

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. 10,150,995

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. 10,150,995 (the “’995 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 ’995 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 that I have reviewed, it is my opinion that claims 1, 3, 5-6, and 10-23 are anticipated by Grunenwald (EX1003).

17. Based on my knowledge, experience, and the materials I have reviewed, it is my opinion that the claims of the '995 Patent would have been obvious to a POSA. Specifically:

- a. Claim 2 would have been obvious to a POSA in view of the combination of Grunenwald (EX1003) with O'Geen (EX1005);
- b. Claims 6-8 would have been obvious to a POSA in view of the combination of Grunenwald (EX1003) with Okino (EX1004);
- c. Claim 9 would have been obvious to a POSA in view of the combination of Grunenwald (EX1003) with Pitt (EX1006);
- d. Claim 17-20 would have been obvious to a POSA in view of Grunenwald (EX1003) with Adey (EX1009).
- e. Claims 1, 3, 5-6, and 10-23 would have been obvious to a POSA in view of Grunenwald (EX1003) with Okino (EX1004)
- f. Claim 2 would have been obvious to a POSA in view of Grunenwald (EX1003) with O'Geen (EX1005) and Okino (EX1004)
- g. Claims 17-20 would have been obvious to a POSA in view of Grunenwald (EX1003) with Ade (EX1009) and Okino (EX1004).

IV. LEGAL STANDARDS

18. 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

19. 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

20. 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.

21. 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

22. 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.

23. 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.

24. 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.

25. 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.

26. 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 '995 PATENT

27. The '995 patent is titled "Transposition Of Native Chromatin For Personal Epigenomics." '995 patent. The '995 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.

28. The claims of the '995 patent are reproduced below:

1. A method for analyzing a biological sample, comprising:

(a) contacting chromatin of a genome region of said biological sample with an insertional enzyme complex to produce tagged nucleic acid molecules, wherein said insertional enzyme complex does not comprise an antibody specific to a protein that is part of said chromatin; and

(b) performing a nucleic acid assay on said tagged nucleic acid molecules or derivatives thereof, to provide sequence information of said tagged nucleic acid molecules or derivatives thereof.

2. The method of claim 1, further comprising generating a representation of epigenetic features of said genome region at least in part by mapping said sequence information to said genome region.

3. The method of claim 1, wherein said biological sample is a cell or a nucleus.

4. The method of claim 3, further comprising lysing said cell or nucleus.

5. The method of claim 3, further comprising permeabilizing said cell or said nucleus to permit said insertional enzyme complex to access said chromatin.

6. The method of claim 5, wherein said permeabilizing is configured to minimally perturb said chromatin of said cell or said nucleus.

7. The method of claim 5, wherein said cell or said nucleus is permeabilized with a reagent configured to permit said insertional enzyme complex to access said chromatin.

8. The method of claim 7, wherein said reagent comprises NP40, digitonin, tween, streptolysin, or a cationic lipid.

9. The method of claim 5, wherein said cell or said nucleus is permeabilized by hypotonic shock or ultrasonication.

10. The method of claim 1, wherein said insertional enzyme comprises a transposase.

11. The method of claim 10, wherein said transposase is a Tn5 transposase or derived from a Tn5 transposase.
12. The method of claim 11, wherein said transposase is a hyperactive Tn5 transposase.
13. The method of claim 1, wherein said insertional enzyme complex comprises a first nucleic acid insert element comprising a first adapter sequence.
14. The method of claim 13, wherein said first adapter sequence comprises a first sequencing adapter sequence.
15. The method of claim 13, wherein said first adapter sequence comprises a barcode sequence.
16. The method of claim 13, wherein said first adapter sequence comprises a first primer sequence.
17. The method of claim 13, wherein said insertional enzyme complex further comprises a second nucleic acid insert element comprising a second adapter sequence.
18. The method of claim 17, wherein said second adapter sequence comprises a second sequencing adapter sequence.
19. The method of claim 17, wherein said second adapter sequence comprises a barcode sequence.
20. The method of claim 17, wherein said second adapter sequence comprises a second primer sequence.
21. The method of claim 1, further comprising subjecting said tagged nucleic acid molecules to one or more reactions.
22. The method of claim 21, wherein said one or more reactions comprise a nucleic acid amplification reaction.
23. The method of claim 22, wherein said nucleic acid amplification reaction is configured to add one or more functional sequences to said

tagged nucleic acid molecules or derivatives thereof, wherein said one or more functional sequences are compatible with a selected next generation sequencing platform.

29. Claim 1 recites two steps: the first relating to tagging a DNA sample comprising chromatin, and the second to sequencing the tagged DNA. This tagging 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”.

30. The aforementioned tagging can be used to investigate chromatin accessibility and understand epigenetic features of chromatin. The '995 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.

31. As I discuss below, chromatin is the DNA-protein complex in which the genome of a cell exists endogenously. The '995 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.

32. Once isolated, the chromatin may be tagged. The '995 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.

33. 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 '995 patent.

34. 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.

35. I do not have an opinion on the priority date of the '995 patent. Based on the face of the patent, I understand that the earliest potential priority date of the '995 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 '995 PATENT

36. On July 24, 2018, Applicant The Board of Trustees of the Leland Stanford Junior University filed Nonprovisional Patent Application No. 16/043,874 titled "Transposition of Native Chromatin for Personal Epigenomics." EX1007.364-371.

37. On December 11, 2018, the '995 patent issued. EX1007.208-209.

38. I have been informed by counsel that during the prosecution of the '995 patent, the Examiner did not issue any non-final rejections, final rejections, or office actions.

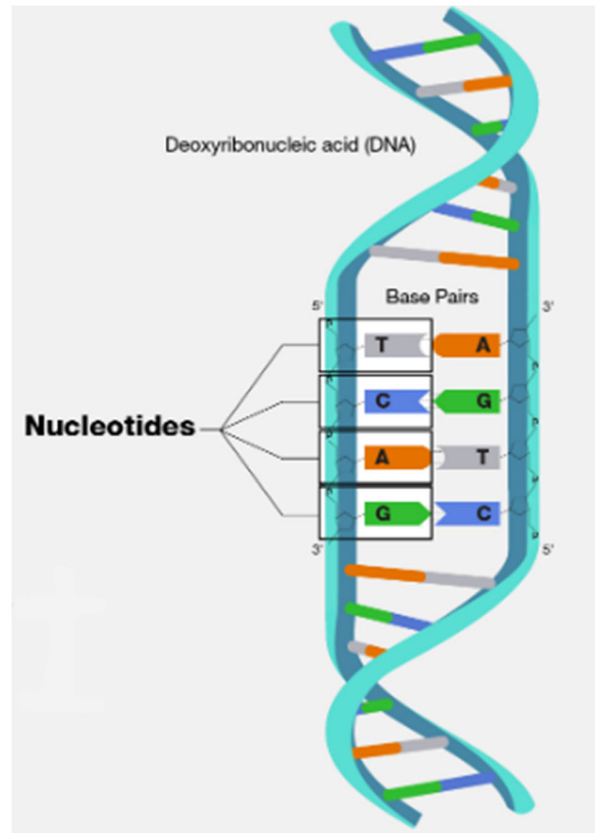
VII. TECHNICAL BACKGROUND AND STATE OF THE ART

A. DNA and RNA

39. 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."

40. 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.

41. 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.

42. 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.

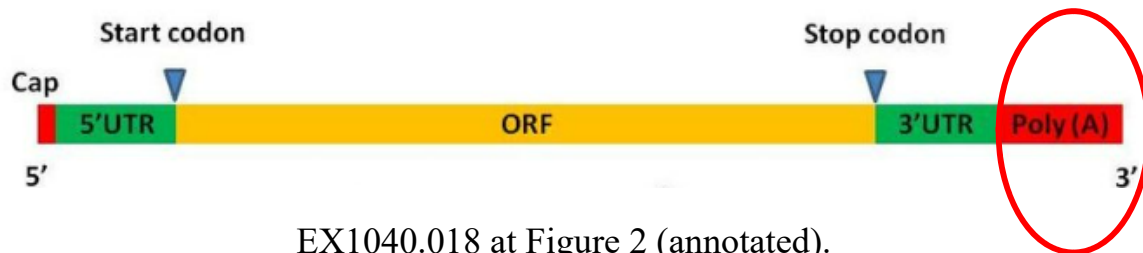
43. Ribonucleic acid (RNA) is a molecule similar to DNA, as both store genetic information and are made up of nucleotides consisting of sugar, nitrogenous bases, and a phosphate backbone. RNA can hybridize to a complementary DNA sequence with the help of specific enzymes. DNA and RNA are molecules that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

44. 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).

45. 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.

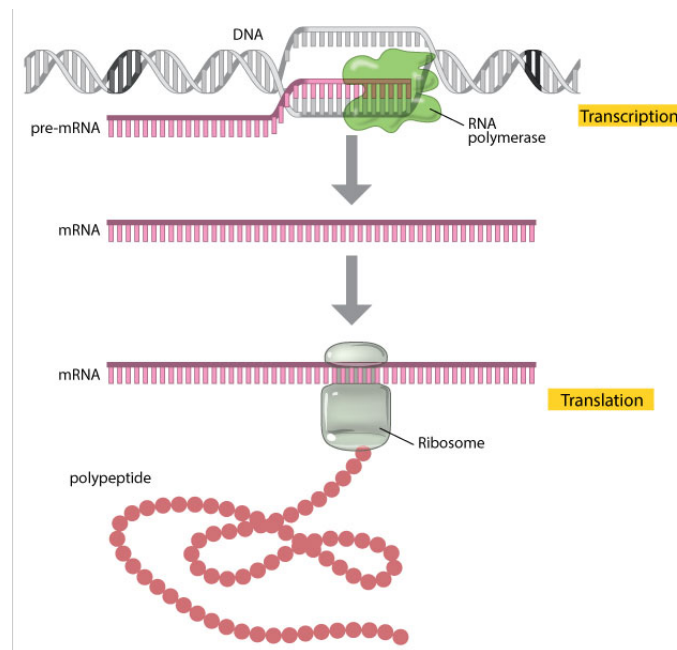
46. Protein-coding gene expression is achieved by two major steps – transcription and translation. Transcription is the copying of genetic information from a DNA sequence into a specific type of RNA – messenger RNA (mRNA), which is made up of nucleotides that are complementary to the DNA sequence. During 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.



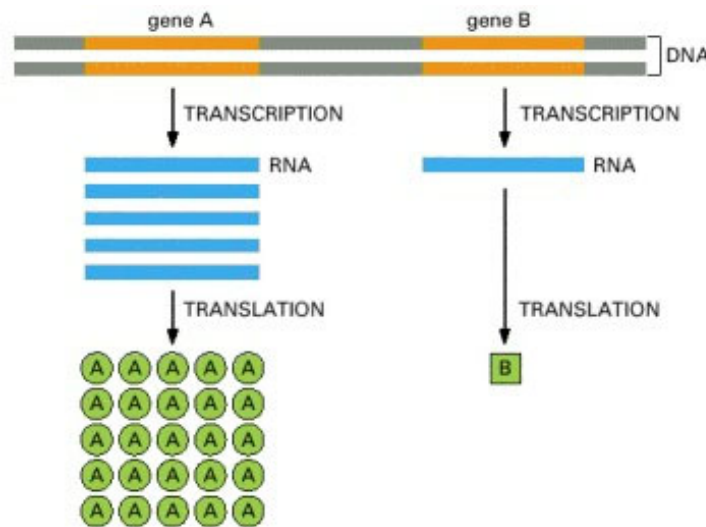
EX1040.018 at Figure 2 (annotated).

47. 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).

48. 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).

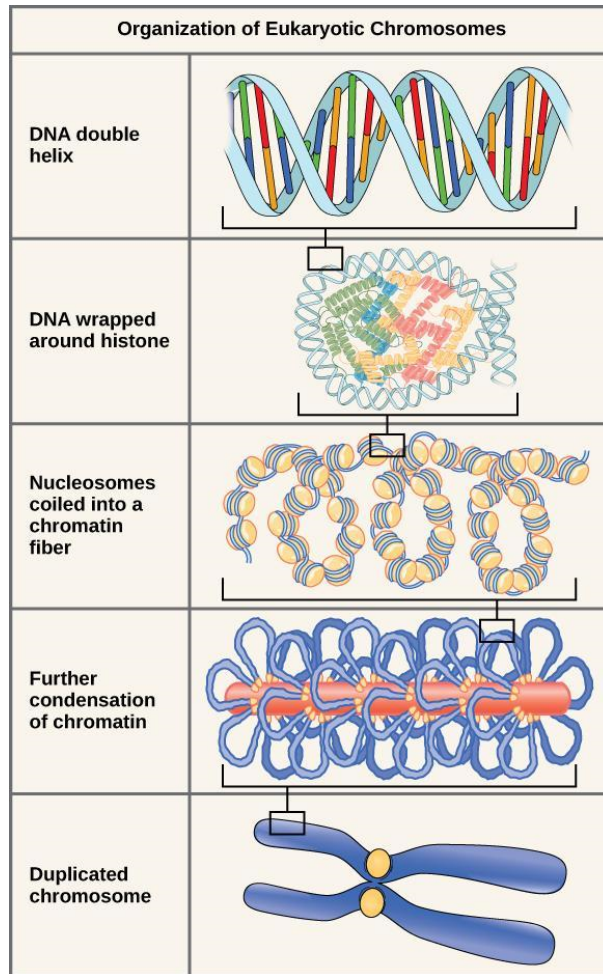
49. Gene expression profiling is the determination of the mRNA content in a cell or tissue to understand the pattern of genes expressed. Thus, detecting mRNA molecules is critical to the study of gene expression because they show the portions of DNA copied for the production of proteins. This is useful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

B. DNA Packaging

50. 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.

51. 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.

52. 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

53. 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.

54. 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.

55. 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.

56. 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.

57. 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.

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

59. 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.

60. 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

61. 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).

62. 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

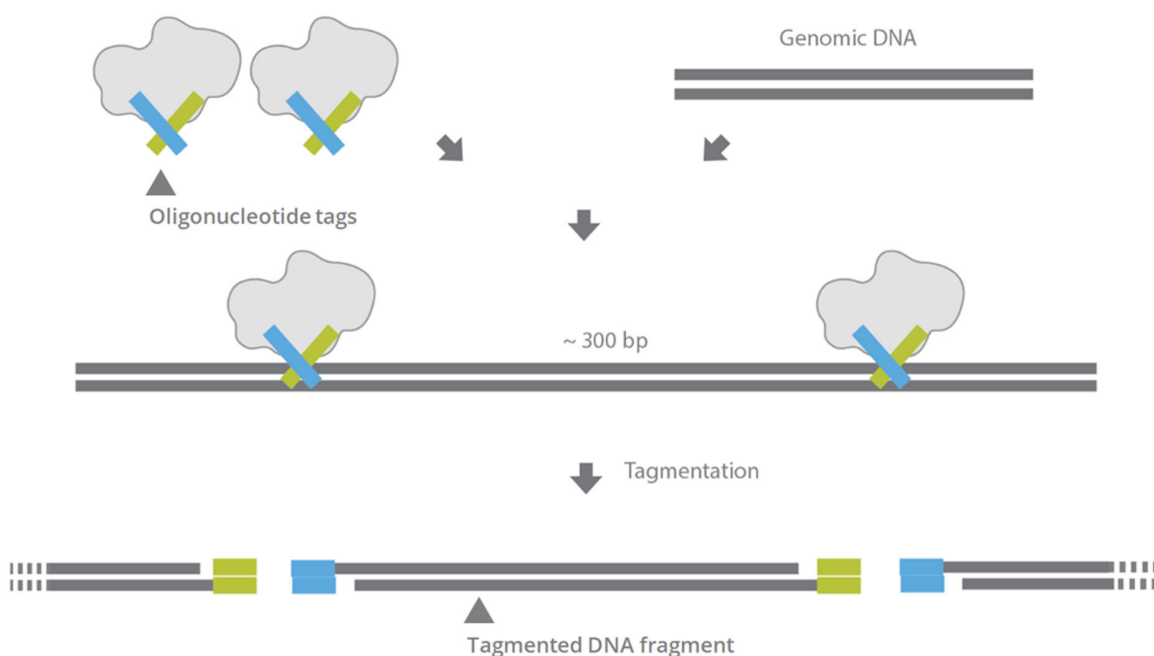
63. Many NGS platforms, including those sold by Illumina, can generate only a short string of DNA sequence information from a nucleic acid molecule, typically only a couple of hundred nucleotides in length. Hence DNA is essentially broken up or “fragmented” into small chunks, prior to sequencing, so 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).

64. 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

(EX1009.002). Oligonucleotide tags can however be used for various purposes.

EX1010.002

65. 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

66. The '995 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

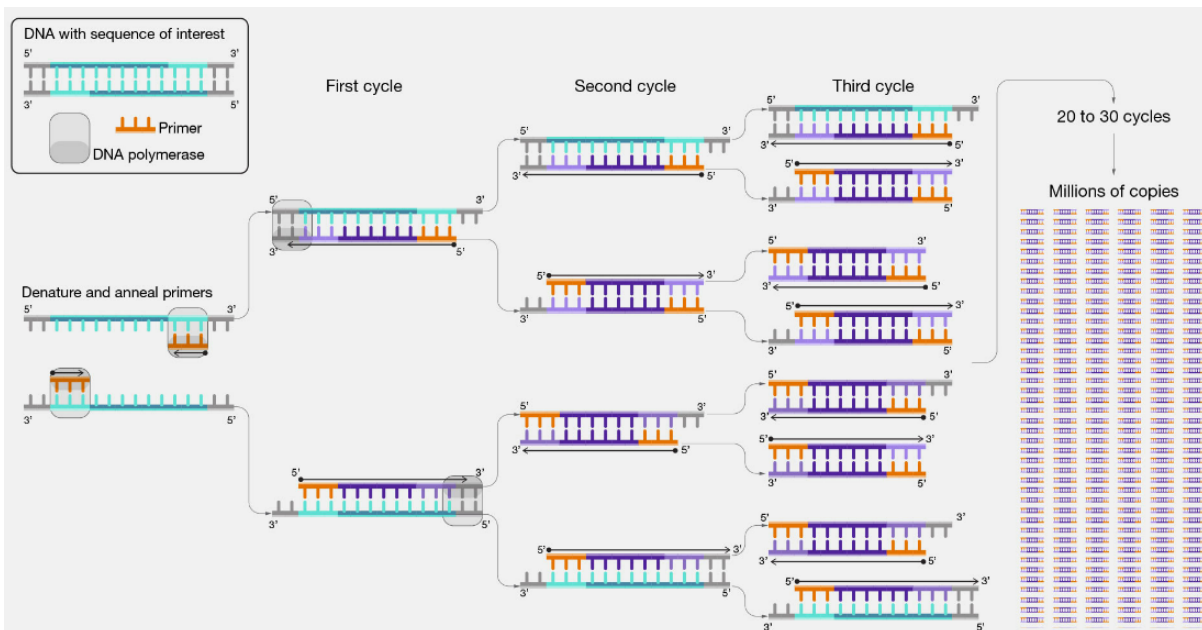
67. DNA sequencing requires a minimum amount of DNA or RNA. Before it can be sequenced, a sample with limited amount of DNA or RNA must sometimes be amplified. Amplification refers to the process of creating multiple copies of a specific polynucleotide. Various methods of amplification were known in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

68. 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).

69. 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.

70. 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.

71. 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.

72. 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).

73. 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

74. 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.

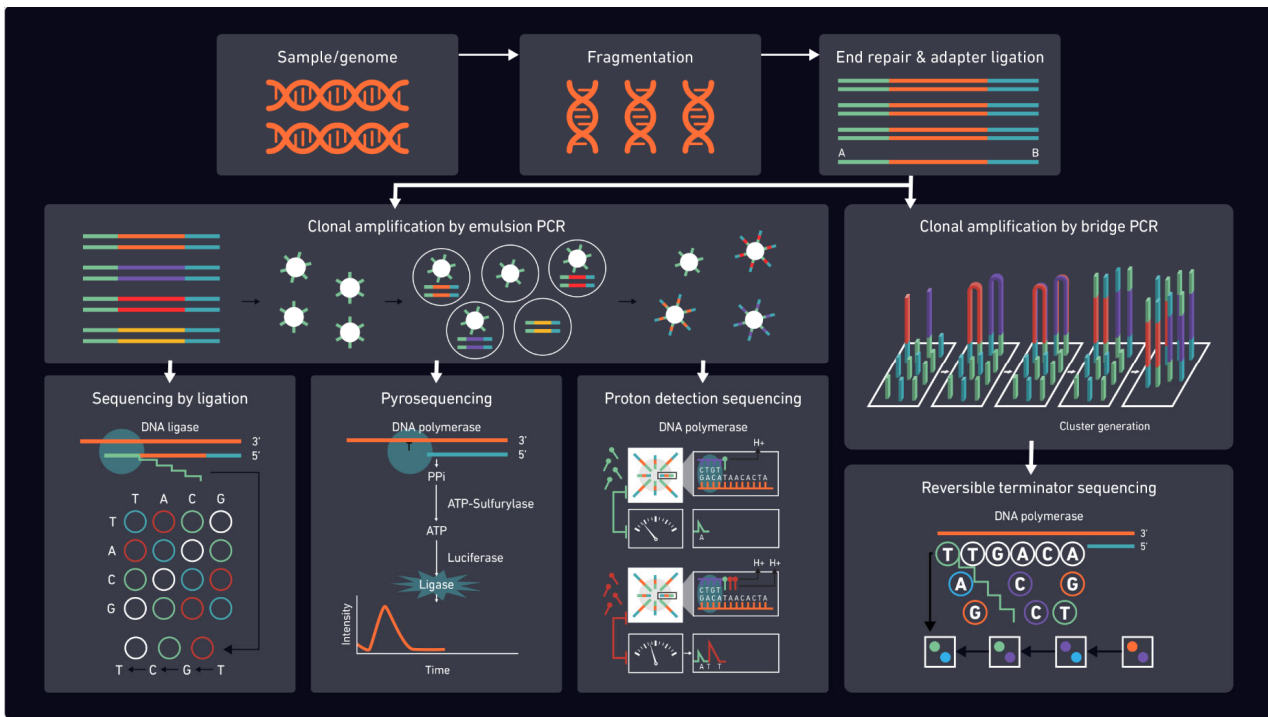


Figure 2, EX1039.005.

75. 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

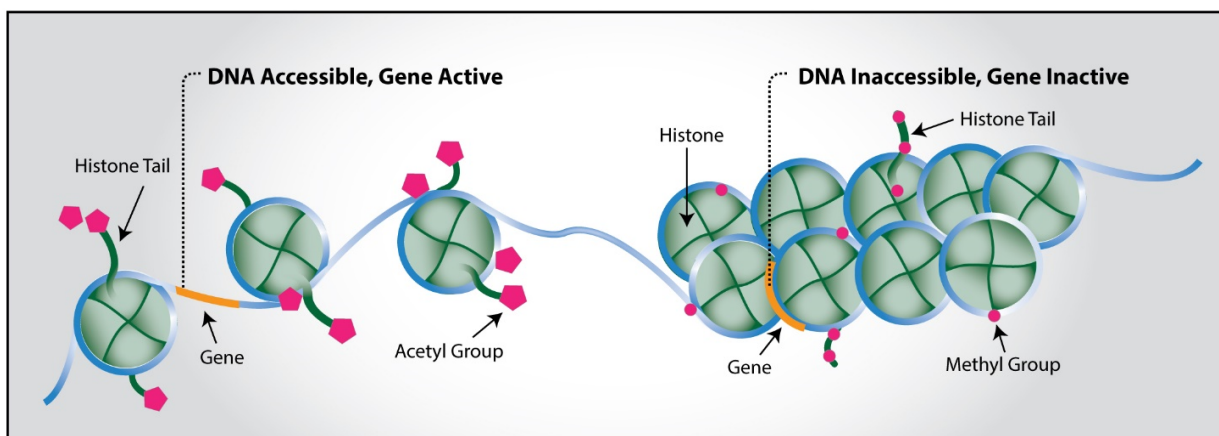
76. “Gene expression,” as explained above, refers to the process of reading instructions encoded in certain portions of DNA (*i.e.*, genes) and manufacturing proteins based on those instructions. Not all portions of the genome are expressed and used to manufacture proteins. In fact, certain genes may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

77. “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.

78. 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.

79. 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.

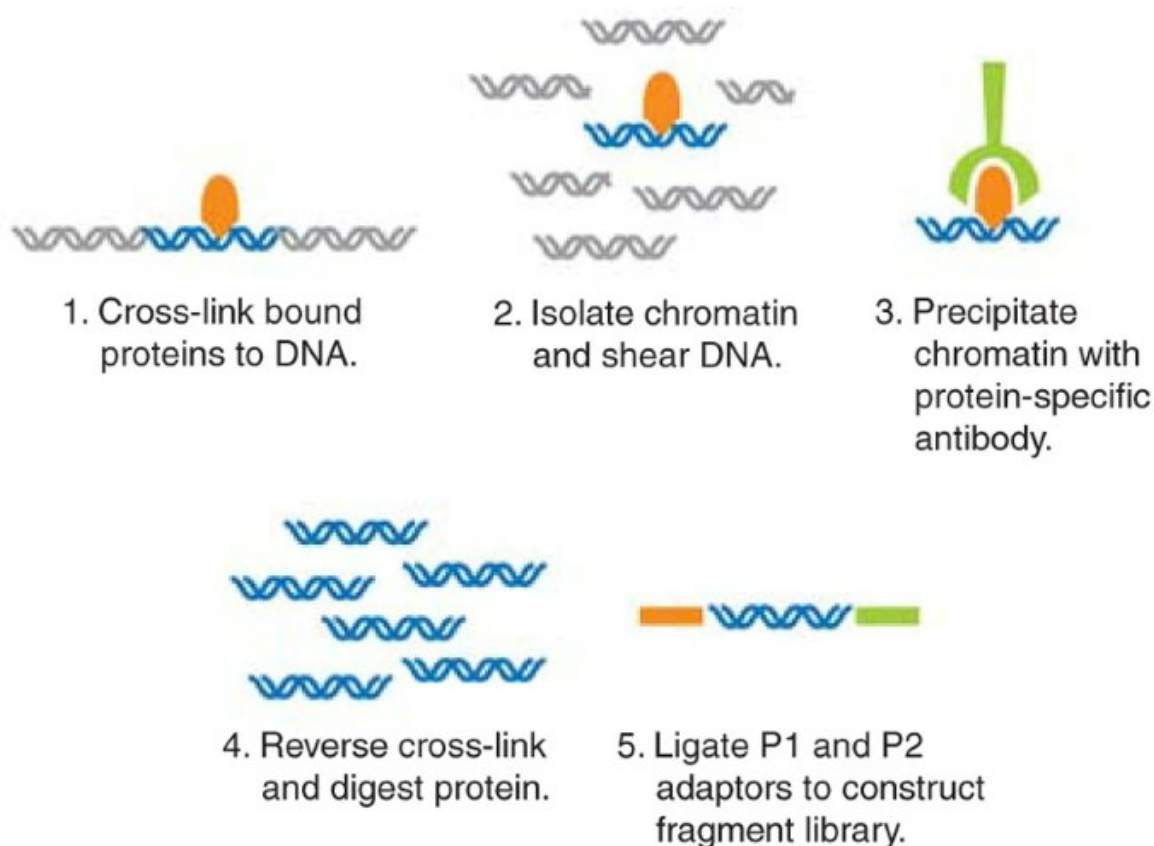
80. An example of histone modification is depicted in the figure below. Covalent modifications such as methylation, addition of a methyl group (pink dot), and acetylation, addition of an acetyl group (pink pentagon), are highly correlated with the structure and accessibility of chromatin. Histone acetylation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



EX1037

81. 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

82. 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)

83. 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).

84. 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).

85. 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)

86. 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).

* * *

87. 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

88. 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.

89. 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.

90. 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).

91. 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)

92. 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.

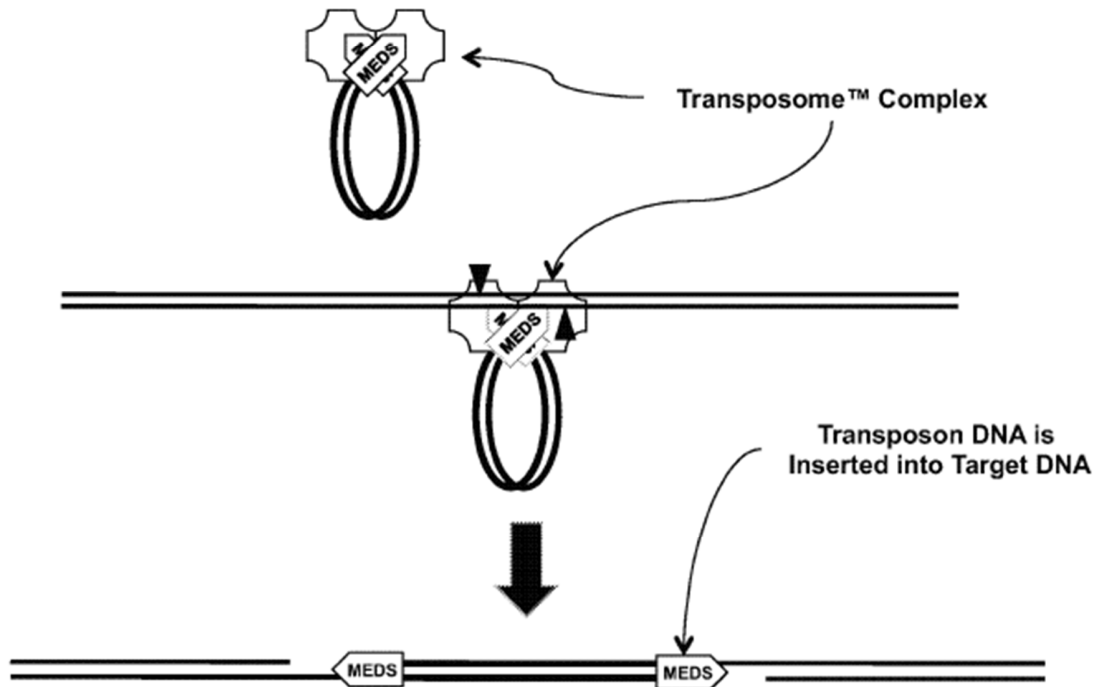
93. Grunenwald teaches the prior art tagmentation process described above. *See supra* ¶¶ 64-66. Grunenwald broadly discloses the methods, compositions, and kits for fragmenting and tagging DNA using transposon compositions. Grunenwald explains the invention is useful for generating libraries of tagged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

94. 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.

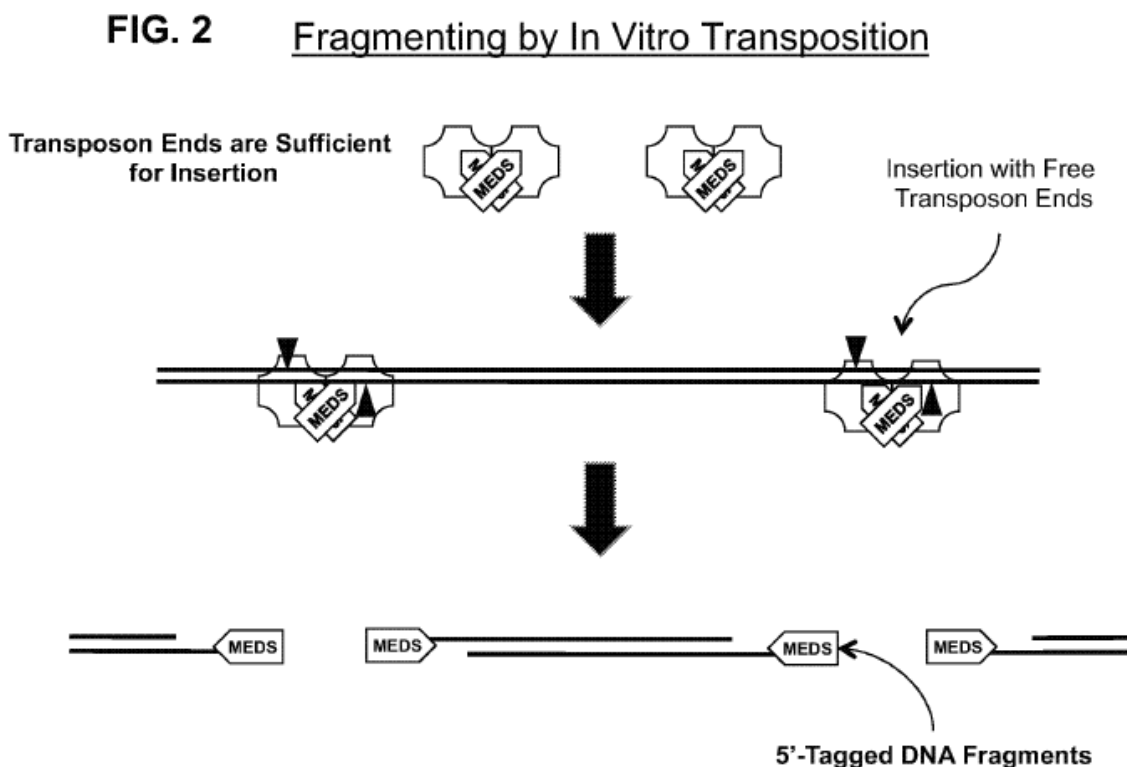
95. The method that Grunenwald teaches is incubating the target DNA with “at least one transposase and one transposon end composition,” where the transposon end composition includes the oligonucleotide tag to be inserted into the target DNA. *Id.* at [0162]. An exemplary transposition reaction tagging 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

96. 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

97. 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].

98. 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. O’Geen (EX1005)

99. *Using ChIP-Seq Technology to Generate High-Resolution Profiles of Histone Modifications* by Henriette O’Geen, Lorigail Echipare, and Peggy J. Farnham (“O’Geen”) was published in *Epigenetics Protocols* in 2011. It is my

understanding that O'Geen is prior art and was not considered by the Examiner in the prosecution of the '995 Patent.

100. O'Geen teaches a prior art method of fragmenting and tagging DNA, sequencing that DNA, and then using that sequence information to determine epigenetic features of the DNA. This method is known as chromatin immunoprecipitation and sequencing, or ChIP-seq. O'Geen at 266 (EX1005.005).

101. The first portion of this method includes fragmenting and tagging steps. Initially, whole cells are prepared for fragmentation and tagging by processing them to crosslink the DNA with the proteins associated with it at the moment of preparation. *Id.* at .009-010. This process permits the capture of a “snapshot” of DNA-protein interactions at the moment the cells are processed. *Id.* at .005.

102. After crosslinking, chromatin is fragmented through the use of ultrasound waves. Fragment size can be checked to ensure that the chromatin has been fragmented sufficiently for analysis and, if needed, the fragmenting step can be repeated to generate smaller fragments. *Id.* at .010-011.

103. Once fragmentation is complete, immunoprecipitation occurs by treating the chromatin with antibodies specific to proteins of interest in the chromatin, which in turn bind those proteins. *Id.* at .011.

104. The complexes formed by the antibodies with the proteins of interest are captured and the DNA can be unbound from the protein. *Id.* at .012.

105. Once the DNA is unbound, it can be purified and prepared for sequencing with a known next generation sequencing platform, such as Illumina. *Id.* at .013-017.

106. The sequencing data collection from NGS can in turn be used to develop a map of epigenetic features, e.g., the locations of sites where the proteins of interest were bound to the target DNA. *Id.* at .017-018.

C. Okino (EX1004)

107. 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 '995 Patent.

108. 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].

109. 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].

110. 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].

111. 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].

112. 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.*

113. 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].

D. Pitt (EX1006)

114. *Ultrasonic Drug Delivery – A General Review* by William G. Pitt, Ghaleb A. Hussein, and Bryant J. Staples (“Pitt”) was published in *Expert Opinion*

on Drug Delivery in 2004. It is my understanding that Pitt is prior art and was not before the Examiner during the prosecution of the '995 Patent.

115. Pitt is a review of the state of the art as early as 2004. It provides an overview on ultrasonic delivery of various types of molecules, including proteins. Pitt at 10 (EX1006.010).

116. Pitt emphasizes, and a POSA would have known, that “proteins have very different transport and solubility characteristics in tissues” from other types of molecules that cross the cell membrane, and “thus their delivery is usually much more complex .” *Id* at .010.

117. Particularly, Pitt discloses the transport of enzymes across the cell membrane. For example, Pitt teaches the transport of “tissue plasminogen activator (tPA) and other lytic proteins such as urokinase” through the use of ultrasound. *Id.* at .011.

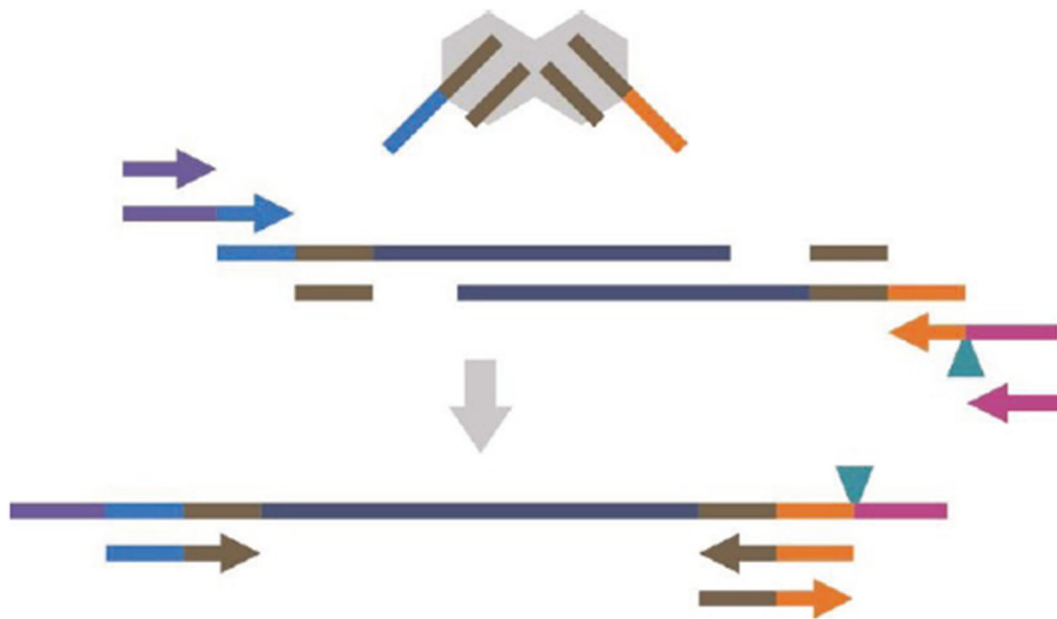
118. Pitt further teaches that low frequency ultrasound may be used to permeabilize cells and allow delivery of the proteins, citing that “1 MHz ultrasound increased the uptake and the depth of penetration of tPA into clots.” *Id.*.

E. Adey (EX1009)

119. *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 ’995 Patent.

120. 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 (EX1009.002). As depicted in Figure 1(b), the transposome complex comprises the transposase enzyme (two grey hexagons), a sequence to assist the transposase to fragment DNA (brown), and two different adaptors (mild blue/orange).



EX1009.002 at Figure 1

121. 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.

122. 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.

X. CLAIM CONSTRUCTION

123. 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).

124. Claim 1 of the '995 patent recites, in part, “contacting chromatin of a genome region of said biological sample with *an insertional enzyme complex* to produce tagged nucleic acid molecules.” 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 '995 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.

125. Claims 13-20 recite the use of a “nucleic acid insert element.” 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 ’995 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.

126. Claims 13-20 recite the use of an “adapter sequence.” While the ’995 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 adaptors 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.

127. Claim 13 recites “wherein said first adapter sequence comprises a barcode sequence.” Here, the specification of the ’995 Patent defines a barcode sequence as a “unique sequence of nucleotides used to a) identify and/or track the source of a polynucleotide in a reaction and/or b) count how many times an initial molecule is sequenced (e.g., in cases where substantially every molecule in a sample

is tagged with a different sequence, and then the sample is amplified).” EX1001 at 12:26-32. I have therefore adopted this definition in my analysis.

XI. PRIOR ART ANTICIPATED OR WOULD HAVE RENDERED OBVIOUS THE CLAIMS OF THE '995 PATENT

A. Ground 1: Claims 1, 3, 5-6, And 10-23 Are Anticipated By Grunenwald

a) Claim 1

i. [1A] A method for analyzing a biological sample, comprising:

128. To the extent that the preamble is deemed limiting, it is my opinion that Grunenwald discloses a method for analyzing a biological sample.

129. First, Grunenwald discloses the analysis of cells as biological samples. For example, Grunenwald discloses that a 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).” EX1003 at [0146].

130. This “target DNA” may be DNA comprising “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.* I apply this definition to my analysis below.

131. Because the target DNA may come from one or multiple cells, and that target DNA is ultimately tagged, fragmented, and sequencing according to the methods of the invention, it is my conclusion that Grunenwald teaches the analysis of cells as a biological sample.

132. Second, and alternatively, Grunenwald’s disclosure of the analysis of “target DNA” would also constitute analysis of a biological sample. *Id.*

133. Grunenwald teaches analyzing a biological sample by first preparing the DNA sample found in a cell by tagging and fragmenting the sample, followed by sequencing the target DNA. As I explained above in Section IX.A and will explain in more detail below in Section XI.A.a.b, Grunenwald teaches a method for analyzing a biological sample by simultaneously tagging and fragmenting a target DNA sample either from or in a cell using a transposase and transposon end composition. *See, e.g., id.* at [0018].

134. Grunenwald teaches that tagging and fragmentation are methods for preparing the target DNA sample for sequencing. *See, e.g., id.* at [0159].

135. Grunenwald further discloses analyzing the target DNA sample by sequencing the target DNA sample following the tagging and fragmentation steps.

Id. (explaining that the tagged fragments may be used as “templates for massively parallel DNA sequencing”). As I explained above in Section VII C, sequencing a DNA sample is determining the sequence of nucleotides present in it.

136. A POSA would have understood that the steps of fragmenting and tagging followed by sequencing a DNA sample constitute a method for analyzing the DNA sample that is isolated from a cell.

ii. **[1B] contacting chromatin of a genome region of said biological sample with an insertional enzyme complex to produce tagged nucleic acid molecules,**

137. It is my opinion that Grunenwald discloses contacting chromatin of a genome region of said biological sample with an insertional enzyme complex to produce tagged nucleic acid molecules.

138. The first portion of the claim recites “*contacting chromatin* of a genome region of said biological sample *with an insertional enzyme complex* .”

139. First, it is my opinion that that Grunenwald explicitly discloses this limitation. Grunenwald teaches tagmentation on “chromosomal DNA.” EX1003 at [0146]. A POSA would understand that chromosomal DNA exists in the form of chromatin, and whether in a cell or isolated if the method disclosed in Grunenwald is being performed on chromosomal DNA it is being performed on chromatin.

140. However, to the extent that the Patent Owner argues that this disclosure does not explicitly teach this limitation, it is further my opinion that it would have been clear to a POSA that Grunenwald necessarily discloses this limitation.

141. Grunenwald contemplates tagging DNA *in vivo*, i.e., in a live host cell. The *in vivo* nature of the transposition reaction is particularly key to my conclusion that Grunenwald anticipates the '995 Patent.

142. Grunenwald discloses a method for fragmenting and tagging DNA that begins by “incubating the target DNA with a transposase and a transposon end or transposon end composition.” *Id.* at [0018]. The transposase and transposon end composition collectively make up an insertional enzyme complex. *Id.* Grunenwald defines a transposase as an “enzyme that is capable of forming a functional complex with transposon end-containing composition” and “catalyzing insertion or transposition of the transposon end-containing composition” into the target DNA. *Id.* at [0093].

143. The transposon end composition that is part of this complex includes a “transposon end sequence,” which is a nucleic acid portion that permits the transposase to perform its cleaving and insertion function, and a tag that “can have one or more other tag portions or tag domains.” *See, e.g., id.* at [0102]. These tags are on the 5’ end of the transferred strand and the 3’ end of the non-transferred strand. *Id.*

144. A POSA would have further understood that Grunenwald necessarily discloses using that transposon complex to contact chromatin. Specifically, Grunenwald teaches using the transposon complex to contact target DNA such as chromosomal DNA and that this method may be performed *in vivo* on a live cell by electroporating the enzyme complex directly into the cell. *Id.* at [0146]; [0157]. In either case, the target DNA would comprise chromatin and this claim limitation would be met.

145. This is because, as noted in the '995 Patent, in a cell *in vivo* chromatin is the complex of DNA and proteins found in the nucleus of the cell. EX1001 at 12:54-60. Chromosomal DNA exists in the form of chromatin naturally, even when isolated, as I note above. *See supra* Section VII.B. Therefore, because Grunenwald teaches using a transposon complex to contact chromosomal DNA or to contact target DNA in the cell *in vivo*, it necessarily teaches “contacting chromatin . . . with an insertional enzyme complex,” as required by this claim limitation.

146. The second portion of the claim recites “contacting chromatin of a ***genome region*** of said biological sample with an insertional enzyme complex.” It is my opinion that Grunenwald discloses this limitation. A POSA would recognize that contacting chromosomal DNA or contacting DNA *in vivo* in a cell would require contacting a genome region.

147. Finally, the claim recites contacting chromatin of a genome region with an insertional enzyme complex “*to produce tagged nucleic acid molecules.*” It is my opinion that Grunenwald recites this limitation. Grunenwald further discloses using its method to generate “a library of tagged DNA fragments of a target DNA.” EX1003 at [0018].

148. 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]. 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. *See infra* Section XI.A.i-k (explaining different tag domain types in detail).

149. 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].

- iii. **[1C] wherein said insertional enzyme complex does not comprise an antibody specific to a protein that is part of said chromatin; and**

150. It is my opinion that Grunenwald discloses “wherein said insertional enzyme complex does not comprise an antibody specific to a protein that is part of said chromatin.”

151. I note that the '995 Patent discloses that “the insertional enzyme can further comprise an affinity tag” which “can be an antibody.” EX1001 at 16:37-39. The '995 Patent further describes that such antibody affinity tag can “bind to [] a transcription factor, a modified nucleosome or a modified nucleic acid.” *Id.* at 16:39-41. It is my opinion that this disclosure is actually an embodiment that cannot practice claim 1. Because a transcription factor would be a protein that is part the chromatin (i.e., it is a protein bound to specific genome regions inside a cell), a POSA would have understood that by binding an antibody to a transcription factor, it is undisputable that the insertional enzyme complex comprising an antibody is comprising an antibody specific to said chromatin.

152. It is my opinion that Grunenwald's disclosures are sufficient to allow someone to practice the method of claim 1 without an antibody specific to a protein that is part of the chromatin; that is, while Grunenwald does not explicitly state that

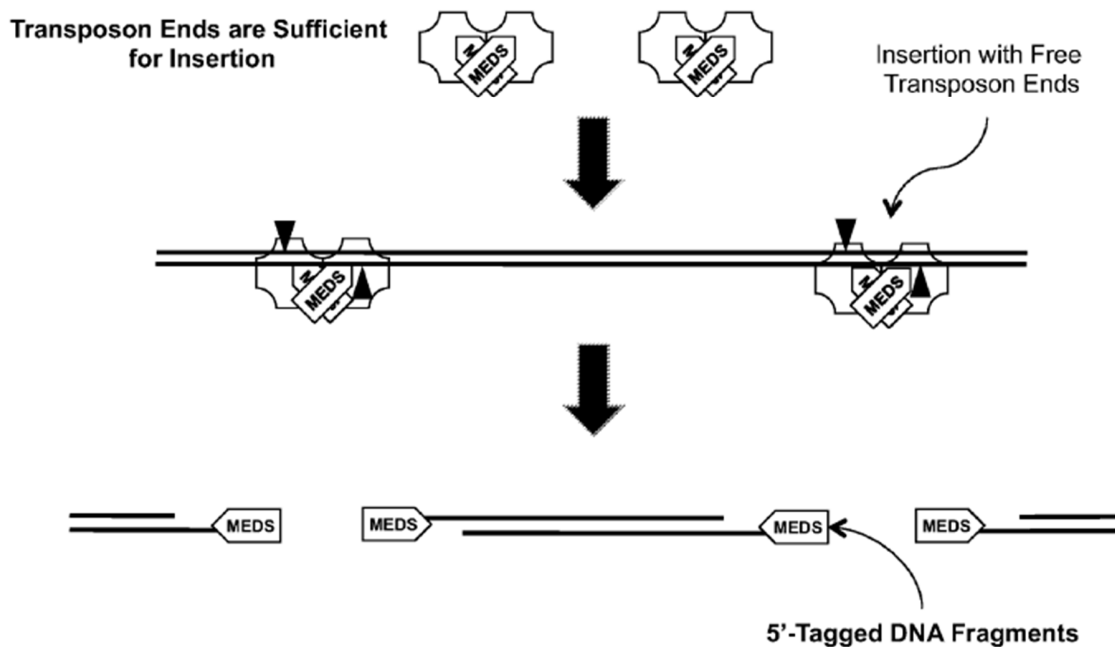
an antibody specific to a protein that is part of the chromatin should not be used, there is nothing in Grunenwald that suggests an antibody *must* be used.

153. For example, as indicated in Grunenwald's Figure 2 below, the transposition reaction is done with a transposase without the use of an antibody specific to a protein that is part of the chromatin. This is further supported by the first embodiment disclosed in Grunenwald, wherein the method for tagging comprises

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

EX1003 at [0018] – [0022].

FIG. 2 Fragmenting by In Vitro Transposition



EX1003 at Figure 2.

154. Figure 2 of Grunenwald, reproduced above, illustrates an example of fragmenting with an *in vitro* transposition reaction. In this example, the “Transposome Complex” is the transposase along with the transferred strand, including the tag with multiple tag domains.

155. This disclosure is sufficient to carry out the method claimed in claim 1 without the use of an antibody specific to a protein that is a part of the chromatin, and so it is my opinion that Grunenwald anticipates this claim limit.

156. I further note that Grunenwald does reference the term “antibody” seven times. *Id.* at [0109], [0117], [0212], [0216], [0217], [0226], [0229]. None of these instances suggest that Grunenwald discloses that the disclosed transposase and transfer strand (i.e., the claimed “insertional enzyme complex”) comprises an antibody specific to a protein that is part of the target DNA (i.e., the claimed “said chromatin”).

157. First, Grunenwald discloses that:

In some other embodiments, the capture tag domain comprises a 5'-portion of the transferred strand that is joined to a chemical group or moiety that comprises or consists of an affinity binding molecule (e.g., wherein the 5'-portion of the transferred strand is joined to a first affinity binding molecule, such as biotin, streptavidin, an antigen, or an ***antibody*** that binds the antigen, that permits capture of the circular tagged ssDNA fragments or the di-tagged linear ssDNA fragments on a surface to which a second affinity binding molecule is attached that forms a specific binding pair with the first affinity binding molecule).

Id. at [0109].

158. I have concluded that this first disclosure does not disclose a complex comprising an antibody specific to a protein that is part of said chromatin. While it does disclose that an antibody may be part of the insertional enzyme complex, the antibody is not for a protein that is part of the chromatin. Rather, this disclosure is an antibody that permits capture of tagged DNA fragments to which a second affinity binding molecule is attached. *Id.* This disclosure contextualizes the use of an

antibody with a “capture tag domain,” or a tag domain that may be added to capture an ssDNA fragment to which the capture tag is joined. *Id.*

159. Second, Grunenwald discloses “biotin and streptavidin, biotin and avidin, or digoxigenin and a specific **antibody** that binds digoxigenin” can form “specific binding pairs.” *Id.* at [0117]. The members of each pair comprise “affinity binding molecules” or “affinity binding substances”. *Id.* Grunenwald defines “[a]ffinity binding substances” to mean “molecules that have affinity for and ‘bind’ to each other under certain conditions, referred to as ‘binding conditions,’ to form a ‘specific binding pairs.’” *Id.* This defines certain terms in the patent, and in my opinion these definitions do not disclose that the complex in Grunenwald comprises “an antibody specific to a protein that is part of said chromatin.”

160. Third, Grunenwald teaches that in some embodiments, an antibody may act as a “detectable molecule.” *Id.* at [0212]. In these embodiments, “a library of labeled amplified tagged DNA fragments is generated by incorporation of one or more modified dNTPs that are labeled with an affinity-binding molecule or a detectable moiety.” *Id.* This label allows the fragments “to be detected, either directly, or indirectly following labeling with any other detectable molecule or combination of molecules known in the art,” including “streptavidin, an antibody”. *Id.* This again does not disclose an insertional enzyme complex comprising an antibody that binds to a protein component of chromatin. Here, the disclosed

antibody plays no role in the insertional enzyme complex. Rather, the antibody is used as a detection tool for labeled fragments. In these embodiments, the target DNA is labeled with a moiety that has an affinity for a secondary molecule, which may be an antibody. *Id.* An antibody containing a detectable molecule may then be used to bind to that moiety and allow detection. *Id.*

161. Fourth, Grunenwald discloses that the method labels tagged DNA fragments, this time with an amplification reaction. *Id.* at [0216]. Here, again, an antibody is used for label detection. *Id.* at [0216]; [0217]. This process is identical to the process listed above for detection with an antibody.

162. Fifth, Grunenwald discloses that “[i]n some other embodiments, the tagged DNA fragments or amplified tagged DNA fragments prepared using a method of the invention are labeled by incorporation of one or more modified dNTPs that has an affinity-binding molecule or a detectable moiety during the amplification reaction”. *Id.* at [0217]. These moieties may be “detected, either directly, or indirectly following labeling with any other detectable molecule or combination of molecules known in the art . . . that is linked to an affinity binding molecule (e.g., as streptavidin, an antibody).” *Id.*

163. As I’ve stated above, this is yet another case in which an antibody does not play a role in the insertional enzyme complex . Rather, the antibody is used to allow for detection of a labeled target DNA.

164. Sixth, Grunenwald discloses an embodiment further comprising the step of:

providing a solid surface that is covalently or non-covalently coated with an affinity binding substance that is capable of specifically binding and forming a specific binding pair with the affinity molecule (e.g., streptavidin or avidin for binding biotin, or *an antibody* for binding digoxigenin); and, either prior to or following the step in which it is involved, contacting the products generated using the transferred transposon end oligonucleotide, the ligation tagging oligonucleotide, or the PCR primer that is chemically joined to the affinity molecule under conditions and for sufficient time wherein it binds to affinity binding substance that is joined to the solid surface.

Id. at [0226].

165. The disclosure above does not disclose an insertional enzyme complex comprising an antibody because the antibody is used as a capture tool and not part of the insertional enzyme complex. Namely, this disclosure has the antibody present on a solid surface, and the target DNA is tagged in such a way to enable the antibody to capture it when contacted. This is done to “permit manipulation (e.g., capture and washing to remove from other molecules in a reaction mixture), isolation, and capture of the transferred transposon end oligonucleotide, the ligation tagging oligonucleotide, or the PCR primer that is chemically joined to the affinity molecule.” *Id.* at [0227].

166. Seventh, Grunenwald discloses that in “some preferred embodiments, the affinity molecule is biotin and the affinity binding substance is avidin or

streptavidin, or wherein the affinity molecule is digoxigenin and the affinity binding substance is *an antibody* that specifically binds digoxigenin.” *Id.* at [0229].

167. This disclosure provides a definition linked to the solid surface embodiment stated above. Grunenwald further defines that the moiety added to the target DNA through a capture tag may be a dioxigenin molecule, and that on the solid surface antibodies are used to specifically bind dioxigenin. This would be performed for the reasons disclosed above, e.g. capture and washing or purification.

168. As explained above, while Grunenwald refers to an “antibody” seven times, none of such reference discloses an insertional enzyme complex comprising an antibody specific to a protein that is part of the target DNA (e.g., chromatin).

169. Because Grunenwald discloses a complex that does not comprise an antibody, and because none of the seven references to antibody in Grunenwald disclose a complex comprising an antibody specific to a protein that is part of the target DNA, it is my conclusion that Grunenwald does disclose the limitation “wherein said insertional enzyme complex does not comprise an antibody specific to a protein that is part of said chromatin.”

- iv. **[1D] performing a nucleic acid assay on said tagged nucleic acid molecules or derivatives thereof, to provide sequence information of said tagged nucleic acid molecules or derivatives thereof.**

170. It is my opinion that Grunenwald discloses “performing a nucleic acid assay on said tagged nucleic acid molecules or derivatives thereof, to provide sequence information of said tagged nucleic acid molecules or derivatives thereof.”

171. Grunenwald discloses “the step of amplifying and/or *sequencing* the library of the *tagged circular ssDNA fragments*.” EX1003 at [0037], [0312] (disclosing using tagged DNA fragments as “templates in a DNA sequencing method or an amplification reaction.”). The tagged circular ssDNA fragments are produced according to the methods discussed in Section XI.A.a.b above. Further, sequencing is a type of nucleic acid assay. Therefore, Grunenwald discloses the “performing a nucleic acid assay on said tagged nucleic acid molecules or derivatives thereof” limitation.

172. Grunenwald additionally discloses the addition of nucleic acid tags that include sequences needed to carry out DNA sequencing, including those selected from a list comprising “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]. These short nucleic acid fragments that facilitate sequencing are attached through the methods discussed in

Section XI.A.a.d above. A POSA would understand that the addition of such a fragment to facilitate sequencing would be for the purpose of sequencing the tagged DNA.

173. Moreover, Grunenwald explains that the purpose of sequencing the tagged DNA is to “determine the sequence of the target DNA.” *Id.* at [0312]. Also, as I explained in Section VII.C above, a POSA would have understood that sequencing is a method for determining sequence information of nucleic acid molecules. Therefore, Grunenwald discloses the limitation “to provide sequence information of said tagged nucleic acid molecules or derivatives thereof” limitation.

b) Claim 3: The method of claim 1, wherein said biological sample is a cell or a nucleus.

174. It is my opinion that Grunenwald discloses the method of claim 1, wherein the biological sample is a cell or a nucleus.

175. As I explained in Section XI.A.a, Grunenwald disclose analyzing one or multiple cells which constitutes the claimed “biological sample.” The target DNA for a transposition reaction may be derived from sources such as “*one or multiple cells*, tissues, organs, or organisms, whether living or dead, or from any biological or environmental source (e.g., water, air, soil).” *Id.* at [0146].

176. Moreover, as I discuss above, Grunenwald teaches that the method of analysis may occur in vivo by “electroporating a synaptic complex of a transposase and a suitable transposon end composition into the host cell.” *Id.* at [0157].

- c) **Claim 5: The method of claim 3, further comprising permeabilizing said cell or said nucleus to permit said insertional enzyme complex to access said chromatin.**

177. It is my opinion that Grunenwald discloses the method of claim 3, further comprising permeabilizing said cell or said nucleus to permit said insertional enzyme complex to access said chromatin.

178. As I discussed in connection with of claims 1 and 3, Grunenwald discloses the use of electroporation to carry out the transposition fragmenting and tagging reaction *in vivo* in a live cell. *See supra* ¶ 67. As I discuss above, electroporation is a type of permeabilization method.

- d) **Claim 6: The method of claim 5, wherein said permeabilizing is configured to minimally perturb said chromatin of said cell or said nucleus.**

179. It is my opinion that Grunenwald teaches the method of claim 5, wherein said permeabilizing is configured to minimally perturb said chromatin of said cell or said nucleus.

180. Electroporation is a well-known and commonly used method of permeabilization in the art. It was commonly performed on cells *in vivo* to allow analysis of chromatin, and a POSA would have understood electroporation as a method that minimally perturbs the chromatin in a cell or nucleus.

181. Particularly, the method of electroporation disclosed in Grunenwald is configured to minimally perturb the chromatin of the cell. Grunenwald discloses a

method of electroporation described in U.S. Patent No. 6,159,736 (“the ’736 Patent”). EX1003 at [0157]. Further, the ’736 Patent incorporates by reference a protocol taught by Dower, et al. (“the Dower protocol”). EX1045 at 7:13-17. For the purposes of my analysis, I treat the Dower protocol as though it were disclosed in full in the Grunenwald publication.

182. The Dower protocol teaches a method for transformation of *E. coli* bacteria. This refers to the process of moving DNA into the bacterial cell by permeabilizing the cell and allowing uptake of external DNA. *See, e.g.*, Dower at 6138 (EX1011.012).

183. The Dower protocol begins with cells that were suspended in a liquid that permits electroporation at high voltages. *Id.* at .002, .012.

184. The cells are then exposed to a pulse that is set at particular parameters to allow for holes to form in the cell membrane, which permits external DNA to enter the cell and thus “transform” the bacterial cell. *Id.* at .012.

185. Dower indicates that the taught protocol is successful, stating that transformation to “extremely high levels” is possible with electroporation. *Id.* at .014. Based on such success, a POSA would be aware that this would be configured to minimally perturb the chromatin of the cell, as the DNA in the protocol suffers few to no adverse effects while successfully transforming the cell.

186. This success can be quantified using a number called the “transformation efficiency.” Transformation efficiency is a quantity defined as a colony forming units/microgram of plasmid DNA added. EX1011.003. In other words, it is a ratio of how many countable colonies of bacteria are able to survive after transformation per a single microgram of the DNA added to transform.

187. The Dower protocol discloses an extremely high transformation efficiency at $2 \text{ to } 3 \times 10^9$ /microgram – in other words, for every microgram of DNA added, there were two to three billion surviving colony forming units. EX 1011.007.

188. Due to the success of the Dower protocol in transforming cells while they remain alive, it is my opinion that the Dower method is configured to minimally perturb the chromatin of the cell or nucleus.

e) **Claim 10: The method of claim 1, wherein said insertional enzyme comprises a transposase.**

189. It is my opinion that Grunenwald discloses the method of claim 1, wherein said insertional enzyme comprises a transposase.

190. The '995 Patent defines an insertional enzyme as “any enzyme capable of inserting a nucleic acid sequence into a polynucleotide.” EX1001 at 15:56-57. The '995 Patent further states that “[e]xamples of insertional enzymes include, but are not limited to, *transposases*, HERMES, and HIV integrase.” *Id.* at 15:61-63. I have applied this definition to my analysis below.

191. Grunenwald defines a transposase as an enzyme capable of “catalyzing insertion or transposition of the transposon end-containing composition into the double-stranded target DNA with which it is incubated.” EX1003 at [0093]. This definition is consistent with a POSA’s interpretation of a transposase, and I have applied this definition to my analysis below.

192. Grunenwald teaches “incubating the target DNA in an in vitro transposition reaction with at least one transposase and a transposon end composition” for “generating a library of tagged DNA fragments from target DNA.” *Id.* at [0162]. Thus, the transposase taught by Grunenwald performs the same function as the transposase taught by the ’995 Patent.

193. Because Grunenwald discloses the use of a transposase for the purpose of tagging and fragmentation consistent with the definition in the ’995 Patent, I have concluded that Grunenwald discloses this element.

f) Claim 11: The method of claim 10, wherein said transposase is a Tn5 transposase or derived from a Tn5 transposase.

194. It is my opinion that Grunenwald discloses the method of claim 10, wherein said transposase is a Tn5 transposase or derived from a Tn5 transposase.

195. As I discuss above in my conclusions regarding claim 10, Grunenwald teaches the use of a transposase as an insertional enzyme. Moreover, in embodiments where the insertion of a transposon end occurs *in vivo*, Grunenwald additionally discloses that “[t]his transposition method is exemplified by employing

a transposition complex formed by a *hyperactive Tn5 transposase and a suitable Tn5-type transposon end composition.*” *Id.* at [0157].

g) Claim 12: The method of claim 11, wherein said transposase is a hyperactive Tn5 transposase.

196. It is my opinion that Grunenwald discloses the method of claim 11, wherein said transposase is a hyperactive Tn5 transposase.

197. Grunenwald discloses specifically using a hyperactive Tn5 transposase. *Id.* (“[T]his transposition method is exemplified by employing a transposition complex formed by a *hyperactive Tn5* transposase and a suitable Tn5-type transposon end composition.”).

h) Claim 13: The method of claim 1, wherein said insertional enzyme complex comprises a first nucleic acid insert element comprising a first adapter sequence.

198. It is my opinion that Grunenwald discloses the method of claim 1, wherein said insertional enzyme complex comprises a first nucleic acid insert element comprising a first adapter sequence.

199. First, Grunenwald discloses the limitation “wherein said insertional enzyme complex comprises a first nucleic acid insert element.” In coming to this conclusion, I understand that the ’995 Patent discloses that a “nucleic acid insert element” is a “nucleic acid comprising a predetermined sequence.” EX1001 at 3:61-63.

200. 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].

201. 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].

202. 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].

203. 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].

204. Based on the definitions in the '995 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.

205. Second, Grunenwald discloses the limitation “wherein said insertional enzyme complex comprises *a first nucleic acid insert element comprising a first adapter sequence.*” Grunenwald discloses that in some embodiments, the transposon end composition comprises “one or more other tag portions or *tag domains.*” EX1003 at [0102].

206. Grunenwald defines a “tag domain” as a “portion or domain of a tag that exhibits a sequence for a desired intended purpose or application.” *Id.* at [0103]. As I describe above, an adapter sequence is a short sequence of DNA added to fragments for a specific purpose. *See supra* Section X.

207. Because Grunenwald’s tag domain is both a nucleic acid insert element and a short sequence added for a specific purpose, it is my opinion that the disclosure of tag domains discloses “a first nucleic acid insert element comprising a first adapter sequence.”

- i) **Claim 14: The method of claim 13, wherein said first adapter sequence comprises a first nucleic acid insert element comprising a first sequencing adapter sequence.**

208. It is my opinion that Grunenwald discloses the method of claim 13, wherein said first adapter sequence comprises a first nucleic acid insert element comprising a first sequencing adapter sequence.

209. The ’995 Patent does not explicitly define a “sequencing adapter sequence.” A POSA would have understood a “sequencing adapter sequence” to

refer to an adapter sequence suited to facilitate sequencing of the tagged DNA fragment. I will be applying that definition to my analysis.

210. Grunenwald discloses that one type of tag domain is a “sequencing tag domain.” EX1003 at [0110]. Grunenwald defines a “sequencing tag domain” as a domain “that exhibits a sequence for the purposes of facilitating sequencing of the ssDNA fragment to which the tag is joined.” *Id.* Therefore, a POSA would recognize that Grunenwald’s sequencing tag domain is a “sequencing adapter sequence,” as recited by claim 14.

j) **Claim 15: The method of claim 13, wherein said first adapter sequence comprises a barcode sequence.**

211. It is my conclusion that Grunenwald discloses the method of claim 13, wherein said first adapter sequence comprises a barcode sequence.

212. As I noted above in discussing claim construction, a barcode sequence is a “unique sequence of nucleotides” that may be used to identify the source of a polynucleotide in a reaction. EX1001 at 12:26-32; *see supra* Section X.

213. Grunenwald discloses the use of an “address tag domain,” which is defined as a tag domain that “exhibits a sequence that permits identification of a specific sample (e.g., wherein the transferred strand has a different address tag domain that exhibits a different sequence for each sample).” EX1003 at [0113].

214. Because the address tag domain is a unique sequence that enables the identification of a specific sample, i.e. the source of a target DNA, it is my opinion that the disclosure of an address tag domain anticipates this claim.

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

215. It is my conclusion that Grunenwald discloses the method of claim 13, wherein said first adapter sequence comprises a first primer sequence.

216. The '995 Patent does not define a “primer sequence.” A POSA would have understood a “primer sequence” to refer to a sequence that facilitates nucleic acid synthesis by acting as a binding site for a DNA or RNA polymerase enzyme. *See, e.g.*, EX1021.003. I will apply that definition to my analysis below.

217. Grunenwald discloses the use of an “amplification tag domain,” which “exhibits a sequence for the purpose of facilitating amplification of a nucleic acid to which said tag is appended.” EX1003 at [0111]. In particular, the amplification tag domain “provides a priming site for a nucleic acid amplification reaction using a DNA polymerase.” *Id.*

218. Because Grunenwald’s amplification tag domain is a binding site for a DNA polymerase, it is my opinion that the amplification tag domain is an adapter sequence that comprises a first primer sequence.

- l) **Claim 17: The method of claim 13, wherein said insertional enzyme complex further comprises a second nucleic acid insert element comprising a second adapter sequence.**

219. It is my conclusion that Grunenwald discloses the method of claim 13, wherein said insertional enzyme complex further comprises a second nucleic acid insert element comprising a second adapter sequence.

220. I incorporate my analysis of claim 13 in its entirety regarding Grunenwald's disclosures of an insertional enzyme complex comprising a nucleic acid insert element. *See supra* Section XI.A.h.

221. As I explained in Section XI.A.h, *supra*, Grunenwald discloses a tag domain, which constitutes a recited "nucleic acid insert element." I further note that as I explained above, it is my understanding that a nucleic acid insert element does not need to be on separate molecules; rather, each tag domain (i.e., each predetermined sequence) is a nucleic acid insert element itself. Therefore, multiple tag domains on a single tag on a single transferred strand would comprise multiple nucleic acid insert elements.

222. In disclosing the addition of tag domains in a tag sequence, Grunenwald further discloses that "in addition to the transferred transposon end sequence, the tag can have *one or more other tag portions or tag domains.*" EX1003 at [0102].

223. As I've noted, in embodiments described by Grunenwald with at least two tag domains, it is my opinion that a second tag domain would comprise a second nucleic acid insert element comprising a second adapter sequence.

- m) **Claim 18: The method of claim 17, wherein said second adapter sequence comprises a second sequencing adapter sequence.**

224. As explained above, Grunenwald discloses this limitation. *See supra* Section XI.A.i.

- n) **Claim 19: The method of claim 17, wherein said second adapter sequence comprises a barcode sequence.**

225. As explained above, Grunenwald discloses this limitation. *See supra* Section XI.A.j.

- o) **Claim 20: The method of claim 17, wherein said second adapter sequence comprises a second primer sequence.**

226. As explained above, Grunenwald discloses this limitation. *See supra* Section XI.A.k.

- p) **Claim 21: The method of claim 1, further comprising subjecting said tagged nucleic acid molecules to one or more reactions**

227. It is my opinion that Grunenwald discloses the method of claim 1, further comprising subjecting said tagged nucleic acid molecules to one or more reactions.

228. I first note that as I discuss above, Grunenwald discloses tagged nucleic acid molecules. *See supra* Section XI.A.a.b.

229. Further, Grunenwald discloses subjecting the tagged nucleic acid molecules to PCR, which is a reaction that leads to nucleic acid amplification. In particular, Grunenwald teaches “amplifying one or more 5’ tagged target DNA fragments and/or di-tagged target DNA fragments,” and that “the amplifying comprises use of one or more of a PCR amplification reaction, a strand-displacement amplification reaction, a rolling circle amplification reaction, a ligase chain reaction, a transcription-mediated amplification reaction, or a loop-mediated amplification reaction.” EX1003 at [0022]; *see also id.* at [0123].

- q) **Claim 22: The method of claim 21, wherein said one or more reactions comprise a nucleic acid amplification reaction.**

230. It is my opinion that Grunenwald discloses the method of claim 21, wherein said one or more reactions comprise a nucleic acid amplification reaction.

231. As I describe above, Grunenwald teaches subjecting tagged nucleic acid molecules to PCR, which is a nucleic acid amplification reaction. *See supra* Section X.A.p.

- r) **Claim 23: The method of claim 22, wherein said nucleic acid amplification reaction is configured to add one or more functional sequences to said tagged nucleic acid molecules or derivatives thereof, wherein said one or more functional sequences are compatible with a selected next generation sequencing platform.**

232. It is my opinion that Grunenwald teaches the method of claim 22, wherein said nucleic acid amplification reaction is configured to add one or more

functional sequences to said tagged nucleic acid molecules or derivatives thereof, wherein said one or more functional sequences are compatible with a selected next generation sequencing platform.

233. As I describe above, Grunenwald discloses both tagged nucleic molecules and a nucleic acid amplification reaction. *See supra* Section X.a.p.

234. Further, Grunenwald teaches that the method of generating tagged DNA fragments to create a library may include “amplifying the library of tagged DNA fragments comprising di-tagged DNA fragments, tagged circular ssDNA fragments, or fantail DNA fragments.” EX1003 at [0198].

235. When amplifying, the 5’ portions of the “first PCR primer” or the “second PCR primer” may comprise or consist of “first or second sequencing tags, respectively, for generation of templates for next-generation sequencing for a particular sequencing platform.” *Id.* at [0201].

236. Additionally, Grunewald discloses that the one or more functional sequences are “compatible with a selected next generation sequencing platform.” Particularly, examples of sequencing tag domains taught in Grunenwald 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].

237. The platforms listed in the exemplary sequencing tag domains, such as Roche, ILLUMINA™ SOLEXA™, Applied Biosystems' SOLID™, etc. would have all been known next generation sequencing platforms as of the critical date of the '995 Patent.

238. These sequences, when added to a DNA fragment, render the sample compatible with a selected next generation sequencing platform.

B. Ground 2: Claim 2 Is Rendered Obvious By The Combination Of Grunenwald With O'Geen

- a) **Claim 2: The method of claim 1, further comprising generating a representation of epigenetic features of said genome region at least in part by mapping said sequence information to said genome region.**

239. It is my opinion that Claim 2 is rendered obvious by the combination of Grunenwald with O'Geen.

240. I begin by describing the methods of O'Geen that permit generating a representation of epigenetic features by mapping sequence information to a genome region.

241. O'Geen discloses the technique of ChIP-seq, short for chromatin immunoprecipitation and sequencing. This technique broadly involves three steps: binding the proteins found in chromatin to the DNA for fragmenting; capturing the protein-DNA complexes using antibodies specific to the protein of interest; and

purifying the DNA and analyzing it to determine the sequence. EX1005.005-.006.

I walk through the procedure O'Geen teaches in greater detail below.

242. First, proteins found in chromatin (such as histones and transcription factors) are bound to the genomic DNA surrounding them while in the living cell. This step allows a scientist to capture a snapshot of protein-DNA interactions at the moment that the proteins are bound. *Id.* at .005, .009.

243. Second, chromatin is prepared through fragmentation. The chromatin is fragmented by the use of sonication, or sound waves, to break it apart into smaller pieces. *Id.* at .010.

244. Third, chromatin size and concentration is determined. This is done by collecting a small amount of the sample, unbinding the DNA and proteins, and purifying the DNA. The purified DNA can then be run on a gel to determine the average size. If it is too large, the sonication step may be repeated until the fragments are of an appropriate size. *Id.* at .010-.011.

245. Fourth, chromatin immunoprecipitation is conducted. This is done by using the DNA fragments produced in the sonication step and contacting them with an antibody specific to a protein of interest, such as a particular histone or a particular transcription factor. This mixture is incubated for several hours to allow the antibodies to successfully pull the chromatin out of solution and solidify. *Id.* at .011.

246. Fifth, the newly created complexes of antibodies with the chromatin are captured and unbound. This is done by incubating the mixture with magnetic beads and using magnetism to separate the captured chromatin, followed by allowing the mixture to incubate in a hot water bath overnight to unbind proteins and DNA. *Id.* at .011-.012.

247. Sixth, DNA is purified from the mixture and the chromatin immunoprecipitation is confirmed before proceeding with sequencing. This is done to ensure that the DNA is prepared for a sequencing analysis downstream. *Id.*

248. Seventh, the DNA fragments are prepared for sequencing according to the protocol of the sequencing platform being used, such as an Illumina sequencing platform. *Id.* at .013-.017.

249. Finally, after sequencing is performed, target sites are read based on the data collected and mapped to epigenetic features of the cell. *Id.* at .017-.018.

250. O'Geen's aforementioned method is an example of a method that would have been well-known in the art. However, it was not without limitations. O'Geen, for example, notes that the "biggest limitations to histone ChIP assays are the specificity of the antibody and the variability between different lot numbers of the same antibody." *Id.* at .023.

251. Given knowledge of fragmenting and sequencing DNA to generate a map of epigenetic features, and the known limitations of the ChIP-seq method taught

in O'Geen, a POSA would have been motivated to use the method of tagmentation taught by Grunenwald to generate an epigenetic map. Additionally, a POSA would have had a reasonable expectation of success in applying Grunenwald's tagmentation to the ChIP-seq method to generate a representation of epigenetic features.

252. I begin by noting that both the method taught in Grunenwald and the method taught in O'Geen are practiced on chromatin. *See supra* Section XI.A.a. A POSA, aware of both, would have been aware of the need to efficiently fragment and tag chromatin for the purpose of epigenetic analysis.

253. Further, a POSA would have been aware of the limitations of O'Geen's method, particularly limitations of using an antibody specific to a chromatin protein. *See, e.g.*, EX1005.020. Knowing those limitations, a POSA would have been motivated to overcome them. Grunenwald discloses a method of overcoming those limitations through the use of tagmentation with a transposase, as opposed to the binding, fragmenting, and unbinding taught in O'Geen. *See supra* Section XI.A.a.

254. Importantly, after fragmenting is completed, both methods teach sequencing as a step to determine sequence information. The use of sequencing to subsequently determine a map of epigenetic features by mapping DNA-protein interactions was well-known in the art as early as 2007. *See, e.g.* EX1022.004-.005; EX1005.017-.018.

255. Thus, a POSA would have been motivated to find a method of fragmenting and tagging that did not have the antibody limitations highlighted in O’Geen. Grunenwald, disclosing tagmentation on chromatin and subsequently sequencing the fragments, would have provided such a method. A POSA would have combined the tagmentation disclosure in Grunenwald with the disclosure of sequencing to produce an epigenetic map of the genome in O’Geen.

256. Further, a POSA would have had a reasonable likelihood of success in doing so. As I note above, the problem presented by ChIP-seq is the limitations that arise from the use of antibodies for immunoprecipitation. The use of an alternative method of fragmenting and tagging through the use of tagmentation that still allowed for sequencing the fragments would be successful in permitting a POSA to build a map of epigenetic features by mapping the sequence information to genome regions.

C. Ground 3: Claims 4 And 6-8 Are Rendered Obvious By The Combination Of Grunenwald With Okino

a) Claim 4: The method of claim 3, further comprising lysing said cell or nucleus

257. As I discuss above, it is my opinion that Grunenwald anticipates claim 3. *See supra* Section XI.A.b. Further, it is my opinion that claim 4 is rendered obvious by the combination of Grunenwald and Okino. Specifically, Okino discloses the use of a cell as a biological sample to study chromatin structure, and discloses a lysis step as a part of the analysis.

258. I begin with a brief summary of Okino's disclosures. Okino is an international patent application published in 2010 that is directed to detecting the structure of chromatin. This is done broadly through two steps: first, permeabilizing a cell and simultaneously contacting it with a "DNA modifying agent," which is a protein or chemical compound that is capable of altering DNA by cutting it or adding a detectable molecule to do it; second, by detecting a physical characteristic of the DNA through a variety of methods, including sequencing. EX1004 at [0005].

259. The first step of this reaction involves simultaneously permeabilizing a cell and contacting the cell with a "DNA modifying agent." *Id.* at [0066]. The extent of the modification is then measured as a reflection to how accessible the chromatin is. *Id.* at [0064]. These modifications may include with a restriction enzyme to cut the DNA at a specific site, a DNase to cut the DNA in any random site, a methyltransferase to add a methyl group to the DNA, or a chemical alteration to cleave or add a methyl group. *Id.* at [0079], [0087], [0092], [0099].

260. When this step involves the use of a restriction enzyme, the modification is the introduction of a site-specific nick or double-stranded cleavage. *Id.* at [0079]. A restriction enzyme is a type of protein that is responsible for cutting DNA based on a specific sequence of nucleotides that it can recognize as a "cut site." *See, e.g.*, EX1021.004.

261. In this method, a restriction enzyme may be added after or simultaneously to the addition of a reagent that permeabilizes a cell, allowing the restriction enzyme access to chromatin. EX1004 at [0084].

262. Okino further discloses that the digestion may be stopped and the cell may be lysed. *Id.* at [0086]. This is done as an initial step in isolating DNA following DNA modification. *Id.* at [0105].

263. After DNA is modified and isolated, the physical characteristic that was modified is detected. *Id.* at [0109]. These characteristics may include “DNA methylation, melting temperature, GC content, nucleotide sequence, and ability to hybridize to a polynucleotide.” *Id.*

264. As I stated earlier, it is my opinion that the claim limitation of “lysing said cell or nucleus” is obvious under the combination of Grunenwald and Okino. Specifically, a POSA would have been motivated to combine the two references with a reasonable likelihood of success.

265. 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.

266. Further, 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.

267. As I mention above, Grunenwald particularly discloses an *in vivo* embodiment of its method, which practices tagmentation on chromatin in a living cell. *See supra* Section XI.A.a; EX1003 at [0157].

268. In such an embodiment, the DNA must be extracted from the cell after the tagmentation process to be further analyzed. A POSA would have been aware of different methods of extraction and isolation of the fragmented DNA.

269. The process of lysing the cell to isolate treated DNA would have been well-known in the art, and a POSA would have been aware of lysing a cell as a step to isolating the DNA found in the cell. *See, e.g.*, EX1015.003.

270. Therefore, a POSA, aware of both Okino's disclosure of DNA modification followed by lysing the cell for extraction and Grunenwald's disclosure of tagmentation would have been motivated to lyse the cell to extract the DNA. Further, because lysing is a well-known technique in the art and because it was disclosed in Okino as a method to extract and isolate DNA after modification, a POSA would have had a reasonable likelihood of success.

b) **Claim 6: The method of claim 5, wherein said permeabilizing is configured to minimally perturb said chromatin of said cell or said nucleus.**

271. In my opinion, Claim 5 is anticipated by Grunenwald. *See supra* Section XI.A.c. As I state above, it is also my opinion that Claim 6, which recites that the permeabilizing is configured to minimally perturb said chromatin of said cell or nucleus, is anticipated by Grunenwald. *See supra* Section XI.A.d.

272. However, to the extent that the Patent Owner contends that Grunenwald does not anticipate Claim 6, it is my opinion that it is rendered obvious by the combination of Grunenwald with Okino.

273. I begin by noting that Okino also teaches electroporation as a method to permeabilize a cell membrane; specifically, Okino discloses that electroporation “can be used to permeabilize a cell membrane such that a DNA modifying agent is introduced into the cell and can thus contact the genomic DNA.” EX1004 at [0075].

274. Further, Okino specifically contemplates that because the methods taught contact genomic DNA prior to its isolation from a cell, “methods of permeabilizing or disrupting the cell membrane will not disrupt the structure of the genomic DNA of the cell such that nucleosomal or chromatin structure is destroyed.” *Id.* at [0071].

275. A POSA familiar with Okino would recognize that Okino was concerned with minimally perturbing the chromatin of the cell because of the *in vivo*

nature of Okino's method, as disclosed explicitly by Okino in the paragraph cited above.

276. A POSA, with knowledge of the disclosures of both Okino and Grunenwald, would further be aware that electroporation is a method of permeabilizing a cell membrane that does "not disrupt the structure of the genomic DNA of the cell," thus minimally perturbing the chromatin. *See* EX1004 at [0071].

277. Therefore, a POSA would have knowledge that electroporation is configured to minimally perturb said chromatin of said cell or said nucleus.

- c) **Claim 7: The method of claim 5, wherein said cell or said nucleus is permeabilized with a reagent configured to permit said insertional enzyme complex to access said chromatin.**

278. As I state above, it is my opinion that Grunenwald anticipates Claim 5 of the '995 Patent. *See supra* Section XI.A.c. Further, it is my opinion that Claim 7 is rendered obvious by the combination of Grunenwald with Okino.

279. I begin with an overview of Okino's disclosure of permeabilization techniques. I incorporate my discussion of the method disclosed in Okino above. As I stated, Okino teaches a method that involves simultaneously permeabilizing the cell and delivering a DNA modifying or cleaving agent. *See supra* Section IX.B; EX1004 at [0074].

280. In addition to electroporation, Okino teaches that the cell membrane can be contacted with an agent that permeabilizes the cell membrane. Specifically,

it discloses two types of permeabilizing agents, lysolipids and non ionic detergents. EX1004 at [0072], [0073].

281. Okino discloses the “simultaneous delivery of the permeabilization agent and the DNA cleaving or DNA modifying agent” with a “buffer comprising both agents” contacting a cell. Okino specifically teaches that the “buffer should be adapted for maintaining activity of both agents while maintaining the structure of the cellular chromatin.” *Id.* at [0074].

282. A POSA would have had the motivation to combine the disclosures of Grunenwald and Okino. First, as I note above, both references are directed to methods of analyzing chromatin structure. A POSA aware of both would have considered the combination in studying chromatin, particularly when that study was conducted *in vivo*. *See supra* Section XI.C.a.

283. Further, Grunenwald discloses that to transmit transposase *in vivo*, electroporation may be used. EX1003 at [0157]. Okino presents electroporation as an alternative to the use of an agent. EX1004 at [0075].

284. There exist only a finite set of methods to permeabilize in the art. *See supra* Section VII.E. A POSA familiar with the various methods of permeabilization, including by electroporation and agent, would recognize that the decision to use one or the other is merely a design choice related to optimization of a protocol to specific applications.

285. Therefore, a POSA would have had the motivation to combine by knowing the disclosures of both references and would have had a reasonable likelihood of success in using an agent as an alternative to electroporation.

d) **Claim 8: The method of claim 7, wherein said reagent comprises NP40, digitonin, tween, streptolysin, or a cationic lipid.**

286. It is my opinion that the combination of Grunenwald with Okino renders Claim 8 obvious. Claim 8 further specifies the types of reagent used in the permeabilization recited in Claim 7.

287. I incorporate my analysis as to Claim 7's obviousness here. *See supra* Section XI.C.c.

288. I add that in addition to the disclosures of the use of a detergent generally, Okino provides an exemplary list of reagents that may permeabilize a cell membrane. This list includes "NP40, Tween 20, and Triton X-100." EX1004 at [0073].

289. For the reasons above, a POSA would have been motivated to combine Okino and Grunenwald due to the interchangeable nature of permeabilization by a reagent and permeabilization by electroporation, which Okino presents as alternatives.

290. Further, a POSA familiar with the disclosures in Okino would have been motivated to use the specific detergents that are listed.

291. Because Okino not only discloses the use of reagents broadly, as I discussed in my analysis of Claim 7 above, but specifically lists NP40 and Tween 20 as exemplary detergents, it is my opinion that this claim is rendered obvious by the combination of Grunenwald and Okino.

D. Ground 4: Claim 9 Is Rendered Obvious By The Combination Of Grunenwald and Pitt

a) The method of claim 5, wherein said cell or said nucleus is permeabilized by hypotonic shock or ultrasonication.

292. I first restate my earlier conclusion that Grunenwald anticipates claim 5 of the '995 Patent. *See supra* Section XI.A.c.

293. Particularly, Grunenwald requires permeabilization to perform the *in vivo* embodiment of the invention. *See supra* XI.A.c.

294. Further, it is my opinion that claim 9 is rendered obvious by the combination of Grunenwald with Pitt.

295. I will begin by discussing the relevant disclosures in Pitt. Pitt is a review that discusses ultrasonic delivery into cells, using ultrasound to permeabilize a cell and permit the delivery of various macromolecule types across the cell membrane. EX1006.001. This includes the delivery of proteins across the cell membrane. *Id.* at .010.

296. Pitt notes that “proteins have very different transport and solubility characteristics in tissues, and thus their delivery is usually much more complex.” *Id.*

Further, to solve this issue, ultrasonication may be used to permeabilize a cell and allow for the entry of proteins. Pitt teaches that as early as 1996, “low frequency ultrasound” was capable of “significant transport of protein.” *Id.*

297. Not only does Pitt disclose the transport of proteins generally, but it specifically discloses the transport of enzymes through ultrasonication. Pitt teaches the transport of tissue plasminogen activator (tPA), an enzyme, into clotting cells. EX1006.011. Particularly, Pitt teaches that “1 MHz ultrasound increased the rate of uptake and the depth of penetration of tPA into clots.” *Id.*

298. Based on the disclosures in Pitt and Grunenwald, I further conclude that it would have been obvious to a POSA to combine the teachings in the two references.

299. As an initial matter, I consider Pitt and Grunenwald to be analogous art. Both references are in the field of molecular biology broadly, and both are directed to the delivery of enzymes into cells to permit an enzyme to perform an activity from within the cell. *See, e.g.*, EX1003 at [0157] (“transposition into the target DNA is preferably achieved by electroporating a synaptic complex of a transposase and a suitable transposon end composition into the [] cell.”); EX1006.011 (“1 MHz ultrasound increased the rate of uptake and the depth of penetration of tPA into clots.”). Thus, a POSA would have been aware of both references.

300. Further, a POSA interested in practicing the *in vivo* embodiment in Grunenwald would have been aware of various methods of permeabilization. As I discuss above in both the background and my discussion of claim 7, the issue of permeabilization is a problem with a finite number of solutions, and the decision to use one solution or another would have been a design choice on the part of the skilled artisan. *See supra* Section VII.E, XI.C.c. Thus, a POSA would have been motivated to combine Grunenwald and Pitt by using the ultrasonication methods disclosed in Pitt to permeabilize a cell and practice the method taught in Grunenwald.

301. A POSA would have also had a reasonable likelihood of success when combining Pitt with Grunenwald. Particularly, as Pitt discloses a method for delivering an enzyme *in vivo*, a POSA would expect that such a method could successfully be used to deliver the insertional enzyme complex into the cell as taught by Grunenwald.

E. Ground 5: Claims 17-20 Are Rendered Obvious By The Combination of Grunenwald with Adey

- a) **Claim 17: The method of claim 13, wherein said insertional enzyme complex further comprises a second nucleic acid insert element comprising a second adapter sequence.**

302. I first restate my opinion that claim 17 is fully anticipated by Grunenwald. *See supra* Section XI.A.1.

303. To the extent that the Patent Owner contends that Grunenwald does not anticipate claim 17, it is my opinion that claim 17 is rendered obvious by the combination of Grunenwald and Adey.

304. It is my opinion that claim 13, from which claim 17 depends, is anticipated by Grunenwald. *See supra* Section XI.A.h.

305. Further, Adey discloses “wherein said insertional complex further comprises a second nucleic acid insert element comprising a second adapter sequence.”

306. Figure 1(b) of Adey, below, illustrates this disclosure. 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.

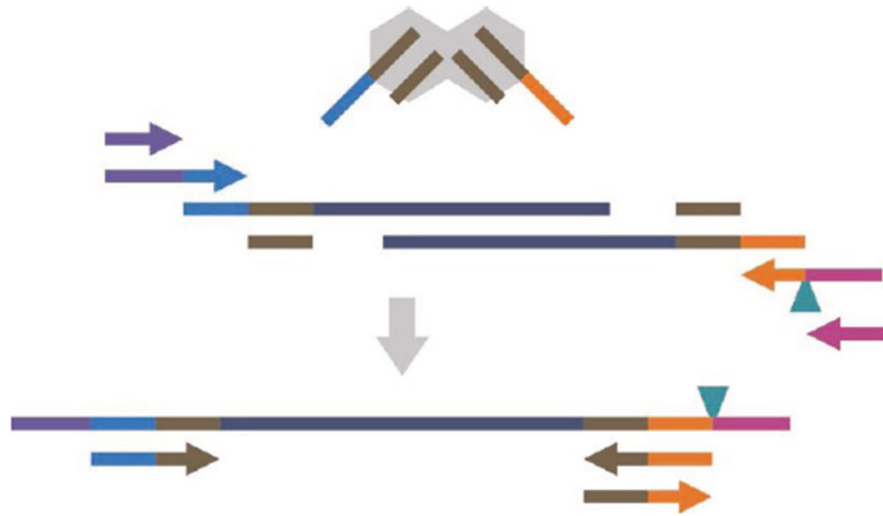


Figure 1(b), EX1009.002

307. 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.*, EX1009.012.

308. 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).

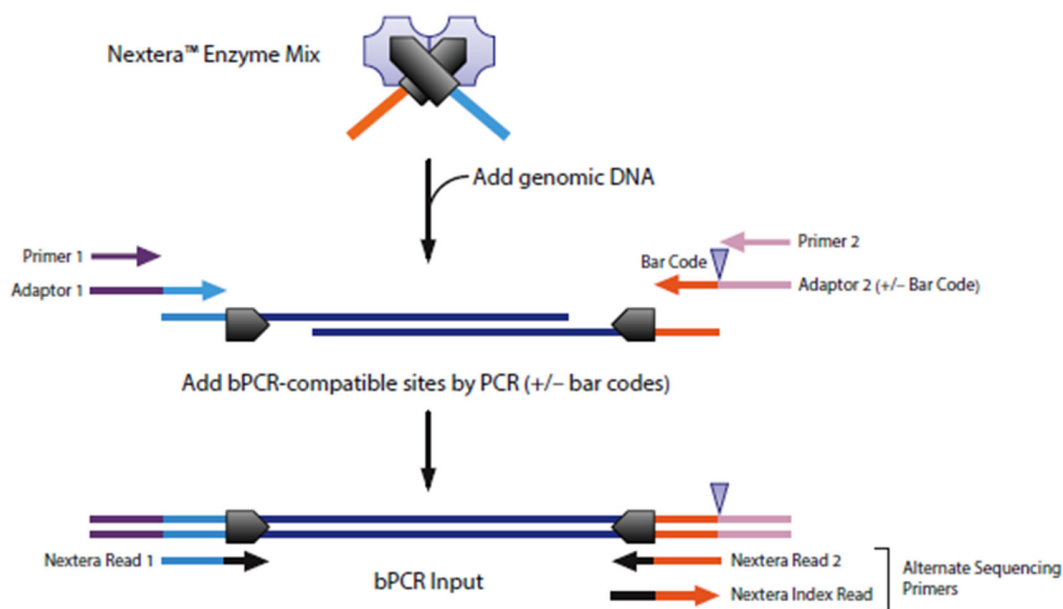


Figure 1, EX1048.002.

309. 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.*

310. 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.*

311. 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.

312. 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.

313. 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.* EX1009.001.

314. 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.

315. 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.*, EX1009.011. 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.

b) Claim 18: The method of claim 17, wherein said second adapter sequence comprises a second sequencing adapter sequence.

316. I first restate my opinion that claim 18 is fully anticipated by Grunenwald. *See supra* Section XI.A.m.

317. To the extent that the Patent Owner contends that Grunenwald does not anticipate claim 18, it is my opinion that claim 18 is rendered obvious by the combination of Grunenwald and Adey.

318. It is my opinion that claim 17, from which claim 18 depends, is anticipated by Grunenwald. *See supra* Section XI.A.

319. Further, Adey discloses “said second adapter sequence comprises a second sequencing adapter sequence.”

320. As I note above, the Nextera™ kit contains adaptors that have multiple types of sequences. Included among these are the Nextera Read 1 and 2 sequence, which would comprise a first and second sequencing adapter sequence. EX1048.002.

321. For the reasons I state above, it would be obvious to a POSA to combine the two references. *See supra* Section XI.E.a.

c) Claim 19: The method of claim 17, wherein said second adapter sequence comprises a barcode sequence.

322. I first restate my opinion that claim 19 is fully anticipated by Grunenwald. *See supra* Section XI.A.n.

323. To the extent that the Patent Owner contends that Grunenwald does not anticipate claim 19, it is my opinion that claim 19 is rendered obvious by the combination of Grunenwald and Adey.

324. It is my opinion that claim 17, from which claim 19 depends, is anticipated by Grunenwald. *See supra* Section XI.A.l.

325. Further, Adey discloses “said second adapter sequence comprises a barcode sequence.”

326. First, I note that Adey teaches a method of barcode design for integration into the Nextera™ adapter sequences, disclosing a barcode design that

yielded “a set of 96 x 9 bp sequences in which each 9 bp sequence contained no homopolymer run of three or more bases, had a GC content of $\leq 60\%$, was a edit distance of at least four away from all other members of the set of 96, and screened negative when compared with other adaptor and primer sequences used here.” EX1009.016.

327. Second, the Nextera™ kit manual points out that the kit allows for the incorporation of “platform-specific tags and optional barcodes.” EX1048.001.

328. Because the adapters disclosed in Adey may have barcodes added to them, Adey discloses that the second adapter sequences comprises a barcode sequence.

329. For the reasons I state above, it would have been obvious to a POSA to combine the two references. *See supra* Section XI.E.a.

d) Claim 20: The method of claim 17, wherein said second adapter sequence comprises a second primer sequence.

330. I first restate my opinion that claim 20 is fully anticipated by Grunenwald. *See supra* Section XI.A.o.

331. To the extent that the Patent Owner contends that Grunenwald does not anticipate claim 20, it is my opinion that claim 20 is rendered obvious by the combination of Grunenwald and Adey.

332. It is my opinion that claim 17, from which claim 20 depends, is anticipated by Grunenwald. *See supra* XI.A.1.

333. Further, Adey discloses “wherein said second adapter sequence comprises a second primer sequence.”

334. As I note above, the adapters in the Nextera™ kit used in Adey include a first and second primer sequence. EX1048.002. The second of these sequences would be a second adapter sequence that comprises a second primer sequence.

335. For the reasons I state above, it would have been obvious to a POSA to combine the two references. *See supra* Section XI.E.a.

F. Grounds 6-8: Claims 1-3, 5-6, and 10-23 Are Rendered Obvious By The Combination of Grunenwald and Okino

336. To the extent that the Patent Owner argues that claims 1-3, 5-6, and 10-23 are not anticipated by Grunenwald, it is further my opinion that those claims are rendered obvious by the combination of Grunenwald with Okino.

337. I first reiterate that electroporation, as a method of permeabilization, can be successfully used to contact the chromatin of the cell with an insertional enzyme complex. *See supra* Section XI.A.a, d.

338. Should Patent Owner contend that electroporation fails to permeabilize the cells in such a way as to contact the chromatin in the cell, a POSA would have been motivated to use the permeabilization techniques taught by Okino and had a reasonable expectation of success in using those techniques. My full analysis of this motivation and reasonable expectation of success is above in Section XI.C. By way of summary, a POSA would have been motivated to combine the references because

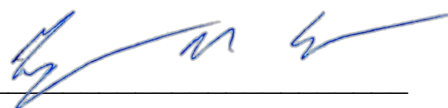
both references teach methods of chromatin analysis *in vivo* in a cell, and a choice of permeabilization technique is merely a design choice from a finite number of solutions.

339. For example, a POSA would have found it obvious to use a detergent to permeabilize the cell and contact the chromatin with an insertional enzyme complex to achieve tagmentation.

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: April 18, 2023

By: _____

Gregory Cooper, Ph.D.