formed between the transposase and the transposon end composition.” EX1003 at [0161] (emphasis added) (further explaining that “it will be understood that any method that describes the use of a transposase and a transposon end composition could also use a transposome composition....”).

Grunenwald explains that a “transposon end composition” may comprise multiple tags:

A “transposon end composition” means a composition comprising a transposon end (*i.e.*, the minimum double-stranded DNA segment that is capable of acting with a

transposase to undergo a transposition reaction), optionally plus additional sequence *or sequences*[] 5'-of the transferred transposon end sequence and/or 3'-of the non-transferred transposon end sequence. For example, a transposon end attached to a tag is a “transposon end composition.”

EX1003 at [0098]. According to this passage, Grunenwald’s transposon end composition may have a “sequence or sequences,” which are tags for the 5’ end of the transferred polynucleotide strand and the 3’ of the non-transferred polynucleotide strand. EX1002 ¶ 153; *see also* EX1003 at [0102] (explaining that the transposon end composition may include a “transposon end sequence” plus a “tag [that] can have one or more other tag portions or tag domains.”).

In addition, Grunenwald discloses that each tag of a transposon end composition “can have one or more other tag portions or tag domains,” which a POSA would have understood also teaches the tags of the ’207 Patent’s insertional enzyme complex. EX1003 at [0102]; *see also id.* at [0103] (defining “tag portion” and “tag domain” as “a portion or domain of a tag that exhibits a sequence for a desired intended purpose or application.”); EX1002 ¶ 156. These tag domains can include, for example, “one or more of a restriction site tag domain, a capture tag domain, a sequencing tag domain, an amplification tag domain, a detection tag domain, an address tag domain, and a transcription promoter domain” EX1003

at [0103]. Further, Grunenwald teaches that “one tag domain can comprise two or more other tag domains....” *Id.* at [0115]. Thus, Grunenwald discloses a transposome complex comprising a transposase and at least two tags.

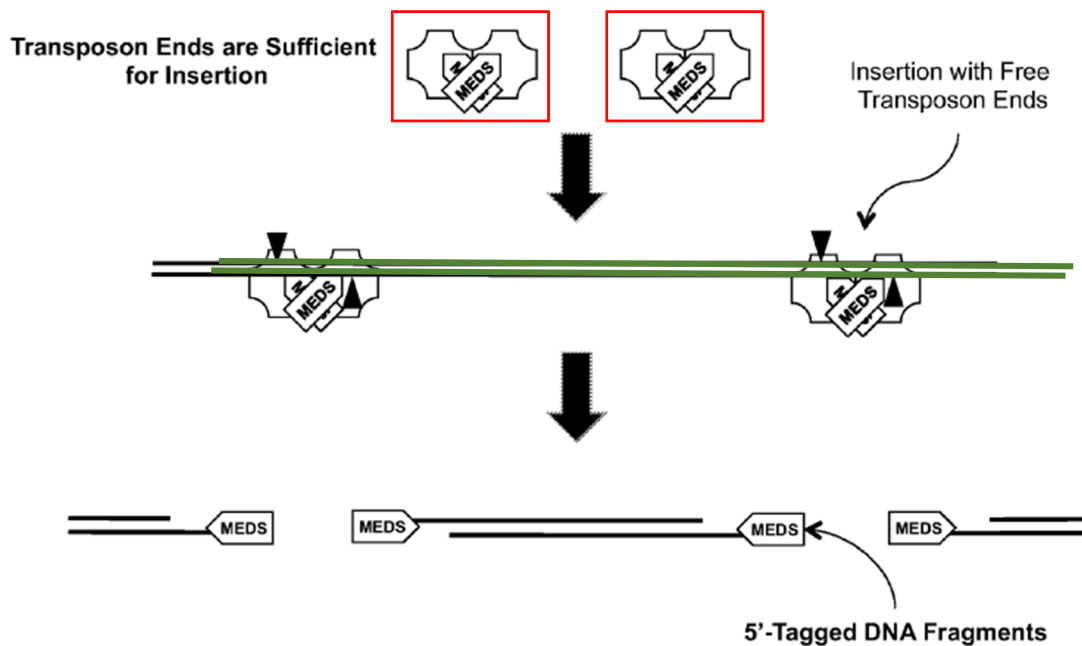
As Grunenwald explains, this transposase complex comprising a transposase and at least two tags is combined with polynucleotides to fragment and add adaptors to the polynucleotides. For example, Grunenwald discloses that one embodiment of the invention is a “method for generating a library of tagged DNA fragments,” with the method comprising in part “incubating the target DNA in an in vitro transposition reaction with at least one transposase and transposon end composition with which the transposase forms a transposition complex...under conditions and for sufficient time wherein multiple insertions into the target DNA occur, each of which results in joining of a first tag comprising or consisting of the transferred strand to the 5’ end of a nucleotide in the target DNA, thereby fragmenting the target DNA and generating a population of annealed 5’-tagged DNA fragments.” EX1003 at [0161].

Grunenwald therefore discloses the claimed transposase complex that comprises a transposase and at least two adaptors (*e.g.*, tags, tag portions, or tag domains) that combine with polynucleotides of a cell nucleus (*e.g.*, target DNA) to tag said polynucleotides.

Second, Grunenwald discloses that the transposase complex contacts cell nuclei “to produce a plurality of tagged fragments.” The explanation of how the transposase complex tags polynucleotides is set forth above, but it is also described throughout Grunenwald’s disclosure. *See supra* Section X.A.1.c; *see, e.g.*, EX1003 at [0017] (summarizing portions of the invention).

For example, Figure 2 of Grunenwald, reproduced below, showcases the disclosure of this limitation. *See* EX1003 at [0060]. In this figure, the two parallel lines highlighted in green represent the target DNA and the transposomes (which are the claimed transposase complex) are boxed in red. This schematic demonstrates the transposase complex contacting the target DNA, and simultaneously fragmenting and tagging it, resulting in 5’-tagged DNA fragments. *See* EX1002 ¶ 158.

FIG. 2 Fragmenting by In Vitro Transposition



EX1003 at Figure 2

Third, Grunenwald teaches that the polynucleotides targeted with the transposase complex are “regions of open chromatin” of the cell nuclei. Grunenwald expressly discloses that the target DNA contacted by the insertional enzyme complex may “comprise[] or consist[] of genomic DNA, subgenomic DNA, [or] *chromosomal DNA*....” EX1003 at [0146] (emphasis added). A POSA would have been aware that chromosomal DNA is chromatin, and so in embodiments wherein the contacted nucleic acid is chromosomal DNA, chromatin is contacted with the transposase complex. EX1002 ¶ 155. This will necessarily

include the regions of “open chromatin” that are accessible to the transposase complex.

To the extent Grunenwald is deemed to not explicitly disclose tagmenting open chromatin, this limitation inherently disclosed. A prior art reference “may anticipate without disclosing a feature of the claimed invention if that missing characteristic is necessarily present, or inherent, in the single anticipating reference.” *Schering Corp. v. Geneva Pharm.*, 339 F.3d 1373, 1377 (Fed. Cir. 2003). Here, Grunenwald discloses that its methods of using an insertional enzyme complex to tag and fragment DNA can be applied to chromosomal DNA *in vivo*, which necessarily requires contacting open chromatin. *See* EX1003 at [1046], [0157].

This *in vivo* embodiment is accomplished by electroporating the cell to allow the transposome to enter the cell:

In some embodiments, the insertion of a transposon end into target DNA according to the present invention can also be carried out *in vivo*. If transposition is carried out *in vivo*, transposition into the target DNA is preferably achieved by electroporating a synaptic complex of a transposase and a suitable transposon end composition into the host cell as described in U.S. Pat. No. 6,159,736 (herein incorporated by reference).

EX1003 at [0157].¹ Because chromosomal DNA in a cell is necessarily packaged in the nuclei in the form of chromatin, and genomic DNA is necessarily part of the chromatin, Grunenwald inherently discloses performing its method of contacting chromatin of a genome region of a biological sample with the insertional enzyme complex to produce tagged polynucleotide fragments. *See* EX1002 ¶¶ 155-157. Moreover, this will necessarily and inherently involve tagging regions of “open chromatin.” This is because, as documented above, during the normal life cycle portions of the chromatin will be “open” and more accessible to enzymes and other cellular machine, other portions will be “closed.” *See supra* Part V.B.

Importantly, the '207 Patent does not disclose any special measures that must be taken to practice the alleged invention other than the use of standard techniques for preparing DNA. In fact, the '207 Patent admits that “the chromatin used in the method may be made by any suitable method.” EX1001 at 14:45-47. To this end, as documented above, the '207 Patent discloses an open-ended laundry list of permeabilization techniques (including “hypotonic shock and/or ultrasonification”) without any special guidance on how they ought to be employed along with a prior

¹ While Grunenwald discloses applying tagmentation to nuclei via electroporating cell, it would have been obvious to simply substitute this with the protocol for nuclei disclosed in Song, as set forth in the immediately preceding section. *See supra* Part X.A.1.b.

art Song protocol for isolating cell nuclei. *See, e.g.*, EX1001 at 3:22-26, 16:52-62, 25:4-15.

Beyond the use of well-known permeabilization and nuclear isolation techniques, the '207 Patent not once suggests that any steps need to be taken to practice tagmentation on chromatin. Thus, the overriding message that is acknowledged in the '207 Patent itself is that so long as one can introduce the tagmentation enzymes to chromatin (using standard techniques), the invention will necessarily and inherently work to tag open chromatin. As inventor Jason Buenrostro stated in his tweet, the alleged invention is “so easy that anyone can do it!” EX1029.

Thus, Grunenwald discloses this limitation. In particular, Grunenwald discloses the use of standard electroporation to introduce the enzymes for a tagmentation into a cell, which will necessarily and inherently lead to contacting cell nucleus with a transposase complex to produce tagged polynucleotide fragments at regions of open chromatin. The '207 Patent does not specify any novel or inventive steps needed to apply the method of tagmentation to open chromatin. In fact, it actually points out that “methods for tagmenting isolated genomic DNA are known in the art” and that those systems “may readily be adapted for use” in the invention, contemplating that if one can introduce the insertional enzyme complex (*e.g.*, a

transposase complex) to cell nuclei, the invention will necessarily work. EX1001 at 14:35-42.

d. [1C] performing one or more nucleic acid reactions on the tagged fragment to produce a sequencing library.

First, as explained above in Section X.A.1.c., Grunenwald discloses “tagged nucleic acid molecules.”

Second, Grunenwald teaches subjecting the tagged nucleic acid molecules to additional reactions after tagmentation and prior to sequencing, to produce a sequencing library. Indeed, Grunenwald is clear the whole point of its method is to prepare nucleic acid libraries for sequencing, stating that “what is needed are improved methods for generating libraries of DNA fragments from target DNA molecules for amplification including amplification of whole or partial genomes from one organism (e.g., from a clinical sample) or from multiple organisms (e.g., metagenomic target DNA from an environmental sample), for further analysis (e.g., by real-time PCR, emulsion PCR, comparative genomic hybridization (CGH), comparative *genomic sequencing* (CGS)....” EX1003 at [0015]. Likewise, Grunenwald explains that the invention is useful for “generating libraries of tagged DNA fragments for use, e.g., in *next generation sequencing methods*.” EX1003 at [0017].

To this end, Grunenwald teaches subjecting the tagged nucleic acid fragments to multiple different reactions to prepare the sequencing library. For instance,

Grunenwald teaches that in “in some embodiments of any of the methods of the invention for generating the library of tagged DNA fragments, the method further comprises: amplifying the library of tagged DNA fragments.” EX1003 at [0198]. That is, the library for sequencing is generated by further amplifying a previously prepared library. Specifically, Grunenwald teaches that “the step of amplifying the tagged DNA fragments comprises performing a polymerase chain reaction (PCR), thereby generating a library of tagged DNA fragments comprising amplified ditagged DNA fragments.” EX1003 at [0194]. Grunenwald further teaches that “the invention is not limited to use of PCR to amplify the library of tagged DNA fragments,” and that “any suitable amplification method” can be used. EX1003 at [0205]. Grunenwald exemplifies this method in working Example 11, where “adaptors compatible with Illumina GAII bridge PCR and sequencing” are added through PCR “performed using adaptor oligos which hybridize to the transposon end.” EX1003 at [0513].

As another example, Grunenwald discloses embodiments wherein a 5'-tagged DNA fragment is generated comprising a first tag that, in turn, “comprises or consists of a sequencing tag.” EX1003 at [0263]. This first tag is added through tagmentation. *Id.*; see also EX1002 ¶ 164. After the 5'-tagged DNA fragments are generated, they are further subjected to a second reaction using a DNA polymerase, which would be an amplification reaction. EX1003 at [0263];

EX1002 ¶ 164. The DNA polymerase is “used to join a second tag that comprises or consists of the other sequencing tag,” in turn generating “a library of DNA fragments comprising 5’-and 3’ tagged DNA fragments with both sequencing tags.” EX1003 at [0263].

These di-tagged DNA fragments can be further subjected to a nucleic acid reaction in the form of an amplification reaction.

2. Claim 2: The method of claim 1, further comprising sequencing the sequencing library to produce a plurality of sequence reads.

Grunenwald discloses performing sequencing reactions and generating reads “in a ‘massively parallel’ or ‘multiplex’ fashion.” EX1003 at [0008]. A POSA would have understood that this disclosure indicates that a plurality of sequence reads are produced. Moreover, specifically for an embodiment based on the Roche/454 FLX Titanium system, Grunenwald discloses the deep sequencing of the library to “provide[] ~80,000 reads,” which Grunenwald describes as being “consistent with the non-selective and massively parallel amplification of a DNA fragment library.” EX1003 at [0507].

3. Claim 3: The method of claim 1, wherein the one or more nucleic acid reactions comprise a nucleic acid amplification reaction.

Disclosed. See Section X.A.1.d, *supra*.

4. **Claim 4: The method of claim 3, wherein said nucleic acid amplification reaction is configured to add one or more functional sequences to said tagged nucleic acid molecules or derivatives thereof, wherein said one or more functional sequences are compatible with a selected next generation sequencing platform.**

Grunenwald discloses claim 4.

First, Grunenwald discloses “tagged nucleic acid molecules.” *See supra* Section X.A.1.c.

Second, Grunenwald discloses a “nucleic acid amplification reaction,” including one using a DNA polymerase to generate a strand based on a template strand. EX1003 at [0123]; *see also* EX1002 ¶¶ 174.

Third, Grunenwald discloses that the “nucleic acid amplification reaction is configured to add one or more functional sequences to said tagged nucleic acid molecules or derivatives thereof.” For example, Grunenwald discloses that the method of generating a library of tagged DNA fragments further comprises “amplifying the library of tagged DNA fragments comprising di-tagged DNA fragments, tagged circular ssDNA fragments, or fantail DNA fragments.” EX1003 at [0198]. In these embodiments, the “5’ portion of the first PCR primer or the 5’ portion of the second PCR primer” may comprise or consist of “first or second sequencing tags, respectively, for generation of templates for next-generation sequencing for a particular sequencing platform.” EX1003 at [0201]; EX1002 ¶ 176.

5. Claim 5: The method of claim 1, wherein a tagged fragment of the plurality of tagged fragments comprises a nucleotide sequence corresponding to the tagged fragment.

Grunenwald discloses claim 5. In the '207 Patent, the term “tagged fragments” is broadly defined to mean “polynucleotide fragments that are attached to tags.” EX1001 at 13:31-32. As detailed above, Grunenwald teaches a method of tagmentation that results in “tagged polynucleotide fragments” that consist of polynucleotide fragments from the original DNA sample along with tags that are part of the transposon end composition. *See supra* Section A.1.c. As such, the tagged fragments generated in Grunenwald will, by their very nature, comprise a nucleotide sequence (*i.e.*, the polynucleotide sequence from the original DNA sample) that corresponds to the tagged fragment. EX1002 ¶ 182.

6. Claim 6: The method of claim 5, wherein the tagged fragment further comprises a primer sequence.

Grunenwald discloses claim 6. Specifically, Grunenwald discloses a tag comprising an amplification tag domain. EX1003 at [0103]. In Grunenwald, an amplification tag domain is a “tag domain that exhibits a sequence for the purpose of facilitating amplification of a nucleic acid to which said tag is appended.” EX1003 at [0111]. For example, the amplification tag domain “provides a priming site for a nucleic acid amplification reaction using a DNA polymerase.” *Id.* The priming site on the tag domain is the claimed primer sequence. EX1002 ¶ 186.

Grunenwald exemplifies this in Figure 13, reproduced below. Figure 13 is a diagram depicting tagmentation to produce an “Illumina/Solexa compatible library by insertion of tagged transposon ends in a transposition reaction, followed by tag-specific PCR +/- barcodes.” EX1003 at [0071]. Grunenwald further teaches that “in order to non-selectively amplify and append the DNA fragment library with adaptors compatible with Illumina GAII bridge PCR and sequencing, PCR was performed using adaptor oligos which hybridize to the transposon end sequence.” EX1003 at [0513]. In these embodiments, the tagged fragment does further comprise a primer sequence, as adaptor oligos used for bridge PCR compatibility are primer sequences. EX1002 ¶ 190.

8. Claim 8: The method of claim 7, wherein said Tn5 transposase is a hyperactive Tn5 transposase.

Disclosed. *See supra* Section X.A.7.

9. Claim 9: The method of claim 1, wherein said transposase complex comprises a first nucleic acid insert element comprising a first adapter sequence.

Grunenwald discloses a transposase complex that comprises a nucleic acid insert element. As explained above in Section X.A.1.c, Grunenwald discloses a transposome comprising a transposase (*i.e.*, an insertional enzyme) and a transposon end composition comprising multiple tags. *See* EX1003 at [0098], [0102], [0161], Figs. 1-2. These tags are nucleic acid insert elements because they are nucleic acids for insertion in a polynucleotide by a transposase complex. EX1002 ¶¶ 198-199.

Further, Grunenwald discloses a nucleic acid insert “comprising a first adapter sequence.” As detailed above, the ’207 Patent uses adaptor/adapter sequence interchangeably with “tag.” *See supra* Section VIII.C. Grunenwald, in turn, discloses a transposome comprising tags, which comprise a sequence. *See* Section X.A.1.b, EX1003 at [0098], [0102], [0161], Figs. 1-2. Further, the ’207 Patent discloses that the molecular tags added to DNA fragments may comprise “sequencing adaptors,” and that they may further comprise a “barcode label.” EX1001 at 16:30-40. Thus, the ’207 Patent contemplates that these tags are functional sequences, akin to the “tag domains” and molecular tags taught in Grunenwald. *See* EX1002 ¶ 201-202; *see supra* Section X.A.1.b. Therefore,

Grunenwald discloses a tag that is a nucleic acid insert element comprising an adapter, or tag, sequence. EX1002 ¶¶ 203-205.

10. Claim 10: The method of claim 9, wherein said first adapter sequence comprises a first sequencer adapter sequence.

Grunenwald discloses claim 10. As discussed in Section X.A.1.c above, Grunenwald discloses that tags (*i.e.*, the claimed adapter sequence) may comprise one or more portions, or domains, “that exhibit[] a sequence for a desired intended purpose or application.” See EX1003 at [0103]. Grunenwald further teaches that one type of tag domain for use with its disclosed methods is “a sequencing tag domain.” EX1003 at [0103], [0110].

Further, Grunenwald teaches that the sequencing tag domain may comprise “Roche 454A and 454B sequencing tags, ILLUMINA™ SOLEXA™ sequencing tags, Applied Biosystems’ SOLID™ sequencing tags, the Pacific Biosciences’ SMRT™ sequencing tags, Pollonator Polony sequencing tags, or the Complete Genomics sequencing tags.” *Id.* at [0041], [0194], Claims 7, 13, 15, 21. This domain, being a short sequence of DNA to facilitate sequencing of the fragmented and tagged DNA, is the sequencing adapter sequence. EX1002 ¶ 208.

11. Claim 11: The method of claim 9, wherein said first adapter sequence comprises a barcode sequence.

Grunenwald discloses an adapter sequence comprising a barcode sequence. Specifically, Grunenwald discloses that the tag inserted with a transposase may

comprise an “address tag domain.” EX1003 at [0103]. An address tag domain “exhibits a sequence that permits identification of a specific sample,” which a POSA would have understood to be the claimed barcode sequence. EX1003 at [0113]; EX1002 ¶ 211; *compare* EX1001 at 12:26-32 (stating that the “barcode sequence” is “used to...identify and/or track the source of a polynucleotide reactions....”).

12. Claim 12: The method of claim 9, wherein said first adapter sequence comprises a first primer sequence.

Grunenwald discloses claim 12. Specifically, Grunenwald discloses a tag comprising an amplification tag domain. EX1003 at [0103]. In Grunenwald, an amplification tag domain is a “tag domain that exhibits a sequence for the purpose of facilitating amplification of a nucleic acid to which said tag is appended.” EX1003 at [0111]. For example, the amplification tag domain “provides a priming site for a nucleic acid amplification reaction using a DNA polymerase.” *Id.* The priming site on the tag domain is the claimed primer sequence. EX1002 ¶ 216.

13. Claim 13: The method of claim 1, wherein said transposase complex comprises a second nucleic acid insert element comprising a second adapter sequence.

Grunenwald discloses a transposase complex that comprises a second nucleic acid insert element comprising a second adapter sequence. For example, Grunenwald teaches that a transposon end composition may comprise, in addition to the transposon end, an “additional sequence *or sequences* 5’-of the transferred

transposon end sequence and/or 3' of the non-transferred transposon end sequence.”
EX1003 at [0098].

Moreover, Grunenwald discloses an insertional enzyme complex (*i.e.*, a transposase complex) comprising at least two tags. *See* EX1003 at [0102], [0161], Figs. 1-2. For example, in one working example Grunenwald teaches that “‘A-MEDS Transposomes’ and ‘B-MEDS Transposomes’ were formed by pre-incubating 12.5 μ M TSase with 12.5 μ M A-MEDS or 12.5 μ M B-MEDS transposon end compositions, respectively, for 60 minutes at 37°C.” EX1003 at [0425]. The second tag is a second nucleic acid insert element comprising a second adapter sequence for the same reasons that the first tag satisfies this limitation. *See supra* Section X.A.9.

Alternatively, in embodiments where the tag comprises multiple tag domains (*i.e.*, a sequencing tag domain and an amplification tag domain), each tag domain would be a separate nucleic acid insert element. Further, the tag domain, being a short sequence of DNA added for a desired intended purpose, is an adapter sequence. EX1002 ¶ 204; *see supra* Section X.A.9. Grunenwald discloses that the tag domain sequences “can be used to accomplish any desired purposes.” EX1003 at [0331]. In these embodiments, where “one or more tag domain sequences” are the additional sequences being used to accomplish a desired purpose, a second tag domain

sequence (*e.g.*, a second nucleic insert element) acts as a second adapter sequence.

EX1003 at [0331]; EX1002 ¶ 221.

14. Claim 14: The method of claim 13, wherein said second adapter sequence comprises a second sequencing adapter sequence.

As discussed above in Section X.A.13, Grunenwald discloses a second adapter sequence. Moreover, for the same reasons that Grunenwald discloses that the first adapter sequence comprises a first sequencing adapter sequence, Grunenwald discloses that the second adapter sequence comprises a second sequencing adapter sequence. *See supra* Section X.A.10.

15. Claim 15: The method of claim 13, wherein said second adapter sequence comprises a barcode sequence.

As discussed above in Section X.A.13, Grunenwald discloses a second adapter sequence. Moreover, for the same reasons that Grunenwald discloses that the first adapter sequence comprises a barcode sequence, Grunenwald discloses that the second adapter sequence comprises a barcode sequence. *See supra* Section X.A.11.

16. Claim 16: The method of claim 13, wherein said first adapter sequence comprises a second primer sequence.

Grunenwald discloses claim 16. As discussed above in Section X.A.12, Grunenwald discloses a second adapter sequence. Moreover, for the same reasons that Grunenwald discloses that the first adapter sequence comprises a first primer

sequence, Grunenwald discloses that the second adapter sequence comprises a second primer sequence. *See* Section *supra* X.A.12.

17. Claim 22: The method of claim 1, where the transposase complex does not comprise an antibody specific to a protein that is part of chromatin.

Grunenwald discloses claim 17. To disclose a negative limitation, a prior art reference need not specify the negative limitation in the disclosure; rather, if a prior art reference does not require the use of that limitation, it is sufficient to disclose a negative limitation. *See, e.g. WAG Acquisition, LLC. V. WebPower, Inc.*, 781 Fed. Appx. 1007, 1013 (Fed. Cir. 2019) (“While [the prior art reference] does not specify that a pointer is *not* used, nothing in the record suggests that a pointer *must* be used.”).

Nothing in Grunenwald suggests that an antibody specific to a protein that is part of the chromatin must be used. On the contrary, Grunenwald discloses multiple embodiments that do not use an antibody specific to a protein that is part of the chromatin. For example, Grunenwald discloses:

incubating the target DNA with a transposase and transposon end or transposon end composition comprising a transferred strand that has a tag domain in its 5' portion, under conditions in which a transposition reaction is catalyzed by the transposase, and wherein the target DNA is fragmented to generate a plurality of target DNA fragments and a transferred strand of the transposon end

or transposon end composition is joined to 5' ends of each of a plurality of the target DNA fragment, to produce a plurality of 5' tagged target DNA fragments.

EX1003 at [0018]. At no point does Grunenwald state that an antibody specific to a protein that is part of said chromatin is required to carry out the method disclosed in the embodiment. This is true for subsequent embodiments disclosed by Grunenwald. *See, e.g.*, EX1003 at [0019]–[0057].

As confirmation that the embodiments in Grunenwald do not involve an antibody specific to a protein that is part of the chromatin, consider the references to an antibody that are included in Grunenwald. Although Grunenwald references the term “antibody” seven times throughout the specification, these disclosures do not describe an antibody that is part of a transposase complex or that is specific to a protein in the target DNA. EX1003 at [0109], [0117], [0212], [0216], [0217], [0229]. As explained in the accompanying declaration of Dr. Cooper, the disclosures of the use of an antibody are for various purposes such as capture of a tagged DNA molecule and assisting in isolation and purification of tagged DNA, not antibodies specific to a protein that is a part of the chromatin. EX1002 ¶¶ 232-244.

Because Grunenwald discloses methods for contacting chromatin with a transposase complex that does not require the use of an antibody specific to a protein that is part of the chromatin, Grunenwald discloses this claim limitation.

18. Claim 23:

- a. [23A] A method for generating a sequencing library from a plurality of cells, comprising:**

Disclosed. *See supra* Section X.A.1.

- b. [23B] lysing a plurality of cells to isolate a plurality of cell nuclei, wherein the plurality of cell nuclei comprise chromatin;**

For the reasons stated above, it would have been obvious to perform cell lysis to isolate nuclei as set forth in Song and apply this technique to the method of Grunenwald. *See supra* Section X.A.1.b.

- c. [23C] contacting a cell nucleus of the plurality of cell nuclei with a Tn5 transposase complex such that polynucleotides of the cell nucleus are tagged at regions of open chromatin to produce a plurality of tagged fragments,**

Disclosed. *See supra* Sections X.A.1.c and XA.7.

- d. [23D] wherein the Tn5 transposase complex comprises a first sequencing adapter sequence and a second sequencing adapter sequence,**

Disclosed. *See supra* Sections X.A.9, X.A.10, and X.A.13.

- e. [23E] wherein the Tn5 transposase complex does not comprise an antibody specific to a protein that is part of chromatin,**

Disclosed. *See supra* Section X.A.22.

- f. [23F] wherein a tagged fragment of the plurality of tagged fragment comprises a nucleotide sequence corresponding to a region of open chromatin,**

Disclosed. *See supra* Section X.A.1.c; *see also* EX1002 ¶¶ 252-254.

- g. [23G] the first sequencing adapter sequence, and**

Disclosed. *See supra* Section X.A.10.

- h. [23H] the second sequencing adapter sequence; and**

Disclosed. *See supra* Section X.A.14.

- i. [23I] performing one or more nucleic acid reactions on the tagged fragment to produce a sequencing library.**

Disclosed. *See supra* Section X.A.1.d.

- 19. Claim 24: The method of claim 23, further comprising sequencing the sequencing library to produce a plurality of sequence reads.**

Disclosed. *See supra* Section X.A.2.

B. Ground 2: Claims 17-21 and 26-29 Are Unpatentable Under 35 U.S.C. § 103 Over O’Geen in View Of Grunenwald And Song And The Knowledge Of A POSA

- 1. Claim 17: The method of claim 2, further comprising analyzing the sequence reads to generate a profile of chromatin accessibility along the polynucleotides of the cell nuclei.**

- a. The Combination of O’Geen And Grunenwald Teaches This Element**

The combination of O’Geen, Grunenwald, and Song discloses and renders obvious claim 17.

First, the combination of Grunenwald and Song in view of the knowledge of a POSA discloses and renders obvious claim 2.

Second, O’Geen discloses “analyzing the sequence reads to generate a profile of chromatin accessibility along the polynucleotides of the cell nuclei.” Specifically, O’Geen teaches that “technologies allow the genome-wide visualization of the information encoded in the epigenome.” EX1005.004. Furthermore, O’Geen “describe[]s in detail the steps involved in performing ChIP assays,” including steps for fragmenting and tagging section of chromatin, *sequencing, and mapping tags to the human genome to identify epigenetic features of the genome*. EX1005.009, .014, .024, Fig. 1 (depicting the steps of a ChIP-seq assay). O’Geen explains that “peak calling methods” can then be used to *identify epigenetic features* such as target sites and binding patterns. EX1005.19.

b. It Would Have Been Obvious To Combine O’Geen With Grunenwald

It would have been obvious to combine O’Geen’s methods of determining epigenetic features of a genome by mapping sequence reads to the genome with Grunenwald’s methods of fragmenting and tagging chromatin.

First, O’Geen and Grunenwald are analogous art. Both references are in the field of genetics and molecular biology, and particularly concern methods of DNA tagging and fragmenting for sequencing and analysis. EX1002 ¶ 272. Further,

both references cover the preparation of chromatin for analysis. *See, e.g.*, EX1003 at Abstract; [0146]; [0157]; EX1005.004.

Second, a POSA would have combined O’Geen with Grunenwald, because O’Geen identifies problems of the ChIP-seq method to which Grunenwald teaches the solution. O’Geen discloses that the “biggest limitations to histone ChIP assays are the specificity of the antibody and the variability between different lot numbers of the same antibody.” EX1005.23. This resulted in a very small number of validated assays for ChIP-seq, and limited its usefulness as a tool to investigate epigenetic features of chromatin. *Id.*; EX1002 ¶ 271. A POSA would have been aware of these limitations and sought to overcome them. EX1002 ¶ 273.

Grunenwald teaches a solution to this problem. Rather than using immunoprecipitation and sonication as taught by O’Geen to fragment and tag chromatin, Grunenwald uses the method of tagmentation described above. *See supra* Section VIII.A. A POSA would have understood that unlike immunoprecipitation, tagmentation as taught by Grunenwald does not face the same issues caused by antibody specific and variability between different lot numbers of the same antibody. EX1002 ¶ 276.

Notably, during the prosecution of the ’207 Patent, the Examiner found that the point of novelty for the ’207 Patent was contacting of cell nuclei with a transposase complex, such that polynucleotides of the cell nucleus are tagmented at

regions of open chromatin to produce a plurality of tag fragments, *i.e.*, tagmentation. EX1007.155. However, as Grunenwald shows, this idea was not new. A POSA would have recognized the advantages of Grunenwald's method of tagging and fragmenting and would have had a reason to modify O'Geen's method of epigenetic analysis with these teachings.

Third, a POSA would have had a reasonable expectation of success in combining the references. Both the ChIP-seq method taught by O'Geen and the tagmentation method taught by Grunenwald ultimately result in sequencing fragmented DNA samples. EX1002 ¶ 275; *see, e.g.*, EX1005.17-18, EX1003 at [0312]. As was taught in O'Geen, the use of sequencing data to generate a map of the epigenetic features of the DNA was well-known in the art. *See, e.g.*, EX1005.005-006. Therefore, a POSA seeking to generate such a map through sequencing would have a reasonable expectation of success applying Grunenwald's preparation methods to build a sequencing library that could be used as taught in O'Geen.

2. Claim 18: The method of claim 2, further comprising analyzing the sequence reads to determine a DNA binding protein occupancy for one or more DNA binding protein binding sites in the polynucleotides of the cell nuclei.

The combination of O’Geen, Grunenwald, and Song in view of the knowledge of a POSA discloses and renders obvious claim 18.

First, the combination of Grunenwald and Song in view of the knowledge of a POSA renders obvious claim 2.

Second, O’Geen discloses “analyzing the sequence reads to determine a DNA binding protein occupancy for one or more DNA binding protein binding sites in the polynucleotides of the cell nuclei.” O’Geen teaches that sequencing libraries are “assessed on a Bioanalyzer using the DNA High Sensitivity chip.” EX1005.018. Furthermore, O’Geen explains generally that ChIP assays have been used “investigators to characterize DNA–protein interactions in vivo.” EX1005.004. O’Geen teaches that “proteins such as histones or transcription are covalently crosslinked to their genomic DNA substrates in living cells” and that “provides an opportunity to take a snapshot of histone or other protein–DNA interactions.” EX1005.005. Protein-DNA interactions take place at binding protein sites of the cell nuclei. EX1002 ¶ 279. Therefore, POSA would understand identifying protein-DNA interactions to refer to binding protein interactions. *Id.* Furthermore, O’Geen discloses that with the taught ChIP-Seq method, “the binding patterns of certain modified histones (H3K9ac and H3K4me3) can be identified as

sharp peaks.” EX1005.019. A POSA would further understand this binding pattern to refer to binding protein occupancy. EX1002 ¶ 280. Therefore, O’Geen discloses identifying DNA binding protein occupancy in the polynucleotides of the cell nuclei. *Id.*

3. Claim 19: The method of claim 2, further comprising analyzing the sequence reads to generate an epigenetic map representing one or more epigenetic features of the polynucleotides of the cell nuclei.

The combination of O’Geen, Grunenwald, and Song in view of the knowledge of a POSA discloses and renders obvious claim 19.

First, the combination of Grunenwald and Song in view of the knowledge of a POSA renders obvious claim 2.

Second, O’Geen discloses “analyzing the sequence reads to generate an epigenetic map representing one or more epigenetic features of the polynucleotides of the cell nuclei.” O’Geen teaches that sequencing libraries are “assessed on a Bioanalyzer using the DNA High Sensitivity chip.” EX1005.018. Furthermore, O’Geen teaches the use of Chip-Seq to generate epigenetic profiles of the cell, where “for the study of primary cells and tissues, epigenetic profiles can be generated using as little as 1 mg of chromatin.” EX1005.004. O’Geen teaches that this is achieved by identifying six markers that “have been selected to provide “roadmaps” of the epigenomic profiles of primary cells by the Roadmap Epigenome Mapping Centers.” EX1005.005. A POSA would have understood the terms of epigenetic

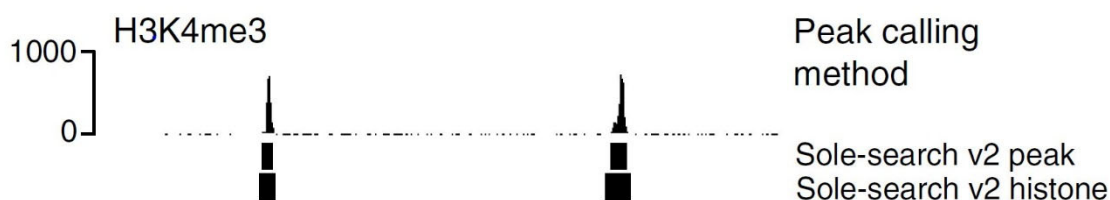
map and epigenetic profile to be interchangeable and, therefore, that O’Geen discloses epigenetic map representing one or more epigenetic features of the polynucleotide of cell nuclei. EX1002 ¶ 285.

4. Claim 20: The method of claim 2, further comprising analyzing the sequence reads to determine a position of one or more transcription start sites (TSSs) in the polynucleotides of the cell nuclei.

The combination of O’Geen, Grunenwald, and Song in view of the knowledge of a POSA discloses and renders obvious claim 19.

First, the combination of Grunenwald and Song in view of the knowledge of a POSA renders obvious claim 2.

Second, O’Geen discloses “analyzing the sequence reads to determine a position of one or more transcription start sites (TSSs) in the polynucleotides of the cell nuclei.” O’Geen teaches that sequencing libraries are “assessed on a Bioanalyzer using the DNA High Sensitivity chip.” EX1005.018. O’Geen explains that a “variety of peak calling methods” can be used to identify target sites. EX1005.019. O’Geen further teaches the sole-search was developed “to identify transcription factor binding sites, which typically display peaks.” *Id.* Notably, O’Geen discloses that “sharp *H3K4me3 peaks are observed proximal to the transcription start sites.*” *Id.* The following Fig. 3 of O’Geen illustrates this disclosure.



5. **Claim 21: The method of claim 2, further comprising analyzing the sequence reads to determine a position of one or more nucleosomes in the polynucleotides of the cell nuclei.**

The combination of O’Geen, Grunenwald, and Song in view of the knowledge of a POSA renders obvious claim 21.

First, the combination of Grunenwald and Song in view of the knowledge of a POSA renders obvious claim 2.

Second, O’Geen discloses “analyzing sequence reads to determine a position of one or more nucleosomes in the polynucleotides of the cell nuclei.” O’Geen teaches that sequencing libraries are “assessed on a Bioanalyzer using the DNA High Sensitivity chip.” EX1005.018. Specifically, O’Geen explains that “ChIP followed by ... *high-throughput sequencing*” is a powerful tool for identifying “genome-wide profiles of transcription factors, histone modifications, DNA methylation, and *nucleosome positioning*.” EX1005.004.

6. **Claim 25: The method of claim 24, further comprising analyzing the sequence reads to generate a profile of chromatin accessibility along the polynucleotides of the cell nuclei.**

Obvious, for reasons stated above. *See supra* Sections X.A.19 and X.B.1

7. **Claim 26:** The method of claim 24, further analyzing the sequence reads to determine a DNA binding protein occupancy for one or more DNA binding protein binding sites in the polynucleotides of the cell nuclei.

Obvious, for reasons stated above. *See supra* Sections X.A.19 and X.B.2.

8. **Claim 27:** The method of claim 24, further comprising analyzing the sequence reads to generate an epigenetic map representing one or more epigenetic features of the polynucleotides of cell nuclei.

Obvious, for reasons stated above. *See supra* Sections X.A.19 and X.B.3.

9. **Claim 28:** The method of claim 24, further comprising analyzing the sequence reads to determine a position of one or more transcription start sites (TSSs) in the polynucleotides of the cell nuclei.

Obvious, for reasons stated above. *See supra* Sections X.A.19 and X.B.4.

10. **Claim 29:** The method of claim 24, further comprising analyzing the sequence reads to determine a position of one or more nucleosomes in the polynucleotides of the cell nuclei.

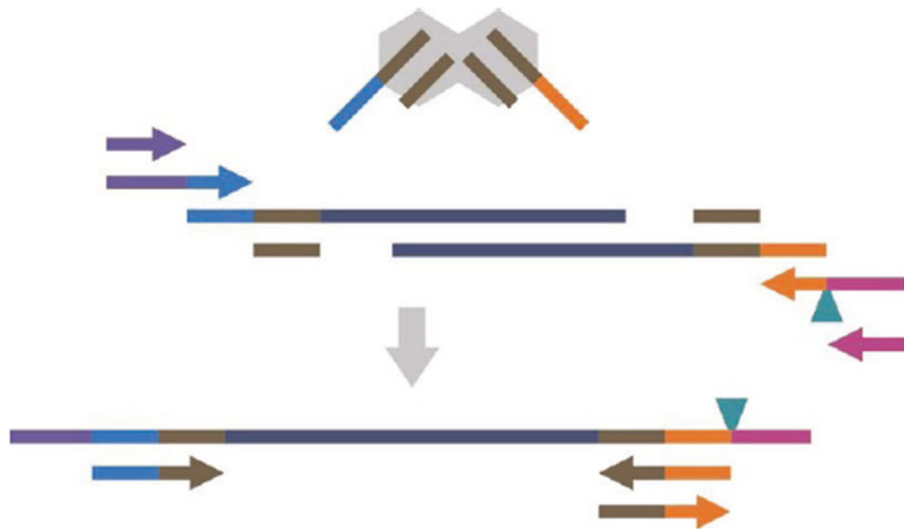
Obvious, for reasons stated above. *See supra* Sections X.A.19 and X.B.5.

C. Ground 3: Claims 13-16 Are Unpatentable Under 35 U.S.C. § 103 Grunenwald And Song In View Of Adey And The Knowledge Of A POSA

Claim 13 recites the method of claim 1, “wherein said transposase complex further comprises a second nucleic acid insert element comprising a second adapter sequence.” Claims 14-16 depend from claim 13, and further recite that the second adapter sequence is a second sequencing adapter sequence, a barcode sequence, or a second primer sequence. As explained above, the combination of Grunenwald and

Song in view of the knowledge of a POSA renders claims 1 and 13 obvious. *See supra* Sections XI.A.1 and *See supra* Section X.A.13. To the extent Patent Owner claims that such a combination does not render claim 13 obvious, it would nonetheless be obvious by further in view of Adey. *See supra* Section X.A.13.

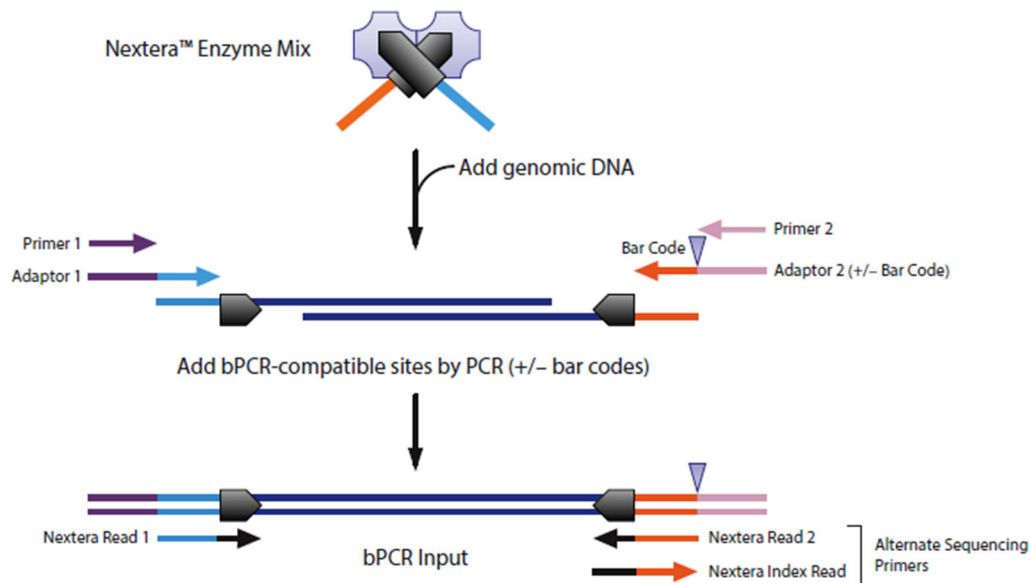
Adey discloses the limitation that “said insertional enzyme complex further comprises a second nucleic acid insert element comprising a second adapter sequence.” For example, in Figure 1(b), reproduced below, the transposome complex is composed of the two grey hexagons, which contain two different adaptors (blue/brown/orange). The complex is capable of simultaneously fragmenting and tagging genomic DNA (navy blue).



EX1009.002 at Figure 1(b) (modified)

As shown in Figure 1(b), Adey discloses the use of a first and a second adapter sequence, in which the two adapter sequences are different (as marked by different colors). Thus, the combination of Grunenwald and Adey discloses each and every limitation of Claim 13.

Adey further discloses that the second adapter sequence comprises a second sequencing adapter sequence, barcode sequence, or second primer sequence. Adey teaches that in the preparation of a library of fragments, genomic DNA is contacted with a Nextera™ enzyme mix. EX1009.014. This mix contains transposon ends with a variety of sequences, illustrated below in Figure 1 from the Nextera™ DNA Sample Prep Kit manual. In Figure 1, the transposase enzyme (two gray crosses) is shown carrying a payload of two adapters (blue and orange) which are sequencing adapters (labeled “Nextera Read 1” and “Nextera Read 2”). EX1048 at .002. In pink and purple, the nucleic acids labeled “Primer 1” and “Primer 2” are amplification primers that permit PCR to be conducted on tagged fragments. The small triangle represents a location for a barcode sequence to be inserted, and in embodiments the adapter would further comprise a barcode sequence. Adey, in its use of the Nextera™ Enzyme kit, did integrate a barcode sequence into the adapters. EX1009.016. Thus, the combination of Grunenwald and Adey discloses each and every limitation of claims 13-16.



EX1048.002 at Figure 1

Further, the combination of Grunenwald and Adey would have been obvious to a POSA.

First, Adey and Grunenwald would have been considered analogous art. Both references disclose the use of a transposase complex to accomplish tagmentation in preparation for sequencing, and a POSA interested in collecting sequence data for the purpose of generating a map of epigenetic features would have been aware of both references. EX1002 ¶ 313.

Second, a POSA would have been motivated to combine the references. Adey discloses a method of tagmentation using a particular kit, the Nextera™ kit, which uses two different adapters that contain different amplification primer sequences, sequencing primer sequences, and, optionally, a barcode sequence.

See, e.g., EX1009.004, .016. Grunenwald, in turn, teaches a method of tagmentation that can be applied directly to chromatin, either on isolated chromosomal DNA or in vivo on chromatin within a living cell. EX1003 at [0146], [0157]. A POSA seeking to prepare chromatin for sequencing would have found it obvious to try using the methods taught in Adey, particularly the use of the Nextera™ kit, for the tagmentation methods taught in Grunenwald. EX1002 ¶ 315.

Third, a POSA would have had a reasonable expectation of success in combining the references. The Nextera™ Enzyme kit disclosed in Adey was commercially available and, as references such as Adey show, the kit was successful in accomplishing tagmentation. *See, e.g.,* EX1009.014. It would have been predictable that combining an effective commercially available kit with Grunenwald's method to fragment and tag chromatin would yield the predictable result of successful tagmentation and preparation of chromatin for subsequent sequencing and analysis. EX1002 ¶ 316.

XI. THE BOARD SHOULD REACH THE MERITS OF THIS PETITION

A. Section 325(d) Does Not Apply Here, and Even if it Does, the Examiner's Material Errors Warrant Reaching the Merits of This Petition

In deciding whether to exercise its discretion to deny institution under 35 U.S.C. § 325(d), the Board applies a two-part framework that considers six non-exclusive factors. *See Becton, Dickinson & Co. v. B. Braun Melsungen AG*,

IPR2017-01586, Paper 8 at 17-18 (P.T.A.B. Dec. 15, 2017) (precedential). As documented below, the same art and arguments presented herein were not previously presented to the Office in any meaningful or relevant way. In addition, the Examiner erred by not considering the relevant teachings in Grunenwald alone or in combination with Song and/or O’Geen. As such, application of *Becton* overwhelmingly confirms that the Board should not exercise its discretion to deny institution under § 325(d) .

1. Factors (a), (b) and (d): The Office Has Not Considered the Same or Substantially the Same Art or Arguments

O’Geen was not in front of the Examiner during prosecution. Although the Applicant identified Grunenwald in an IDS, the Examiner did not cite or discuss it during prosecution. Grunenwald is also mentioned in the specification of the ’207 Patent. Mention of a reference in a specification alone, however, does not justify denial under § 325(d) , particularly where, as here, neither were cited or discussed by the Examiner during prosecution. *See 3Shape A/S v. Align Tech., Inc.*, IPR2019-00160, Paper 9 at 39-42 (P.T.A.B. June 11, 2019) (declining to apply § 325(d) where there was no evidence that the examiner substantively considered references that were of record during prosecution); *Limelight Networks, Inc. v. Akamai Tech., Inc.*, 2016 WL 8115506, at *5 (P.T.A.B. Dec. 30, 2016) (declining to exercise § 325(d) discretion because the Board was “not persuaded that merely referencing [references] in the ’102 patent’s specification necessarily is the same as presenting

arguments with respect to those references”); *Microsoft Corp. v. Parallel Networks Licensing, LLC*, IPR2015-00486, Paper 10 at 15 (P.T.A.B. July 15, 2015); *Weber, Inc. v. Provisur Tech., Inc.*, 2020 WL 755049, at *4 (P.T.A.B. Feb. 14, 2020);.

2. Factors (c), (e), and (f): The Examiner Erred by Not Considering the Art and Relevant Teachings Herein

Even if the Board determines that the same or substantially the same art or arguments were before the Office, the Examiner materially erred by not considering the relevant teachings in Grunenwald and Song.

In the Notice of Allowance, the Examiner did not discuss or cite any passage or disclosure of Grunenwald or Song. Notably, the Examiner issued the Notice of Allowance without outlining the Reasons for Allowance. EX1007.209. In such a case, where “the record of the Office’s previous consideration of the art is not well developed...a petitioner may show the Office erred by overlooking something persuasive under factors (e) and (f).” *Advanced Bionics, LLC v. Med-El Elektromedizinische Gerate GMBH*, IPR2019-01469, Paper 6 (P.T.A.B. Feb. 13, 2020) at 10; see *Kayak Software Corp. v. Int’l Bus. Machs. Corp.*, CBM2016-00075, Paper 16 at 11 (P.T.A.B. Dec. 15, 2016) (informative) (“where the prior art at issue was only cursorily considered that can weigh against exercising the discretion”). Moreover, the fact that Grunenwald and Okino “w[ere] not the basis of a rejection weighs strongly against exercising [the Board’s] discretion to deny institution.”

Cellco P'ship v. Huawei Device Co., Ltd., IPR2020-01117, Paper 10 at 13 (P.T.A.B. Feb. 3, 2021).

Here, the Examiner erred by failing to acknowledge the teachings in Grunenwald regarding application of tagmentation to living cells *in vivo* that disclose transposition tagging on chromosomal target DNA and Song that disclose cell lysis and isolation of nuclei. *Amber.IO, Inc. v. 72LUX, Inc.*, IPR2020-00015, Paper 8 at 20 (P.T.A.B. Apr. 1, 2020) (finding that the Examiner materially erred by failing to consider prior art references listed on IDS, when the Petitioner demonstrated that the references teach significant elements of the challenged claims). The Board should reach the merits of this petition and consider these relevant teachings in the first instance.

B. The *Fintiv* Factors Favor Institution

The factors set forth in *Apple Inc. v. Fintiv, Inc.*, IPR2020-00019, Paper 11 (P.T.A.B. Mar. 20, 2020) (“*Fintiv*”), for weighing discretionary denial when there is parallel, co-pending district court litigation also overwhelmingly favor institution.

The **first *Fintiv* factor**—whether the court granted a stay or evidence exists that one may be granted if a proceeding is instituted—is neutral. If instituted, Petitioner will seek stay in the district court litigation. As the court’s decision will come after institution, and is “based on a variety of circumstances and facts beyond [the Board’s] control and to which the Board is not privy.” *Sand Revolution II*,

LLC v. Continental Intermodal Grp. – Trucking, LLC, IPR2019-01393, Paper 24 at 7 (P.T.A.B. June 16, 2020) (informative).

Moreover, the District of Delaware grants majority of motions to stay pending IPR. EX1049; *see also, e.g., International Business Machines Corp. v. Rakuten Inc.*, No. 21-461-GBW, 2022 WL 17848779 (D. Del. Dec. 22, 2022) (granting defendant’s motion to stay after the Board instituted IPRs for the asserted patents). Thus, this factor is at least neutral.

The **second *Fintiv* factor**—proximity of the court’s trial date to the Board’s projected statutory deadline for a final written decision—favors institution. Currently, the trial date in the district court litigation is scheduled to start on December 2, 2024. As such, there is sufficient time for a final written decision in advance of the scheduled trial. EX1046.

But more realistically, it is far from certain that the district court trial will proceed as planned. There is a reasonable chance that the litigation may be stayed upon institution and/or ordinary delays in district court litigation may occur over the next 18 months. Indeed, the median time to trial for civil cases in the District of Delaware is 34.9 months, which would place trial in July 2025. EX1047. Thus, this factor favors institution.

The **third *Fintiv* factor**—investment in the parallel proceeding by the court and the parties—favors institution. The Board has found that the third factor favors

institution where in the parallel litigation, “the District Court has not issued any substantive orders,” “no claim construction orders have issued,” and “discovery is not complete, because...neither party had taken any depositions.” *Snap, Inc. v. SRK Tech. LLC*, IPR2020-00820, Paper 15, at 10–11 (P.T.A.B. Oct. 21, 2020). This is all true for the district court litigation.

Fact discovery is ongoing. Further, expert discovery or dispositive motion briefing will not be completed until after the institution deadline. EX1046.012. Moreover, no claim construction brief has been filed by either party, and the claim construction hearing will not occur until February 21, 2024. *Id.* Furthermore, “the most burdensome parts of the case—filing and responding to pretrial motions, preparing for trial, going through the trial process, and engaging in post-trial motions practice—all lie in the future.” *CyWee Grp. Ltd. v. Samsung Elecs. Co. Ltd.*, No. 2:17-cv-00140-WCB-RSP, 2019 WL 11023976, at *6 (E.D. TEX Feb. 14, 2019). Thus, this third factor favors institution.

The **fourth *Fintiv* factor**—overlap between issues raised in the petition and in the parallel proceeding—favors institution. If instituted, Petitioner will stipulate to not pursuing, in the district court litigation, invalidity arguments based on any instituted IPR ground.

The **fifth *Fintiv* factor**—whether the Petitioner and the defendant in the parallel proceeding are the same party—is neutral. Petitioner is the defendant in

the district court litigation, but that is often the case for a patent asserted in district court and challenged at the Board.

Finally, the **sixth *Fintiv* factor**—other circumstances that impact the Board’s exercise of discretion, including the merits—also favors institution. This Petition presents a compelling merits case that all claims of the ’207 Patent are unpatentable, and therefore this factor weighs in favor of institution. *Fintiv*, IPR2020-00019, Paper 11 at 14–15.

Thus, in view of the facts presented in this Petition, the *Fintiv* factors do not support discretionary denial.

XII. CONCLUSION

For the reasons set forth above, Petitioner Parse Biosciences, Inc. respectfully requests *inter partes* review of the ’207 Patent.

Dated: June 6, 2023

Respectfully submitted,

/Derek C. Walter/

Derek C. Walter
Lead Counsel for Petitioner
Reg. No. 74,656
Weil, Gotshal & Manges LLP
201 Redwood Shores Parkway
Redwood Shores, CA 94065

CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24 et seq., the undersigned certifies that this document complies with the type-volume limitations. This document contains 13,986 words as calculated by the “Word Count” feature of Microsoft Word 2016, the word processing program used to create it.

Respectfully submitted,

WEIL, GOTSHAL AND MANGES LLP

Dated: June 6, 2023

By: /Derek C. Walter/
Derek C. Walter
Lead Counsel for Petitioner
Reg. No. 74,656

CERTIFICATE OF SERVICE

I hereby certify that a true and correct copy of **PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 10,619,207 WITH PARSE BIOSCIENCES, INC'S EXHIBIT LIST, POWER OF ATTORNEY AND EXHIBITS 1001-1057** are being served on June 6, 2023, via Priority Express Mail (Fed Ex) pursuant to 37 C.F.R. § 42.105 and § 42.6(e) to the Patent Owner at the address below:

Casimir Jones, S.C.
2275 Deming Way, Suite 310
Middleton, WI 53562

Patent Owner's Address of Record

Nicholas Groombridge
Jennifer H. Wu
Josephine Young
Jennifer Rea Deneault
PAUL, WEISS, RIFKIND,
WHARTON & GARRISON LLP
1285 Avenue of the Americas
New York, NY 10019

*Additional Addresses Known as Likely to
Effect Service*

Dated: June 6, 2023

By: /Derek C. Walter/
Derek C. Walter
Lead Counsel for Petitioner
Reg. No. 74,656