

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PARSE BIOSCIENCES, INC.
Petitioner,

v.

10X GENOMICS, INC.
Patent Owner.

IPR _____
U.S. PATENT NO. 10,738,357

DECLARATION OF GREGORY COOPER, PH.D. IN SUPPORT OF
PETITIONER FOR *INTER PARTES* REVIEW

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I. INTRODUCTION

1. My name is Gregory Cooper. I have been retained by Parse Biosciences, Inc. (“Parse” or “Petitioner”) in the above-captioned *inter partes* review (“IPR”) to provide my independent evaluation and opinions regarding U.S. Patent No. 10,738,357 (the “’357 patent”) and the related prior art.

2. This Declaration sets forth my analyses and opinions based on my technical background, skills, knowledge, training, education and experience in the relevant art, and the materials I considered. As I explain below, it is my opinion that all claims of the ’357 patent are anticipated or rendered obvious by the combination of prior art references discussed herein.

II. QUALIFICATIONS AND COMPENSATIONS

3. I am currently a Faculty Investigator at the HudsonAlpha Institute for Biotechnology (“HudsonAlpha”) in Huntsville, Alabama. I have held this position since 2010. HudsonAlpha, established in 2008, is an independent, non-profit institution dedicated to improving human health and welfare through biological research, genomic medicine, education, and economic development. Since coming to HudsonAlpha, I have set up an independent human genomics research lab. My lab works on projects related to the genetics of human disease, with a particular emphasis on research and clinical applications of genome sequencing for diagnosing children with developmental delays and intellectual disabilities. We also study the

genetics and biochemistry of gene expression regulation, develop new computational approaches to analysis of human genomes and related datasets, and pursue other applications of genomic technologies to understand human biology.

4. I am also concurrently an Associate Professor Adjunct in the Department of Genetics at the University of Alabama at Birmingham (“UAB”). I have held a UAB adjunct faculty position since 2010.

5. I earned a Bachelor of Arts in Microbiology and Bachelor of Science in Mathematics and Statistics, summa cum laude, from Miami University (Oxford, OH) in 2001. As an undergraduate student, I performed molecular biology and genetic research in research laboratories at Miami University, the University of Illinois at Urbana-Champaign, and the University of Cincinnati.

6. I earned a Ph.D. in Genetics from Stanford University in 2006 under the mentorship of Professor Arend Sidow, Ph.D. My thesis work was mainly comprised of computational biology work, with a focus on evolutionary comparisons of mammalian genome assemblies to better annotate functional elements in human genomes and assess whether mutations in those elements might contribute to disease. While there, I worked and/or received training in areas including: theoretical and applied human genetics, molecular evolution, functional genomics, developmental biology, bioinformatics, genomic technologies, and

analysis of datasets produced by high-throughput experimental platforms, especially DNA sequencers.

7. From 2006 to 2009, I was a Senior Fellow in the Department of Genome Sciences at the University of Washington working with Professors Evan Eichler, Ph.D., and Deborah Nickerson, Ph.D. While there, I conducted my postdoctoral research. I was primarily interested in applying genomic technologies to better understand the genetic basis for human disease. One series of projects centered on analytical and algorithmic developments to identify large deletion and duplication events, also known as “copy-number variants” (“CNVs”), from microarray data generated to perform Single Nucleotide Polymorphism (“SNP”) genotyping. This process involved extensive processing, normalization, and analysis of Illumina SNP array data. A second series of projects was aimed at better understanding the genetic basis for a variety of complex traits. These included genetic analyses of transcript levels, for example, and also a genome-wide association study of patient response to the commonly prescribed anticoagulant warfarin. Finally, another series of projects focused on analysis of high-throughput DNA-sequencing data, in particular the locations of genetic variants, from individuals with Mendelian diseases. I completed my postdoctoral studies in 2009.

8. From 2009 to 2010, I was an Acting Assistant Professor in the Department of Genome Sciences at the University of Washington.

9. I am an author or contributor to more than 100 publications in the areas of human genetics and genomics, including functional and evolutionary annotations of mammalian genomes, DNA sequencing, and DNA analysis, as they relate to human diseases, especially, but not exclusively, intellectual disabilities and other developmental issues. As of March 23, 2023, these publications have been collectively cited more than 42,000 times, according to Google Scholar estimates.

10. Among the awards and honors I have earned are: Harrison Scholar, Miami University (Oxford, OH) (1997-2001), Goldwater Scholar (1999-2001), Howard Hughes Medical Institute Doctoral Fellowship (2001-2006), a University of Washington Genome Training Grant Postdoctoral Fellowship (2007), and a Merck, Jane Coffin Childs Memorial Fund Postdoctoral Fellowship (2007-2010).

11. I am a Principal Investigator (“PI”) on two current human genomic research projects funded by the National Institutes of Health (“NIH”) and a PI or co-Investigator on several additional projects funded by non-NIH sources, including both governmental and philanthropic sources. I have also been a PI or co-Investigator on multiple additional grants related to human genetics and genomics, from NIH and elsewhere, that have been completed.

12. In general, I have knowledge and experience with both the general scientific contexts relevant to this matter and specific details of the technologies employed. For example, I have extensive experience with DNA sample preparation

techniques (including electroporation and permeabilization), tagmentation, amplification methods, and DNA sequencing technologies (including next generation sequencing).

13. I have also previously served as an expert witness in three now concluded patent disputes and one ongoing patent dispute. The three completed cases, in which I worked on behalf of the plaintiffs, are Illumina/Verinata vs Ariosa/Roche (C.A. No. 3:12-5501-SI, N.D. Cal.), Guardant Health vs FMI (C.A. No. 17-1616-LPS-CJB, D. Del.), and Guardant Health vs PGDx (17-1623-LPS-CJB, D. Del.). The ongoing case is Natera vs ArcherDX/Invitae (C.A. No. 20-125-GBW, D. Del.), in which I am working on behalf of the defendants.

14. A copy of my Curriculum Vitae, attached as Exhibit 1023, contains further details on my education, experience, publications, and other qualifications to render an expert opinion in this matter.

15. For this service, I am paid my standard hourly consulting fee of \$750/hour, and I am reimbursed for actual expenses. My compensation in no way depends on the outcome of this matter or the substance of this declaration.

III. SUMMARY OF OPINIONS

16. Based on my knowledge, experience, and the materials I have reviewed, it is my opinion that the claims of the '357 Patent would have been obvious to a POSA in view of a combination of Grunenwald (EX1003) with Okino (EX1004).

IV. LEGAL STANDARDS

17. I am not a lawyer, and I offer no legal opinion in this declaration. I have, however, been advised by counsel as to various legal standards that apply to the technical issues I address in this declaration. I have applied those standards in arriving at my conclusions expressed in this declaration.

A. Claim Construction

18. I have been informed that the words of the claims of a patent are generally given the ordinary and customary meaning as interpreted by a POSA at the time of the invention. I have been informed that patent claims are construed in light of the claim language, the patent specification (including the drawings), and the prosecution history (including the references cited during the prosecution of the patent). For this declaration, I have laid out my opinions on what terms should be construed in the section on Claim Construction below; I have given all other terms their plain and ordinary meaning as would have been understood by a POSA at the time of the invention.

B. Anticipation

19. I understand that a prior art reference “anticipates” an asserted claim and renders that claim invalid if all elements of the claim are disclosed in that prior art reference and arranged in the prior art reference as they are in the claims.

20. I also understand that even if a prior-art reference does not expressly disclose every limitation of a claim, the reference may still anticipate the element if

the missing descriptive element is “inherent” in the disclosure. A claim element is inherent if it is necessarily present in, or is the natural result of, subject matter disclosed in the reference. I further understand that inherency may not be established by probabilities or possibilities, and the mere fact that a certain thing might result from a given set of circumstances is insufficient.

C. Obviousness

21. I understand that even if a patent is not anticipated, a claimed invention is “obvious” if the difference between the claimed subject matter and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person of ordinary skill in the art.

22. I understand that a person of ordinary skill in the art provides a reference point from which the prior art and claimed invention should be viewed. This reference point prevents a person of ordinary skill from using one’s insight or hindsight in deciding whether a claim is obvious.

23. Also, I understand that a claim can be obvious in light of a combination of multiple prior art references. I understand that such a combination must meet certain requirements. I understand that the prior art must teach or suggest all the requirements of the invention as recited in the claim. I understand that the prior art, coupled with knowledge generally available in the art at the time of invention, must contain some suggestion or incentive that would have motivated a POSA to modify a

reference or to combine the references. I also understand that this proposed modification must have had a reasonable expectation of success.

24. I understand that only analogous art may be considered in an obviousness analysis. Art is considered analogous if it is in the same field of endeavor as the patent or if it is reasonably pertinent to the particular problem with which the patent is involved.

25. I also understand that in evaluating whether patent claims are obvious, objective indicia of non-obviousness can be considered, such as but not limited to, commercial success, long-felt but unsolved needs, praise, unexpected results, industry acceptance, failure of others, and skepticism by experts.

V. OVERVIEW OF THE '357 PATENT

26. The '357 patent is titled "Transposition Of Native Chromatin For Personal Epigenomics." '357 patent. The '357 patent states that it describes "a method for analyzing polynucleotides such as genomic DNA." EX1001 at Abstract. This is accomplished through treating the chromatin (e.g., genomic DNA) isolated from a population of cells with a transposase and molecular tags, producing tagged fragments. EX1001 at 1:56-58. These tagged fragments may then be analyzed to determine the sequence of the fragments, and that data may be used to map information about the sequences to epigenetic features of the chromatin. EX1001 at 1:58-62.

27. The claims of the '357 patent are reproduced below:

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.

2. A composition comprising:

A permeabilized cell nucleus comprising:

(a) chromatin comprising genomic DNA and a plurality of DNA binding proteins, wherein the chromatin comprises a plurality of open chromatin regions; and

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

An insert element comprising a predetermined sequence, wherein the insertional enzyme complex is bound to one of said open chromatin regions.

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

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

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

6. The composition of claim 2, wherein the insertional enzyme comprises a nuclear localization signal.
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.
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.
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.
10. The composition of claim 9, wherein the insertional enzyme complex comprises a transposase.
11. The composition of claim 10, wherein the transposase is a Tn transposase.
12. The composition of claim 11, wherein the Tn transposase is a Tn5 transposase.
13. The composition of claim 2, wherein the insert element comprises one or more molecular tags.
14. The composition of claim 13, wherein one or more of the molecular tags comprises a sequencing adaptor.

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

16. A composition comprising:

A permeabilized cell nucleus comprising:

(a) an insertional enzyme complex comprising a transposase enzyme; and

(b) a plurality of tagged nucleic acid fragments, wherein each tagged nucleic acid fragment comprises a first sequencing adapter and a second sequencing adapter;

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

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

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

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

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

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

22. A composition comprising:

A permeabilized cell nucleus comprising:

(a) an insertional enzyme complex comprising a transposase enzyme; and

(b) a plurality of tagged nucleic acid fragments, wherein each tagged nucleic acid fragment comprises a first sequencing adapter and a second sequencing adapter;

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.

- 23. The composition of claim 22, further comprising a plurality of divalent cations.
- 24. The composition of claim 23, wherein the divalent cations are not chelated.
- 25. The composition of claim 23, wherein the divalent cations are selected from the group consisting of: magnesium, manganese, or any combination thereof.
- 26. The composition of claim 22, wherein the transposase is a Tn transposase.
- 27. The composition of claim 26, wherein the Tn transposase is a Tn5 transposase.
- 28. The composition of claim 2, wherein (a) and (b) are contained within the permeabilized cell nucleus.
- 29. The composition of claim 2, wherein the permeabilized cell nucleus is isolated from a cell.
- 30. The composition of claim 2, wherein the permeabilized cell nucleus is contained within a cell.

28. Claim 1 recites a composition that comprises a permeabilized cell nucleus, which in turn comprises an insertional enzyme complex and tagged polynucleotides. Thus, it can be understood that to generate the claimed composition, there is a step requiring some form of permeabilization of the cell nucleus, and a tagmentation step for the tagged polynucleotides. This tagmentation is done with an “insertional enzyme complex ,” e.g. a transposase and associated tags, to fragment large DNA molecules into smaller molecules and add tags to the ends of those molecules in one step. The smaller, tagged DNA fragments may then be analyzed to determine the sequence information of those molecules. This process is sometimes referred to as “tagmentation”.

29. The aforementioned tagging can be used to investigate chromatin accessibility and understand epigenetic features of chromatin. The ‘357 patent defines “chromatin accessibility” as “how accessible a nucleic acid site is within a polynucleotide, such as in genomic DNA, i.e., how ‘open’ the chromatin is.” EX1001 at 13:54-57. Additionally, a “nucleic acid site associated with a polypeptide, such as with genomic DNA in nucleosomes, is usually inaccessible. A nucleic acid site not complexed with a polypeptide is generally accessible, such as with genomic DNA between nucleosomes (with the exception of nucleic acid sites complexed with transcription factors and other DNA binding proteins).” EX1001 at

13:57-63. By fragmenting the accessible chromatin and determining sequence information, data about chromatin accessibility can be collected.

30. As I discuss below, chromatin is the DNA-protein complex in which the genome of a cell exists endogenously. The '357 Patent, however, specifically contemplates the isolation of chromatin from the cell. It discloses some embodiments in which “nuclei may be isolated, lysed, and the chromatin may be further purified” and other embodiments in which “the chromatin may be isolated by contacting the isolated nuclei with a reaction buffer.” EX1001 at 14:41-56.

31. Once isolated, the chromatin may be tagged. The '357 Patent discloses that methods for “tagging isolated genomic DNA are known in the art (see, e.g., Caruccio *Methods Mol. Biol.* 2011 733: 241-55; Kaper et al, *Proc. Natl. Acad. Sci.* 2013 110: 5552-7; Marine et al, *Appl. Environ. Microbial.* 2011 77: 8071-9 and US20100120098) and are commercially available from Illumina (San Diego, Calif.) and other vendors. Such systems may be readily adapted for use herein.” EX1001 at 14:31-38.

32. After the “chromatin has been fragmented and tagged to produce tagged fragments of genomic DNA, at least some of the adaptor tagged fragments are sequenced to produce a plurality of sequence reads. The fragments may be sequenced using any convenient method.” EX1001 at 14:59-63. The specification lists examples of sequencing methods known in the art, including “Illumina’s

reversible terminator method, Roche's pyrosequencing method (454), Life Technologies' sequencing by ligation (the SOLiD platform) or Life Technologies' Ion Torrent platform." EX1001 at 14:64-67. Further, the specification points out that:

Examples of such methods are described in the following references: Margulies et al (Nature 2005 437: 376-80); Ronaghi et al (Analytical Biochemistry 1996 242: 84-9); Shendure et al (Science 2005 309: 1728-32); Imelfort et al (Brief Bioinform. 2009 10:609-18); Fox et al (Methods Mol Biol. 2009; 5 553:79-108); Appleby et al (Methods Mol Biol. 2009; 513:19-39) and Morozova et al (Genomics. 2008 92:255-64), which are incorporated by reference for the general descriptions of the methods and the particular steps of the methods, including all starting products, methods for library preparation, reagents, and final products for each of the steps. As would be apparent, forward and reverse sequencing primer sites that are compatible with a selected next generation sequencing platform can be added to the ends of the fragments during the amplification step.

EX1001 at 14:67-15:15. This shows that sequencing methods were known in the art and incorporated by reference in the '357 patent.

33. The sequencing data gained from the above disclosure may then be used to generate an epigenetic map of a region of the genome. This map may be used show "(i) a profile of chromatin accessibility along the region; (ii) DNA binding protein occupancy for a binding site in the region; (iii) nucleosome-free DNA in the region; (iv) positioning of nucleosomes along the region; and/or (v) chromatin states." EX1001 at 2:6-11. This map may be used for a variety of purposes, such as

comparing two samples or to aid in providing a diagnosis for a patient. *See, e.g.*, EX1001 at 2:30-44.

34. I do not have an opinion on the priority date of the '357 patent. Based on the face of the patent, I understand that the earliest potential priority date of the '357 patent is May 23, 2013 based on the filing date of U.S. Provisional Patent Application No. 61/826,728. I have considered May 23, 2013 to be the relevant date for assessing prior art invalidity.

VI. PROSECUTION HISTORY OF THE '357 PATENT

35. I have been informed of the following facts regarding the prosecution history of the '357 Patent.

36. The application resulting in the '357 Patent was filed on May 21, 2019. At the time that the application was filed, Track One priority was requested. It is my understanding that this is a priority system that grants an application expedited review. Track one priority was granted on July 11, 2019.

37. I understand that on August 30, 2019, the Examiner issued a non-final rejection based on, in part, 35 U.S.C. § 103. The Examiner found that the claims of the '357 Patent were obvious based on the combination of Grunenwald in view of two other prior art references, Jouvenot and Audit.

38. Specifically, the Examiner pointed to a disclosure in Grunenwald teaching "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." EX1007.152-153.

39. However, the Examiner further stated that Grunenwald does not expressly teach that the composition or reaction mixture "comprise[s] a permeabilized cell nucleus." EX1007.155.

40. The Applicant, in responding to the rejection, argued that none of the cited references teach or suggest a composition comprising a permeabilized cell nucleus. EX1007.198.

41. I understand that on April 3, 2020, the Examiner allowed all claims based on the arguments raised by the Applicant.

42. However, it is my understanding that the Examiner, in analyzing Grunenwald, did not provide any analysis of Grunenwald's disclosure of an *in vivo* embodiment, electroporating a complex with a transposase enzyme directly into a cell.

43. Further, it is my understanding that while Okino was before the Examiner, Okino did not form the basis of any rejection and was not cited by the Examiner in the Notice of Allowance. EX1007.151-158, 224-225.

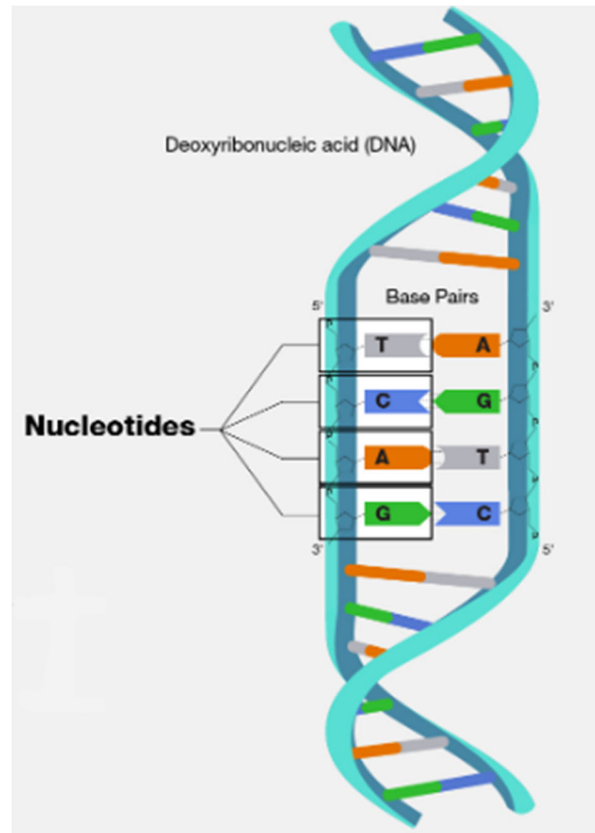
VII. TECHNICAL BACKGROUND AND STATE OF THE ART

A. DNA and RNA

44. DNA is an informational molecule, containing the information necessary to specify many aspects of development and maintenance of the cells of organisms. This information is stored in the sequence of adjacent bases in the DNA strand, or a “nucleotide sequence.”

45. DNA is a double-stranded molecule composed of monomeric units called nucleotides. A polynucleotide is a linear polymer comprised of several nucleotides. An oligonucleotide or oligo is a short polynucleotide. Oligonucleotides may be used in various biological techniques such as probing, DNA sequencing, polymerase chain reaction (“PCR”), or molecular cloning.

46. In DNA, each nucleotide comprises a deoxyribose sugar molecule, one of four nitrogenous bases, and one or more phosphate groups. The four nitrogenous bases of DNA are: adenine (“A”), which is complementary to thymine (“T”), and cytosine (“C”), which is complementary to guanine (“G”). The two strands of DNA are connected through complementary base pairing via chemical bonds or hybridization – adenine bonds with thymine and cytosine bonds with guanine, as depicted below:



EX1035.002.

47. Each strand of the DNA molecule has two ends – one end is referred to as 5' (five prime) and the other end is referred to as 3' (three prime). DNA is synthesized in the 5' to 3' direction.

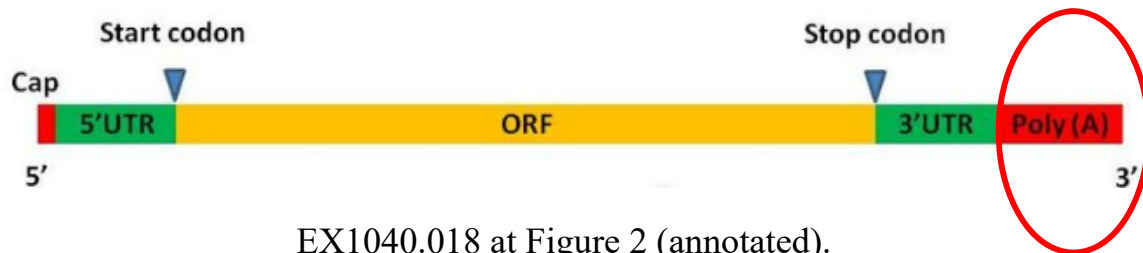
48. Ribonucleic acid (RNA) is a molecule similar to DNA, as both store genetic information and are made up of nucleotides consisting of sugar, nitrogenous bases, and a phosphate backbone. RNA can hybridize to a complementary DNA sequence with the help of specific enzymes. DNA and RNA are molecules that are known in the art as “nucleic acids.”

49. Unlike DNA, RNA is mostly single-stranded. Also, the four nucleotides that make up RNA are adenine, guanine, cytosine, and uracil (U), wherein G hybridizes to C, and A hybridizes to U (as oppose to T in DNA). Despite the structural difference, uracil and thymine are functionally equivalent because they both form base pairs with adenine, and denote the same genetic information. Vértessy at 1 (EX1050.001).

50. Gene expression is the process by which information encoded in a gene is used by a cell, often by converting the nucleic acid sequence information into a protein. Proteins are made up of amino acids, which serve critical functions of cellular life including repairing and building body's tissues, carrying out metabolic reactions, and coordinating bodily functions.

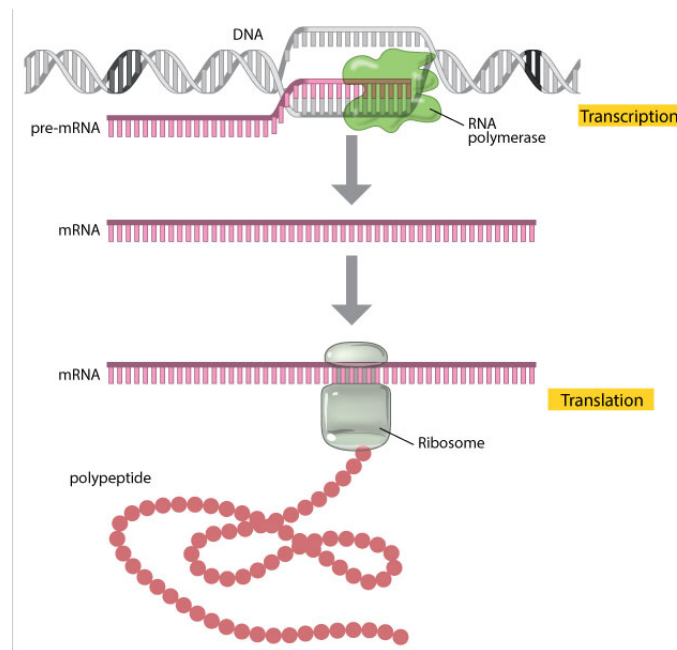
51. Protein-coding gene expression is achieved by two major steps – transcription and translation. Transcription is the copying of genetic information from a DNA sequence into a specific type of RNA – messenger RNA (mRNA), which is made up of nucleotides that are complementary to the DNA sequence. During transcription, an enzyme called RNA polymerase catalyzes the formation of a pre-mRNA molecule using the DNA sequence as a template for complementary base-pairing. The pre-mRNA is then processed to form mature mRNA. Clancy at 1 (EX1042.001). A poly(A) tail, which is a sequence of adenine nucleotides, is added to the 3' end of the mature mRNA (circled in red, below) and makes the

mRNA molecule more stable and allows it to be exported from the nucleus to carry out the next step, translation.



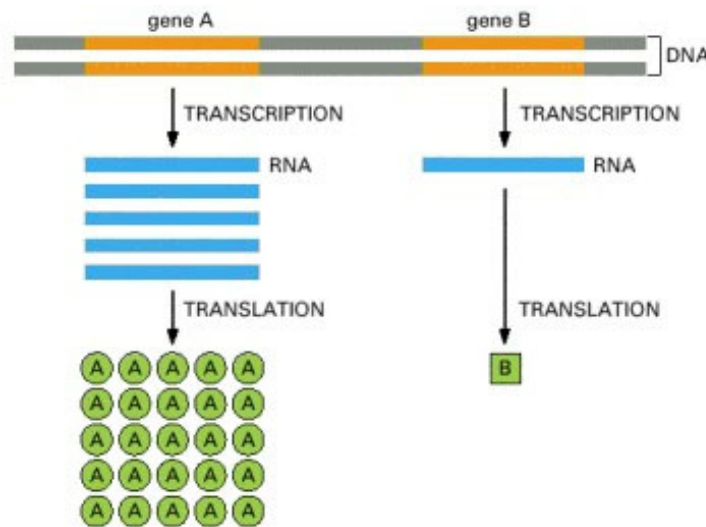
EX1040.018 at Figure 2 (annotated).

52. Translation is the decoding of genetic information carried by the mRNA to make proteins. This process takes place within a specialized organelle called the ribosome, which reads the mRNA in a group of three bases, referred to as a codon, which correspond to a particular amino acid. EX1042.001.



Clancy at 2, Figure 1 (EX1042.001).

53. Genes can be expressed at different levels. In the figure below, Gene A is transcribed and translated at much higher levels than gene B. This allows the amount of protein A in the cell to be much greater than that of protein B. Alberts at 302 (EX1025.005).



Alberts at Fig. 6-3 (EX1025.005).

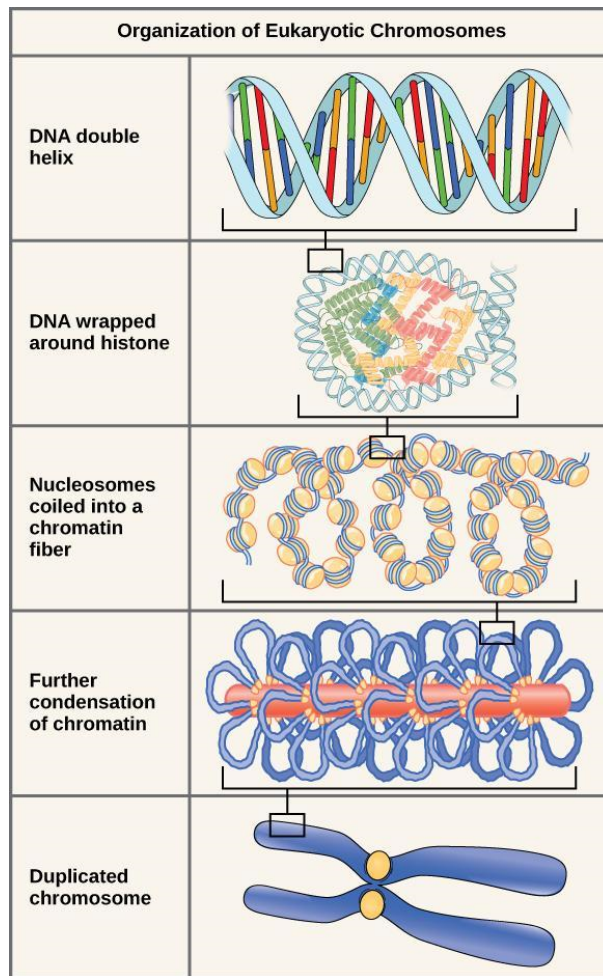
54. Gene expression profiling is the determination of the mRNA content in a cell or tissue to understand the pattern of genes expressed. Thus, detecting mRNA molecules is critical to the study of gene expression because they show the portions of DNA copied for the production of proteins. This is useful to understand aberrant gene activity and identify potential drug targets that alter gene expression, among other research and clinical applications.

B. DNA Packaging

55. A “genome” describes all the genetic information of an organism. A “genome region” consists of specific portions of DNA sequences within a chromosome, some of which may act as the template from which mRNA is transcribed and/or influence the amount of transcription that occurs at transcribed regions. The genome region acts as the template from which mRNA is created.

56. As the genome must fit within the nucleus of a cell, it must be tightly packaged. However, this packaging must be balanced against the need to readily access the DNA so that its encoded information may be expressed. To accomplish this balance, there is a scheme of DNA packaging that I describe further below.

57. First, a molecule of DNA in a cell with the associated structural proteins is known as a chromosome. A chromosome may be more tightly or loosely packaged depending on the cell’s growth cycle and other factors. In humans, there are normally twenty-three pairs of chromosomes in the nucleus of each cell.



EX1036.004

58. A chromosome is packaged according to the scheme depicted above. The first level of packaging is no packaging at all. This is the DNA existing as a double helix, with no associated proteins.

59. The second level of packaging is where the double-stranded DNA wraps around spools of histone, a family of spherical proteins that provides a scaffolding structure for DNA packaging, to form nucleosomes. A single nucleosome is a short stretch of DNA that wraps around a core of eight histones.

60. The aforementioned complex of DNA and protein is known as chromatin. DNA in the nucleus of a cell generally exists in the form of chromatin, where it is subject to varying levels of packaging. Even outside of a cell, however, chromatin would be known as chromatin.

61. The third level of packaging is where nucleosomes are linked to each other with small sections of DNA known as linker DNA, and coiled into a chromatin fiber. This level of packaging has a characteristic “beads on a string” appearance.

62. The fourth level of packaging is where the chromatin, in the form of “beads on a string,” is further condensed around structural proteins (red) to form more tightly wound fibers.

63. The final level is the classical “X” shape of the chromosome, at which point the DNA is fully condensed and tightly packaged.

64. It is important to note that DNA packaging is dynamic, and changes in the cell depending on the cell’s phase in the cell’s life cycle. For example, chromatin is at its most condensed directly prior to cell division, and tends to be less condensed when the cell is going through a growth phase or when the cell is in a resting state. The level of DNA packaging generally correlates to the need for DNA to be accessible to the machinery of the cell for gene expression.

65. Accessibility to chromatin is directly associated with gene expression and is a widely studied characteristic of the eukaryotic genome. Open chromatin

regions are more accessible to DNA regulatory regions and more loosely packaged. Closed chromatin regions are less accessible and more tightly packed.

C. Nucleic Acid Sequencing

66. Nucleic acid sequencing is the process of determining the exact sequence of nucleotides in a nucleic acid molecule. The development of sequencing technologies can be traced to at least 1977 when Frederick Sanger developed DNA sequencing technology based on a chain termination method. Hutchinson at 6227, 6299 (EX1030.001, 003). DNA sequencing technologies went through significant improvements in subsequent years. In the early 2000s, NGS technologies emerged which were different from the Sanger method in terms of throughput and cost. Mardis at 388 (EX1031.002). By approximately 2008, techniques and platforms for NGS were commercially available and in widespread use including the Roche/454 FLX, Illumina/Solexa Genome Analyzer and Applied Biosystems SOLiD System. Mardis at 388 (EX1031.002); Shendure at 1136 (EX1032.002).

67. NGS is a type of high-throughput sequencing technology that uses parallel sequencing of many fragments of DNA. Parallel sequencing enables these sequencers to process and detect millions of sequence reads in parallel. Mardis 2 at 133 (EX1033.001). The platforms carrying out NGS are diverse in sequencing and detection biochemistry but generally have a common workflow to produce sequence

reads including steps of – library preparation, amplification and sequencing. Shendure at 1136 (EX1032.002).

1. Library Preparation and Tagmentation

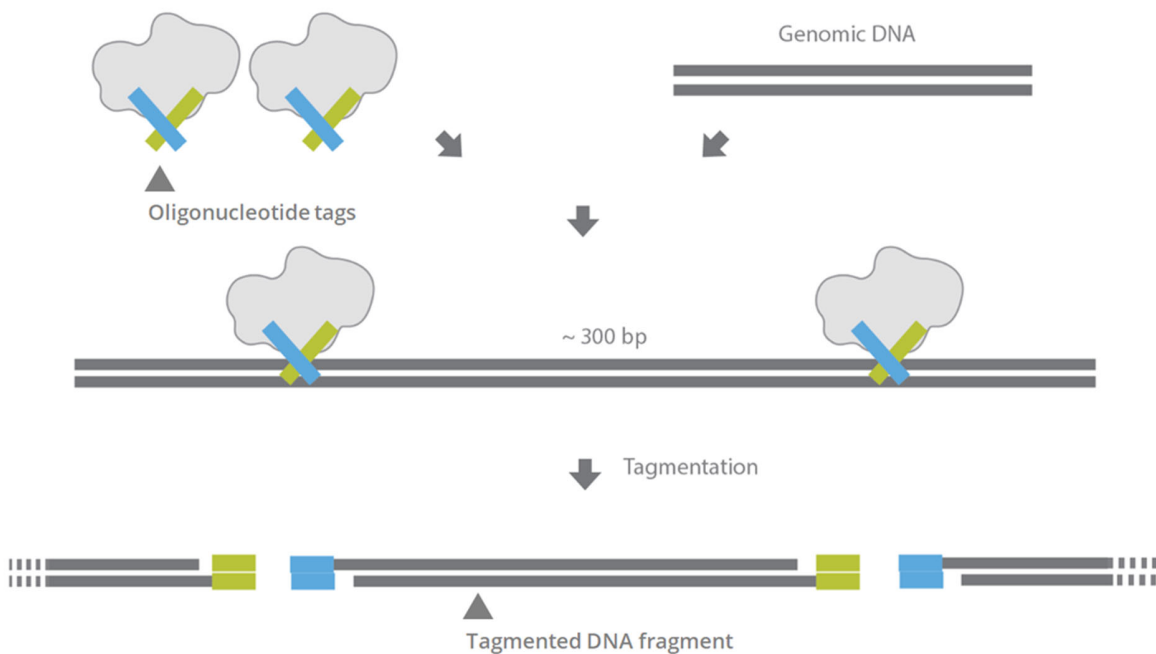
68. Many NGS platforms, including those sold by Illumina, can generate only a short string of DNA sequence information from a nucleic acid molecule, typically only a couple of hundred nucleotides in length. Hence DNA is essentially broken up or “fragmented” into small chunks, prior to sequencing, so that it is suitable for the sequencing instrument. Library preparation includes fragmentation of DNA and ligation of common adapter sequences to both ends of each DNA fragment, also referred to as “tagmentation” when performed in a single step. Shendure at 1136 (EX1032.002).

69. Tagmentation was developed by researchers as early as 2010 in order to reduce the time and cost of preparing DNA libraries for next-generation sequencing. EX1008.003. It is a one-step reaction where accessible genomic DNA is fragmented and tagged for analysis. A specific type of enzyme—transposase—simultaneously fragments a target DNA portion and attaches oligonucleotide tags at the cleaved site. Oligonucleotide tags are discrete segments of DNA with pre-defined lengths and sequences such that each tag is identifiable during sequencing and can be used in downstream applications like amplification. Adey at 2

(EX1006.002). Oligonucleotide tags can however be used for various purposes.

EX1010.002

70. The tagmentation process is shown in the figure below: transposase (light grey) is loaded with two oligonucleotide tags (green, blue), forming a complex. The complexes bind to the target DNA (dark grey) at high density, that is, one complex around every three hundred base pair. The complexes then cleave the target DNA backbone on both strands, and append the oligonucleotide tags at the 3' and 5' ends of the fragmented DNA at each of the cut sites.



EX1043.001

71. The '357 Patent acknowledges that methods for “tagmenting isolated genomic DNA are known in the art (see, e.g., Caruccio Methods Mol. Biol. 2011 733: 241-55; Kaper et al, Proc. Natl. Acad. Sci. 2013 110: 5552-7; Marine et al,

Appl. Environ. Microbial. 2011 77: 8071-9 and US20100120098) and are commercially available from Illumina (San Diego, Calif.) and other vendors. Such systems may be readily adapted for use herein.” EX1001 at 14:31-38.

2. Amplification

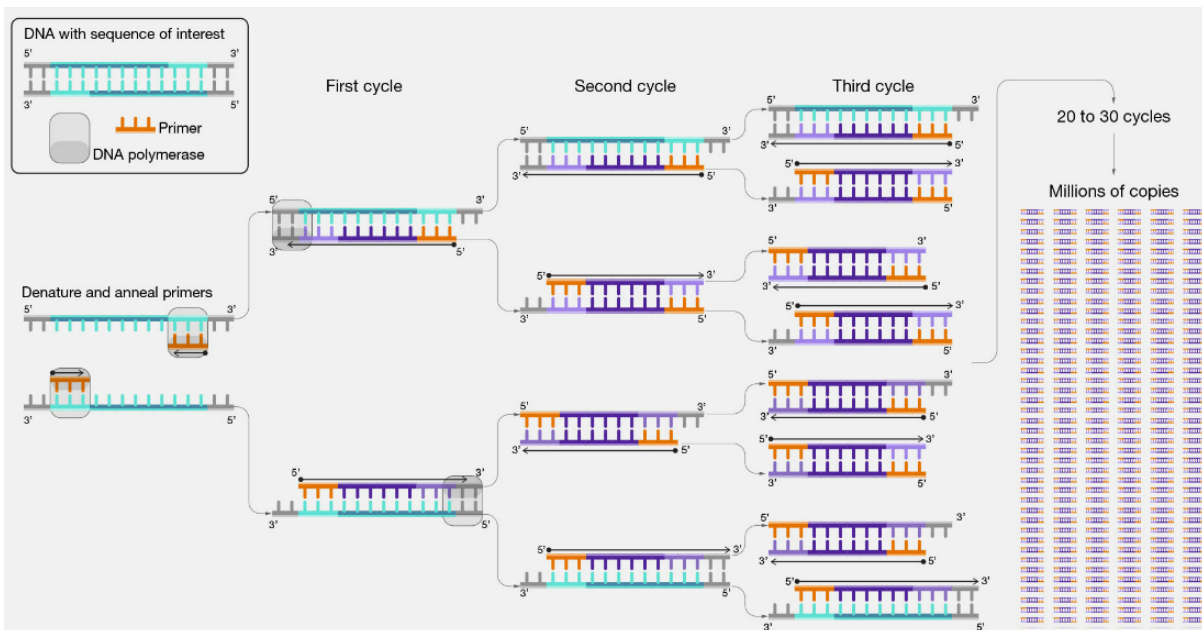
72. DNA sequencing requires a minimum amount of DNA or RNA. Before it can be sequenced, a sample with limited amount of DNA or RNA must sometimes be amplified. Amplification refers to the process of creating multiple copies of a specific polynucleotide. Various methods of amplification were known in the art before 2009, such as Polymerase Chain Reaction (PCR), Ligase chain reaction (LCR), Nucleic acid based amplification (NASBA), and Transcription-mediated amplification, etc.

73. PCR is a technique using repeated cycles of oligonucleotide-directed DNA synthesis to carry out amplification of target nucleic acid sequences. Persing at 1281 (EX1026.001). PCR requires generally involves three steps: (1) denaturation of the double-stranded DNA, (2) annealing of primers, and (3) primer extension. Schochetman at 1154 (EX1012.002).

74. Denaturation is the separation of the complementary strands of DNA by using heat or through other chemical or physical means of dissociation. Schochetman at 1154 (EX1012.002). This results in two complementary single-stranded DNA template strands, which can then be replicated.

75. Annealing is the attachment of primers or probes which are oligonucleotides complementary to one of the original DNA strands. When the reaction mixture comprising denatured DNA template strands is cooled to a certain temperature, the primers hybridize, that is bind through base pairing interactions to either the left (5') or right (3') side of the sequence of interest. Schochetman at 1154 (EX1012.002). These portions to which the primers bind are called priming sites.

76. During the primer extension step, a thermostable enzyme catalyzes the synthesis of new strands of DNA through primer extension. The enzyme called "DNA polymerase" adds nucleotides complementary to those in the single-stranded DNA template starting at the 3' end of the annealed primer. Schochetman at 1154 (EX1012.002). The result of one cycle of PCR is to duplicate a double-stranded DNA. Upon completion of one cycle, the DNA strands are subjected to additional cycles, i.e., the same steps of denaturation, primer annealing, and primer extension. This results in an exponential amplification of target DNA sequences, as demonstrated below.



EX1038.001.

77. Methods for the application of PCR amplification to small amounts of sample polynucleotide, such as from single cells, were well established and known in the prior art. Kurimoto (EX1027); Esumi (EX1028).

78. In NGS platforms, DNA fragments to be sequenced may be subjected to clonal amplification which, depending on the platform, includes approaches of emulsion PCR or bridge PCR. These are PCR techniques that cause the amplified sequences derived from a single library molecule to be spatially clustered either to a single location as in the case of bridge PCR or to the surface of micron-scale beads in case of emulsion PCR, thus facilitating sequencing and detection. Shendure at 1136 (EX1032.002).

3. Sequencing

79. After the amplification step, the sequencing step is performed depending on the selected platform, which consists of alternating cycles of enzyme-driven processes and imaging steps as depicted below.

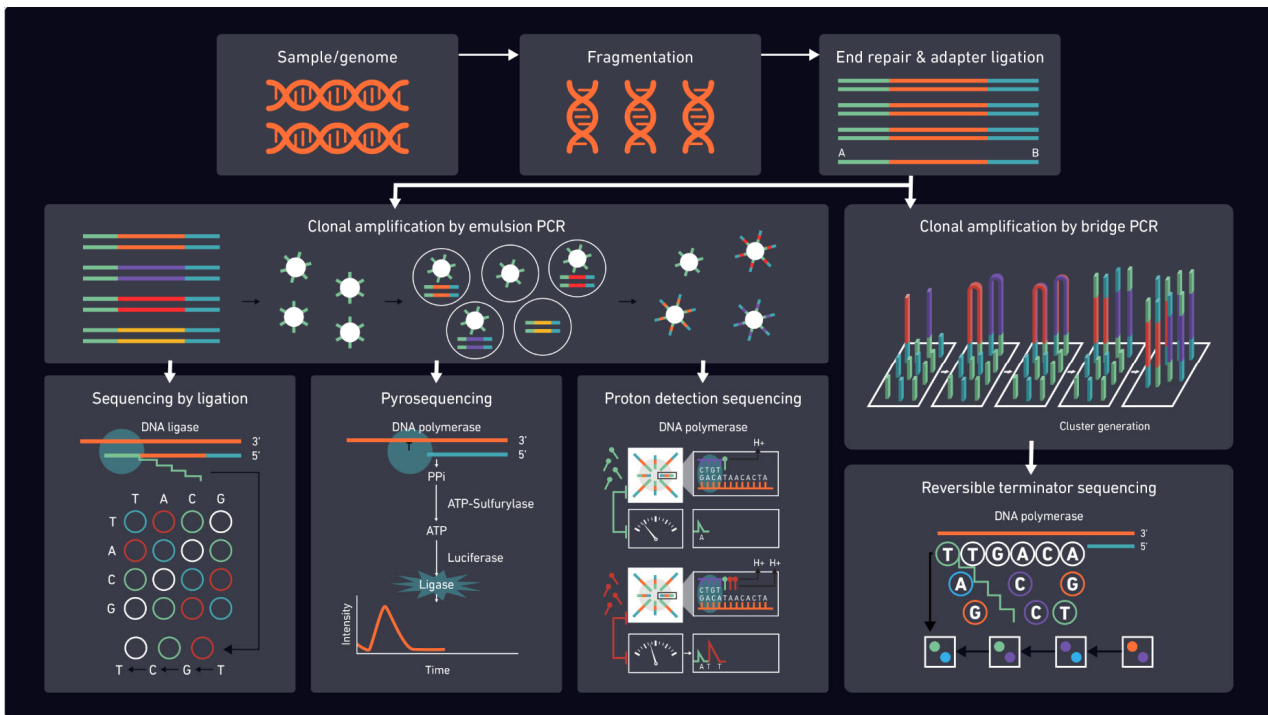


Figure 2, EX1039.005.

80. For example, the Illumina Genome Analyzer, utilizes a sequencing-by-synthesis approach, also called reversible terminator sequencing. Mardis at 391 (EX1031.005); Shendure at 1139 (EX1032.005). In this sequencing platform, starting with the fragmentation and adapter ligation steps as described above, the sequenced library is added to a “flow cell” which are sample surfaces that have channels coated with oligonucleotides complementary to the sequencing adapters of

the library, so that single-stranded adapter-ligated DNA fragments can attach through hybridization. Holt at 839 (EX1034.001). After hybridization, an isothermal polymerase creates a complementary strand of the hybridized template. The double stranded molecule is then denatured and the single stranded template is clonally amplified through bridge amplification, repeatedly. Shendure at 1138 (EX1032.004). This results in production of multiple DNA copies or clusters, each representing the single molecule that initiated the cluster amplification. Mardis 2 at 135 (EX1033.003). These DNA clusters are again made single-stranded in order to be used as a template to make copies by the help of “reverse terminators.” Shendure at 1139 (EX1032.005). Reverse terminators are nucleotides that carry a base-unique fluorescent label and have their 3'-OH group chemically inactivated such that only that single base is incorporated which corresponds to its respective base (A, G, T, or C) at the position on the single stranded template. Shendure at 1139 (EX1032.005). After the addition of each nucleotide, an imaging step is performed which excites the clusters by a light source resulting in emission of a characteristic fluorescent signal. The number of cycles determines the length of the read and the emission wavelength determine the base call. Mardis at 392 (EX1031.006). For a given cluster, all identical strands are read simultaneously in a computer and millions of clusters are processed in a massively parallel process. Mardis 2 at 135 (EX1031.003), Shendure at 1140 (EX1032.006).

D. Gene Expression and Epigenetics

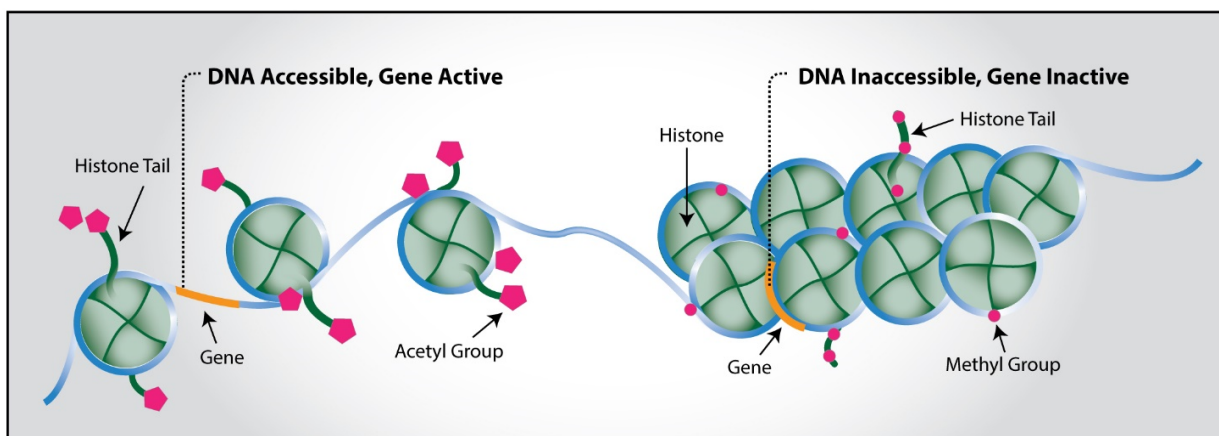
81. “Gene expression,” as explained above, refers to the process of reading instructions encoded in certain portions of DNA (*i.e.*, genes) and manufacturing proteins based on those instructions. Not all portions of the genome are expressed and used to manufacture proteins. In fact, certain genes may be turned “on” or “off,” affecting which proteins are made and at what levels those proteins are made.

82. “Epigenetics” is the study of how environmental influences affect gene expression. During development of an organism, environmental influences can induce chemical changes that “mark” DNA to affect expression. This collection of chemical marks is sometimes referred to as the epigenome. Frequently studied types of epigenetic changes, or marking, include DNA methylation and histone modification.

83. DNA methylation involves adding a chemical group, called a methyl group, to specific places on the DNA. Methylation at some sites can reduce or prevent expression of a gene. Typically, methylation of a gene’s promoter (*i.e.*, the DNA sequence at or near the transcriptional start site of the gene) turns genes “off,” and demethylation of promoters —removal of the blocking chemical group—is associated with turning genes “on.” Thus, methylation changes the structure of the DNA and can result in more or less transcriptional activity of a gene.

84. Histone modification involves altering chromatin structure by binding chemical compounds to histone proteins. As I explained above in the section on DNA Packaging, histones are the proteins around which a DNA strand wraps to form chromatin. Information encoded in a DNA is less accessible when histones are tightly packaged and more accessible when histones are loosely packaged. Environmental influences may cause the addition or removal of certain chemical groups from histones, which in turn causes the histones to become more tightly or loosely packaged, which often correlates with turning genes “off” or “on,” respectively.

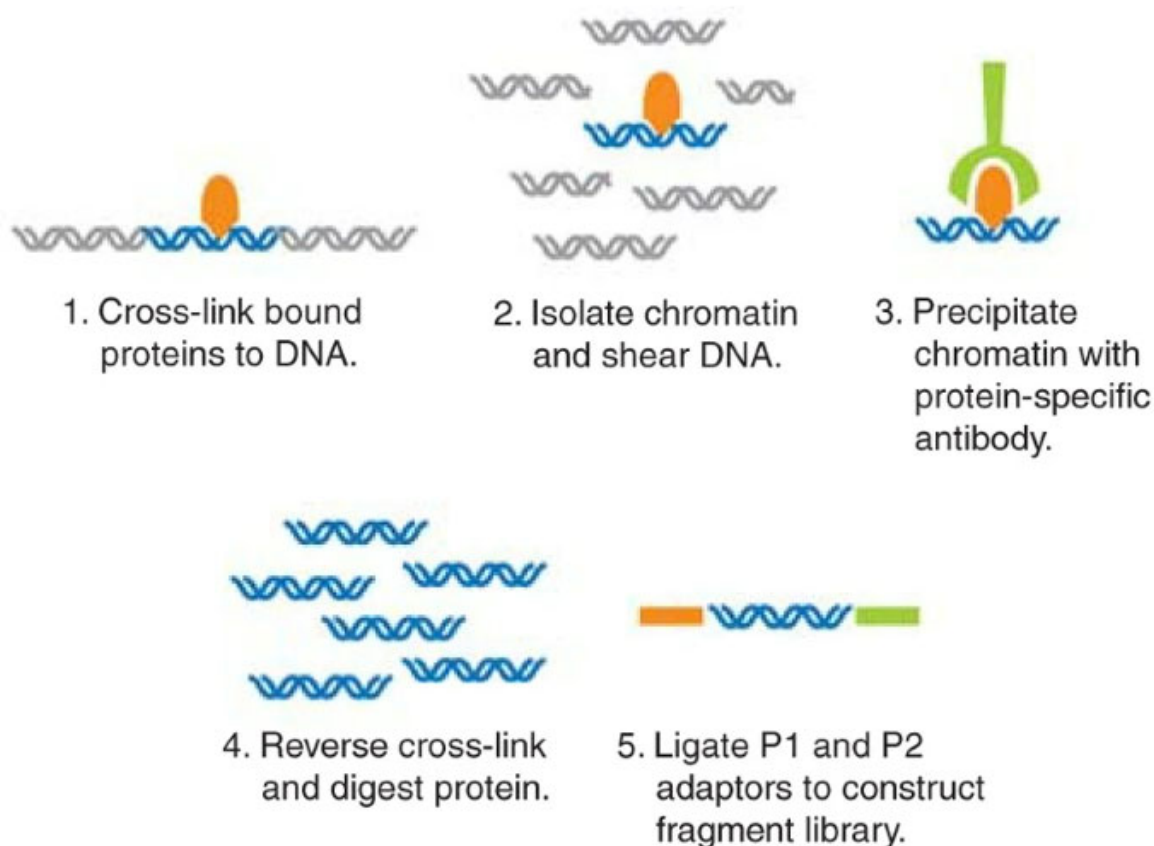
85. An example of histone modification is depicted in the figure below. Covalent modifications such as methylation, addition of a methyl group (pink dot), and acetylation, addition of an acetyl group (pink pentagon), are highly correlated with the structure and accessibility of chromatin. Histone acetylation tends to occur where DNA is open and accessible, while histone methylation has been correlated to tightly packaged and less accessible DNA.



EX1037

86. NGS, as early as 2007, has enabled analysis of DNA-protein interactions and obtaining profiles of epigenetic changes by using the method of chromatin immunoprecipitation followed by sequencing (“ChIP-seq”). Johnson at 1 (EX1022.001). This method typically starts with a formaldehyde treatment to crosslink or fix proteins directly to the DNA. Shah at i. (EX1044.001). Chromatin is isolated from the cells and fragmented through sonication or by using restriction enzymes. The chromatin fragments are then enriched using antibodies specific to the proteins or histone modifications of interest. Shah at i. (EX1044.001). The target protein-DNA interactions are separated or purified from the other protein-DNA interactions with the use of magnetic beads present on the antibodies. Then, crosslinks are reversed by extensive heat incubation or digestion of the protein component and the DNA is extracted. Shah at i. (EX1044.001). The next step involves using the purified ChIP DNA as a template for high-throughput sequencing, which utilizes the same workflow of NGS as described above – library preparation,

amplification and sequencing. This process helps in finding the genomic sites to which the protein of interest is bound. A step by step process of DNA enrichment by ChIP is depicted below.



Shah at Figure 1 (EX1044.001)

E. Permeabilization

87. Permeabilization is the process of creating openings in a cell membrane in order to allow entry of reagents into the cell. This process helps in gaining access to chromatin inside of a cell which is guarded by the cell membrane by making the cell membrane permeable. It is important that DNA of interest is still intact and

remains entrapped and associated with the cell after permeabilization, therefore researchers had developed permeabilization techniques that minimally perturbs the genomic material inside the cell. Becker at 1313 (EX1014.003)

88. Permeabilization techniques can be physical or chemical. Electroporation is a physical method of permeabilization established at least as early as 1988. Brodelius at 186 (EX1013.001). The process involves the use of short high-voltage pulses to temporarily create pores in the cell membrane. Gehl at 438 (EX1016.002). By applying external electric fields that just exceeds the capacitance of a cell membrane, a transient and reversible breakdown of the cell membrane can be triggered. This transient state of permeability can be, and had been widely used, to introduce many types of molecules into the cell, including antibodies, oligonucleotides, DNA, dyes, and drugs. Electroporation had proven to be a useful method of permeabilization both in vitro and in vivo, and researchers had optimized electroporation protocols to ensure delivery of molecules while preserving cell viability. Gehl at 437 (EX1016.001).

89. Similar to electroporation, ultrasonication is also an established, physical method of permeabilization as early as 1998. Liu at 918 (EX1017.001). Ultrasonication employs ultrasound to transiently permeabilize cell membranes. Application of ultrasound temporarily increases the permeability of cell membrane by acoustic cavitation, where acoustic cavitation involves the creation, oscillation,

and collapse of gas bubbles. Research had demonstrated that ultrasonication can facilitate transportation of large molecules, like DNA and proteins, into cells. Liu at 918 (EX1017.001).

90. Hypotonic shock is also another well-known method of permeabilization as early as 1992. Márián at 291 (EX1018.001). Hypotonic shock induces an osmotic gradient across the cell membrane by concentration difference. The osmotic stress triggers formation of pores or cracks at weak spots without completely rupturing the cell, increasing permeability of the cell membrane while maintaining its integrity. Márián at 291 (EX1018.001)

91. Chemical permeabilization techniques have included detergents such as saponin, Triton X-100, and NP40, commonly used as permeabilization agents as early as 1994. Melan at 61 (EX1019.007). Detergents act on membrane by dissolving the components of the membranes, thereby creating openings on the membranes 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. Melan at 60 (EX1019.006).

* * *

92. Thus, tagmentation, sequencing, epigenetic studies of chromatin, sequencing, and permeabilization were all well-known techniques in the field of genetic analysis no later than 2013. In fact, the inventors of the '995 patent

acknowledge that methods of tagmentation are known in the art and have been subject of commercial product offerings which can be readily adapted for the alleged invention. EX1001 at 14:23-28.

VIII. PERSON OF ORDINARY SKILL IN THE ART

93. I understand that whether a patent is invalid as obvious in light of the prior art is considered from the perspective of a person of ordinary skill in the art (“POSA”) at the time of the alleged invention. I am knowledgeable about what the level of ordinary skill in the art would have been at the time of the alleged invention based on my education and experience. *See supra* Section II.

94. In determining the definition of a POSA, I have considered the following factors: (1) the level of education and experience of those in the field; (2) the complexity of the technology at issue; (3) the rate of innovation; (4) the kinds of problems faced by those in the art; and (5) solutions to such problems known in the prior art.

95. In my opinion, a POSA would have been someone with 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. Alternatively, a POSA would have been someone with a Bachelor of Science degree in Molecular Biology, Genetics, Chemistry, Engineering, or a related discipline 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).

96. Further, a POSA would have been familiar with several well-known and established principles and techniques in molecular biology and genetics. For example, a POSA would have familiarity with the molecular structure of nucleic acids such as DNA and RNA, as well as the structure of chromatin. Additionally, a POSA would be familiar with epigenetics, particularly the connection between sequences and epigenetic features such as DNA methylation. In terms of techniques, a POSA would have been aware of tagmentation as a method to simultaneously fragment and tag DNA in preparation for further assays, such as sequencing and amplification, as well as tagging for identification. A POSA would have also been familiar with next-generation sequencing, including the various platforms using NGS to determine sequencing data and how those platforms operate.

IX. THE PRIOR ART REFERENCES OF THE GROUNDS

A. Grunenwald (EX1003)

97. U.S. Patent No. 9,080,211 (“Grunenwald”) was filed on October 24, 2009 by Epicentre Technologies Corporation and was published as an application on May 13, 2010 entitled “Transposon End Compositions And Methods For

Modifying Nucleic Acids.” The application claims priority to a provisional application filed on October 24, 2008.

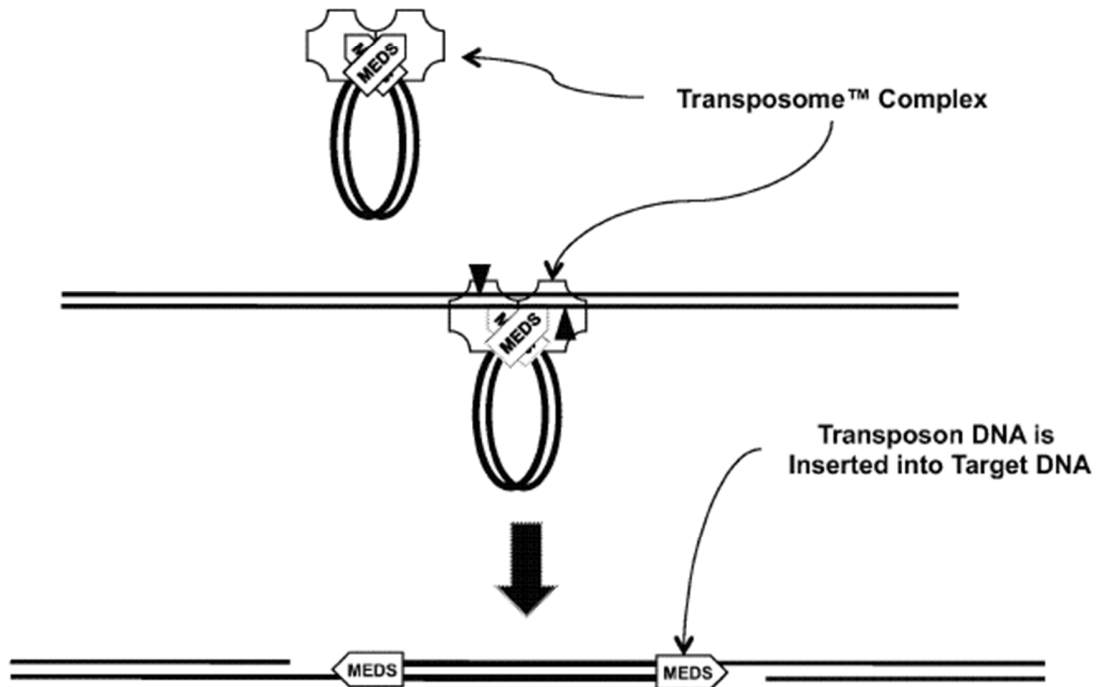
98. Grunenwald teaches the prior art tagmentation process described above. *See supra* ¶¶ 64-66. Grunenwald broadly discloses the methods, compositions, and kits for fragmenting and tagging DNA using transposon compositions. Grunenwald explains the invention is useful for generating libraries of tagged DNA fragments for use in “in next generation sequencing methods, fluorescence in situ hybridization, and the like.” EX1003 at [0017].

99. As an initial matter, Grunenwald teaches a method for fragmenting and targeting a “target DNA.” *Id.* at [0146]. The target DNA 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.* The DNA derived from these sources may be selected from a variety of types of DNA, including “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.

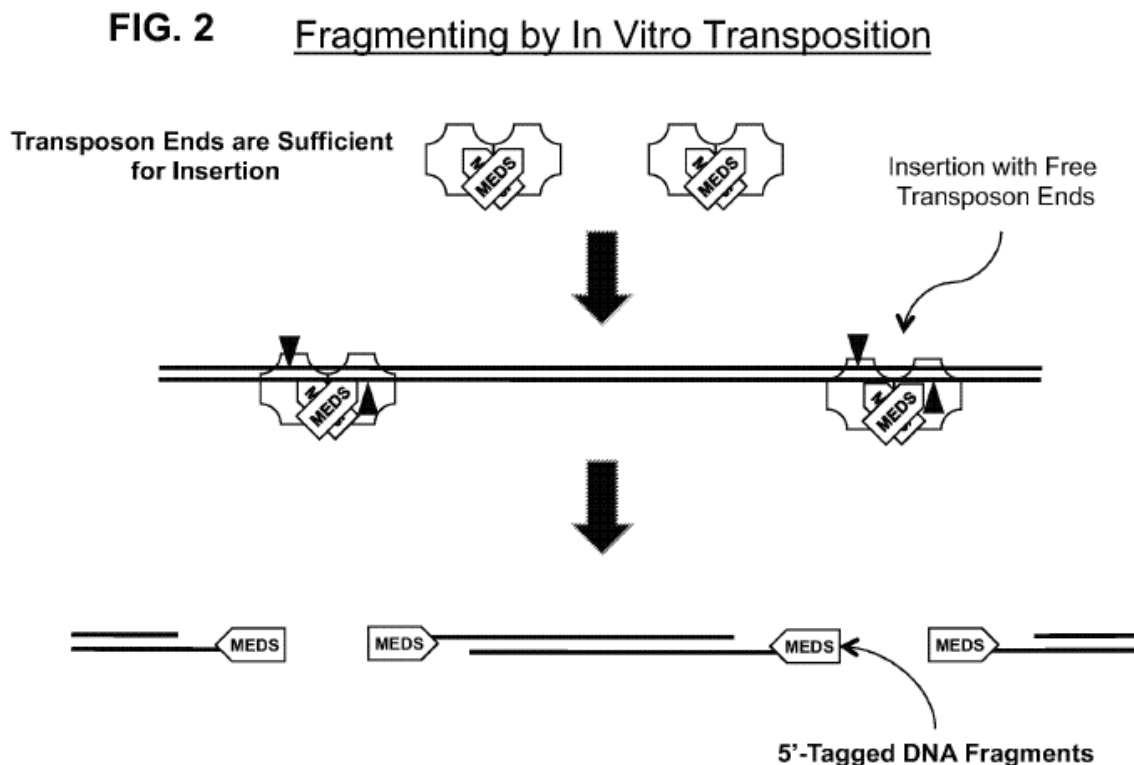
100. The method that Grunenwald teaches is incubating the target DNA with “at least one transposase and one transposon end composition,” where the transposon end composition includes the oligonucleotide tag to be inserted into the target DNA. *Id.* at [0162]. An exemplary transposition reaction tagging a double-stranded target DNA is depicted in FIG. 1 below. In this figure, the transposome complex is composed of a transposase enzyme (the two cross-shaped structures), a transferred end (the looping black lines below the enzyme), and two transposon ends (labeled MEDS). FIG. 1 demonstrates the transposase contacting a target DNA (two black horizontal parallel lines) and inserting the transferred strand into the target DNA.

FIG. 1*In Vitro* Transposition

EX1003 at Figure 1

101. Further, Grunenwald explains that a transposase and a target DNA are incubated under conditions to catalyze a transposition reaction. This transposition reaction can be used to simultaneously fragment and tag the target DNA to generate a plurality of 5' tagged target DNA fragments. *See Id.* at [0018]. An exemplary 5'-tagging is depicted in FIG. 2 below. In the figure, the transposase enzymes are again represented by the two cross shapes, with a tag labeled MEDS. The transposase

enzymes bind to the target DNA, and simultaneously fragment and tag the DNA to create 5'-tagged DNA fragments.



EX1003 at Figure 2

102. The tag joined to a target DNA as part of this tagmentation process is “a non-target nucleic acid component, generally DNA that provides a means of addressing a nucleic acid fragment to which it is joined.” *Id.* at [0088]. Grunenwald further discloses the tag sequence “comprises one or more of a restriction site tag domain, a capture tag domain, a sequencing tag domain, an amplification tag domain, a detection tag domain, an address tag domain, and a transcription promoter

domain.” *Id.* at [0103]. Grunenwald specifically discloses that the tag sequence can comprise “sequencing tags selected from 55 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,” all of which are of predetermined sequence. *Id.* at [0021].

103. Importantly, Grunenwald teaches that tagmentation can be applied not just to DNA that has been isolated from living cells, but directly to living cells. Specifically, Grunenwald teaches that the reaction may be carried out in vivo by “electroporating a synaptic complex of a transposase and a suitable transposon end composition into the host cell.” *Id.* at [0157]. To be tagmented with a transposase, chromatin must be open and accessible. As I explained above in Section VII.D on Gene Expression and Epigenetics, accessible DNA exists in the form of open chromatin regions in living cells. Therefore, tagmentation reaction in vivo necessarily entails application of tagmentation to open chromatin

B. Okino (EX1004)

104. PCT Application Publication No. WO 2010/065266 A1 titled “Chromatin Structure Detection” (“Okino”) was filed on November 13, 2009 and published on June 10, 2010. Okino claims priority to U.S. Provisional Application

No. 61/119,280. It is my understanding that Okino is prior art and was not before the Examiner during the prosecution of the '357 Patent.

105. Okino teaches the study of chromatin structure using a “DNA modifying agent,” which the application defines as any molecules that “alters DNA in a detectable matter.” This may be an enzyme that cleaves or nicks the DNA, creating a break in the strand, or an enzyme that adds or removes a moiety that is bound to the DNA. EX1004 at [0005].

106. The DNA modification occurs in two steps. First, a cell is simultaneously permeabilized and contacted with a DNA modifying agent. Second, the DNA sequence information is detected. EX1004 at [0005].

107. In performing the first step, Okino teaches two methods of permeabilization that may be used. One method is permeabilization by reagent, which includes “non ionic detergents” such as “NP40, Tween20, and Triton X-100.” EX1004 at [0072-73]. Alternatively, as in Grunenwald, electroporation may be used to permeabilize the cell. EX1004 at [0075].

108. Simultaneously while the cell is permeabilized, a DNA modifying agent is permitted to enter the cell. Okino discloses different types of DNA modifying agents, but many of them are enzymes. EX1004 at [0079-95]. These enzymes may cleave or nick the DNA strands or add some form of detectable moiety, such as a methyl group, to the DNA. EX1004 at [0079, 87, 92].

109. Following modification, the DNA must be isolated for analysis. Okino discloses that “[e]ssentially any DNA purification procedure can be used so long as it results in DNA of acceptable purity” for the following steps. EX1004 at [0105]. To isolate the DNA, “standard cell lysis reagents can be used to lyse cells.” *Id.*

110. Finally, after the DNA has been analyzed, sequence information (or other physical characteristic) can be detected and analyzed to determine chromatin structure information. EX1004 at [0109].

C. Saleh (EX1005)

111. *An Efficient Chromatin Immunoprecipitation (ChIP) Protocol For Studying Histone Modifications In Arabidopsis Plants* by Abdelaty Saleh, Raul Alvarez-Venegas, and Zoya Avramova (“Saleh”) was published in Nature Protocols in 2008. It is my understanding that Saleh is prior art and was not before the Examiner during prosecution of the ’357 Patent.

112. Saleh describes a prior art method for fragmenting and tagging DNA known as chromatin immunoprecipitation (ChIP). ChIP permits the study of protein and DNA interactions by targeting proteins of interest in chromatin with antibodies, precipitating them out of solution, and then analyzing the associated DNA.

113. The first step in ChIP protocols is a cross-linking step. In this step, formaldehyde is used to bind to bind the DNA found in chromatin to any associated

proteins. This freezes the interactions and generates a “snapshot” of the chromatin at the time of cross-linking. EX1005.005.

114. In ChIP protocols generally, the next step is to actually shear the DNA into fragments of an appropriate size for tagging and subsequent analysis. This may generally be done by sonication, which uses sound waves to shear the DNA into smaller fragments. *See, e.g.*, EX1005.005-006.

115. Saleh, however, teaches an additional step prior to sonication. Saleh teaches that nuclei can be isolated prior to the DNA shearing step. EX1005.005. In teaching this nuclei isolation step, Saleh describes it as “important for the purity and yield of DNA.” EX1005.002. Thus, Saleh teaches that in the context of DNA preparation for sequencing, particularly in fragmenting and tagging, it would have been known that nuclei could be isolated to aid in the process.

116. After shearing the DNA, fragment size is checked through gel electrophoresis, which allows visualization of relative fragment sizes of DNA. EX1005.006. This is important because next-generation sequencing platforms are limited in the size of a fragment that may be sequenced, and so the DNA fragments must be of a size compatible with the sequencing platform of choice. *See supra* Section VII.C.

117. Once fragment size is confirmed, the bound proteins are subjected to immunoprecipitation. This step involves contacting the proteins bound to DNA with antibodies specific to those proteins to pull them out of solution. EX1005.006.

118. After immunoprecipitation, proteins are un-linked from the DNA and digested. This permits precipitation of the DNA itself, which can then be subjected to further analysis (such as amplification and sequencing). EX1005.007.

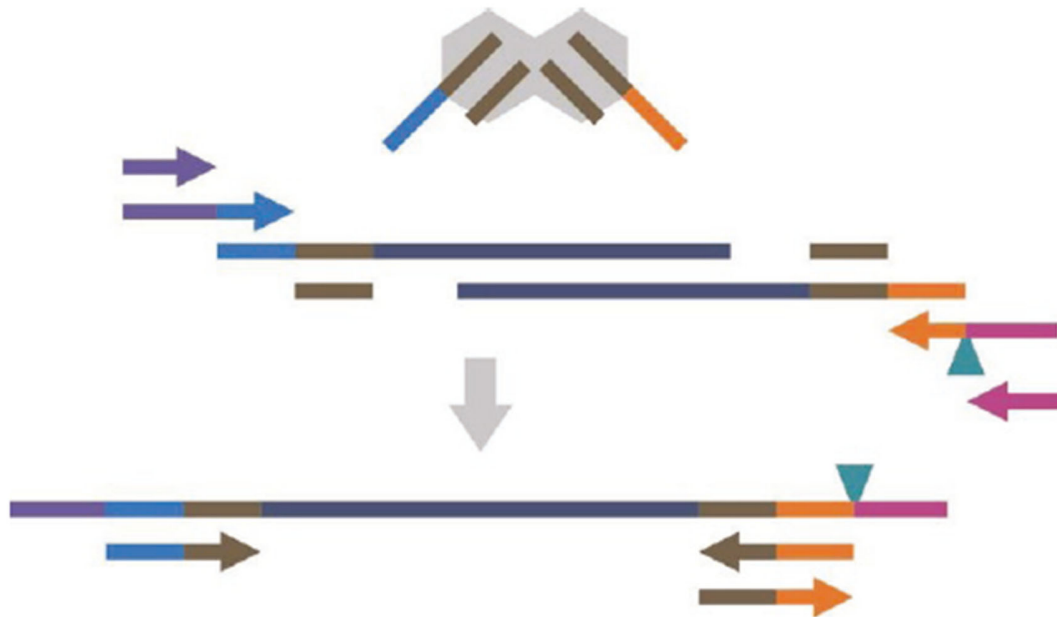
119. At the time of the alleged invention of the '357 Patent, it is my opinion that the techniques detailed in Saleh would have been well-known and routine to a POSA.

D. Adey (EX1006)

120. *Rapid, Low-input, Low-bias Construction of Shotgun Fragment Libraries by High-density In vitro Transposition* by Andrew Adey, Hilary G Morrison, Asan, Xu Xun, Jacob O Kitzman, Emily H Turner, Bethany Stackhouse, Alexandra P MacKenzie, Nicholas C Caruccio, Xiuqing Zhang and Jay Shendure (“Adey”) was published in Genome Biology in 2010. It is my understanding that Adey is prior art and was not before the Examiner during the prosecution of the '357 Patent.

121. 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. Particularly, Adey

discloses using a transposase to insert distinct adapter sequences. Adey at 2 (EX1006.002). As depicted in Figure 1(b), the transposome complex comprises the transposase enzyme (two grey hexagons), a sequence to assist the transposase to fragment DNA (brown), and two different adaptors (mild blue/orange).



EX1006.002 at Figure 1

122. These adaptor sequences are composed of various subsequences. They contain PCR-compatible primer sites which act as a site to initiate amplification reactions. *Id.* at .002. The adaptor sequences also include sequencing primers, which are short sequences of DNA that permit sequencing of a DNA sample - the Nextera™ Kit is specifically tailored to the use of Illumina's NGS platform. *Id.*

Optionally, a barcode sequence can be added to the adaptor sequence to permit identification of the sample being studied. *Id.* at .012.

123. Adey provides a review of the state of the art and a POSA would have known that simultaneous tagmentation and fragmenting of DNA in NGS processes can be facilitated by using two different adapter sequences that contain primer sequences and barcodes.

E. Song (EX1060)

124. *DNase-seq: A High-Resolution Technique For Mapping Active Gene Regulatory Elements Across The Genome From Mammalian Cells* by Lingyun Song and Gregory E. Crawford (“Song”) was published in Cold Spring Harbor Protocols in 2010. It is my understanding that Song is prior art and was not before the examiner during the prosecution of the ’207 Patent.

125. Song is a paper directed to “identification of active gene regulatory elements,” i.e., understanding the relationship between DNA histone proteins and packaging and gene expression. Song teaches a prior art protocol of isolating, digesting, and sequencing DNA to collect and analyze this data. EX1004.001.

126. The first step of this protocol is a nucleus isolation step. Song teaches the isolation of nuclei through the use of a lysis buffer, which can be used to burst the cells. This lysis buffer is composed of RSB buffer and 0.1% IGEPAL CA-630, which is a surfactant that can interact with the cell membrane to cause it to lyse.

EX1004.003. The Cold Spring Harbor protocols further teach that RSB buffer is composed of 10 mM Tris-Cl, pH 7.4, 10 mM NaCl, and 3mM MgCl₂. EX10XX.001 (RSB Buffer). To confirm that lysis was successful, the sample is stained with Trypan blue, a dye that stains nuclei but is excluded by intact cell membranes. EX1004.003.

127. This isolation is followed by a digestion step with DNase I. DNase I is an enzyme capable of breaking down large DNA molecules into smaller fragments, and would have been understood to digest DNA in a sequence independent manner. EX1004.005. After digestion, electrophoresis is used to confirm that digestion was optimal and fragments are the appropriate size for preparation for next generation sequencing. EX1004.0005.

128. After fragmenting, the fragments are blunt-ended. This process removes overhangs and prepares the DNA fragments to have sequencing adapters added to them. EX1004.006-007.

129. The final step is the construction of sequencing library, which is the ligating of sequencing adaptors to blunt ended DNA fragments, purifying the DNA, and sequencing the library. EX1004.007-009.

130. The techniques taught in Song would have been well-known, routine, and common in the art and known to a POSA at the time of the alleged invention of the '357 Patent.

X. CLAIM CONSTRUCTION

131. I understand that the words of the claims of a patent are generally given the ordinary and customary meaning as interpreted by a person of ordinary skill in the art at the time of invention. I have been informed that patent claims are construed in light of the claim language, the specification (including the drawings), and the prosecution history (including references cited during the prosecution of the patent).

132. Claim 1 of the '357 patent describes, in part, a composition comprising ***“an insertional enzyme complex*** comprising an insertional enzyme.” The specification provides an explicit definition for an insertional enzyme complex, which is 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.” This definition is consistent with how a POSA would view the term. In an exemplary embodiment of the invention, the '357 patent teaches the use of a “transposase and molecular tags” as the insertional enzyme complex. EX1001 at 1:55-58. A POSA would have understood that a transposase is an example of an insertional enzyme. *See, e.g.*, EX1021.005.

133. Claims 1-30 recite the use of an “insert element comprising a predetermined sequence.” For my analysis, I use the plain and ordinary meaning of this term, which is a nucleic acid for insertion into a polynucleotide by an insertional enzyme. Further, this plain and ordinary meaning is consistent with the use of “insert

element” in the ’357 Patent, where it is described as a nucleic acid sequence and can be part of a composition also including a polynucleotide and an insertional enzyme. *See* EX1001 at 3:59-67, 4:19-26, 22:24-30, 22:44-48, 22:64-67.

134. Claims 16-21 recite the use of a “sequencing adapter.” While the ’357 Patent does not provide an explicit definition to this term, it references that an insertional enzyme complex comprises an “insertional enzyme and two adaptor [*sic*] molecules.” *Id.* at 13:20-21. These adapters may be added to fragmented DNA to tag the DNA. *Id.* at 14:51-56. This use of adaptor, or adapter, is consistent with its common usage and a POSA’s understanding as a portion of a nucleic acid that has a specific purpose, such as a DNA sequence used to facilitate sequencing, act as a molecular barcode, or act as a primer.

XI. PRIOR ART WOULD HAVE RENDERED OBVIOUS THE CLAIMS OF THE ’357 PATENT

A. Ground 1: Claims 1-30 Are Rendered Obvious By The Combination Of Grunenwald With Okino

1. Claim 1:

a. A composition comprising:

135. It is my opinion that Claim 1 is rendered obvious by the combination of Grunenwald with Okino.

136. As an initial matter, it is my opinion that this preamble is not limiting. I have been informed by counsel that a preamble is only limiting when recites an essential structure or a step to the claim.

137. The preamble of claim 1, however, does not recite anything further than a general composition. There is no mention in this preamble of any substantive features of the claimed invention.

138. Based on my understanding of the law as explained by counsel and the generic recitation of a composition, I do not believe this preamble is limiting.

b. a permeabilized cell nucleus comprising:

139. It is my opinion that the combination of Grunenwald and Okino teaches this claim element.

140. As an initial matter, I note that the '357 Patent does not actually refer to a "permeabilized cell nucleus" in the specification, and it does not teach any techniques that are designed to permeabilize the nuclear envelope specifically. Rather, the '357 Patent's disclosure that the "chromatin used in the method may be made by any suitable method," leads me to understand that the Patent presumes that a POSA would already know the appropriate methods for DNA preparation, including the permeabilization of a cell nucleus. EX1001 at 14:48-50.

141. Lacking any particular disclosure of a nuclear permeabilization, I note that the specification does explain that nuclei may be used for tagmentation reactions after the cells are lysed upon contact with a reaction buffer capable of cell lysis. EX1001 at 14:52-60. Lysis is mentioned a second time in the Patent's discussion of kit embodiments, wherein it discloses a kit that refers to a process by which cells

and nuclei are lysed. EX1001 at 22:60-23:3. However, this kit only discloses a general reaction buffer, and does not explain the lysis in any further detail.

142. A specific lysis protocol is mentioned only a single time in the Patent, in column 25. There, the Patent teaches a protocol to prepare the nuclei of the cell for tagmentation. This is done through the addition of a lysis buffer that comprises 10 mM Tris-Cl, pH7.4, 10mM NaCl, 3 mM MgCl₂, and 0.1% IGEPAL CA-630. EX1001 at 25:8-9.

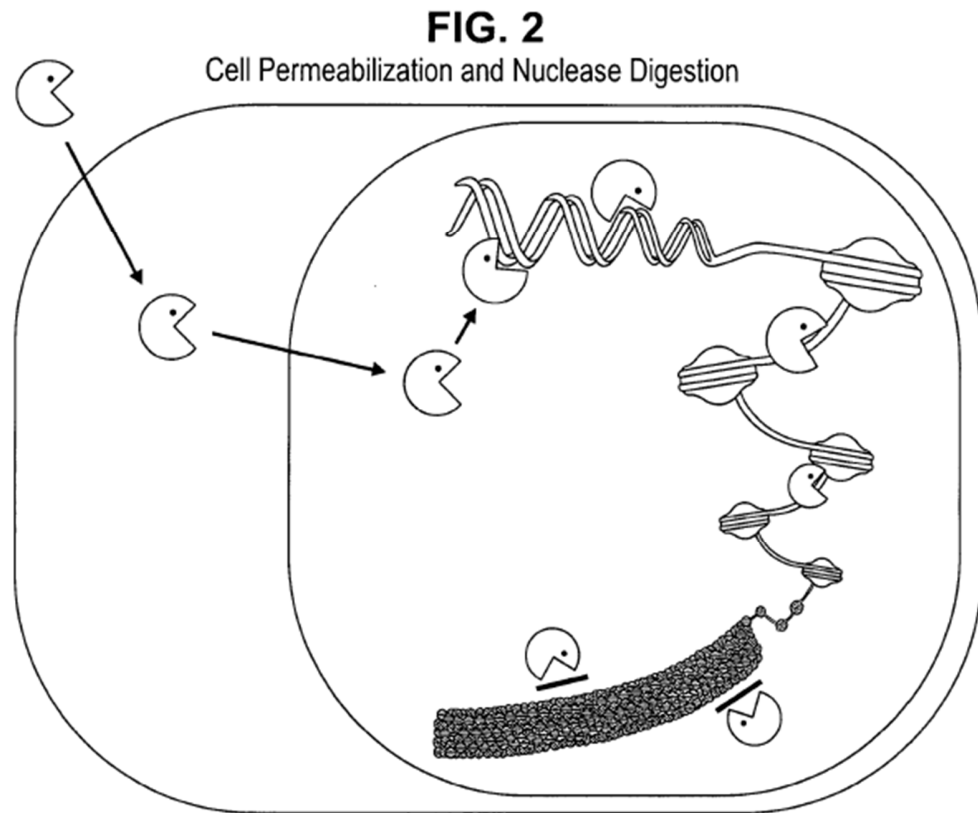
143. This formulation for a lysis buffer is not a novel formulation nor is it unique to the '357 Patent. In fact, I've found this exact formulation verbatim in the 2010 volume of the Cold Spring Harbor Protocols, which are a common source for routine laboratory protocols. EX1060.003.

144. Based on these disclosures, it is my opinion that a POSA would understand the reference to a "permeabilized cell nucleus" in this claim and other claims of the '357 Patent to refer to cell nuclei resulting from the treatment of cells with standard lysis buffers that lyse the cell membrane and permeabilize the nuclear envelope. Further, the disclosures make clear to me that the use of a permeabilized cell nucleus in this context is a routine and well-known step resulting from common techniques well-established in the art at the time of the alleged invention contained in the '357 Patent.

145. Thus, I turn to the teachings in Okino. It is my opinion that Okino discloses a permeabilized cell nucleus.

146. Okino teaches simultaneously permeabilizing a cell membrane and contacting it with DNA modifying agents, which may be enzymes that are able to perform some sort of modification to the chromatin found within. EX1004 at [0005]. The methods of permeabilizing taught are designed such that they “will not disrupt the structure of the genomic DNA of the cell such that nucleosomal or chromatin structure is destroyed.” EX1004 at [0071].

147. This simultaneous permeabilization and modification step is exemplified in Figure 2 of Okino (reproduced below). Okino explains that in Figure 2, a “permeabilization/digestion buffer” is used with the cell. EX1004 at [0044]. This buffer is formulated to both permeabilize the cell chemically while still maintaining the activity of a digestion enzyme (shown as the pac-man-like shape).



EX1004 at Figure 2

148. Importantly, Figure 2 depicts the enzyme entering not only the cell, but specifically entering the nucleus. The accompanying description in Okino does not describe any unique method to permeabilize the nucleus and permit the enzyme to enter, teaching only the permeabilization methods mentioned above. *See* EX1004 at [0044]. A POSA would be aware that such methods would allow for the permeabilization of the nucleus to permit the entry of the enzyme.

149. The permeabilization in Okino is conducted with a detergent-based lysis buffer. Particularly, Okino teaches the use of NP40 or Tween detergents, both of which are common detergents for lysis buffers. EX1004 at [0012], [0073], claims 10-11. In fact, these detergents are specifically named in the '357 Patent as detergents used to treat cells for use with the alleged invention. EX1001 at 3:24-26.

150. NP40, in particular, was long known in the art not only as a detergent capable of disrupting cell membranes, but doing so while causing minimal harm to structures in the cell. *See, e.g.* EX1011, Wang at 7063 (1015.001). For example, prior art sources teach that the use of an NP40 lysis buffer is more effective when trying to capture a “conformation epitope” of a protein located inside a cell, referring specifically to the ability of the detergent to lyse the cell without disrupting the structure of the protein found within the cell. Ji at 1 (EX1062.001).

151. It is also my opinion that it would have been obvious to combine this disclosure of permeabilized cell nuclei with Grunenwald. This is because, in my opinion, Okino and Grunenwald are analogous art, a POSA would have been motivated to combine them, and in combining them would have had a reasonable expectation of success.

152. First, it is my opinion that Grunenwald and Okino are analogous art. Both Okino and Grunenwald are in the same field with disclosures directed to the analysis of chromatin, particularly the structure of chromatin. Additionally, both

ultimately use sequencing as a method to collect data after the initial treatment of chromatin.

153. Several of the physical modifications taught in Okino, such as cleaving and nicking, are analogous to the tagmentation process taught in Grunenwald. Both processes use enzymes to break down the DNA into smaller fragments for subsequent analysis.

154. Second, it is my opinion that a POSA would have had a motivation to combine the two references. This is because a skilled artisan interested in tagmenting the DNA in a living cell would have been aware of the need to permeabilize the cell and nucleus, in turn allowing the insertional enzyme complex to actually contact the chromatin.

155. Grunenwald teaches a wide breadth of DNA sources, as “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 from any biological or environmental source (e.g. water, air, soil).” EX1003 at [0145], [0146].

156. Grunenwald also provides an exemplary list of the types of DNA that may be target DNA, which includes 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].

157. Further, Grunenwald teaches an embodiment wherein “the insertion of a transposon end into a target DNA according to the present invention” can be carried out in a cell. EX1003 at [0157]. This is done by electroporating “a synaptic complex of a transposase and a suitable transposon end composition” into the cell. EX1003 at [0157]. As I detail in my discussion of the state of the art, electroporation was well-established as a method of permeabilization, particularly to permit an enzymatic complex to enter the cell. *See supra* Section VII.E.

158. I understand that during the prosecution of the ’357 Patent, the Examiner found that Grunenwald “does not expressly teach wherein the composition . . . comprise[s] a permeabilized cell nucleus. EX1007.151-158. However, I disagree with the Examiner’s conclusion that Grunenwald does not teach this. It is my understanding, based on my review of the prosecution history, that the Examiner neither recognized nor analyzed this cellular embodiment of Grunenwald. In my opinion, this is an oversight. The cellular embodiment would have been understood by a POSA to include permeabilizing the cell nucleus and allowing the insertional

enzyme complex taught in Grunenwald to enter the nucleus and tagment the chromatin present within.

159. Further, given Grunenwald's disclosure of electroporation, it is my opinion that a POSA would have considered other alternatives to gain access to the cell nucleus. This is because of the several well-known permeabilization techniques I detail in VII.E, and because a POSA would have been motivated to better permit an insertional enzyme complex to enter the cell nucleus and tagment DNA in a live cell. Okino is one such source a POSA would have been motivated to look to for alternatives.

160. Indeed, looking at Okino's teachings, Okino presents electroporation and permeabilization by detergent as alternatives. EX1004 at [0072] – [0075]. Based on both Okino's teachings and the skill of a POSA at the time of the alleged invention, a POSA would have viewed a choice between the two as a mere design choice.

161. It is my opinion that a POSA would recognize that given the aforementioned permeabilization techniques and allowing the insertional enzyme of Grunenwald to contact chromatin within a permeabilized cell nucleus, the result is necessarily the creation of the claimed composition. That is, Grunenwald's insertional enzyme complex (described in detail in Section XI.A.1.d, *infra*) contains an insertional enzyme comprising an insert element comprising a predetermined

sequence, and that the permeabilized cell nucleus would also, necessarily, be a part of this complex.

162. Third, it is my opinion that a POSA would have had a reasonable expectation of success in combining the references. As I state above, Grunenwald and Okino's methods of DNA modification are analogous as both tagmentation and Okino's modification requires the use of enzyme activity in the nucleus of the cell directly on chromatin. Therefore, it would be expected that permeabilization using Okino's methods to accomplish tagmentation would be successful.

163. To confirm that a POSA would be successful, one need only look to the '357 Patent's disclosures of permeabilization. The '357 Patent does not disclose any methods to permeabilize a cell membrane that are novel, inventive, or unique to the composition claimed in the patent.

164. In fact, the '357 Patent 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. Agents such as NP40 are disclosed in Okino, and were well-known and routine prior to 2013. *See, e.g.*, EX1004 at [0012].

165. Looking to the examples listed in the '357 Patent, there is a single example that mentions permeabilization. This mention is only that "a group of cells

were permeabilized and the chromosomal DNA was transposed with Tn5 transposase.” EX1001 at 34:36-38.

166. As I describe above, the only mention of a protocol to prepare nuclei for use in tagmentation actually found in the '357 Patent is the protocol titled “Prepare Nuclei” found in column 25. This protocol describes only a prior art protocol, the one disclosed in the Song reference from the 2010 volume of the Cold Spring Harbor Protocols. As I have mentioned, these protocols are a common source to find routine protocols for laboratory experiments.

167. Thus, the '357 Patent teaches no methods of permeabilization that are unique, novel, inventive, or particularly required to achieve a composition comprising a permeabilized cell nucleus. A skilled artisan reading the Patent would understand that routine methods of permeabilization could be used to achieve the composition comprising a permeabilized cell nucleus. Thus, a POSA would have a reasonable expectation of success that the routine methods of permeabilization taught in Okino and Grunenwald could be used to achieve a permeabilized cell nucleus and that tagmentation would be successful in generating a plurality of tagged polynucleotides.

168. For all of the above-listed reasons, it is my opinion that it would have been obvious to a POSA to combine Grunenwald and Okino. Thus, this combination renders this claim limitation obvious.

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

169. It is my opinion that Grunenwald discloses plurality of polynucleotide molecules.

170. Grunenwald teaches a tagmentation method to be used on “target DNA,” which is DNA that can be derived from any “in vivo or in vitro source, including from one or multiple cells.” EX1003 at [0146]. Further, Grunenwald discloses a list of possible types of DNA, including 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), mitochondria DNA, chloroplast DNA, plasmid or other episomal-derived DNA (or recombinant DNA contained therein), or double-stranded cDNA made by reverse transcriptase” *Id.* at [0146].

171. Additionally, I discuss above the cellular embodiment taught in Grunenwald. In this embodiment, tagmentation is occurring on DNA found directly in the cell. EX1003 at [0157]. Grunenwald thus teaches tagmentation directly on DNA found in a cell, which may, and in the case of cells with nuclei, often does, contain more than one chromosome. Thus, it would be inherent that in any embodiment wherein the cell has more than one chromosome, the method is being performed on a plurality of polynucleotide molecules.

172. Grunenwald also provides an exemplary list of the types of organisms that the DNA may be derived from. These include “humans, animals, plants, fungi (e.g. molds or yeasts), bacteria, viruses, viroids, mycoplasma, or other microorganisms.” EX1003 at [0146]. From this exemplary list, deriving DNA from even a single human, animal, plant, or fungus cell would inevitably result in deriving a plurality of polynucleotides.

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

173. It is my opinion that Grunenwald discloses “an insertional enzyme complex comprising an insertional enzyme.”

174. As I explain above in Section X, 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 add adaptors to the polynucleotides.” EX1001 at 13:26-33. Specifically, the '357 Patent states that the “insertional enzyme can be a transposase.” *Id.* at 3:46-47. Based on the '357 Patent’s definitions, an insertional enzyme complex as used in the Patent refers to a complex of a transposase and two transposon tags, which in turn are combined with a sample polynucleotide to fragment and tag said sample.

175. Grunenwald discloses this insertional enzyme complex and insertional enzyme because it teaches the use of “a single transposome composition comprising a stable complex formed between the transposase and the transposon end

composition.” EX1003 at [0161]. Thus, Grunenwald both teaches an insertional enzyme complex broadly (the transposome complex) but also specifically the use of an insertional enzyme (the transposase).

176. An important part of this insertional enzyme complex is the transposon end composition, which comprises the tags that are ultimately used in tagmentation. A transposon end composition may have a “sequence or sequences,” which are tags for the 5’ end of the transferred polynucleotide strand and the 3’ end of the non-transferred strand. In other words, the result of the transposition reaction is that polynucleotide fragments will have tags at the 5’ end.

177. Thus, a transposon end composition comprises “a transposon end (i.e., the minimum double-stranded DNA segment that is capable of acting with a transposase to undergo a transposition reaction),” as well as “additional sequence or sequences[] 5’-of the transferred transposon end sequence.” Grunenwald even explicitly states that “for example, a transposon end attached to a tag is a transposon end composition.” EX1003 at [0098].

178. Each tag of a transposon end composition in Grunenwald is composed of at least one “tag portion” or “tag domain.” It is my opinion that a POSA would have understood this as analogous to the tags of the ’357 Patent’s insertional enzyme complex, as it is a “portion or domain of a tag that exhibits a sequence for a desired intended purpose or application.” EX1003 at [0103].

179. Grunenwald includes an exemplary list of the purposes of these tag domains, including , “one or more of a restriction site tag domain, a capture tag domain, a sequencing tag domain, an amplification tag domain, a detection tag domain, an address tag domain, and a transcription promoter domain” EX1003 at [0103]. Additionally, “one tag domain can comprise two or more other tag domains” *id.* at [0115]. Based on the disclosure of both multiple tags and multiple tag domains, it is my opinion that Grunenwald discloses an insertional enzyme complex comprising both an insertional enzyme (i.e., a transposase) and at least two tags.

180. This complex can be used to fragment and add adaptors to polynucleotides. Grunenwald, for example, teaches 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].

181. For the foregoing reasons, it is my opinion that Grunenwald discloses an insertional enzyme complex, comprising an insertional enzyme (i.e. a transposase). This insertional enzyme complex further comprises at least two tags, which is why it is also my opinion that Grunenwald discloses tagmentation, using the tags to fragment and tag sample DNA.

e. an insert element comprising a predetermined sequence.

182. It is my opinion that Grunenwald discloses an insert element comprising a predetermined sequence.

183. As I discuss above, Grunenwald discloses the use of a “transposon end.” This transposon end exhibits the sequences that are necessary for the DNA to form a complex with an enzyme, such as a transposase, and facilitate the transposition reaction. EX1003 at [0018].

184. A portion of the transposon end is the “transferred strand.” This is a strand of DNA that is transferred to a target DNA during a transposition reaction. EX1003 at [0093].

185. Grunenwald discloses that a transferred strand may have multiple parts to it. One part of the transferred strand is the sequence that facilitates the transposition reaction, described above. Another part of the transferred strand is the tag, which may have one or more tag domains. *Id.* at [0094] – [0096]. This tag, being added to the 5’ end of a DNA fragment, is an insert element.

186. Further, as I describe above, a tag domain is a portion of a tag that “exhibits a sequence for a desired intended purpose or application.” EX1003 at [0103].

187. Based on the definitions in the '357 Patent and the construction of a transferred strand disclosed by Grunenwald, it is my opinion that each individual tag domain is a nucleic acid insert element. This is because each tag domain is a “predetermined sequence” of nucleotides. This also means that a given tag may have multiple nucleic acid insert elements, and so a single transferred strand being carried by a transposase may contain multiple nucleic acid insert elements.

188. Grunenwald also teaches that one type of tag domain particularly is a “sequencing tag domain.” EX1003 at [0103], [0110].

189. 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.

190. It is my opinion that these sequences would be a predetermined sequence, as they are selected for the purpose of being compatible with a particular next-generation sequencing system.

191. Thus, it is my opinion that the combination of Grunenwald and Okino disclose each and every limitation of claim 1. Further, for the aforementioned reasons listed in Section XI.A.1.b, *supra*, it is my opinion that the combination of Grunenwald and Okino would have been obvious to a POSA.

2. Claim 2

a. A composition comprising:

192. For the reasons detailed in Section XI.A.1.a, it is my opinion that this limitation is disclosed.

b. a permeabilized cell nucleus comprising:

193. For the reasons detailed in Section XI.A.1.b, it is my opinion that this limitation is disclosed.

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

194. It is my opinion that Grunenwald discloses chromatin comprising genomic DNA and a plurality of DNA binding proteins.

195. As I discuss above in Section VII.B, *supra*, chromatin is the endogenous form of DNA and associated DNA binding proteins in the cell. Chromatin is composed of genomic DNA, i.e., the DNA that composes the genome of the cell, and DNA binding proteins, e.g., transcription factors and histones.

196. Turning to Grunenwald, the target DNA of the invention may comprise chromosomal DNA, which is DNA “from an isolated chromosome or a portion of a

chromosome, e.g., from one or more genes or loci from a chromosome.” EX1003 at [0146].

197. As I discuss above in the technical background, a chromosome is one level of packaging for chromatin, which contains both genomic DNA and associated proteins. *See supra* VII.B. Thus, by definition, Grunenwald teaches that the invention may be practiced on genomic DNA and a plurality of DNA binding proteins when it is being practiced on chromosomal DNA.

198. Additionally, and tellingly, Grunenwald teaches an embodiment of the invention in which the nucleus of the cell is directly contacted with a transposase complex. *See supra* Section XI.A.1.b. DNA in the cell nucleus inherently exists in the form of chromatin, which would contain the claimed genomic DNA and any associated DNA binding proteins.

199. I understand that even if a prior art reference does not explicitly disclose a claim element, it may do so inherently when that claim element is a necessary characteristic of the disclosure taught in the prior art reference. *See supra* Section IV.B.

200. Thus, even if Patent Owner contends that the disclosure of chromosomal DNA is somehow insufficient to disclose chromatin as claimed here, the disclosure of the cellular embodiment necessarily requires a permeabilized cell

nucleus, as discussed above, comprising chromatin which in turn comprises genomic DNA and a plurality of DNA binding proteins. *See supra* Section XI.A.1.b.

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

201. It is my opinion that Grunenwald discloses that the chromatin “comprises a plurality of open chromatin regions.”

202. As I describe in Section VII.B and VII.D, *supra*, chromatin existing in a cell nucleus contains both regions of “open” chromatin, which is chromatin that is accessible to the cellular machinery for gene expression, and “closed” chromatin, which is chromatin that is bound to DNA binding proteins and thus inaccessible to the cellular machinery for gene expression.

203. Grunenwald teaches a method for contacting a permeabilized cell nucleus directly with an insertional enzyme complex. EX1003 at [0157]. Because the chromatin in that nucleus would, by definition, have regions of open and closed chromatin, Grunenwald discloses the chromatin comprising a plurality of open chromatin regions.

e. an insertional enzyme complex comprising an insertional enzyme and

204. For the reasons stated above in Section XI.A.1.d, this is disclosed.

f. an insert element comprising a predetermined sequence,

205. For the reasons stated above in Section XI.A.1.e, this is disclosed.

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

206. It is my opinion that Grunenawald discloses “wherein the insertional enzyme complex is bound to one of said open chromatin regions.”

207. Grunenwald teaches a method of tagmentation using a transposase to catalyze a transposition reaction, resulting in the simultaneous fragmenting and tagging of DNA. EX1003 at [0017]. Specifically, that transposase can be a Tn5 or Mu transposase. EX1003 at [0045].

208. These are particular types of transposases. Prior to 2013, it would have been known and well-established in the art that these types of transposases preferentially bind to open chromatin. *See, e.g.*, Liu 2 at 1 (Ex. 1052.001). Thus, binding to one of the open chromatin regions in the cell is an inherent characteristic of the transposases taught in Grunenwald.

209. Additionally, this is supported in the cellular embodiment of Grunenwald, which is exemplified “by employing a transposition complex formed by a hyperactive Tn5 transposase and a suitable Tn5-type transposon end composition.” EX1003 at [0157].

210. For the foregoing reasons, it is my opinion that the combination of Grunenwald and Okino discloses each and every limitation of claim 2. For the reasons detailed in Section XI.A.1.b, it is my opinion that it would have been obvious to a POSA to combine these references.

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

211. It is my opinion that Grunenwald and Okino disclose and render obvious the composition of claim 2, “wherein the insertional enzyme comprises one or more enzymatic moieties.”

212. *First*, as discussed above in Section XI.A.2, the combination of Grunenwald and Okino disclose claim 2.

213. *Second*, it is my opinion that Grunenwald discloses an insertional enzyme with one or more enzymatic moiety. For example, Grunenwald teaches “the insertional enzyme comprises one or more enzymatic moieties” through the disclosure of a transposase. *See supra* Section IX.A.1.d.

214. The '357 Patent uses the phrase “enzymatic moieties” broadly; for example, the Patent states that the “the enzymatic moieties can comprise transposases,” but also that they can comprise “derivatives thereof.” EX1003 at 3:5-6; 17:15-16. Thus, I treat not only a transposase as a whole as an enzymatic moiety, but the constituent active parts of a transposase as enzymatic moieties themselves, being “derivatives” of a complete transposase.

215. The transposase used in Grunenwald is described as “an enzyme capable of forming a functional complex with a transposon end or transposon end sequences needed in a transposition reaction.” EX1003 at [0153]. Thus, a

transposase is an enzymatic moiety, and Grunenwald teaches an insertional enzyme comprising one or more enzymatic moieties.

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

216. It is my opinion that Grunenwald and Okino disclose and render obvious the composition of claim 3, “wherein the insertional enzyme comprises two or more enzymatic moieties.”

217. *First*, as discussed above in Section XI.A.2, the combination of Grunenwald and Okino disclose claim 3.

218. *Second*, it is my opinion that Grunenwald discloses “wherein the insertional enzyme comprises two or more enzymatic moieties.”

219. For example, Grunenwald discloses using a Tn5 transposase with an accompanying transposon end. *See, e.g.*, EX1003 at [0045]. A Tn5 transposase is made up of a dimer of transposase subunits. Rice at 303 (Ex 1053.002). A protein dimer is a protein that is composed of two subunits, or smaller functional or structural units. In the case of Tn5 transposase, both these subunits are functional subunits that work to catalyze a transposition reaction. *Id.* The dimer is linked to permit the Tn5 transposase to act as a functional unit. *Id.*

220. Because these subunits are both functional, it is my opinion that they act as two enzymatic moieties that form a synaptic complex by linking. Thus, the

Tn5 transposase is, by nature, comprised of two or more enzymatic moieties and Grunenwald teaches this claim limitation.

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

221. It is my opinion that Grunenwald and Okino disclose the composition of claim 4, “wherein the two or more enzymatic moieties are linked together.” This is for the aforementioned reason that the two subunits are linked to form a functional transposase. *See supra* Section XI.A.4.

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

222. It is my opinion that the combination of Grunenwald and Okino renders claim 6 obvious.

223. **First**, it is my opinion that the combination of Grunenwald and Okino renders claim 2 obvious. *See supra* Section XI.A.2.

224. **Second**, it is my opinion that Grunenwald discloses “wherein the insertional enzyme comprises a nuclear localization signal.”

225. A nuclear localization signal is a short stretch of amino acids that mediates the transport of proteins into the nucleus. Cokol at 411 (Ex. 1054.001). Thus, an enzyme that would be directed to activity in the nucleus would require one to permit the enzyme to easily enter the nucleus and operate efficiently.

226. Grunenwald teaches the use of a Tn5 transposase. EX1003 at [0145]. A Tn5 transposase is a specific type of transposase, known as a Tnp transposase, derived from bacterial genomes. *See, e.g.*, Naumann at 233 (Ex. 1051.001).

227. Prior to the alleged invention date of the '357 Patent, it would have been known in the art that a Tnp transposase possesses a nuclear localization signal, and so this is an inherent property of the Tn5 transposase taught in Grunenwald. Moon at 1 (Ex 1045.001).

228. Thus, because a nuclear localization is an inherent property, Grunenwald discloses this claim limitation.

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.

229. It is my opinion that the combination of Grunenwald and Okino renders claim 7 obvious.

230. **First**, it is my opinion that the combination of Grunenwald and Okino renders claim 2 obvious. *See supra* Section XI.A.2.

231. **Second**, it is my opinion that Grunenwald discloses “wherein in the insertional enzyme complex is configured to insert the insert element into the genomic DNA at an open chromatin region.

232. Grunenwald teaches a transposition reaction wherein the transposase is part of an insertional enzyme complex with transposon ends. These transposon ends

are DNA sequences that, in turn, play a role in the insertion of tags in the tagmentation process. EX1003 at [0052], [0094]-[0098].

233. A transposon end is a “double-stranded DNA that exhibits only the nucleotide sequences (the ‘transposon end sequences’) that are necessary to form the complex with the transposase.” EX1003 at [0094]. A transposon end is composed of a “transferred strand,” which permits insertion into target DNA, and a “non-transferred strand,” which permits the DNA to be inserted to form a complex with a transposase enzyme. *See, e.g.* EX1003 at [0094]-[0098].

234. Because of the role these transposon ends play in the insertion of tags into a target DNA, and the construction of “insert element” I detail above in Section X, it is my opinion that the transposon ends are the claimed “insert element” of the ’357 Patent.

235. Further, Grunenwald explicitly teaches insertion into genomic DNA. EX1003 at [0146].

236. Given that Grunenwald teaches insertion into genomic DNA as directly applicable to a cell nucleus, it is my opinion that this limitation is an inherent characteristic of the cellular embodiment that is taught in Grunenwald. EX1003 at [0157].

237. Grunenwald also teaches that this insertion may be conducted a Mu transposase, a particular type of transposase enzyme. EX1003 at [0145]. It would

have been known in the art prior to the alleged priority date of the '357 Patent that a Mu transposase binds preferentially to regions of open chromatin. Liu 2 at 1 (Ex. 1052.001).

238. Thus, to the extent that Grunenwald's disclosure is not considered to explicitly teach this limitation, it is my opinion that a POSA would have understood from the disclosure of a Mu transposase that Grunenwald teaches preferential insertion at regions of open chromatin.

239. For the foregoing reasons, it is my opinion that Grunenwald teaches this limitation.

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.

240. It is my opinion that the combination of Grunenwald and Okino renders claim 8 obvious.

241. *First*, it is my opinion that the combination of Grunenwald and Okino renders claim 7 obvious. *See supra* Section XI.A.7.

242. *Second*, it is my opinion that Grunenwald discloses "wherein the insertional enzyme complex is configured to insert the insert element into the genomic DNA in a substantially sequence independent manner.

243. I detail in Section XI.A.7 that Grunenwald discloses inserting an insert element into genomic DNA. For this discussion of claim 8, I turn specifically to the

recited limitation that the insertional enzyme complex is configured to insert that insert element “in a substantially sequence independent manner.”

244. Grunenwald teaches that the transposition reaction that comprises its disclosed tagmentation reaction is one “wherein one or more transposon ends are inserted into a target DNA *at random sites or almost random sites.*” EX1003 at [0154].

245. That insertions are happening at random sites means that, by definition, insertion is happening in a sequence-independent manner as the transposase enzyme is not targeting a particular sequence for insertion.

246. Further, it was well-known in the art at the time that a Tn5 transposase, as taught in Grunenwald, operated with sequence-independent insertion. Zhou at 1 (Ex. 1057.001).

247. Thus, it is my opinion that Grunenwald discloses this claim limitation.

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.

248. It is my opinion that Grunenwald and Okino render obvious 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.” *See supra* Section XI.A.3.

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

249. It is my opinion that Grunenwald and Okino render obvious the composition of claim 9, “wherein the insertional enzyme complex comprises a transposase.” *See supra* Section XI.A.3.

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

250. It is my opinion that Grunenwald and Okino disclose and render obvious the composition of claim 10, “wherein the transposase is a Tn transposase.”

251. *First*, as described above in Section XI.A.2, the combination of Grunenwald and Okino disclose and render obvious claim 2.

252. *Second*, Grunenwald further discloses that the transposase is a Tn transposase. For example, Grunenwald teaches that the transposition reaction may be achieved by employing a “hyperactive Tn5 transposase and a Tn5-type transposon end.” EX1003 at [0154]. This Tn5 transposase is a specific type of Tn transposase.

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

253. It is my opinion that Grunenwald and Okino render obvious the composition of claim 11, “wherein the Tn transposase is a Tn5 transposase.” *See supra* Section XI.A.11.

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

254. It is my opinion that Grunenwald and Okino disclose and render obvious the composition of claim 2, “wherein the insert element comprises one or more molecular tags.”

255. *First*, as discussed above in Section XI.A.2, the combination of Grunenwald and Okino disclose claim 2.

256. *Second*, it is my opinion that Grunenwald discloses “wherein the insert element comprises one or more molecular tags.” *See* EX1003 at [0102] (“in some embodiments, the transposon end composition comprises a transferred strand that exhibits one or more other nucleotide sequences 5'-of the transferred transposon end sequence, which one or more other nucleotide sequences are also exhibited by the tag.”), [0161], Figs. 1-2.

257. For example, Grunenwald provides an exemplary embodiment where “‘A-MEDS Transposomes’ and ‘B-MEDS Transposomes’ were formed by pre-incubating 12.5 μ M TSase with 12.5 μ M A-MEDS or 12.5 μ M B-MEDS transposon end compositions, respectively, for 60 minutes at 37°C.” EX1003 at [0425]. The A-MEDS and B-MEDS transposomes are each molecular tags.

258. Alternatively, Grunenwald provides embodiments where the tag comprises multiple tag domains, for example a sequencing tag domain and an

amplification tag domain. In such embodiments, a POSA would have understood that each of these tag domains would constitute a separate molecular tag.

259. For example, Grunenwald provides that a tag domain is “a portion or domain of a tag that exhibits a sequence for a desired intended purpose or application.” EX1003 at [0103]. Similarly, the ’357 Patent uses the term “tags” as synonymous with “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”). Thus, the short sequence of DNA inserted for a desired intended purpose taught by Grunenwald is an adapter sequence and therefore a tag.

260. Finally, Grunenwald provides embodiments where “one or more tag domain sequences” are the additional sequences being used to accomplish a desired purpose. In such embodiments, the second tag domain sequence is a second tag. EX1003 at [0331].

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

261. It is my opinion that Grunenwald and Okino disclose and render obvious the composition of claim 13, “wherein one or more of the molecular tags comprises a sequencing adaptor.”

262. *First*, as discussed above in Section XI.A.13, the combination of Grunenwald and Okino renders obvious claim 13.

263. *Second*, it is my opinion that Grunenwald discloses “wherein one or more of the molecular tags comprises a sequencing adaptor.” For example, Grunenwald teaches that tags may comprise one or more domains “that exhibit[] a sequence for a desired intended purpose or application.” *See* EX1003 at [0103].

264. Grunenwald provides examples of tag domains that may be utilized, such as “a sequencing tag domain.” EX1003 at [0103], [0110]. Additionally, Grunenwald provides exemplary sequencing tag domains, which include “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.

265. Because a sequencing adapter sequence is a short sequence of DNA which facilitates sequencing of the tagged DNA, this disclosure of a sequencing tag domain is a sequencing adapter sequence.

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

266. It is my opinion that Grunenwald and Okino disclose and render obvious the composition of claim 14, “wherein one or more of the sequencing adaptors comprises a barcode label.”

267. **First**, as discussed above in Section XI.A.14, the combination of Grunenwald and Okino renders obvious claim 14.

268. **Second**, it is my opinion that Grunenwald discloses “wherein one or more of the sequencing adaptors comprises a barcode label.” For example, Grunenwald teaches that the tag inserted with a transposase may comprise an “address tag domain.” EX1003 at [0103]. Grunenwald further provides that such an address tag domain “exhibits a sequence that permits identification of a specific samples.” *Id.* at [0113]. The specification of the ’357 Patent provides that a barcode sequence is “used to ...identify and/or track the source of a polynucleotide in a reaction....” Thus, a POSA would understand that this address tag domain identifying a specific sample is the claimed barcode sequence.

269. As discussed above, Grunenwald teaches that a tag may comprise multiple tag domains. *See supra* Section XI.A.13. Specifically, Grunenwald teaches that 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).

270. A POSA would understand this embodiment, wherein a tag has both a sequencing tag and an address tag domain, to be a sequencing adapter that comprises a barcode label, as required by this claim.

16. Claim 16

a. A composition comprising:

271. For the reasons detailed in Section XI.A.1.a, it is my opinion that this limitation is disclosed.

b. a permeabilized cell nucleus comprising:

272. For the reasons detailed in Section XI.A.1.b, it is my opinion that Grunenwald and Okino render this claim limitation obvious.

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

273. It is my opinion that Grunenwald and Okino render this limitation obvious. *See supra* Section XI.A.1.d.

d. a plurality of tagged nucleic acid fragments

274. It is my opinion that Grunenwald discloses a plurality of tagged nucleic acid fragments.

275. For example, Grunenwald discloses “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). Thus, Grunenwald teaches generating “tagged DNA fragments.”

276. Specifically, Grunenwald teaches using a transposase to insert a transferred stand comprising a “tag domain” into the target DNA. *Id.* Grunenwald

defines “tag domain” as “a portion or domain of a tag that exhibits a sequence for a desired intended purpose or application.” *Id.* at [0103].

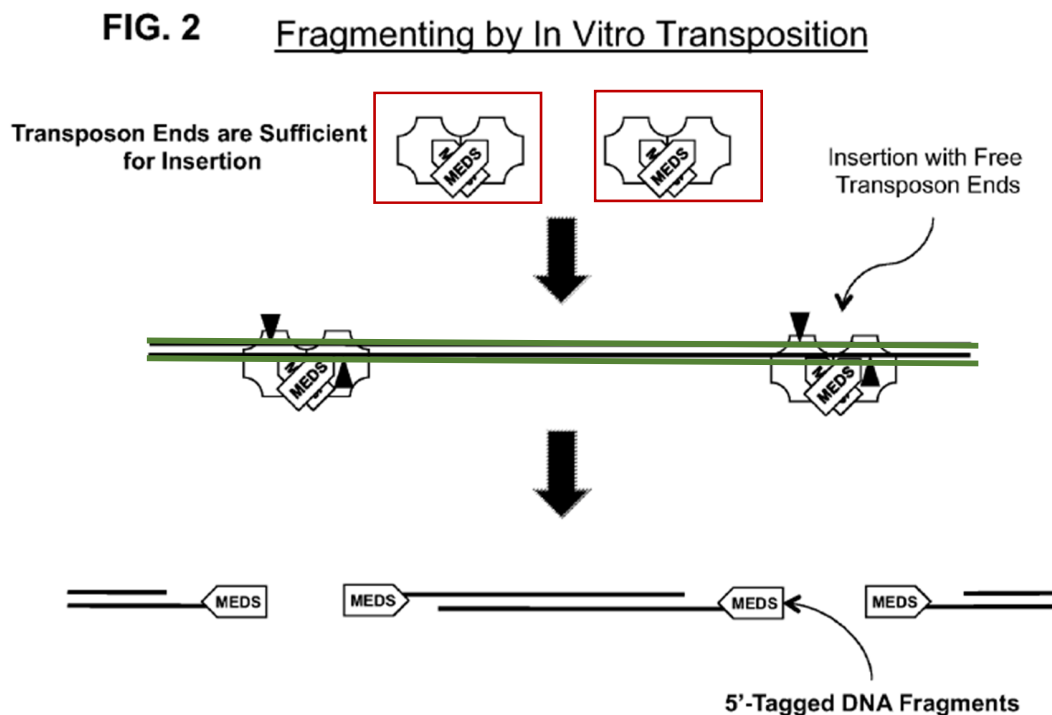
277. Grunenwald further provides an exemplary list of tag domains, including a “restriction site tag domain, a capture tag domain, a sequencing tag domain, an amplification tag domain, a detection tag domain, an address tag domain, and a transcription promoter domain.” *Id.* These tag domains all provide various functions, such as facilitating sequencing, amplification, or identification.

278. Particularly, the methods described above for fragmenting and tagging may be used to “produce tagged circular ssDNA fragments,” which can be used as a template for sequencing. EX1003 at [0100]. In these methods, after the fragmentation and tagging process, double-stranded DNA is denatured and incubated with a different enzyme, a nucleic acid ligase, which forms circular ssDNA fragments that exhibit a portion of the transferred strand and a portion of the target DNA. *Id.* at [0026].

279. Additionally, Grunenwald provides graphical representations of a method for generating a library of tagged DNA fragments. For example, Figure 2 of Grunenwald, reproduced below, demonstrates a method of fragmenting by *in vitro* transposition, which generates a plurality of tagged nucleic acid fragments.

280. In this figure, the green highlighted parallel lines represent target DNA and the transposomes are boxed in red. These transposomes are the claimed

insertional enzyme complex or transposase complex. Grunenwald's Figure 2 demonstrates the insertional enzyme complex contacting the target DNA, which simultaneously tags and fragments the DNA. This generates the claimed plurality of tagged nucleic acid fragments, seen below labeled "5'-Tagged DNA Fragments."



EX1003 at Figure 2

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

281. It is my opinion that Grunenwald discloses "each tagged nucleic acid fragment comprises a first sequencing adapter and second sequencing adapter."

282. *First*, Grunenwald discloses the use of a first and second adapter.

283. Grunenwald teaches tags that may comprise one or more portions, or domains, that exhibit “a sequence for a desired intended purpose or application.” EX1003 at [0103]. This would be an adapter sequence.

284. Grunenwald then teaches that there can be more than one tag associated with any given transposome complex. Grunenwald does this in two ways.

285. First, for example, Grunenwald teaches that “‘A-MEDS Transposomes’ and ‘B-MEDS Transposomes’ were formed by pre-incubating 12.5 μ M TSase with 12.5 μ M A-MEDS or 12.5 μ M B-MEDS transposon end compositions, respectively, for 60 minutes at 37°C.” EX1003 at [0425].

286. In this example, the A-MEDS and B-MEDS transposomes represent an insertional enzyme complex with at least two tags, as the A and B represent two different tags.

287. In the alternate, Grunenwald teaches embodiments where a tag comprises multiple tag domains, which would be separate nucleic acid insert elements and separate adapter sequences.

288. Grunenwald teaches that these tag domains exhibit “a sequence for a desired intended purpose or application,” and so “can be used to accomplish any desired purposes.” *See* EX1003 at [0103], [0331].

289. Thus, it is my opinion embodiments wherein there is a first tag domain and a second tag domain would act as a first and second adapter sequence.

290. ***Second***, it is my opinion that Grunenwald discloses that these two tags may be a first and second sequencing adapter.

291. Grunenwald provides that these tags may contain a domain that is a “sequencing tag domain.” EX1003 at [0103], [0110]. Grunenwald further provides that such a 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 sequencing tag domain is the claimed sequencing adapter sequence because it is a short sequence of DNA used to facilitate sequencing of the fragmented and tagged DNA.

292. Because Grunenwald discloses that both tags may contain tag domains that are, in turn, sequencing tag domains, it is my opinion that Grunenwald discloses multiple sequencing adapters.

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

293. It is my opinion that Grunenwald and Okino disclose this limitation. *See supra* Sections XI.A.2.g; XI.A.16.d.

294. Thus, it is my opinion that the combination of Grunenwald and Okino disclose and render obvious each and every limitation of claim 16. Further, as

discussed above in Section XI.A.1.b., it is my opinion that it would be been obvious to a POSA to combine Grunenwald and Okino.

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.

295. It is my opinion that Grunenwald discloses “wherein the plurality of tagged nucleic acid fragments correspond to two or more regions of a genome.”

296. Example 2 of Grunenwald describes the efficacy of the tagmentation process, describing the range of fragment sizes generated by the tagmentation taught. This was determined through gel electrophoresis, the process of running DNA fragments through a gel matrix alongside a ladder of known DNA fragment sizes. By comparison with the ladder, fragment sizes can be determined.

297. Grunenwald discloses that depending on the concentration of Tn5 used in the reaction mixture, the size of the fragmented DNA ranges from 150 bp to 1.5 Kbp, or 400 bp to 3.5 Kbp. EX1003 at [0421]. Thus, a POSA would understand that tagmentation can produce fragments ranging in size from 150 bp to approximately 3,500 bp.

298. Grunenwald teaches that target DNA can be derived from many organisms, including from cells from “humans, animals, plants, [or] fungi (e.g., molds or yeasts).” EX1003 at [0146].

299. Given the size of chromosomes from organisms from this exemplary list (for example, the human genome is over 3 billion base pairs long), even the largest fragment disclosed in Grunenwald must correspond to at least two regions of the genome. EX1056.002.

300. Indeed, even assuming the largest possible size at 3.5 Kbp, at over 3 billion total bases there would be over 800,000 unique fragments corresponding to different regions of the genome.

301. Further, as I describe above, Grunenwald operates at “random or almost random” sites in the genome and interrogates “whole” genomes. Thus, the application of Grunenwald’s method to, for example, a human cell will inevitably generate tagged fragments from thousands of regions of the genome. It is my opinion that this would have been obvious to a POSA in view of Grunenwald.

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.

302. It is my opinion that Grunenwald and Okino disclose the composition of claim 16, “wherein the plurality of tagged nucleic acid fragments correspond to at least 1,000 regions of a genome.” *See supra* Sections XI.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.**

303. It is my opinion that Grunenwald discloses “wherein the plurality of tagged nucleic acid fragments correspond to genome-wide regions of open chromatin.”

304. Grunenwald states that the whole purpose of the taught method is to generate a library of DNA fragments that correspond to genome-wide regions of open chromatin. In the background to the invention, Grunenwald states that “what is needed are improved methods for generating libraries of DNA fragments from target DNA molecules for amplification including amplification of *whole or partial genomes from one organism* (e.g., from a clinical sample) *or from multiple organisms* (e.g., metagenomic target DNA from an environmental sample), for further analysis (e.g., by real-time PCR, emulsion PCR, comparative genomic hybridization (CGH), comparative *genomic sequencing* (CGS)....” EX1003 at [0015].

305. A POSA would have understood that generating a library from a whole genome of one organism necessarily requires the tagged fragments to correspond to genome-wide regions of the chromatin.

306. Further, given the dynamic nature of DNA packing (which I describe in detail in Section VII.B), a POSA would understand that by tagmenting a whole

genome, the tagged fragments must correspond to regions of open chromatin. This is further supported by the nature of a transposase, which is to insert primarily in areas of open chromatin.

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

307. It is my opinion that Grunenwald and Okino disclose the composition of claim 18, “wherein the transposase is a Tn transposase.” *See supra* Section XI.A.11.

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

308. It is my opinion that Grunenwald and Okino disclose the composition of claim 20, “wherein the Tn transposase is a Tn5 transposase.” *See supra* Section XI.A.12.

22. Claim 22

a. A composition comprising:

309. For the reasons detailed in Section XI.A.1.a, it is my opinion that this limitation is disclosed.

b. a permeabilized cell nucleus comprising:

310. It is my opinion that Grunenwald and Okino disclose this limitation. *See supra* Section XI.A.1.b.

- c. **an insertional enzyme complex comprising a transposase enzyme**

311. For the reasons detailed in Section XI.A.1.d, it is my opinion that Grunenwald and Okino render this claim limitation obvious.

- d. **a plurality of tagged nucleic acid fragments**

312. It is my opinion that Grunenwald discloses this limitation. *See supra* Section XI.A.1.d.

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

313. It is my opinion that Grunenwald discloses this limitation. *See supra* Section XI.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**

314. It is my opinion that Grunenwald discloses “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.”

315. **First**, as discussed above, it is my opinion that Grunenwald discloses “the insertional enzyme complex and the tagged nucleic acid fragments are bound

to one or more regions of open chromatin with the cell nucleus.” *See supra* Section XI.A.2.ag.

316. **Second**, it is my opinion that Grunenwald discloses that that the insertional enzyme complex and tagged nucleic acid fragments are bound “in a substantially sequence independent manner.” Grunenwald teaches a cellular embodiment, as discussed above, which utilizes a Tn5 transposase. EX1003 at [0157]. It was well-known in that art at the earliest priority date of the ’357 patent that a Tn5 transposase binds nonspecifically to target DNA. Reznikoff at 4-6 (Ex. 1058.004-006) (describing how the Tn5 transposase binds in a nonspecific manner to DNA).

317. Therefore, the cellular embodiment taught by Grunenwald, which utilizes a nonspecific Tn5 transposase, inherently discloses that the insertional enzyme complex and the tagged DNA generated by the transposase would be sequence independent.

318. **Third**, it is my opinion that Grunenwald discloses that the insertional enzyme complex and the tagged nucleic acids “form a high affinity macromolecule complex.”

319. I first note that the specification of the ’357 Patent describes that after 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.

320. However, it was a well-known property of the Tn5 transposase that it would remain “tightly bound to the ends of the resulting fragment.” Kwon at 253 (Ex.1059.266). Thus, this characteristic is inherent to the enzyme.

321. A POSA would have understood that in practicing the methods taught in Grunenwald, the Tn5 transposase would have remained tightly bound to form a high affinity macromolecule complex after the tagging and fragmenting step.

322. Thus, it is my opinion that the combination of Grunenwald and Okino disclose and render obvious each and every limitation of claim 22.

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

323. It is my opinion that Grunenwald and Okino disclose the composition of claim 22, “further comprising a plurality of divalent cations.”

324. **First**, as discussed above, the combination of Grunenwald and Okino discloses and renders claim 22 obvious. *See supra* Section XI.A.22.

325. **Second**, it is my opinion that Grunenwald discloses that the composition further comprises “a plurality of divalent cations.” For example, Grunenwald discloses that a Transposase Reaction Buffer may be used to conduct a transposition reaction. *See, e.g.* EX1003 at [0408]-[0414]. The Transposase Reaction Buffer contains magnesium acetate. A POSA would have understood that

this magnesium acetate would naturally dissociate and yield the divalent cation Mg^{2+} . Thus, Grunenwald teaches the composition of claim 22 further comprising a plurality of divalent magnesium cations.

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

326. It is my opinion that Grunenwald and Okino disclose the composition of claim 23, “wherein the divalent cations are not chelated.”

327. *First*, as discussed above, the combination of Grunenwald and Okino discloses and renders claim 23 obvious. *See supra* Section XI.A.23.

328. *Second*, it is my opinion that Grunenwald discloses the composition of claim 23, “wherein the divalent cations are not chelated.” The above described Transposase Reaction Buffer does not contain any chelating agents.

329. Further, a POSA would understand there would not be chelating agents present since they would know that divalent cations are required for transposase activity and that chelating agents would therefore inhibit transposase function. Thus, a POSA would have understood that the disclosure in Grunenwald teaches divalent cations which are not chelated.

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.**

330. It is my opinion that Grunenwald and Okino disclose the composition of claim 23, “wherein the divalent cations are selected from the group consisting of: magnesium, manganese, or any combination thereof.” *See supra* Section XI.A.23.

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

331. It is my opinion that Grunenwald and Okino disclose the composition of claim 22, “wherein the transposase is a Tn transposase.” *See supra* Section XI.A.11.

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

332. It is my opinion that Grunenwald and Okino disclose the composition of claim 26, “wherein the Tn transposase is a Tn5 transposase.” *See supra* Section XI.A.12.

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

333. It is my opinion that Grunenwald and Okino render obvious the composition of claim 2, “wherein (a) and (b) are contained within the permeabilized cell nucleus.”

334. ***First***, Grunenwald and Okino render claim 2 obvious. *See supra* Sections XI.A.2.

335. **Second**, the claimed (a) is “chromatin comprising genomic DNA and a plurality of DNA binding proteins, wherein the chromatin comprises a plurality of open chromatin regions” and the claimed (b) is “an insertional enzyme complex comprising an insertional enzyme. Thus, this claim recites the limitation that an insertional enzyme complex (e.g., transposase complex) and the chromatin are both found within the permeabilized cell nucleus.

336. For the reasons that I detail in Section XI.A.1.b, the combination of Grunenwald and Okino disclose this claim element. Chromatin is the endogenous form of genomic DNA and associated proteins found in the cell, and thus Grunenwald in the *in vivo* embodiment necessarily discloses this. This is because by contacting a cell nucleus through the electroporation method disclosed in Grunenwald, a permeabilized cell nucleus containing chromatin is created that is contacted with the complex being electroporated in.

337. Further, for the reasons detailed in Section XI.A.1.b, the detergent permeabilization method would have been a mere design choice rendering the combination of Grunenwald and Okino obvious. This method is exemplified in Figure 2 of Okino, where an enzyme can be seen entering both the cell and the nucleus. Thus, a POSA would understand that the methods taught in Okino create a permeabilized cell nucleus contained within a permeabilized cell, allowing the enzyme to enter and contact the open chromatin.

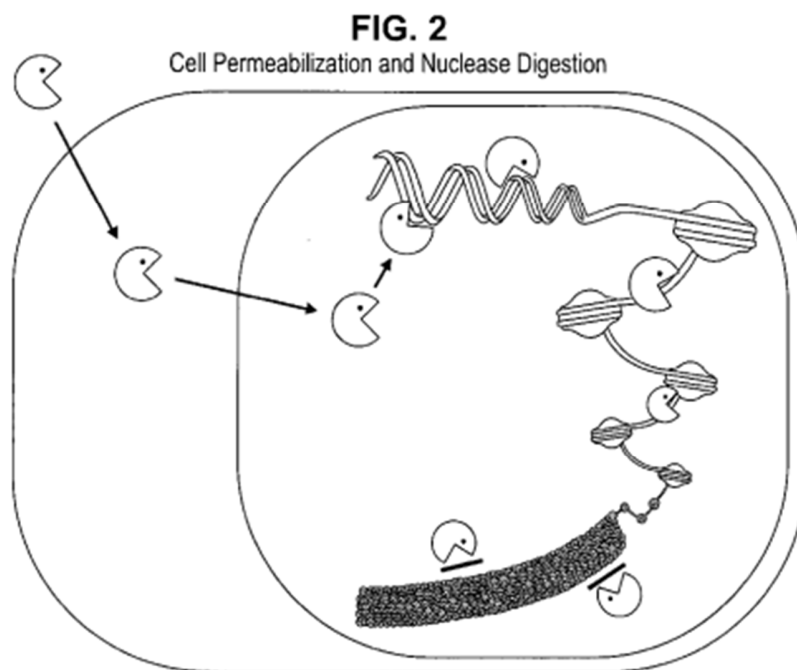
338. Based on the disclosures in Okino and Grunenwald, it is my opinion that a POSA interested in practicing tagmentation on a cell nucleus and so motivated to combine the reference would inevitably create a composition wherein the insertional comprising a transposase and plurality of tagged nucleic acid fragments, with a first and second sequencing adapter, would be contained in a permeabilized cell nucleus.

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

339. It is my opinion that Grunenwald and Okino disclose the composition of claim 2, “wherein the permeabilized cell nucleus is contained within a cell.”

340. **First**, it is my opinion that Grunenwald and Okino render claim 2 obvious. *See supra* Section XI.A.2.

341. **Second**, again looking at Figure 2 of Okino, the permeabilized cell nucleus is still within an intact, living cell. A POSA would have understood this Figure as disclosing a permeabilized cell nucleus contained within a cell.



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.

342. It is my opinion that the combination of Grunenwald, Okino, and Saleh and/or Song renders claim 29 obvious.

a. The combination of Grunenwald, Okino, and Saleh and/or Song discloses each and every limitation of claim 29

343. **First**, it is my opinion that Grunenwald and Okino disclose and render obvious claim 2. *See supra* Sections XI.A.2.

344. **Second**, it is my opinion that both Saleh and Song teach isolation of cell nuclei.

345. Saleh teaches a method for chromatin immunoprecipitation (ChIP), which is a precursor to tagmentation that prepared chromatin in a cell for tagging and sequencing.

346. ChIP is used to understand the relationship between DNA associated proteins and genomic DNA to determine whether specific proteins are associated with specific genomic regions.

347. To accomplish this, DNA and associated proteins are first cross-linked in the living cell to evaluate the chromatin as it exists at the moment of cross-linking. EX1005.001.

348. This is generally followed by “isolation of the chromatin complex, shearing DNA along with bound proteins into small fragments,” and using antibodies to precipitate the DNA-bound proteins, pulling them out of solution. EX1005.001.

349. Then, the DNA co-precipitated with the proteins of interest is released from the complex, amplified via PCR, and tagged for sequencing. EX1005.001.

350. Saleh teaches an improvement on the general ChIP method through the addition of a nuclei isolation step, which is “important for the purity and yield of DNA.” EX1005.002.

351. Thus, Saleh teaches an additional step wherein the nucleus of a cell is isolated to isolate the chromatin prior to shearing and tagging. EX1004.004-005.

352. Song is another prior art reference that features a nuclear isolation step to achieve analogous goals to Okino and Grunenwald.

353. Song teaches a method to digest regions of DNA that are not “tightly wrapped in nucleosome and higher-order structures” so that the resulting fragments can be tagged and subject to sequencing. EX1060.001. In other words, Song’s method digests chromatin at open, or accessible, regions, and sequencing is used to collect data about those open chromatin regions.

354. Song discloses that this digestion is conducted using DNase I. EX1060.001. The first step of Song’s protocol is the isolation of nuclei from the cell, using a routine method based on an IGEPAL detergent lysis buffer and subsequent centrifugation. EX1060.001.

355. The lysis buffer disclosed in Song is formulated with 10 mM Tris-Cl, pH7.4, 10mM NaCl, 3 mM MgCl₂, and 0.1% IGEPAL CA-630. EX1060.003. This formulation is identical to method of nuclei isolation disclosed in ’357 Patent, which uses the same protocol as that disclosed in Song with the same detergent. EX1001 at 25:4-15.

356. Based on the Patent’s disclosure, a POSA would understand that the method for isolating nuclei is nothing novel or inventive. On the contrary, the approach was merely a prior art approach. Further, Song was published in the 2010

volume of the Cold Spring Harbor protocols; a POSA would know that Cold Spring Harbor was a common source for protocols for routine experiments.

b. It would have been obvious to a POSA to combine Saleh and/or Song with the teachings of Grunenwald and Okino

357. It is my opinion, as stated above, that the combination of Grunenwald and Okino would have been obvious to a POSA. *See supra* Section XI.A.1.b. Additionally, it is my opinion that it would be been obvious to further modify the combination of Grunenwald and Okino with the addition of the nuclei isolation method taught in Saleh and/or Song.

358. **First**, Saleh and Song are both analogous art to Grunenwald and Okino. As discussed above, Grunenwald and Okino both are in the field of genetics and molecular biology. *See supra* Section XI.A.1.b.

359. The Saleh and Song references are also in the field of genetics and molecular biology. All articles concern methods of DNA modification for subsequent sequencing and analysis. Specifically, all references teach methods for the preparation of chromatin for analysis. Thus, they are analogous art in the same specific field of endeavor.

360. It is my opinion that given how close all references are, a POSA with Grunenwald and Okino would have been inclined to also consider the teaches in Saleh and/or Song in isolating nuclei.

361. ***Second***, a POSA studying DNA in a living cell would have been aware of both the successes and limitations of the available methods of preparing DNA for sequencing.

362. As described in Section XI.A.1.b, a POSA would have been motivated to combine the teachings of Grunenwald and Okino. Specifically, a POSA would combine the insertional enzyme complex taught by the cellular methodology in Grunenwald with the permeabilization methodology taught in Okino to effectively permit the insertional enzyme complex to enter the cell and nucleus. As discussed, these methods are effective for the study of chromatin.

363. Further, a POSA interested in studying chromatin after fragmentation and tagging would have been aware of nuclei isolation methods, such as the ones taught in Saleh and/or Song, and the benefits that a nuclei isolation step can provide. EX1005.002.

364. Grunenwald teaches a cellular *in vivo* method of tagmentation, which does not include a nuclei isolation step. Thus, the inclusion of a nuclei isolation is merely a design choice in the finite methods available to process chromatin for subsequent sequencing and analysis.

365. Both Saleh and Song, in my opinion, provide routine methods of nucleus isolation that show the process was already well-established in the art. Thus,

to the extent a design choice of isolating nuclei is to be made, a POSA would have been aware of the taught techniques.

366. *Third*, a POSA would have had a reasonable expectation of success in combining the references. As discussed above in Section XI.A.1.b, a POSA would have had a reasonable expectation of success combining the teachings of Grunenwald and Okino.

367. A POSA would have had a reasonable expectation of success combining the teachings of Saleh and/or Song with the methods of Grunenwald and Okino. As discussed at length above in Section XI.A.2, the combination of Grunenwald and Okino disclose a method of generating a composition comprising a permeabilized cell nucleus and an insertional enzyme complex, which can be utilized to generate a plurality of tagged polynucleotides for the goal of evaluating chromatin.

368. A POSA utilizing these methods to prepare DNA for sequencing would have known to expect success in isolating the permeabilized cell nucleus with the insertional enzyme complex because Saleh teaches a method which allows for the isolation of a cell nucleus containing DNA and associated proteins.

369. The method of nuclei isolation taught by Saleh would have been a standard method in the art when isolating DNA in preparation for sequencing. Thus,

a POSA would have had a motivation to combine the teachings of Saleh with Grunenwald and Okino, and a reasonable expectation of success in doing so.

370. Turning to Song, it is my opinion that a POSA would have had a reasonable expectation of success on the basis that the combined references provide compatible methods to accomplish analogous goals.

371. Cell lysis, long prior to 2013, was used to disrupt a cell membrane, breaking it apart and allowing access to the organelles and proteins present in the interior of the cell. Prior art references have referred to lysis as “perhaps the most crucial step” in the context of other assays that require access to the interior of a cell. *See, e.g.*, EX1055.004.

372. Song teaches precisely this combination of lysing a cell to provide nuclei and contacting the nuclei with an enzyme capable of modifying the chromatin present.

373. Thus, it is my opinion that a POSA performing the *in vivo* tagmentation method disclosed in Grunenwald with nuclei isolation procedure taught in Song, applying a lysis buffer, would have had a reasonable expectation that they would be able to ultimately isolate the DNA and modify it with tags in preparation for sequencing.

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

374. As I detail above, it is my opinion that Claim 16 is rendered obvious by the combination of Grunenwald with Okino. However, to the extent that the Patent Owner contends that Grunenwald does not disclose both a first and second sequencing adapter, it is my opinion that this would be disclosed by combining the teaching of Grunenwald and Okino with Adey.

375. Figure 1(b) of Adey, below, illustrates the disclosure of a first and second sequencing adapter.

376. In this figure, the transposase enzyme is represented by two grey hexagons. In that hexagon, there are two adaptor sequences, the lines that are brown and blue/orange. This figure illustrates the use of both a first and a second adapter sequence in which the adapter sequences are different from each other.

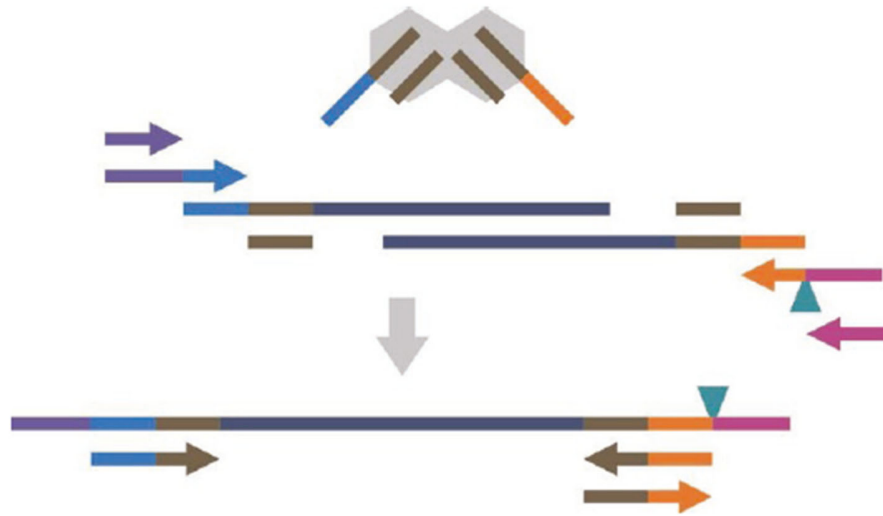


Figure 1(b), EX1006.002

377. Specifically, the fragmentation process shown in Figure 1(b) is executed using the Illumina-compatible Nextera™ DNA Sample Prep Kit from Epicentre. *See, e.g.*, EX1006.012.

378. This kit contains adapters that comprise a first and second adaptor sequence, illustrated in Figure 1 of the Nextera™ DNA Sample Prep Kit manual (reproduced below).

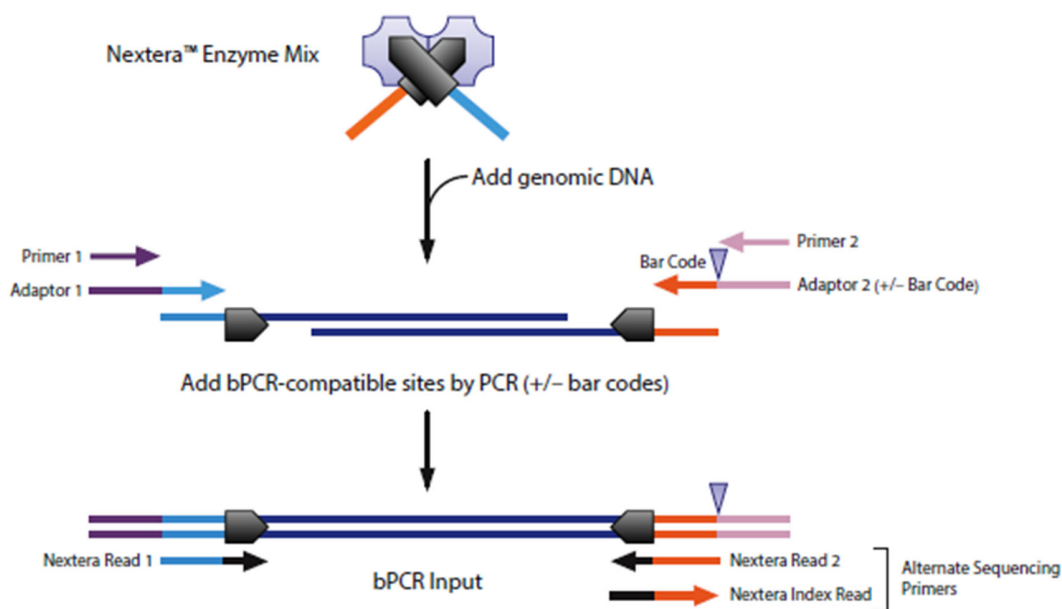


Figure 1, EX1048.002.

379. In the above figure, the Nextera™ Enzyme mix contains both a transposase enzyme (gray crosses) and transposon ends with two sequencing primer sites, or sequencing adaptors (blue and orange). EX1048.002. Once the transposase tags and fragments the DNA, the adaptors are paired with amplification primers (purple and pink). *Id.*

380. The Nextera™ manual further includes a listing of the sequences that are contained on the adapters. These include a Primer 1 and 2 sequence, displayed above in purple and pink, which are sequences compatible with bPCR, an amplification technique. *Id.* The sequences further include the Nextera™ Read 1

and 2 sequences, shown above in blue and orange, which are sequences compatible with Illumina next generation sequencing platforms. *Id.*

381. In my opinion, the combination of Grunenwald and Adey would have been obvious to a POSA at the time of the priority date of the '995 Patent.

382. **First**, I note that Adey and Grunenwald would be considered analogous art. Both are references that disclose the use of a transposase complex for tagmentation. A POSA studying epigenetic features of chromatin would have been aware of both references as teaching methods for tagmentation of genomic DNA and subsequent analysis.

383. **Second**, a POSA would have been motivated to combine the references. As discussed above, Grunenwald teaches a method of tagmentation that can be applied to chromatin, either in the form of isolated chromosomal DNA or tagmentation on chromatin *in vivo* in a cell. *See supra* Section XI.A.a. Adey, in turn, teaches tagmentation broadly with the use of a kit to prepare a genomic DNA sample for sequencing. *See, e.g.* EX1006.001.

384. A POSA desiring to prepare chromatin for sequencing and subsequent analysis would have knowledge of commercially available kits, such as the Nextera™ kit disclosed in Adey, and would have been aware of the two adapter system that can be used in tagmentation to prepare a DNA sample for sequencing. Further, a POSA would have had knowledge of the tagmentation techniques applied

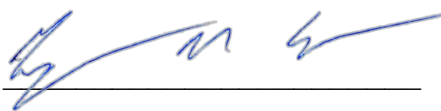
to chromatin as taught in Grunenwald. This would be a case of simple substitution; that is, the use of the Nextera™ kit to complete the process that Grunenwald already teaches.

385. **Third**, a POSA would have had a reasonable expectation of success combining the two references. The Nextera™ kit was a commercially available kit, and references such as Adey demonstrate the success in using the kit to accomplish tagmentation. *See, e.g.*, EX1006.011. Combining an effective, commercially available kit with a method to fragment and tag chromatin, either *in vitro* chromosomal DNA or directly on chromatin *in vivo*, would yield a predictable result of successful tagmentation and preparation of the chromatin for sequencing.

XII. DECLARATION

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: June 1, 2023

By: _____

Gregory Cooper, Ph.D.