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. 10,738,357

_____

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 10,738,357

Mail Stop **"PATENT BOARD"**
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

II. MANDATORY NOTICES 5

A. Real Party-in-Interest 5

B. Related Matters 5

C. Grounds for Standing 6

D. Lead and Back-Up Counsel and Service Information 6

III. RELATIONSHIP BETWEEN THIS IPR AND RELATED PROCEEDINGS 7

IV. IDENTIFICATION OF CHALLENGE 7

V. TECHNOLOGICAL BACKGROUND 8

A. DNA and RNA 8

B. DNA Packaging 8

C. Sequencing and Tagmentation 10

D. Permeabilization 12

VI. OVERVIEW OF THE ’357 PATENT 12

A. Priority Date of the ’357 Patent 12

B. Overview Of The Claims 13

C. Prosecution History Overview 14

VII. LEVEL OF ORDINARY SKILL IN THE ART OF THE ’357 PATENT 15

VIII. THE CITED PRIOR ART 16

A. Grunenwald 16

B. Okino 18

C. Saleh 18

D. Adey 19

E. Song 20

IX. CLAIM CONSTRUCTION 21

A. "Insertional Enzyme Complex" 21

B. "Nucleic Acid Insert Element" 21

X. IDENTIFICATION OF HOW THE CHALLENGED CLAIMS ARE UNPATENTABLE 22

A. Ground 1: Claims 1-28, 30 Are Rendered Obvious by the combination of Grunenwald and Okino 22

1. Claim 1 22

a. A composition comprising: 22

b. a permeabilized cell nucleus comprising: 22

(1) Okino Discloses A Permeabilized Cell Nucleus 24

(2) It Would Have Been Obvious To A POSA To Combine Okino's Permeabilized Cell Nuclei With Grunenwald 27

c. (a) a plurality of polynucleotide molecules; and 32

d. (b) an insertional enzyme complex comprising an insertional enzyme and 33

e. an insert element comprising a predetermined sequence. 36

2. Claim 2 37

a. A composition comprising: 37

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00003

b. a permeabilized cell nucleus comprising:.....37

c. chromatin comprising genomic DNA and a plurality of DNA binding proteins 37

d. wherein the chromatin comprises a plurality of open chromatin regions; and.....38

e. an insertional enzyme complex comprising an insertional enzyme and 38

f. an insert element comprising a predetermined sequence,.....38

g. wherein the insertional enzyme complex is bound to one of said open chromatin regions.....38

3. Claim 3: The composition of claim 2, wherein the insertional enzyme comprises one or more enzymatic moieties. 39

4. Claim 4: The composition of claim 3, wherein the insertional enzyme comprises two or more enzymatic moieties. 39

5. Claim 5: The composition of claim 4, wherein the two or more enzymatic moieties are linked together.....40

6. Claim 6: The composition of claim 2, wherein the insertional enzyme comprises a nuclear localization signal. 40

7. Claim 7: The composition of claim 2, wherein the insertional enzyme complex is configured to insert the insert element into the genomic DNA at an open chromatin region. 40

8. Claim 8: The composition of claim 7, wherein the insertional enzyme complex is configured to insert the insert element into the genomic DNA in a substantially sequence independent manner. 42

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00004

9. Claim 9: The composition of claim 2, wherein the insertional enzyme complex comprises an enzyme selected from the group consisting of: transposases, Hermes transposase, and human immunodeficiency virus (HIV) integrases. 43

10. Claim 10: The composition of claim 9, wherein the insertional enzyme complex comprises a transposase. 43

11. Claim 11: The composition of claim 10, wherein the transposase is a Tn transposase. 43

12. Claim 12: The composition of claim 11, wherein the Tn transposase is a Tn5 transposase. 43

13. Claim 13: The composition of claim 2, wherein the insert element comprises one or more molecular tags. 43

14. Claim 14: The composition of claim 13, wherein one or more of the molecular tags comprises a sequencing adaptor. 45

15. Claim 15: The composition of claim 14, wherein one or more of the sequencing adaptors comprises a barcode label. 45

16. Claim 16 46

a. A composition comprising: 46

b. a permeabilized cell nucleus comprising: 46

c. an insertional enzyme complex comprising a transposase enzyme; and 46

d. a plurality of tagged nucleic acid fragments 47

e. wherein each tagged nucleic acid fragment comprises a first sequencing adapter and second sequencing adapter 48

f. wherein each tagged nucleic acid fragment is derived from a region of open chromatin 50

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00005

17. Claim 17: The composition of claim 16, wherein the plurality of tagged nucleic acid fragments correspond to two or more regions of a genome. 50

18. Claim 18: The composition of claim 16, wherein the plurality of tagged nucleic acid fragments correspond to at least 1,000 regions of a genome.....51

19. Claim 19: The composition of claim 18, wherein the plurality of tagged nucleic acid fragments correspond to genome-wide regions of open chromatin.....51

20. Claim 20: The composition of claim 18, wherein the transposase is a Tn transposase.....52

21. Claim 21: The composition of claim 20, wherein the Tn transposase is a Tn5 transposase.....52

22. Claim 22 52

a. A composition comprising: 52

b. a permeabilized cell nucleus comprising:.....53

c. (a) an insertional enzyme complex comprising a transposase enzyme 53

d. (b) a plurality of tagged nucleic acid fragments.....53

e. wherein each tagged nucleic acid fragment comprises a first sequencing adapter and a second sequencing adapter 53

f. wherein the insertional enzyme complex and the tagged nucleic acid fragments are bound to one or more regions of open chromatin within the cell nucleus in a substantially sequence independent manner to form a high affinity macromolecule complex.....53

23. Claim 23: The composition of claim 22, further comprising a plurality of divalent cations.....55

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00006

24. Claim 24: The composition of claim 23, wherein the divalent cations are not chelated. 55

25. Claim 25: The composition of claim 23, wherein the divalent cations are selected from the group consisting of: magnesium, manganese, or any combination thereof. 55

26. Claim 26: The composition of claim 22, wherein the transposase is a Tn transposase. 55

27. Claim 27: The composition of claim 26, wherein the Tn transposase is a Tn5 transposase. 55

28. Claim 28: The composition of claim 2, wherein (a) and (b) are contained within the permeabilized cell nucleus. 56

29. Claim 30: The composition of claim 2, wherein the permeabilized cell nucleus is contained within a cell. 56

B. Ground 2: Claim 29 Is Rendered Obvious By The Combination Of Grunenwald, Okino, And Saleh And/Or Song 57

1. Claim 29: The composition of claim 2, wherein the permeabilized cell nucleus is isolated from a cell. 57

a. Both Saleh And Song Teach Isolation Of Cell Nuclei 57

b. It Would Have Been Obvious To Combine Saleh And/Or Song With Grunenwald and Okino 59

C. Ground 3: Claim 16 Is Rendered Obvious By The Combination of Grunenwald, Okino, and Adey 62

XI. THE BOARD SHOULD REACH THE MERITS OF THIS PETITION 66

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 66

1. Factors (a), (b) and (d): The Office Has Not Considered the Same or Substantially the Same Art or Arguments 66

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00007

2. Factors (c), (e), and (f): The Examiner Erred by Not Considering the Art and Relevant Teachings Herein 67

B. The *Fintiv* Factors Favor Institution 68

XII. CONCLUSION.....71

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00008

TABLE OF AUTHORITIES

Page(s)

Cases

10x Genomics v. Parse Biosciences, Inc.,
22-cv-01117 (D. Del. filed Aug. 8, 2022) 5

Advanced Bionics, LLC v. Med-El Elektromedizinische Gerate GMBH,
IPR2019-01469, Paper 6 (P.T.A.B. Feb. 13, 2020).....67

Amber.IO, Inc. v. 72LUX, Inc.,
IPR2020-00015, Paper No. 8 (P.T.A.B. Apr. 1, 2020).....68

Apple Inc. v. Fintiv, Inc.,
IPR2020-00019, Paper 11 (P.T.A.B. Mar. 20, 2020).....68, 69, 70, 71

Becton, Dickinson & Co. v. B. Braun Melsungen AG,
IPR2017-01586, Paper 8 (P.T.A.B. Dec. 15, 2017) 66

Catalina Mktg. Int'l. v. Coolsavings.com,
289 F.3d 801 (2002).....22

Cellco P'ship v. Huawei Device Co., Ltd.,
IPR2020-01117, Paper 10 (P.T.A.B. Feb. 3, 2021).....68

CyWee Grp. Ltd. v. Samsung Elecs. Co. Ltd.,
No. 2:17-cv-00140-WCB-RSP, 2019 WL 11023976 (E.D. TEX Feb. 14, 2019) 70

International Business Machines Corp. v. Rakuten Inc.,
No. 21-461-GBW, 2022 WL 17848779 (D. Del. Dec. 22, 2022) 69

Kayak Software Corp. v. Int'l Bus. Machs. Corp.,
CBM2016-00075, Paper 16 (P.T.A.B. Dec. 15, 2016).....67

Sand Revolution II, LLC v. Continental Intermodal Grp. – Trucking, LLC,
IPR2019-01393, Paper 24 (P.T.A.B. June 16, 2020) 69

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00009

Snap, Inc. v. SRK Tech. LLC,
IPR2020-00820, Paper 15 (P.T.A.B. Oct. 21, 2020) 70

Statutes

35 U.S.C. § 102(a) ..... *passim*

35 U.S.C. § 103 ..... 7, 14, 67

35 U.S.C. § 325(d) 66, 67, 68

37 C.F.R. § 42.100(b) 21

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00010

PETITIONER'S EXHIBIT LIST

Exhibit #	Description
EX1001	U.S. Patent No. 10,738,357 (the "'357 Patent")
EX1002	Declaration of Gregory Cooper, Ph.D.
EX1003	U.S. Patent Application Publication No. US 2010/0120098 A1, issued to Grunenwald *et al*. ("Grunenwald").
EX1004	PCT Patent Publication No. WO 2010/065266 A1 to Okino *et al.* ("Okino").
EX1005	Abdelaty Saleh et. al, *An Efficient Chromatin Immunoprecipitation (ChIP) Protocol For Studying Histone Modifications In Arabidopsis Plants*, 3 Nature Protocols 1018-1025 (2008) ("Saleh")
EX1006	Intentionally left blank
EX1007	Prosecution File History of U.S. Patent No. 10,738,357
EX1008	Fraz Syed, 19 Second-Generation Sequencing Library Preparation: In Vitro Tagmentation via Transposome Insertion, *Tag-based Next Generation Sequencing* 311-321 (1st Ed. 2012) ("Syed").
EX1009	Andrew Adey et al., *Rapid, Low-input, Low-bias Construction of Shotgun Fragment Libraries by High-density in vitro Transposition*, Genome Biology 1-17 (2010) ("Adey").
EX1010	Megan L McCloskey et al., *Encoding PCR Products with Batch-Stamps and Barcodes*, 45 Biochemical Genetics, 761-767 (2007) ("McCloskey").
EX1011	CSH Protocols, NP-40 lysis buffer (2006) at https://cshprotocols.cshlp.org/content/2006/1/pdb.rec10423.short
EX1012	Gerald Schochetman et al, *Polymerase Chain Reaction*, 158 J. of Infectious Diseases 1154-1157 (1988) ("Schochetman").
EX1013	Peter E. Brodelius, Christoph Funk, and Raymond D. Shillito, Permeabilization of Cultivated Plant Cells by Electroporation for Release of Intracellularly Stored Secondary Products, 7 Plant Cell Reports 186-188 (1988) ("Brodelius").

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00011

Exhibit #	Description
EX1014	Jeffrey M. Becker, 15 Determination of β-Galactosidase in Permeabilized Yeast Cells, *Biotechnology A Laboratory Course* 131-134 (2nd Ed. 1996) ("Becker").
EX1015	Hong-Gang Wang et. al, *Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf-1*, 93 Proc. Natl. Acad. Sci. USA 7063-7068 (1996) ("Wang")
EX1016	J. Gehl, *Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research*, 177 Acta Physiologica Scandinavica 437-447 (2003) ("Gehl").
EX1017	Jin Liu et al., *Non-invasive Assessment and Control of Ultrasound-Mediated Membrane Permeabilization*, 15 Pharmaceutical Research 918-924 (1998) ("Liu").
EX1018	Terez Márián et al., Hypo-osmotic Shock Induces an Osmolality-dependent Permeabilization and Structural Changes in the Membrane of Carp Sperm, 41.2 Journal of Histochemistry & Cytochemistry 291-297 (1993) ("Márián").
EX1019	Melissa A. Melan, *Overview of Cell Fixation and Permeabilization*, 34 Immunocytochemical Methods and Protocols 55-66 (1995) ("Melan").
EX1020	Defendant Parse Biosciences Inc.'s Supplemental Brief In Support of Motion to Dismiss, *10X Genomics, Inc. and The Board of Trustees of the Leland Stanford Junior University v. Parse Biosciences, Inc.*, C.A. No. 22-1117-JHS (Dec 15, 2022 D. Del.)(No.33)
EX1021	Henderson's Dictionary of Biology (Eleanor Lawrence ed., 14th ed., 2008)
EX1022	David S. Johnson et al., Genome-Wide Mapping of in Vivo Protein-DNA Interactions, Science Express 1-70 (2007) ("Johnson").
EX1023	Curriculum Vitae of Gregory Cooper
EX1024	Transcript of Motion Hearing Before Honorable Joel H. Slomsky United States District Court Judge, *10X Genomics, Inc. and The Board of Trustees of the Leland Stanford Junior University v. Parse Biosciences, Inc.*, C.A. No. 22-1117-JHS (Nov. 29, 2022 D. Del.) (No. 27)

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00012

Exhibit #	Description
EX1025	Bruce Alberts, et al., "Chapter 6 How Cells read the Genome: From DNA To Protein", *Molecular Biology of the Cell*, Garland Science 299-335 (4th ed. 2002)
EX1026	David H. Persing, *Polymerase Chain Reaction: Trenches to Benches*, J. of Clinical Microbiology 1281-1285 (1991) ("Persing").
EX1027	Kazuki Kurimoto et al., *An improved single-cell cDNA amplification method for efficient high-density oligonucleotide microarray analysis*, 34(5) Nucleic Acids Research 1-17 (2006) ("Kurimoto").
EX1028	Shigeyuki Esumi et al., *Method for single-cell microarray analysis and application to gene-expression profiling of GABAergic neuron progenitors*, 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, *DNA sequencing: bench to bedside and beyond*, 35(18) Nucleic Acids Research 6227-6237 (2007) ("Hutchinson").
EX1031	Elaine R. Mardis, *Next-Generation DNA Sequencing Methods*, 9 Annual Review Genomics and Human Genetics 387-402 (2008) ("Mardis").
EX1032	Jay Shendure and Hanlee Ji, *Next-Generation DNA Sequencing*, 26(10) Nature Biotechnology 1135-1145 (2008) ("Shendure").
EX1033	Elaine R. Mardis, *The impact of next-generation sequencing technology on genetics*, 24(3) Trends in Genetics 133-141 (2008) ("Mardis 2").
EX1034	Robert A. Holt and Steven J.M. Jones, *The new paradigm of flow cell sequencing*, 18(6) Genome Research 839-846 (2008) ("Holt").
EX1035	National Human Genome Research Institute, *Nucleotide* (March 21, 2023) at https://www.genome.gov/genetics-glossary/Nucleotide.
EX1036	Lumen, *DNA Packaging in Eukaryotes and Prokaryotes* (March 15, 2023) at https://courses.lumenlearning.com/wm-biology1/chapter/reading-dna-packaging-in-eukaryotes-and-prokaryotes/

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00013

Exhibit #	Description
EX1037	Epigentek, Antibodies for Histone Modifications (March 15, 2023) at https://www.epigentek.com/catalog/antibodies-for-histone-modifications-lp-49.html
EX1038	National Human Genome Research Institute, *Polymerase Chain Reaction* (March 21, 2023) at https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction.
EX1039	Athina Gkazi, *An Overview of Next-Generation Sequencing*, Technology Networks Genomics Research at Figure 2 (March 17, 2021) at https://www.technologynetworks.com/genomics/articles/an-overview-of-next-generation-sequencing-346532.
EX1040	Rami Sukarieh, *Relocalization of elF4E by its binding partners upon stress*, Figure 2 (August 2009) at https://www.researchgate.net/publication/41663010_Relocalization_of_eIF4E_by_its_binding_partners_upon_stress
EX1041	Dr. Samanthi, *Difference Between Chromatin and Nucleosome*, Figure 01 (March 31, 2023) at https://www.differencebetween.com/difference-between-chromatin-and-vs-nucleosome/#:~:text=Nucleosome%20can%20be%20defined%20as,a%20basic%20unit%20of%20chromatin.
EX1042	S. Clancy et al., *Translation: DNA to mRNA to Protein*, Nature Education (2008), at https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393/.
EX1043	Illumina. (2017). Nextera XT Troubleshooting Guide. Technical Note. Illumina. Retrieved from https://www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/nextera-xt-troubleshooting-technical-note.pdf
EX1044	Anjali Shah, *Chromatin Immunoprecipitation Sequencing (ChIP-Seq) on the SOLiD™ system*, Nature America, Inc. I-III (2009) ("Shah")
EX1045	Dong Chan Moon et. al, *Nuclear Translocation of Acinetobacter baumannii Transposase Induces DNA Methylation of CpG Regions in the Promoters of E-cadherin Gene*, 7 Plos One 1-8 (2012) ("Moon")

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00014

Exhibit #	Description
EX1046	Scheduling Order, *10X Genomics, Inc. and The Board of Trustees of the Leland Stanford Junior University v. Parse Biosciences, Inc.*, C.A. No. 22-1117-JHS (Mar. 2, 2023 D. Del.) (No. 48)
EX1047	Federal Judicial Caseload Statistics (2022), at https://www.uscourts.gov/statistics-reports/analysis-reports/federal-judicial-caseload-statistics
EX1048	Epicentre Biotechnologies, Nextera™ DNA Sample Prep Kit (Illumina-compatible) (June 12, 2012), at https://web.archive.org/web/20120622102355/http://nextgen.mgh.harvard.edu/attachments/Nextera%20Protocol.pdf
EX1049	Docket Navigator, *Motion Success for Stay Pending IPR in Delaware District* (March 31, 2023)
EX1050	Beáta G Vértessy and Judit Tóth, *Keeping Uracil Out of DNA: Physiological Role, Structure and Catalytic Mechanism of dUTPases*, Acc Chem Res. 1-20 (2009) ("Vértessy")
EX1051	Todd A. Naumann and William S. Reznikoff, *Tn5 Transposase with an Altered Specificity for Transposon Ends*, 184 Journal of Bacteriology 233-240 (2001) ("Naumann")
EX1052	Sanzhen Liu et. al, *Mu Transposon Insertion Sites and Meiotic Recombination Events Co-Localize with Epigenetic Marks for Open Chromatin across the Maize Genome*, 5 Plos Genetics 1-13 (2009) ("Liu 2")
EX1053	Phoebe A. Rice and Tania A. Baker, *Comparative architecture of transposase and integrase complexes*, 8 Nature Structural Biology 302-307 (2001) ("Rice")
EX1054	Murat Cokol, et al., Finding Nuclear Localization Signals, 1 EMBO Reports 411-415 (2000) ("Cokol")
EX1055	J. Sambrook, E.F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Ford et al, eds., 2d ed. 1989)
EX1056	Meena Kishore Sakharkar, Vincent T.K. Chow, and Pandjassarame Kangueane, *Distributions of Exons and Introns in the Human Genome*, 4 In Silico Biology 387-393 (2004)

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00015

Exhibit #	Description
EX1057	Fuchun Zhou et. al, *A sequence-independent in vitro transposon-based strategy for efficient cloning of genomes of large DNA viruses as bacterial artificial chromosomes*, 37 Nucleic Acids Research 1-8 (2009) ("Zhou")
EX1058	William S. Reznikoff, *Tranposon Tn5*, 42 Annu. Rev. Genet. 269-286 (2008) ("Reznikoff")
EX1059	Young Min Kwon and Steven C. Ricke, *High-Throughput Next Generation Sequencing*, Methods in Molecular Biology Humana Press 1-308 (2011) ("Kwon")
EX1060	Lingyun Song and Gregory E. Crawford, *DNase-seq: A High-Resolution Technique for Mapping Active Gene Regulatory Elements across the Genome from Mammalian Cells*, 2 Cold Spring Harbor Laboratory Press 1-11 (2010) ("Song")
EX1061	James H Keith et. al, *Analysis of the piggyBac transposase reveals a functional nuclear targeting signal in the 94 c-terminal residues*, 9:72 BMC Molecular Biology 1-13 (2008) ("Keith")
EX1062	Hong Ji, *Lysis of Cultured Cells for Immuniprecipitation*, 8 Cold Spring Harbor Laboratory Press 1-4 (2010) ("Ji")

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00016

I. INTRODUCTION

Parse Biosciences, Inc. ("Petitioner") requests review of claims 1-30 of United States Patent No. 10,738,357 (the "'357 Patent"), which issued on August 11, 2020 and is assigned to 10x Genomics, Inc. ("Patent Owner").

The '357 Patent pertains to a technique known as "tagmentation." Briefly, "tagmentation" entails using an enzyme, called a transposase, to fragment DNA 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 '357 Patent is directed to applying this tagmentation process to a "permeabilized cell nucleus." 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 '357 Patent are little different from those of the '995 Patent, except, as noted, the former requires cell nuclei that have been permeabilized.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00017

Importantly, however, neither "tagmentation" nor the analysis of permeabilized cell nuclei were new or novel. In fact, the '357 Patent admits that these were well-established prior art processes. The '357 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 14:38-44.

Likewise, as to the analysis of permeabilized cell nuclei, the '357 Patent does not teach any new or special protocols, but rather admits that "the chromatin used in the method may be made by any suitable method." *Id.* at 14:45-47. In fact, the '357 Patent never once refers to a "permeabilized cell nucleus." To the extent the specification provides any guidance on preparing such "permeabilized cell nuclei," the sole disclosure is of prior art protocols for lysing cells using detergents so that one can access the cellular interior.

What, then, is the alleged invention of the '357 Patent, if not tagmentation itself? To the extent there is any alleged novelty in the '357 Patent, it is nothing more than the trivial idea of using "tagmentation" with cell nuclei to fragment and tag DNA as it exists in nuclei in its naturally-occurring form. 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 nucleus:

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00018

> Although engineered insertional enzymes having sequencing adaptor molecules had been used to tagment isolated DNA fragments, prior to the ATAC-seq Patents, such engineered insertional enzymes had not been introduced into a cell nucleus to perform tagmentation inside the cell nucleus to interrogate areas of open chromatin.

EX1020 at 19 (emphasis in original); *see also* EX1024 at 106:6-13 ("[T]agmentation 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?").

Yet, there is nothing inventive or novel about applying tagmentation to a cell nucleus. Any such contention is negated by the earliest patent filings on the tagmentation process, such as the Grunenwald reference, which teaches applying tagmentation to cells 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).

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00019

EX1003 at [0157].

As a matter of commonsense, and as proven herein and in the accompanying declaration of Dr. Gregory Cooper, the application of tagmentation to cells will unavoidably involve application of tagmentation to cell nuclei because genetic material is stored in nuclei in the cell. And, as noted above, the '357 Patent effectively admits that further permeabilizing cell nuclei was not inventive by failing to state as much and instead simply disclosing prior art cell lysis protocols.

As a result, Grunenwald in view of Okino renders obvious every claim of the '357 Patent. While the Examiner did analyze Grunenwald in issuing a non-final rejection, the Examiner's analysis did not include any consideration of the embodiment disclosed in Grunenwald based on applying tagmentation directly to cells. *See* EX1007.152-157. Likewise, the Examiner performed no analysis of Okino in the non-final rejection, nor in allowing the claims. *Id.*; *see also* EX1007.224-225. The Board should now take this opportunity to correct these oversights and cancel the claims.

Indeed, any suggestion that the claimed invention of the '357 Patent is novel or inventive is put to rest by the primary inventor, Dr. Jason Buenrostro. He and the other inventors termed their alleged invention "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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00020

lawsuit against Petitioner based on *inter alia* the ’357 Patent, he lamented the litigation and confirmed that the alleged invention was “so easy that anyone can do it!”

EX1055. Because the alleged inventions in the ’357 Patent are not new and reflect nothing more than the previously disclosed application of tagmentation to cell nuclei, the claims of the ’357 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 ’357 Patent is involved in litigation in *10x Genomics v. Parse Biosciences, Inc.*, 22-cv-01117 (D. Del. filed Aug. 8, 2022).

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00021

C. Grounds for Standing

Petitioner certifies that the ’357 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) Postal & Hand-Delivery Address: Weil, Gotshal & Manges LLP 201 Redwood Shores Parkway Redwood Shores, CA 94065 T: 650-802-3934 F: 650-802-3100	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: 650-802-3124 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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00022

III. RELATIONSHIP BETWEEN THIS IPR AND RELATED PROCEEDINGS

In addition to seeking review of the ’357 Patent in the instant petition, Petitioner is also seeking review of U.S. Patent Nos. 10,150,995 (the “’995 Patent”) and 10,619,207 (the “’207 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 ’357 Patent at issue here are highly similar to those of the ’995 and ’207 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 ’207 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 ’357 Patent:

- **Ground 1:** Claims 1-28 and 30 of the ’357 Patent are unpatentable under 35 U.S.C. § 103 as being rendered obvious by Grunenwald (US 2010/0120098 (EX1003)) and Okino (WO 2010/065266 A1 (EX1004)).

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00023

- **Ground 2:** Claim 29 is rendered obvious by the combination of Grunenwald, Okino, and Saleh ("An Efficient Chromatin Immunoprecipitation (ChIP) Protocol For Studying Histone Modifications In Arabidopsis Plants" (EX1005)).
- **Ground 3:** Claim 16 is rendered obvious by the combination of Grunenwald, Okino, and Adey ("Rapid, Low-input, Low-bias Construction of Shotgun Fragment Libraries by High-density *in vitro* Transposition" (EX1009))

V. TECHNOLOGICAL BACKGROUND

A. DNA and RNA

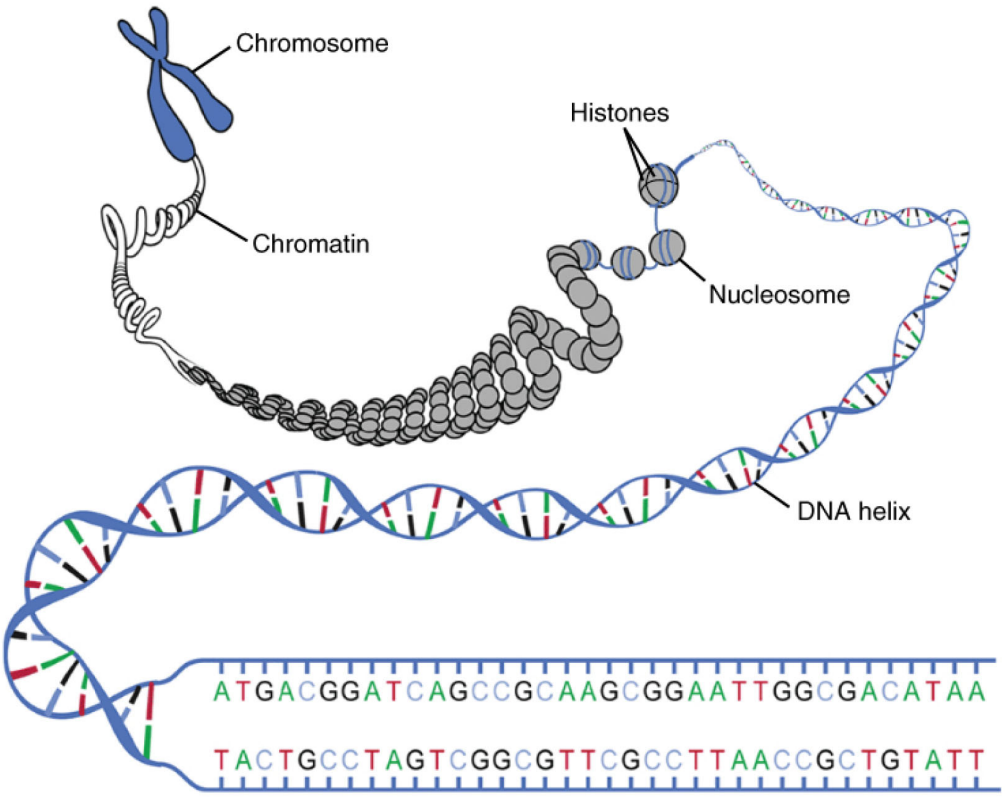
Deoxyribonucleic acid, or DNA, is a double-stranded molecule having a chain of bases or "nucleotides." EX1002 ¶ 45. Each nucleotide comprises of four types of bases: adenine ("A"), cytosine ("C"), guanine ("G"), and thymine ("T"). *Id.* ¶ 46. The order of these bases constitute genetic information, which can be copied to a ribonucleic acid ("RNA") strand, by a process called transcription. *Id.* ¶ 51.

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 ¶ 56. This packaging is achieved in living cells with the aid of proteins called "histones," which act as scaffolding for the DNA to be coiled around. Eight core histone subunits along with approximately 146 base pairs of DNA constitute a "nucleosome." The collection of nucleosomes with DNA form a complex called "chromatin." *Id.* ¶¶ 59-60.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00024

Chromatin is further packaged and condensed into chromosomes, which typically exist as pairs in humans and some other organisms. *Id.* ¶¶ 57; 63. 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:



EX1041 at Figure 01

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 ¶ 64. 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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00025

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.* ¶ 64.

The level of DNA packaging determines the accessibility of DNA for gene expression. Accessibility of chromatin is directly associated with gene expression. *Id.* ¶ 65. 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 ¶ 66. 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 ¶ 67.

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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00026

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.

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. EX1005.001.

However, library preparation, including the necessary fragmentation, can also be, and often is, accomplished by the process of "tagmentation," which was developed by researchers as early as 2010 to reduce the time and cost of preparing DNA libraries for NGS. EX1002 ¶¶ 69-71; EX1006.002; EX1008.001. Tagmentation is a one-step reaction where accessible DNA is fragmented and tagged for analysis. EX1008.001. A specific type of enzyme—transposase—simultaneously fragments a target DNA portion and attaches oligonucleotide tags at the cleaved site. EX1008.001. 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 ¶ 69; EX1006.002; EX1010.002.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00027

D. Permeabilization

To gain access to chromatin nested inside of a cell and guarded by the cell membrane, the cell membrane has to first be made permeable. The process by which openings are created in the cell membrane to allow entry of reagents into the cell is generally known as "permeabilization." EX1002 ¶ 87. Such permeabilization techniques can be chemical in nature. Detergents such as saponin, Triton X-100, and NP40 were commonly used permeabilization agents as early as 1994. EX1019.007. Detergents act on membranes by dissolving the components of the membranes, thereby creating openings and rendering the membranes permeable. Among them, saponin acts by interacting with membrane cholesterol, and Triton X-100 and NP40 act by solubilizing lipids and integral membrane proteins. *Id.* at 006.

VI. OVERVIEW OF THE '357 PATENT

A. Priority Date of the '357 Patent

The '357 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 '357 Patent is no earlier than the date of this provisional application—May 23, 2013.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00028

B. Overview Of The Claims

The ’357 Patent pertains to applying prior art tagmentation techniques to open chromatin, claiming a composition generated through such prior art techniques. Exemplary Claim 1 of the ’357 Patent is as follows:

> 1. A composition comprising:
> a permeabilized cell nucleus comprising:
> (a) a plurality of polynucleotide molecules; and
> (b) an insertional enzyme complex comprising an insertional enzyme; and an insert element comprising a predetermined sequence.

Claim 1 of the ’357 Patent recites only the composition created through the application of an “insertional enzyme complex” to a permeabilized cell nucleus to generate fragmented and tagged genomic nucleic acid molecules.

The ’357 Patent defines “insertional enzyme complex” to mean a complex comprising an insertional enzyme (e.g., a transposase) and a payload of DNA tag sequences. EX1001 at 13:26-33; *see also* 15:67-16:13 (explaining that the insertional enzyme can be a transposase). As explained above, however, this had been known and widely used in the art as of the filing of the ’357 Patent. *See supra* Section IV.C (explaining tagmentation has been known since 2010). Thus, the composition recited in independent claim 1 is nothing more than the composition generated through the application of well-known, widely available tagmentation techniques to permeabilized cell nuclei.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00029

As further documented herein, the ’357 Patent’s other claims further recite nothing more than aspects of the composition formed through the application of well-known techniques.

C. Prosecution History Overview

The ’357 Patent was filed on May 21, 2019. Track One priority was requested and granted on July 11, 2019.

On August 30, 2019, the Examiner issued a non-final rejection. This rejection was based, in part, on § 103. The Examiner cited the combination of Grunenwald in view of Jouvenot and further in view of Audit as a combination that rendered the claims obvious. EX1007.152. The Examiner pointed Grunenwald’s teaching of “a composition comprising target DNA with a transposase and a transposon end or transposon end comprising a transferred strand that has a tag domain in its 5’ portion.” EX1007.152-53. Further, the Examiner stated that “Grunenwald differs from the instant invention” because it does not expressly teach that the composition or reaction mixture “comprise of permeabilized cell nucleus.” EX1007.155.

On December 2, 2019, the Applicant responded to the non-final rejection, stating that “none of the cited references teach or suggest a composition comprising a permeabilized cell nucleus, in addition to the other elements listed in the claims.” EX1007.198. Applicant further argued that unlike the alleged invention of the ’357 Patent, the cited references teach a “multi-step approach” and that there is never a

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00030

"composition comprising a permeabilized cell nucleus comprising the elements recited in the pending claims."

On April 3, 2020, the Examiner issued a Notice of Allowance. While Grunenwald formed the basis for the non-final rejection, there is no evidence that the Examiner cited or analyzed Grunenwald's embodiments based on application of tagmentation to cells. EX1007.151-158. Likewise, the Examiner neither cited nor analyzed Okino as a part of a rejection or in allowing the claims. EX1007.151-158, 224-225.

VII. LEVEL OF ORDINARY SKILL IN THE ART OF THE '357 PATENT

A person of ordinary skill in the art ("POSA") relating to the '357 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 '357 Patent reflect techniques, competencies, and compositions that would have been part of the toolkit

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00031

of the ordinary skilled artisan. 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.
- Epigenetic analysis and techniques therefore.

See EX1002 ¶¶ 93-96.

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* 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]-[0084].

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00032

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 out in vivo, 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 [1057]. A POSA reading these disclosures would have understood that performing transposition fragmentation and tagging on chromosomal target DNA within a host call would mean that

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00033

Grunenwald's methods were applicable to chromatin in the nucleus of the cell. EX1002 ¶ 103.

B. Okino

Okino was filed on November 13, 2009 and published on June 10, 2010. EX1004.001. Okino is prior art under 35 U.S.C. § 102(a).

Okino is directed to the study of chromatin structure through the use of a "DNA modifying agent," which may be any molecule that "alters DNA in a detectable matter," such as an enzyme that breaks the DNA, or an enzyme that introduces or removes a moiety bound to the DNA. EX1004.035. Okino teaches that this is done in two steps: first, a cell is simultaneously permeabilized and contacted with a DNA modifying agent, and second, the DNA sequence information is detected. EX1004.005.

C. Saleh

Saleh was published in Nature Protocols in 2008. EX1005.001. Saleh is prior art under 35 U.S.C. § 102(a).

Saleh describes a prior art method for fragmenting and tagging DNA in preparation for sequencing known as chromatin immunoprecipitation, or ChIP. ChIP allows for the study of protein-DNA interactions through DNA preparation and subsequent sequencing or array study. In ChIP, the first step is "cross-linking" DNA and proteins *in vivo* in a cell.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00034

This consists of using formaldehyde to bind the DNA to associated proteins and freeze the interactions in a "snapshot." EX1005.004. Next, the now-bound chromatin is isolated and sheared. In Saleh, this is done first by isolating nuclei of the cell. EX1005.004. Once the nuclei are isolated, DNA can be extracted and sheared using sonication, or sound waves, to fragments of an appropriate length for tagging and sequencing. EX1005.004-005. Fragment size may be checked through gel electrophoresis, which allows seeing the relative size of DNA fragments. EX1005.005. After ensuring fragments are appropriately sized, the bound proteins are contacted with antibodies to permit immunoprecipitation, or the pulling of proteins out of solution. EX1005.005. Proteins are then un-linked from DNA and digested, and the DNA is precipitated and subject to further analysis. EX1005.006. The prior art techniques disclosed in Saleh would have been conventional and routine at the time of the alleged invention in the '357 Patent. EX1002 ¶ 119.

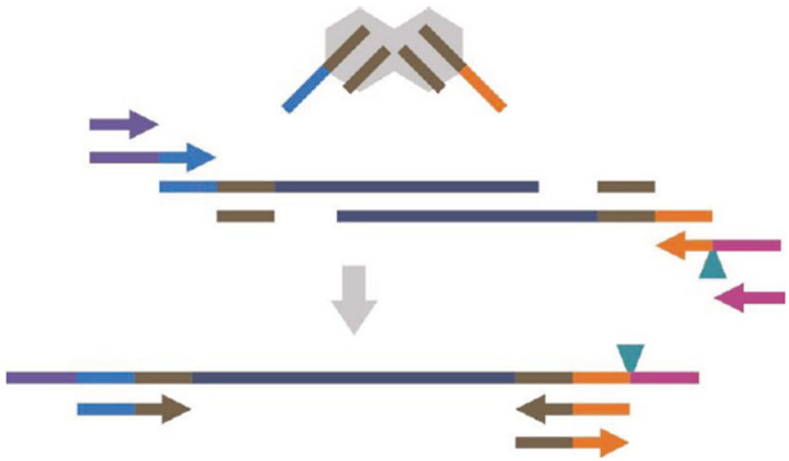
D. Adey

Adey was published in Genome Biology in 2010. EX1009.001; EX1006.001. 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. EX1006.002. As

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00035

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):



EX1006.002 at Figure 1

E. 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). EX1051.040

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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00036

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.

IX. CLAIM CONSTRUCTION

A. "Insertional Enzyme Complex"

The '357 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 13:26-33.

B. "Nucleic Acid Insert Element"

The plain and ordinary meaning of a "nucleic acid insert element" in the context of the '357 Patent is a nucleic acid for insertion in a polynucleotide by an insertional enzyme, such as transposase. EX1002 ¶ 133. This meaning is consistent with plain language of the claim term. EX1001 at claims 1, 2, 7, 13. Moreover, the '357 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 3:64-4:1; 4:2-5; 4:13-15; 4:25-29, 22:31-37; 22:51-55. The insert element can also be "bound to the insertional enzyme and the insertional enzyme bound to the polynucleotide." EX1001 at 4:13-14.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00037

X. IDENTIFICATION OF HOW THE CHALLENGED CLAIMS ARE UNPATENTABLE

A. Ground 1: Claims 1-28, 30 Are Rendered Obvious by the combination of Grunenwald and Okino

1. Claim 1

a. A composition comprising:

A preamble is only limiting if it "recites essential structure or steps or if it is necessary to give life, meaning, and vitality to the claim." *Catalina Mktg. Int'l. v. Coolsavings.com*, 289 F.3d 801, 808 (2002) (internal quotations omitted). The preamble of claim 1 recites nothing more than a generic composition, and is thus is not limiting.

b. a permeabilized cell nucleus comprising:

The specification of the '357 Patent does not once refer to a "permeabilized cell nucleus," nor does it discuss treating the nuclei specifically to permeabilize the nuclear envelope. In fact, there are no specific disclosures throughout the entirety of the specification focusing on permeabilization of cell nuclei. Just the opposite, the '357 Patent assumes the skilled artisan would already know and understand the appropriate methods for DNA preparation, stating generally that the "chromatin used in the method may be made by any suitable method." EX1001 at 14:48-50.

Rather than teaching any form of nuclear permeabilization, the specification explains that nuclei may be used after cells are contacted with a reaction buffer that will lyse the cells. EX1001 at 14:52-56. The specification mentions cell lysis again

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00038

in discussing kit embodiments of the invention, where a kit is disclosed that generally refers to a process where the cells and nuclei are lysed. *Id.* at 22:60-23:3. Even in this kit embodiment, however, the specification teaches nothing more than a general "reaction buffer" that is not otherwise described or explained in any further detail. *Id*.

The only specific mention of nuclei preparation in the working examples is in column 25:5-15, which presents a protocol titled "Prepare Nuclei." This protocol, however, again teaches only a standard lysis protocol based on the use of a cell lysis buffer including a detergent to lyse the cells that was standard and known in the art before the alleged invention of the '357 Patent. EX1001 at 25:5-15; EX1002 ¶¶ 142-43. Specifically, the '357 Patent teaches the addition of a lysis buffer that comprises 10 mM Tris-Cl, pH7.4, 10mM NaCl, 3 mM $MgCl_2$, and 0.1% IGEPAL CA-630. EX1001 at 25:8-9. This formulation based on the use of the IGEPAL detergent was not new or special, but was instead taken verbatim from the Song reference, which appeared in the 2010 volume of the Cold Spring Harbor Protocols, a common source for routine laboratory protocols. EX1002 ¶ 143.

Thus, given the disclosures in the specification of the '357 Patent regarding how to obtain nuclei for use with the invention, one would understand the reference to a "permeabilized cell nucleus" to encompass, *inter alia*, cellular nuclei that result from treatment of cells with standard lysis buffers so as to lyse the cells and

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00039

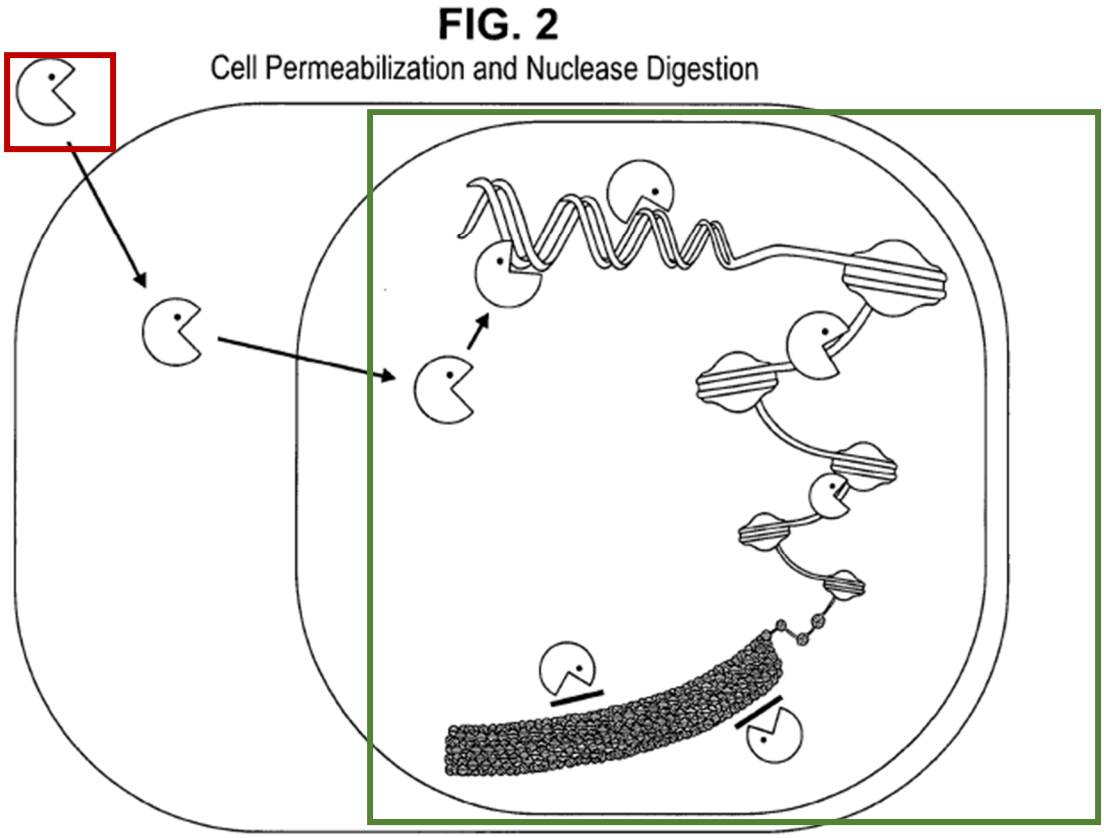
permeabilize the nuclear envelope. In this regard, the '357 Patent makes clear that the use of "permeabilized cell nuclei" is not inventive or novel, but rather a routine step that results from applying known cell lysis buffers to cells that incorporate detergents.

In view of the foregoing, and for the reasons stated below, a POSA would understand that Okino discloses the use of a permeabilized cell nucleus in exactly the same way as the '357 Patent. Further, the POSA would have been motivated to combine this disclosure with Grunenwald's tagmentation method and would have had a reasonable expectation of successfully doing so.

(1) Okino Discloses A Permeabilized Cell Nucleus

Okino teaches "methods of permeabilizing" a cell membrane that "will not disrupt the structure of the genomic DNA of the cell such that nucleosomal or chromatin structure is destroyed." EX1004 at [0071]. These methods are based on the use of an "agent that permeabilizes or disrupts the cell membrane," or, alternatively, "electroporation or biolistic methods can be used to permeabilize a cell membrane such that a DNA modifying agent is introduced into the cell and can thus contact the genomic DNA." *Id*. at [0072], [0075]. Regardless of the method used, an advantage to the method taught in Okino is the "simultaneous delivery of the permeabilization agent and the DNA cleaving or modifying agent." *Id*. at [0075]. This method is exemplified in Figure 2 of Okino, reproduced below.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00040



EX1004 at Figure 2

In this figure, a "permeabilization/digestion buffer," i.e., a buffer that is capable of both permeabilizing the cell by agent and allowing a digestion enzyme to function in the cell is added to allow the reaction to occur. *Id*. at [0044]. The pac-man-like symbol, boxed in red, is a nuclease, or an enzyme that is capable of breaking down DNA. *Id*. In this schematic, the nuclease is able to diffuse into the cell ***and can also*** enter the nucleus of the cell, highlighted in green, and then digest open chromatin while not digesting inaccessible chromatin. *Id*. This figure

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00041

establishes that the methods of permeabilization disclosed in Okino permeabilize the cell nucleus, as the nucleus is permeabilized to permit the entry of the nuclease or other enzyme. EX1002 ¶ 148.

Critically, the permeabilization agent used in Okino is a detergent, such as the common NP40 or Tween detergents. *See* EX1004 at [0012] ("In some embodiments, the permeabilizing or disrupting step comprises disrupting the cell membrane with a nonionic detergent. In some embodiments, the nonionic detergent is selected from the group of NP40, Tween20 and Triton X-100."); *see also id.* at [0073], claims 10-11. Such detergents set forth in Okino are, in fact, included among the exact class of compounds that are used in the '357 Patent to treat cells for use with the alleged invention. *See* EX1001 at 3:24-26 ("The cell sample can be permeabilized using a permeabilization agent including, but not limited to, ***NP40***, digitonin, ***tween***, streptolysin, and/or cationic lipids.").

While the '357 Patent includes the IGEPAL detergent for cell lysis in its working example, *see* EX1001 at 25:5-15, it was well known that other detergents, such as the NP40 detergent identified in both Okino and the '357 Patent, also work to disrupt membranes. *See, e.g.*, EX1011 ("NP-40 lysis buffer"); EX1015 at 7063 (lysing cells using NP40 buffer). In fact, it was known that such detergents could be used to lyse cells while only causing minimal harm to structures inside the cell. *See, e.g.*, EX1062 ("If low detergent concentrations are sufficient to cause cell lysis

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00042

(e.g., 1% Nonidet P-40 [NP-40] or 1% Triton X-100), this method can be gentler to the protein of interest than mechanical homogenization methods.”).

Thus, the skilled artisan would recognize that Okino teaches the exact approaches (*i.e.*, application of a detergent-containing buffer to the cell sample) that are set forth in the ’357 Patent for providing permeabilized cell nuclei. The use of these methods to provide permeabilized cell nuclei, is shown, for instance, in Figure 2 of Okino (reproduced above).

(2) It Would Have Been Obvious To A POSA To Combine Okino’s Permeabilized Cell Nuclei With Grunenwald

It would have been obvious to combine Grunenwald’s disclosures of tagmentation with Okino’s disclosures of detergent-based permeabilization methods and a permeabilized cell nucleus.

First, Grunenwald and Okino are analogous art. Both references are in the field of genetics and molecular biology, and both teach methods to study chromatin through DNA modification and subsequent sequencing. EX1002 ¶¶ 152-53; *see, e.g.*, EX1003 at Abstract; EX1004 at [0005]. Thus, a person of skill in the art with Grunenwald in hand would naturally look to a reference such as Okino.

Second, for several reasons, a person of skill in the art would have been motivated to combine Okino’s permeabilized cell nuclei with Grunenwald. As an initial matter, Grunenwald teaches that just about any source of DNA may be used

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00043

in its tagmentation method. Grunenwald teaches, for instance, that "target DNA" refers to any double-stranded "DNA of interest that is subjected to transposition," and can be "derived from any in vivo or in vitro source, including from one or multiple cells, tissues, organs, or organisms, whether living or dead, or form any biological or environmental source (e.g. water, air, soil)." EX1003 at [0145], [0146]. The target DNA may comprise or consist 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 [0146].

Consistent with its teaching that the method can be carried out on "chromosomal DNA," Grunenwald further teaches that its method can be carried out by introducing the transposase directly into a cell. EX1003 at [0157]; EX1002 ¶ 157. In Grunenwald, to carry out this transposition reaction, a "synaptic complex of a transposase and a suitable transposon end composition" is electroporated into the cell. EX1003 at [0157].

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00044

As documented above, electroporation was well-established in the art as a method of permeabilization. *See supra* Section IV.C. While the Examiner stated during prosecution that Grunenwald "does not expressly teach wherein the composition…comprise[s] a permeabilized cell nucleus," the Examiner did not recognize and performed no analysis of this embodiment contacting a cell directly to achieve this tagmentation reaction. *See* EX1007.151-158. In view of Grunenwald's disclosure of electroporation to permit a transposase to access the cell, a POSA would have considered other alternatives to gain access and/or improve access to the genetic material in the cell nucleus. These include well-known permeabilization techniques that were routine in the art. EX1002 ¶¶ 158-59; *see supra* Section IV.D.

Indeed, a POSA interested in the tagmenting DNA in a live cell would have been motivated to permeabilize the cell and nucleus to better permit an insertional enzyme complex to contact the cellular genetic material. EX1002 ¶ 159. As such, the skilled artisan would have been motivated to look to Okino. Okino's methods of permeabilization were well-known and routine, and a POSA would have been motivated to apply those methods to the *in vivo* method taught in Grunenwald.

Namely, Okino presents electroporation and permeabilization by detergent as alternatives, and a POSA would recognize that to permeabilize a cell and cell nucleus by either option would be a mere design choice. EX1004 at [0072]–[0075]; EX1002

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00045

¶¶ 160-61. Further, a POSA would recognize that in permeabilizing a cell nucleus as taught in Okino, and allowing an insertional enzyme to contact chromatin within the permeabilized cell nucleus, the result would necessarily be the creation of a composition comprising a permeabilized cell nucleus comprising a plurality of polynucleotide molecules and an insertional enzyme complex with an insert element comprising a predetermined sequence. EX1002 ¶ 161.

Third, a POSA would have had a reasonable expectation of success in combining Okino's permeabilized cell nuclei with Grunenwald. Grunenwald teaches a method for modifying chromatin through tagmentation, analogous to the methods of chromatin modification taught in Okino. *See supra* Sections IV.C, VIII.C; *see also* EX1002 ¶ 162. A POSA practicing Grunenwald's *in vivo* tagmentation method with the methods of permeabilization would have a reasonable expectation of producing a composition comprising a permeabilized cell nucleus comprising a plurality of polynucleotide molecules with an insertional enzyme complex and an insert element comprising a predetermined sequence. EX1002 ¶ 162. In fact, as noted above, such detergent-based lysis techniques had been used not just by Okino, but by numerous other researchers for years. *See, e.g.*, EX1011; EX1015.001; EX1062.

Tellingly, the '357 Patent does not disclose any methods to permeabilize the cell nucleus that are not routine or conventional. The '357 Patent instead simply

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00046

discloses that a cell sample can be permeabilized using a "permeabilization agent including, but not limited to, NP40, digitonin, tween, streptolysin, and/or cationic lipids." EX1001 at 3:21-27; 16:55-65. As noted above, the only specific mention of nuclei preparation in the working examples is in column 25:5-15, which presents a protocol titled "Prepare Nuclei." This protocol, however, is just a prior art method taken verbatim from the Song reference, which appeared in the 2010 volume of the Cold Spring Harbor Protocols, a common source for routine laboratory protocols. EX1002 ¶ 166. Disclosing nothing other than prior art detergent-based methods for disrupting cellular membranes that were tried and true as of the priority date, the '357 Patent effectively acknowledges that the skilled artisan would have had a reasonable expectation of successfully applying such methods to Grunenwald.

For the foregoing reasons, a POSA would have found it obvious to take the routine methods of permeabilization taught in Okino and apply them to the cellular embodiment of tagmentation found in Grunenwald to achieve the claimed methods of the '357 Patent. EX1002 ¶ 168.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00047

c. (a) a plurality of polynucleotide molecules; and

Grunenwald teaches that the tagmentation method taught is used on "target DNA" that can be derived from any "in vivo or in vitro source, including from one or multiple cells." EX1003 at [0146]. This DNA can be derived from cells from "humans, animals, plants, [or] fungi (e.g., molds or yeasts)." *Id*. Grunenwald likewise teaches that the target DNA may comprise or consist 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." *Id.* at [0146].

Further, as detailed above, Grunenwald discloses tagmentation occurring on DNA found directly in cells. *Id*. at [0157]. Applied to cells having more than one chromosome (*i.e.*, the cells of almost all eukaryotic organisms), Grunenwald's method will necessarily involve a composition with a plurality of polynucleotide molecules (*i.e.*, the different chromosomes). EX1002 ¶¶ 169-72. Alternatively, in such embodiments, the insertional enzyme complex will promptly fragment and tag

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00048

the chromosomal DNA in the cell so as to yield a plurality of polynucleotide molecules (*i.e.*, fragments of the chromosomes). *See supra* Part VII.A.

d. (b) an insertional enzyme complex comprising an insertional enzyme and

Grunenwald discloses an insertional enzyme complex comprising an insertional enzyme. As explained in Section VIII.A, *supra*, the '357 Patent defines the "insertional enzyme complex" 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 13:26-33. The '357 Patent further states that this "insertional enzyme can be transposase." *Id*. at 3:46-47. Thus, "insertional enzyme complex" encompasses a complex comprising transposase and two transposon tags that are combined with polynucleotides to fragment and tag the polynucleotides. *See* EX1002 ¶ 174.

Grunenwald discloses this 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:

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00049

> 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 ¶ 177; *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 '357 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 ¶¶ 178-79. These tag domains can include, for example, "one or more of a restriction site tag domain, a capture tag

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00050

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 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 "insertional enzyme complex" (e.g., the transposome) that comprises a transposase and at least two adaptors (*e.g.*, tags, tag portions, or tag domains) that combine with polynucleotides (e.g., target DNA) to fragment and tag it.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00051

e. an insert element comprising a predetermined sequence.

Grunenwald discloses an insert element comprising a predetermined sequence.

First, Grunenwald discloses an insert element. As explained above in Section IX.A.1.d, Grunenwald discloses a transposome (*i.e.*, an insertional enzyme complex) 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 an insertional enzyme—*i.e.*, transposase. EX1002 ¶ 185.

Second, Grunenwald discloses that the insert element comprises a predetermined sequence. Grunenwald teaches that tags (*i.e.*, the claimed insert element) may comprise 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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00052

Genomics sequencing tags." *Id*. at [0021], [0194], Claims 7, 13, 15, 21. These sequences would be a predetermined sequence, as they would be sequences that are selected for the purpose of compatibility with a particular next-generation sequencing system. EX1002 ¶ 190.

2. Claim 2

a. A composition comprising:

The preamble is not limiting. *See supra* Section IX.A.1.a.

b. a permeabilized cell nucleus comprising:

This claim limitation is taught by Okino, and as documented above, a POSA would have been motivated to apply Okino's teaching of a permeabilized cell nucleus to Grunenwald. *See supra* IX.A.1.b.

c. chromatin comprising genomic DNA and a plurality of DNA binding proteins

As detailed in the technical background and in the accompanying declaration of Dr. Cooper, a cell nucleus necessarily and inherently contains chromatin, the natural form that genomic DNA takes in a cell. *See supra* Section V.B; EX1002 ¶ 195. This chromatin is composed of genomic DNA and a plurality of DNA binding proteins. *See supra* Section V.B; EX1002 ¶ 195.

Grunenwald discloses contacting a cell, and thus a cell nucleus, directly with an insertional enzyme complex to accomplish tagmentation. *See* EX1003 at [0157].

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00053

Because of this disclosure and the inherent nature of a cell nucleus, Grunenwald discloses this limitation.

d. wherein the chromatin comprises a plurality of open chromatin regions; and

As detailed in the technical background and in the accompanying declaration of Dr. Cooper, chromatin existing in a cell inherently comprises both regions of "open," or accessible, chromatin and "closed," or inaccessible, chromatin. *See supra* Section V.B; EX1002 ¶ 202. Grunenwald, teaching use of its method with a cell directly, thus discloses this claim limitation. *See* EX1003 at [0157]; EX1002 ¶ 203. Indeed, the '357 Patent never once suggests that open chromatin is anything more than a natural form of chromatin that exists in cells, nor does it identify any special steps that must be taken to prepare open chromatin.

e. an insertional enzyme complex comprising an insertional enzyme and

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

f. an insert element comprising a predetermined sequence,

Disclosed. *See supra* Section IX.A.1.e.

g. wherein the insertional enzyme complex is bound to one of said open chromatin regions.

As discussed above, Grunenwald teaches a method of tagmentation using a transposase to conduct a transposition reaction. *See, e.g.*, EX1003 at [0017]. Grunenwald specifically teaches the use of a Tn5 transposase or a Mu transposase.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00054

EX1003 at [0045]. When Grunenwald's method is applied directly to a cell, it is "exemplified by employing a transposition complex formed by a hyperactive Tn5 transposase and a suitable Tn5-type transposon end composition." EX1003 at [0157].

Further, as documented above, during the natural life cycle of a cell, chromatin exists in both open and closed forms, the former of which is accessible to the cellular machinery and other enzymes. *See supra* Section V.B. As such, the Tn5 transposase or Mu transposase will bind to these open chromatin regions when contacting chromatin. This element is thus disclosed as an inherent characteristic of the disclosed compositions in Grunenwald. EX1002 ¶ 208. Indeed, the '357 Patent never once suggests that open chromatin is anything more than a natural form of chromatin that exists in cells, nor does it identify any special steps that must be taken to prepare open chromatin.

3. Claim 3: The composition of claim 2, wherein the insertional enzyme comprises one or more enzymatic moieties.

Grunenwald teaches a transposase, which is an enzymatic moiety. *See supra* Section IX.A.1.d; EX1002 ¶ 215. Thus, Grunenwald discloses this claim limitation.

4. Claim 4: The composition of claim 3, wherein the insertional enzyme comprises two or more enzymatic moieties.

Grunenwald teaches the use of a Tn5 transposase as an insertional enzyme, and an insertional enzyme complex comprising a Tn5 transposase with an

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00055

accompanying transposon end. *See, e.g.*, EX1003 at [0045]. A Tn5 transposase is comprised of a dimer of transposase subunits, which act as two enzymatic moieties that form a synaptic complex by linking. EX1002 ¶ 219. Thus, through its use of the Tn5 transposase, Grunenwald discloses an insertional enzyme that comprises two or more enzymatic moieties. EX1002 ¶ 220.

5. Claim 5: The composition of claim 4, wherein the two or more enzymatic moieties are linked together.

Disclosed. *See supra* Section IX.A.4.

6. Claim 6: The composition of claim 2, wherein the insertional enzyme comprises a nuclear localization signal.

Grunenwald teaches the use of a Tn5 transposase, which is a type of a Tn transposase. EX1003 at [0045]; EX1002 ¶ 226. It was well-known in the art prior to 2013 that such a transposase contains a nuclear localization signal. EX1002 ¶¶ 227-28.

7. Claim 7: The composition of claim 2, wherein the insertional enzyme complex is configured to insert the insert element into the genomic DNA at an open chromatin region.

Grunenwald teaches a method of transposition using a transposase. *See, e.g.*, EX1003 at [0045]. This transposase is part of an insertional enzyme complex with transposon ends, which are DNA sequences that play a role in the insertion of tags in the tagmentation process. EX1003 at [0052], [0094-0098]. These transposon ends comprise the claimed "insert element" in the '357 Patent. EX1002 ¶¶ 232-34.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00056

Further, Grunenwald teaches insertion into genomic DNA, as the methods taught include a transposition reaction in which one or more transposon ends are inserted into a target DNA, which may comprise genomic DNA. EX1003 at [0146]. As noted above, Grunenwald even teaches application of its method to cells. *Id.* at [0157]. During the natural life cycle of a cell, chromatin exists in both open and closed forms, the former of which is accessible to the cellular machinery and other enzymes. *See supra* Section V.B. As such, the Tn5 transposase or Mu transposase will bind to these open chromatin regions when contacting chromatin. This element is thus disclosed as an inherent characteristic of the disclosed compositions in Grunenwald. EX1002 ¶ 237. Indeed, the '357 Patent never once suggests that open chromatin is anything more than a natural form of chromatin that exists in cells, nor does it identify any special steps that must be taken to prepare open chromatin.

To the extent this claim is interpreted as requiring some sort of preferential insertion into open chromatin, Grunenwald teaches the use of a Mu transposase. EX1003 at [0145]. A Mu transposase is a transposase that was known in the art prior to 2013 to bind preferentially to regions of open chromatin. EX1002 ¶ 238. Thus, Grunenwald discloses this limitation.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00057

8. Claim 8: The composition of claim 7, wherein the insertional enzyme complex is configured to insert the insert element into the genomic DNA in a substantially sequence independent manner.

Grunenwald teaches a method of transposition using a transposase. *See, e.g.*, EX1003 at [0045]. This transposase is part of an insertional enzyme complex with transposon ends, which are DNA sequences that play a role in the insertion of tags in the tagmentation process. EX1003 at [0052], [0094-0098]. These transposon ends comprise the claimed "insert element" in the '357 Patent. EX1002 ¶¶ 232-34.

Further, Grunenwald teaches insertion into genomic DNA, as the methods taught include a transposition reaction in which one or more transposon ends are inserted into a target DNA, which may comprise genomic DNA. EX1003 at [0146]. This insertion is substantially sequence-independent; Grunenwald's transposition reaction is "a reaction wherein one or more transposon ends are inserted into a target DNA ***at random sites or almost random sites***." EX1003 at [0154]. Insertion at random sites is sequence-independent insertion. EX1002 ¶ 245. To the extent that Patent Owner contends that this disclosure is insufficient, sequence-independent insertion is an inherent property of Tn5 transposase that would have been well-known to a skilled artisan at the time of invention. EX1002 ¶ 246.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00058

9. Claim 9: The composition of claim 2, wherein the insertional enzyme complex comprises an enzyme selected from the group consisting of: transposases, Hermes transposase, and human immunodeficiency virus (HIV) integrases.

Disclosed. *See supra* Section X.A.3.

10. Claim 10: The composition of claim 9, wherein the insertional enzyme complex comprises a transposase.

Disclosed. *See supra* Section X.A.3.

11. Claim 11: The composition of claim 10, wherein the transposase is a Tn transposase.

Grunenwald teaches that the disclosed transposition reaction may be exemplified by employing a "hyperactive Tn5 transposase and a Tn5-type transposon end." EX1003 at [0154]. The disclosed Tn5 transposase is a specific type of Tn transposase. EX1002 ¶ 252.

12. Claim 12: The composition of claim 11, wherein the Tn transposase is a Tn5 transposase.

Disclosed. *See supra* Section X.A.11.

13. Claim 13: The composition of claim 2, wherein the insert element comprises one or more molecular tags.

Grunenwald discloses "wherein the insert element comprises one or more molecular tags." *See* EX1003 at [0102], [0161], Figs. 1-2. For example, Grunenwald teaches that "'A-MEDS Transposomes' and 'B-MEDS Transposomes' were formed by pre-incubating 12.5 μM TSase with 12.5 μM A-MEDS or 12.5 μM

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00059

B-MEDS transposon end compositions, respectively, for 60 minutes at 37°C." EX1003 at [0425].

Alternatively, in embodiments where the tag comprises multiple tag domains (*i.e.*, a sequencing tag domain and an amplification tag domain), each tag domain constitutes a molecular tag. EX1002 ¶ 238. In Grunenwald, a tag domain refers to "a portion or domain of a tag that exhibits a sequence for a desired intended purpose or application." EX1003 at [0103]. This use of "tag domain" is no different from how the '357 Patent uses "tags," which is synonymous with an adapter molecule. *See, e.g.*, EX1001 at 13:26-30 ("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 adapters to the polynucleotides"). Grunenwald's tag domain, being a short sequence of DNA inserted for a desired intended purpose, is an adapter sequence. EX1002 ¶ 259. In Grunenwald's embodiments where "one or more tag domain sequences" are the additional sequences being used to accomplish a desired purpose, a second tag domain sequence is a second tag. EX1003 at [0331]; EX1002 ¶ 260.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00060

14. Claim 14: The composition of claim 13, wherein one or more of the molecular tags comprises a sequencing adaptor.

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].

Indeed, 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 [0021], [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 ¶ 265.

15. Claim 15: The composition of claim 14, wherein one or more of the sequencing adaptors comprises a barcode label.

Grunenwald teaches 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. *Id. at* [0113]; EX1002 ¶ 268;

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00061

compare EX1001 at 12:33-51 (stating that the "barcode sequence" is "used to …identify and/or track the source of a polynucleotide in a reaction….").

Additionally, in Grunenwald, a given tag may have multiple tag domains. EX1003 at [0103] ("In some embodiments wherein the transferred strand also exhibits one or more other nucleotide sequences 5'-of the transferred transposon end sequence, ***the tag also has one or more other tag domains*** in said 5'-portion") (emphasis added). Thus, in an embodiment wherein a tag has both a sequencing tag domain and an address tag domain, a POSA would understand that to be a sequencing adaptor that comprises a barcode sequence. EX1002 ¶ 270.

16. Claim 16

a. A composition comprising:

The preamble is not limiting. *See supra* Section IX.A.1.a.

b. a permeabilized cell nucleus comprising:

This claim limitation is taught by Okino, and as documented above, the POSA would have been motivated to apply Okino's teaching of a permeabilized cell nucleus to Grunenwald. *See supra* IX.A.1.b.

c. an insertional enzyme complex comprising a transposase enzyme; and

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

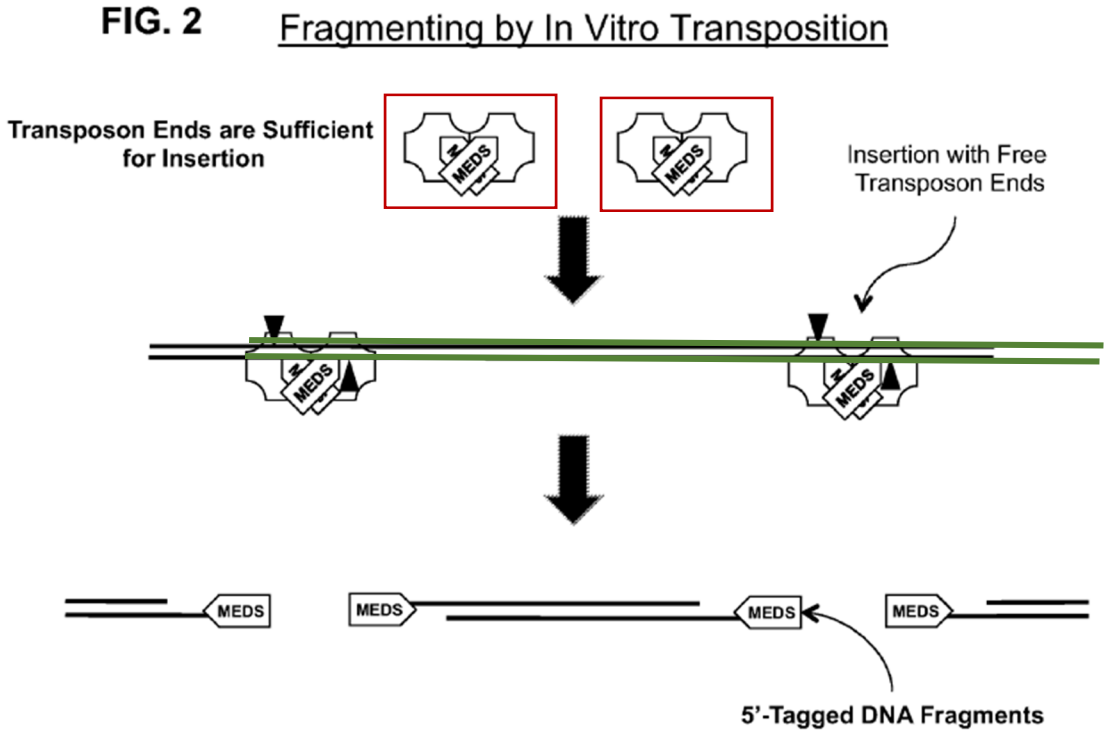
10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00062

d. a plurality of tagged nucleic acid fragments

Grunenwald teaches "methods, compositions and kits for generating a library of tagged DNA fragments from target DNA comprising double-stranded DNA (dsDNA) contained in any sample of interest." EX1003 at [0160]; *see also* EX1003 at [0017] (summarizing portions of the invention).

Further, Figure 2 of Grunenwald, reproduced below, showcases the disclosure of a plurality of tagged nucleic acid fragments. *See* EX1003 at [0060]. In this figure, the two parallel lines highlighted in green represent target DNA and the transposomes (i.e., the claimed insertional enzyme complex or transposase complex) are boxed in red. This schematic demonstrates the insertional enzyme complex contacting the target DNA and simultaneously fragmenting and tagging it, resulting in a plurality of tagged nucleic acid fragments. *See* EX1002 ¶ 280.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00063



EX1003 at Figure 2

e. wherein each tagged nucleic acid fragment comprises a first sequencing adapter and second sequencing adapter

First, Grunenwald discloses the use of a first and second adapter. Grunenwald discloses that tags may comprise one or more portions, or domains, that exhibit "a sequence for a desired intended purpose or application." *See* EX1003 at [0103]. There can be more than one tag associated with a transposome complex. *See* EX1003 at [0102], [0161], Figs. 1-2. For example, in one working example Grunenwald teaches that "'A-MEDS Transposomes' and 'B-MEDS Transposomes'

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00064

were formed by pre-incubating 12.5 μM TSase with 12.5 μM A-MEDS or 12.5 μM B-MEDS transposon end compositions, respectively, for 60 minutes at 37°C." EX1003 at [0425].

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 ¶ 287; *see supra* Section IX.A.8. 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 first and second tag domain would act as a first and second adapter sequence. EX1003 at [0331]; EX1002 ¶ 289.

Second, Grunenwald discloses that these two tags may be a first and second sequencing adapter. Grunenwald teaches that one type of tag domain is a "sequencing tag domain." EX1003 at [0103], [0110]. This 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 [0021], [0194], Claims 7, 13, 15, 21. This domain, being a short sequence of DNA to facilitate sequencing of the

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00065

fragmented and tagged DNA, is the sequencing adapter sequence. EX1002 ¶ 291. Because both tags may contain tag domains that are sequencing tag domains, Grunenwald discloses multiple sequencing adapters. EX1002 ¶ 292.

f. wherein each tagged nucleic acid fragment is derived from a region of open chromatin

For the reasons discussed above, Grunenwald discloses that tagged nucleic acids necessarily and inherently are derived from a region of open chromatin. *See supra* Sections IX.A.2.g; IX.A.16.d.

17. Claim 17: The composition of claim 16, wherein the plurality of tagged nucleic acid fragments correspond to two or more regions of a genome.

Grunenwald teaches that the size of the fragmented DNA that results from its method ranges from 150 bp to 1.5 Kbp, or 400 bp to 3.5 Kbp, depending on the concentration of Tn5 transposase added to the reaction mixture. EX1003 at [0421]. As discussed above, Grunenwald further teaches that the target DNA can be derived from cells from "humans, animals, plants, [or] fungi (e.g., molds or yeasts)" and that its method operates "at random sites or almost random sites." *Id.* at [0146], [0154]. Importantly, Grunenwald teaches that the purpose of its method includes interrogating "whole" genomes of such organisms. *See id.* at [0015].

Given the size of a genome of an organism from this exemplary list (for example, the human genome includes over 3 billion base pairs) and the fact that even the largest disclosed fragment size in Grunenwald is approximately 3,500 bp, by

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00066

definition the tagged fragments resulting from the application of the cellular embodiments include two or more regions of the genome. EX1002 ¶ 299; EX1056.002.

In fact, since Grunenwald operates at "random or almost random" sites and is intended to interrogate "whole" genomes, the application of its method to chromosomal DNA from, for instance, a human cell will necessarily result in tagged fragments from ***thousands*** of regions of the genome. Certainly, given the fragment lengths generated in Grunenwald relative to the size of the genomes disclosed, and the fact that Grunenwald's method operates in a "random" fashion, it would have been obvious based on Grunenwald to generate a composition with thousands of polynucleotide fragments. EX1002 ¶ 301.

18. Claim 18: The composition of claim 16, wherein the plurality of tagged nucleic acid fragments correspond to at least 1,000 regions of a genome.

Obvious, for the reasons stated above. *See supra* Section IX.A.17.

19. Claim 19: The composition of claim 18, wherein the plurality of tagged nucleic acid fragments correspond to genome-wide regions of open chromatin.

Grunenwald discloses that its whole purpose is to generate a library of DNA fragments that correspond to genome-wide regions of open chromatin. For example, Grunenwald that "what is needed are improved methods for generating libraries of DNA fragments from target DNA molecules for amplification including

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00067

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]. Further, given the dynamic nature of DNA packing detailed in Section V.B, *supra*, and the inherent nature of transposase enzymes binding to open chromatin regions detailed in Section X.A.2.g, a POSA would have been aware that these genome-wide regions would necessarily correspond to open chromatin. EX1002 ¶¶ 305-06.

20. Claim 20: The composition of claim 18, wherein the transposase is a Tn transposase.

Disclosed. *See supra* Section IX.A.11.

21. Claim 21: The composition of claim 20, wherein the Tn transposase is a Tn5 transposase.

Disclosed. *See supra* Section IX.A.12.

22. Claim 22

a. A composition comprising:

The preamble is not limiting. *See supra* Section IX.A.1.a.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00068

b. a permeabilized cell nucleus comprising:

This claim limitation is taught by Okino, and as documented above, the POSA would have been motivated to apply Okino's teaching of a permeabilized cell nucleus to Grunenwald. *See supra* IX.A.1.b.

c. (a) an insertional enzyme complex comprising a transposase enzyme

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

d. (b) a plurality of tagged nucleic acid fragments

Disclosed. *See supra* Section IX.A.16.d.

e. wherein each tagged nucleic acid fragment comprises a first sequencing adapter and a second sequencing adapter

Disclosed. *See supra* Section IX.A.16.e.

f. wherein the insertional enzyme complex and the tagged nucleic acid fragments are bound to one or more regions of open chromatin within the cell nucleus in a substantially sequence independent manner to form a high affinity macromolecule complex

First, for the reasons discussed above, Grunenwald discloses "the insertional enzyme complex and the tagged nucleic acid fragments are bound to one or more regions of open chromatin within the cell nucleus." *See supra* Section X.A.2.g.

Second, Grunenwald discloses that that the insertional enzyme complex and tagged nucleic acid fragments are bound "in a substantially sequence independent manner." As described above, Grunenwald's *in vivo* embodiment is exemplified

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00069

through the use of a Tn5 transposase. EX1003 at [0157]. It was well-known in the art that a Tn5 transposase binds in a nonspecific manner to target DNA. *See supra* Section X.A.8; EX1003 at [0154]; EX1002 ¶ 316. Therefore, in practicing the *in vivo* embodiment in Grunenwald, it would be an inherent characteristic of the insertional enzyme complex and the tagged DNA generated by the transposase to be sequence independent. EX1002 ¶ 317.

Third, Grunenwald discloses that that insertional enzyme complex and the tagged nucleic acids "form a high affinity macromolecule complex." The specification of the '357 Patent describes in one working example that "after in vitro insertion of sequencing adapter, the Tn5 transposase remained tightly bound to the DNA and formed a high-affinity macromolecular complex that prevented dissociation of the generated ATAC-seq DNA fragments." EX1001 at 34:20-24. Thus, the patent is clear that the ability of the Tn5 transposase to form a "high-affinity macromolecular complex" was nothing but a property inherent to the Tn5 enzyme. EX1002 ¶ 320. A POSA practicing the *in vivo* embodiment taught in Grunenwald using the Tn5 transposase that it recommends would have thus formed a high affinity macromolecule complex after the tagging and fragmenting step. EX1002 ¶ 321.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00070

23. Claim 23: The composition of claim 22, further comprising a plurality of divalent cations.

Grunenwald teaches the use of a "Transposase Reaction Buffer" to conduct a transposition reaction. *See, e.g.* EX1003 at [0408]-[0414]. A component of this buffer is magnesium acetate, which would dissociate to form the divalent cation Mg^{2+} and two monovalent acetate anions. EX1002 ¶ 325. Thus, Grunenwald teaches the composition of claim 22 further comprises a plurality of divalent magnesium cations. EX1002 ¶ 325.

24. Claim 24: The composition of claim 23, wherein the divalent cations are not chelated.

In the disclosed buffer in Grunenwald that contains divalent magnesium cations, there are no chelating agents. EX1003 at [0411]-[0414]; *see also* EX1002 ¶ 328. A POSA would have understood the disclosure as teaching the divalent cations are not chelated. EX1002 ¶¶ 328-29.

25. Claim 25: The composition of claim 23, wherein the divalent cations are selected from the group consisting of: magnesium, manganese, or any combination thereof.

Disclosed. *See supra* Section IX.A.23.

26. Claim 26: The composition of claim 22, wherein the transposase is a Tn transposase.

Disclosed. *See supra* Section IX.A.11.

27. Claim 27: The composition of claim 26, wherein the Tn transposase is a Tn5 transposase.

Disclosed. *See supra* Section IX.A.12.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00071

28. Claim 28: The composition of claim 2, wherein (a) and (b) are contained within the permeabilized cell nucleus.

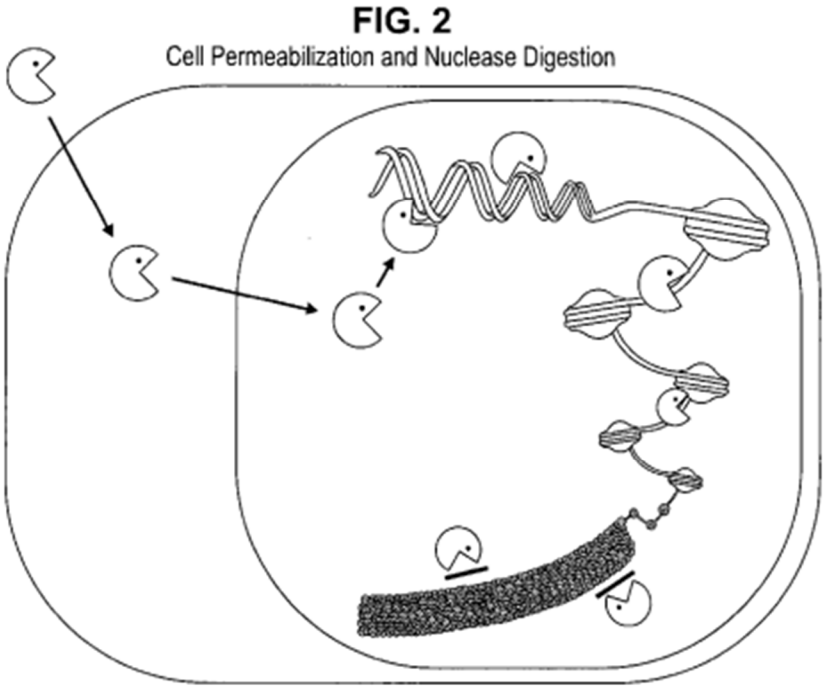
As documented above, Figure 2 of Okino demonstrates the presence of an enzyme with open chromatin contained within a permeabilized cell nucleus. *See supra* Section X.A.1.b. As further discussed above, it would have been obvious to use an insertional enzyme complex as taught in Grunenwald with the methods of permeabilization taught in Okino, achieving the result that both the chromatin and the insertional enzyme complex are contained within the permeabilized cell nucleus. *See, e.g.*, *supra* Section IX.A.1. Thus, a POSA interested in practicing tagmentation on a cell nucleus and combining the disclosures of Okino and Grunenwald would necessarily create a composition wherein the insertional enzyme comprising a transposase and the plurality of tagged nucleic acid fragments with a first and second sequencing adapter are contained within the permeabilized cell nucleus. EX1002 ¶ 338.

29. Claim 30: The composition of claim 2, wherein the permeabilized cell nucleus is contained within a cell.

Figure 2 of Okino demonstrates the presence of a permeabilized cell nucleus contained within a cell. Figure 2, reproduced below for convenience, displays both a cell that is permeabilized to allow an enzyme to enter, and a nucleus that is permeabilized to permit the enzyme to further enter the nucleus and access the

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00072

chromatin. *See supra* Section X.A.1.b.(1). As depicted in Figure 2, the permeabilized cell nucleus is contained within the cell. EX1002 ¶ 341.



EX1004 at Figure 2

B. Ground 2: Claim 29 Is Rendered Obvious By The Combination Of Grunenwald, Okino, And Saleh And/Or Song

1. Claim 29: The composition of claim 2, wherein the permeabilized cell nucleus is isolated from a cell.

The combination of Grunenwald, Okino, and Saleh and/or Song renders claim 29 obvious.

a. Both Saleh And Song Teach Isolation Of Cell Nuclei

Saleh teaches a protocol for chromatin immunoprecipitation (ChIP), a precursor to tagmentation that prepared chromatin in a cell for tagging and

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00073

sequencing. EX1002 ¶ 345; EX1005.001. In ChIP, DNA and associated proteins are first cross-linked in the living cell to capture a "snapshot" of the chromatin as it exists at the moment of cross-linking. EX1005.001; EX1002 ¶ 347. This is generally followed by a step isolating the now-linked chromatin complex, shearing the chromatin into smaller fragments, and using antibodies to precipitate the DNA-bound proteins, pulling them out of solution. EX1005.001. The DNA co-precipitated with the proteins of interest is released from the complex, amplified by PCR, and tagged for sequencing. *Id*. Saleh improved on this general method through the addition of a nuclei isolation step, which is "important for the purity and yield of DNA." EX1005.002. Saleh thus teaches an additional step wherein the nucleus of a cell is isolated to isolate the chromatin prior to shearing and tagging. EX1005.004-005.

Song, analogous to Saleh, teaches a method for digesting regions of DNA that that are not "tightly wrapped in nucleosome and higher-order structures" so that they can be subject to DNA sequencing. EX1060.001. Song particularly discloses the use of DNase I, which is an enzyme that is also disclosed in Okino, to modify DNA by digesting it at areas of open chromatin. EX1060.001; EX1004 at [0081]. The first step of Song's protocol is the isolation of nuclei from cells; this method is routine and based on cell lysis using the IGEPAL detergent and subsequent centrifugation. EX1004.003. This lysis buffer comprises 10 mM Tris-Cl, pH7.4,

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00074

10mM NaCl, 3 mM $MgCl_2$, and 0.1% IGEPAL CA-630. EX1060.003. Indeed, this method is so routine that it is the exact method disclosed in the ’357 Patent, which uses the same protocol based on the same detergent. EX1001 at 25:4-15. Thus, the ’357 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 ¶ 356.

b. It Would Have Been Obvious To Combine Saleh And/Or Song With Grunenwald and Okino

As discussed above, the combination of Grunenwald and Okino would have been obvious to a POSA. *See supra* Section IX.A.1.b. Further, it would have been obvious to additionally modify the combination of Grunenwald and Okino with the addition of the nuclei isolation step taught in Saleh and/or Song.

First, Saleh is analogous art to Grunenwald and Okino. All three references are in the field of genetics and molecular biology, and particularly concern methods of DNA modification for subsequent sequencing and analysis. EX1002 ¶¶ 358-360. Further, all three references cover the preparation of chromatin for analysis. *See, e.g.*, EX1003 at Abstract, [0146], [0157]; EX1004 at [0005]; EX1005.001. Song is also analogous art; it is also in the field of genetics and molecular biology, and covers the study of DNA modification and subsequent sequencing. EX1002 ¶¶ 358-360;

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00075

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 and Okino in hand would have naturally been inclined to consider Song.

Second, a POSA interested in studying DNA in a living cell would have been aware of both the successes and limitations of various methods of preparing the DNA for sequencing. Namely, as described above in Section IX.A.1.b, a POSA would have been motivated to combine the insertional enzyme complex as used *in vivo* in Grunenwald with the permeabilization techniques taught in Okino to permit the insertional enzyme complex to enter the cell and nucleus. A POSA interested in the study of chromatin after fragmentation and tagging would have further been aware of the nuclei isolation methods taught in Saleh and/or Song and the quality improvements that nuclei isolation provides. EX1005.002; EX1002 ¶ 363. Further, the prior art establishes that the tagmentation techniques taught in Grunenwald may be performed *in vivo* in a cell without the nuclei isolation step; thus, the decision to include a step isolating nuclei is a mere design choice in the finite methods to prepare chromatin for sequencing and analysis. EX1002 ¶ 364. To the extent that such a choice is to be made, both Saleh and Song provide routine methods of nucleus

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00076

isolation that show such a process is well-established in the art. EX1060.003; EX1005.005-006; EX1002 ¶ 365.

Third, a POSA would have had a reasonable expectation of success in combining the references. For the reasons discussed above in Section IX.A.1.b, a POSA would have had a reasonable expectation of success in combining Grunenwald and Okino. Further, a POSA would have had a reasonable expectation of success in combining the teachings of Saleh and/or Song with those of Grunenwald and Okino. Grunenwald and Okino, together, teach a method for generating a composition comprising a permeabilized cell nucleus and an insertional enzyme complex, with the resulting plurality of tagged polynucleotides. *See supra* Section IX.A.2. Saleh's disclosure of a method of nuclei isolation would have been a standard method in the art when isolating DNA in preparation for sequencing, and a POSA attempting to prepare DNA for sequencing would expect success in isolating the permeabilized cell nucleus with the insertional enzyme complex, as the methods taught in Saleh allow for the isolation of a cell nucleus containing DNA and associated proteins. EX1002 ¶ 369.

Further, regarding Song, a POSA combining the references would have a reasonable expectation of success on the basis that the references provide compatible methods of accomplishing analogous goals. EX1002 ¶ 370. Additionally, cell lysis had been a long-used technique to break open a cell and access the interior; indeed,

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00077

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 ¶ 371; *see also* EX1055.004. A POSA would also have a reasonable expectation of success because Song teaches this combination; lysing a cell to provide nuclei and subsequently contacting the nuclei with an enzyme to modify the chromatin present. EX1002 ¶ 372. A POSA performing Grunenwald's *in vivo* tagmentation method, combined with the nuclei isolation procedure based on application of a lysis buffer from Song, would have had a reasonable expectation of successfully isolating and modifying DNA in preparation for sequencing. EX1002 ¶ 373.

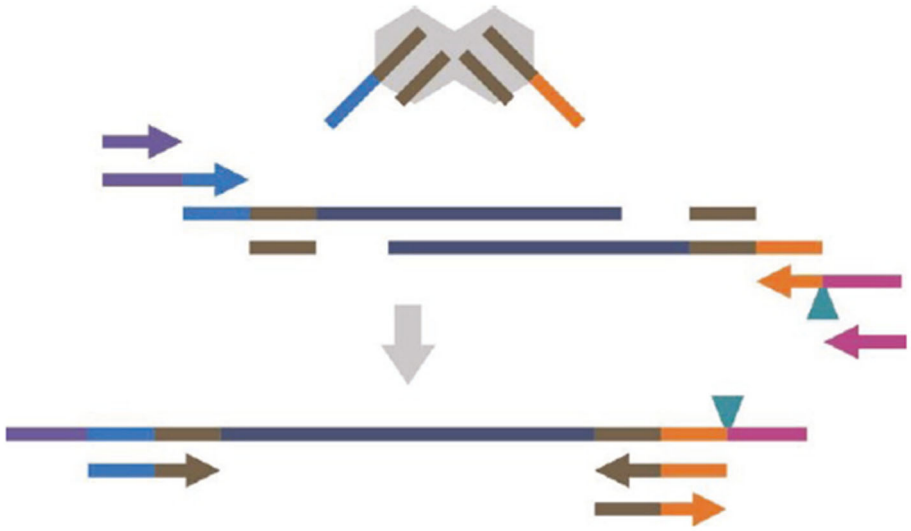
C. Ground 3: Claim 16 Is Rendered Obvious By The Combination of Grunenwald, Okino, and Adey

As detailed above, the combination of Grunenwald and Okino renders claim 16 obvious. *See supra* Section IX.A.16. To the extent that Patent Owner contends that Grunenwald does not disclose both a first and second sequencing adapter, however, this would be disclosed by combining Grunenwald and Okino with the teachings of Adey.

Claim 16 recites, in part, "wherein each tagged nucleic acid fragment comprises a first sequencing adapter and second sequencing adapter." The use of a first and second sequencing adapter is disclosed in Adey. For example, in Figure 1(b), reproduced below, the transposome complex is composed of the two grey

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00078

hexagons, which contain two different adaptors (blue/brown/orange). The complex is capable of simultaneously fragmenting and tagging genomic DNA (navy blue).



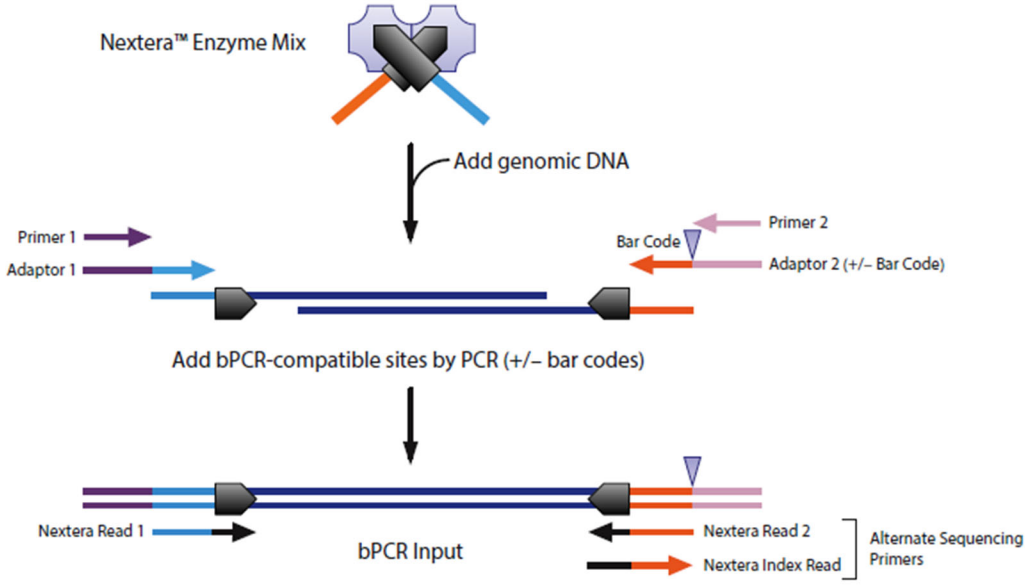
EX1006.002 at Figure 1(b) (modified)

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, Okino, and Adey discloses each and every limitation of Claim16.

Adey further discloses that the second adapter sequence comprises a second sequencing adapter sequence. Adey teaches that in the preparation of a library of fragments, genomic DNA is contacted with a Nextera™ enzyme mix. EX1006.014. 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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00079

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.



EX1048.002 at Figure 1

Further, the combination of Grunenwald, Okino, 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 ¶ 382.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00080

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.*, EX1006.004, 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 ¶¶ 383-84.

Third, a POSA would have had a reasonable expectation of success in combing 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.*, EX1006.014. 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 ¶ 385.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00081

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 in combination with Okino. 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

While the Applicant identified Grunenwald and Okino, only Grunenwald was analyzed in any meaningful way during the prosecution of the '357 Patent. Okino was not cited in any way during prosecution. Saleh was not considered by the Examiner at all during prosecution.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00082

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 Okino, or in Grunenwald and Okino in combination with Saleh.

The Examiner did not discuss or cite any passage of Okino in any rejection, nor in the Notice of Allowance. EX1007.151-158, 224-225. In the only non-final rejection, the Examiner did analyze Grunenwald in rejecting the claims of the '357 Patent under 35 U.S.C. § 103. However, the Examiner did not discuss the *in vivo* embodiment disclosed in Grunenwald, nor did the Examiner consider the teachings in the *in vivo* embodiment in combination with Okino. EX1007.151-158. 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 at 10 (P.T.A.B. Feb. 13, 2020); *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 these references "w[ere] not the basis of a rejection weighs strongly against exercising [the Board's] discretion to deny institution." *Cellco*

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00083

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 Okino that disclose permeabilization of the cell and nuclear membrane by the use of nonionic detergents, particularly NP40 and Tween 20. *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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00084

[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 June 2025. EX1047.014. Thus, this factor favors institution.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00085

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.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00086

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 '357 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

The claims of the'357 Patent should be cancelled.

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00087

Respectfully submitted,

WEIL, GOTSHAL AND MANGES LLP

Dated: June 6, 2023

/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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00088

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 14,000 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. 10,738,357 WITH PARSE BIOSCIENCES, INC'S EXHIBIT LIST, POWER OF ATTORNEY AND EXHIBITS 1001-1062** 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
10x-parse@groombridgewu.com
GROOMBRIDGE, WU, BAUGHMAN & STONE LLP
565 Fifth Avenue, Suite 2900
New York, NY 10017
(332) 269-0030

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

10x Genomics, Inc. Exhibit 2011
Parse Biosciences, Inc. v. 10x Genomics, Inc.
IPR2023-01030
Page 00090