

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Natera Inc.

Petitioner

v.

Illumina, Inc.

(record) Patent Owner

Case No. IPR2018-01317

U.S. Patent No. 9,493,831

DECLARATION OF MICHAEL L. METZKER, Ph.D.

TABLE OF CONTENTS

I.	INTRODUCTION.....	4
II.	MATERIALS REVIEWED	4
III.	SUMMARY OF OPINIONS	9
IV.	EXPERIENCE AND QUALIFICATIONS	10
V.	TECHNICAL BACKGROUND.....	16
A.	Introduction to the '831 Patent	16
B.	DNA Basics	20
C.	Amplification and detection	23
D.	Creating a cell-free DNA library from maternal for aneuploidy detection was well known in the art.....	32
E.	Use of indexed DNA library for multiplexed next generation sequencing was wide spread in the art	38
F.	By 2010, skilled artisans were focused on creating target-specific DNA libraries	45
VI.	BASIS OF MY OPINION	46
VII.	THE '831 PATENT	47
VIII.	RELEVANT LAW	48
A.	Anticipation	48
B.	Obviousness.....	48
IX.	LEVEL OF ORDINARY SKILL IN THE ART	52
X.	RELEVANT TIMEFRAME FOR DETERMINING OBVIOUSNESS	53
XI.	CLAIM CONSTRUCTION.....	53
A.	Claims 1, 6, 7, 14, 18 and 9—"Aneuploid"	53
I.	GROUNDS OF UNPATENTABILITY	53
Ground 1.	Claims 1-7, 10, 14-19, and 22 are obvious over Fluidigm in view of the knowledge of one of ordinary skill.....	53
Ground 2.	Claims 1-10, 13-22 and 24 are obvious as in Ground 1 in further view of Sequenom.	102
Ground 3.	Claim 12 is obvious as in Ground 1 in further view of Harismendy	110

Ground 4. Claims 12 are obvious as in Ground 2 in further view of	
Harismendy.....	116
XII. OATH.....	118

I, Michael L. Metzker, Ph.D., declare as follows:

I. INTRODUCTION

1. I, Michael L. Metzker, have been retained as an expert witness on behalf of Natera, Inc. (the “Petitioner”) in connection with the above-captioned *Inter Partes* review (“IPR”). I understand that the petition for IPR involves U.S. Patent No. 9,493,831 (the “’831 Patent,” (Ex. 1001)), which resulted from U.S. Application Ser. No. 14/677,854, (the “’854 Application”) filed on April 2, 2015, on behalf of Yue-Jen Chuu and Richard P. Rava.

II. MATERIALS REVIEWED

2. I have reviewed the materials cited herein, including the following exhibits. The materials are publications that were typically read and reasonably relied upon by persons of ordinary skill in this field, including the bibliographic information presented therein.

Exhibit No.	Description
1001	U.S. Patent No. 9,493,831 (“ the ’831 Patent ”).
1002	Declaration of Dr. Michael Metzker, Ph.D.
1003	U.S. Pat. App. Pub. US 2010/0120038 A1 (“ Fluidigm ”).
1004	U.S. Pat. App. Ser. No. 12/548,132 (“ Fluidigm App ”).
1005	WO 2009/032781 A2 (“ Sequenom ”).
1006	Fan, et al., <i>Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood</i> , Proc. Natl. Acad. Sci. USA, Vol. 105:16266–16271 (October 21, 2008)(“ Fan 2008 ”).

1007	Harismendy, et al., <i>Evaluation of next generation sequencing platforms for population targeted sequencing studies</i> , Genome Biology, Vol. 10:R32 (March 27, 2009)(“ Harismendy ”).
1008	Bentley, et al., <i>Accurate whole human genome sequencing using reversible terminator chemistry</i> , Nature, Vol. 456:53-59 (November 2008)(“ Bentley ”).
1009	Curriculum vitae of Dr. Michael Metzker, Ph.D.
1010	File History of U.S. Pat. App. Ser. No. 14/677,854.
1011	U.S. Pat. No. 8,318,430.
1012	Quail, et al., <i>A large genome center’s improvements to the Illumina sequencing system</i> , Nature Methods, Vol. 5:1005-1010 (Dec. 2008)(“ Quail ”).
1013	Chiu, et al., <i>Noninvasive Prenatal Diagnosis of Fetal Chromosomal Aneuploidy by Massively Parallel Genomic Sequencing of DNA in Maternal Plasma</i> , Proc. Natl. Acad. Sci. USA, Vol. 105:20458-20563 (Dec. 23, 2008)(“ Chiu ”).
1014	Illumina, Inc., <i>Multiplexed Sequencing with the Illumina Genome Analyzer System</i> ”, Illumina, 2008 (“ Illumina Datasheet ”).
1015	Declaration of Sylvia Hall-Ellis.
1016	Illumina, Inc., <i>Preparing Samples for Multiplexed Paired-End Sequencing</i> (December 2008)(“ Illumina Guide ”).
1017	IDS filing of March 11, 2013 in U.S. App. Ser. No. 13/792,661.
1018	Saiki et al., <i>Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia</i> , Science, Vol. 230:1350-1354 (Dec. 20, 1985)(“ Saiki 1985 ”).
1019	Saiki et al., <i>Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase</i> , Science, Vol. 239:487-491 (Jan. 29, 1988)(“ Saiki 1988 ”).

1020	Gibbs et al., <i>Detection of single DNA base differences by competitive oligonucleotide priming</i> , Nucleic Acids Research, Vol. 17:2437-2448 (Apr. 11, 1989)(“ Gibbs 1989A ”).
1021	Newton et al., <i>Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS)</i> , Nucleic Acids Research, Vol. 17:2503-2516 (1989)(“ Newton ”).
1022	Chamberlain et al., <i>Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification</i> , Nucleic Acids Research, Vol. 16:11141-11156 (1988)(“ Chamberlain ”).
1023	Gibbs et al., <i>Multiplex DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families</i> , Genomics, Vol. 7:235-244 (1990)(“ Gibbs 1990 ”).
1024	Grompe et al., <i>The rapid detection of unknown mutations in nucleic acids</i> , Nature Genetics, Vol. 5:111-117 (1993)(“ Grompe ”).
1025	Ferre et al., <i>Quantitative or semi-quantitative PCR: Reality versus myth</i> , Genome Research, Vol. 2:1-9 (1992)(“ Ferre ”).
1026	Heid et al., <i>Real time quantitative PCR</i> , Genome Research, Vol. 6:986-994 (1996)(“ Heid ”).
1027	Simmonds et al., <i>Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers</i> , Journal of Virology, Vol. 64:864-872 (1990)(“ Simmonds ”).
1028	Zhang et al., <i>Detection, quantification and sequencing of HIV-1 from the plasma of seropositive individuals and from factor VIII concentrates</i> , AIDS, Vol. 5:675-681 (1991)(“ Zhang ”).
1029	Lee et al., <i>Quantitative assessment of HIV-1 DNA load by coamplification of HIV-1 gag and HLA-DQ-α genes</i> , AIDS, Vol. 5:683-691 (1991)(“ Lee ”).

1030	Sykes et al., <i>Quantitation of target for PCR by use of limiting dilution</i> , Biotechniques, Vol. 13:444-449 (1992)(“ Sykes ”).
1031	Brisco et al., <i>Outcome prediction in childhood acute lymphoblastic leukaemia by molecular quantification of residual disease at the end of induction</i> , Lancet, Vol. 343:196-200 (1994)(“ Brisco ”).
1032	Holland et al., <i>Detection of specific polymerase chain reaction product by utilizing the 5'→3' exonuclease activity of Thermus aquaticus DNA polymerase</i> , Proc. Natl. Acad. Sci. USA, Vol. 88:7276–7280 (August 1991)(“ Holland ”).
1033	Livak et al., <i>Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization</i> , Biotechniques, Vol. 4:357-362 (1995)(“ Livak ”).
1034	Vogelstein & Kinzler, <i>Digital PCR</i> , Proc. Natl. Acad. Sci. USA, Vol. 96:9236-9241 (1999)(“ Vogelstein ”).
1035	Metzker, <i>Sequencing technologies — the next generation</i> , Nature Reviews Genetics, Vol. 11: 31-46 (January 2010)(“ Metzker 2010 ”).
1036	Margulies et al., <i>Genome sequencing in microfabricated high-density picolitre reactors</i> , Nature, Vol. 437:376-380 (2005)(“ Margulies ”).
1037	Droege & Hill, <i>The Genome Sequencer FLX™ System—Longer reads, more applications, straight forward bioinformatics and more complete data sets</i> , Journal of Biotechnology, Vol. 136: 3-10 (2008)(“ Droege ”).
1038	Rothberg et al., <i>An integrated semiconductor device enabling non-optical genome sequencing</i> , Nature, Vol. 475:348-352 (2011)(“ Rothberg ”).
1039	Jain et al., <i>Improved data analysis for the MinION nanopore sequencer</i> , Nature Methods, Vol. 12:351-356 (2015)(“ Jain ”).

1040	Lo & Rossa, <i>Prenatal diagnosis: progress through plasma nucleic acids</i> , Nature Reviews Genetics, Vol. 8:71-77 (2007)(“ Lo 2007A ”).
1041	Lo et al., <i>Presence of fetal DNA in maternal plasma and serum</i> , Lancet Vol. 350:485-487 (1997)(“ Lo 1997 ”).
1042	Poon et al., <i>Presence of fetal RNA in maternal plasma</i> , Clinical Chemistry, Vol. 46:1832-1834 (2000)(“ Poon ”).
1043	Lo et al., <i>Digital PCR for the molecular detection of fetal chromosomal aneuploidy</i> . Proc. Natl. Acad. Sci. USA, Vol. 104:13116-13121 (2007)(“ Lo 2007B ”).
1044	Fan & Quake, <i>Detection of aneuploidy with digital polymerase chain reaction</i> , Analytical Chemistry, Vol. 79:7576-7579 (2007)(“ Fan 2007 ”).
1045	Binladen et al., <i>The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing</i> , PLoS ONE, Vol. 2:e197 (2007)(“ Binladen ”).
1046	Huber et al., <i>Microbial population structures in the deep marine biosphere</i> , Science, Vol. 318:97-100 (2007)(“ Huber ”).
1047	McKenna et al., <i>The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis</i> , PLOS Pathogens, Vol. 4:e20 (2008)(“ McKenna ”).
1048	Hamady et al., <i>Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex</i> , Nature Methods, Vol. 5:235-237 (2008)(“ Hamady 2008 ”).
1049	Andersson et al., <i>Comparative analysis of human gut microbiota by barcoded pyrosequencing</i> , PLoS ONE, Vol. 3:e2836 (2008)(“ Andersson ”).
1050	Fierer et al., <i>The influence of sex, handedness, and washing on the diversity of hand surface bacteria</i> , Proc. Natl. Acad. Sci. USA, Vol. 105:17994-17999 (2008)(“ Fierer ”).

1051	Dethlefsen et al., <i>The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing</i> , PLoS Biology, Vol. 6:e280 (2008)(“ Dethlefsen ”).
1052	Hamady & Knight, <i>Microbial community profiling for human microbiome projects: Tools, techniques, and challenges</i> , Genome Research, Vol. 19:1141-1152 (2009)(“ Hamady 2009 ”).
1053	Parameswaran et al., <i>A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing</i> , Nucleic Acids Research, Vol. 35:e130 (2007)(“ Parameswaran ”).
1054	Tewhey et al., <i>Microdroplet-based PCR enrichment for large-scale targeted sequencing</i> , Nature Biotechnology, Vol. 27:1025-1031 (2009)(“ Tewhey ”).
1055	U.S. Provisional Application No. 61/297,755.
1056	Craig, et al., <i>Identification of genetic variants using bar-coded multiplexed sequencing</i> , Nature Methods, Vol. 5:887-893 (Oct. 2008)(“ Craig ”).
1057	International Human Genome Sequencing Consortium, <i>Finishing the euchromatic sequence of the human genome</i> . Nature Vol. 431:931-945 (2004)(“ IHGSC ”).

III. SUMMARY OF OPINIONS

3. In my opinion, claims 1-10, 12-22 and 24 of the '831 Patent were unpatentable as obvious. My opinion is based on the following four grounds:

- a. Ground 1: Claims 1-7, 10, 14-19 and 22 were obvious over Fluidigm in view of the knowledge of one of ordinary skill;
- b. Ground 2: Claims 1-10, 13-22, and 24 were obvious over as in Ground 1 taken together with Sequenom

c. Ground 3: Claim 12 was obvious as in Ground 1, together with Harismendy.

d. Ground 4: Claim 12 was obvious as in Ground 2, together with Harismendy.

IV. EXPERIENCE AND QUALIFICATIONS

4. I am currently Founder, President, and Chief Executive Officer of RedVault Biosciences, LP (“RedVault”). RedVault is a new biotechnology company committed to creating innovative technologies to advance genomic medicine, which I founded in April 2013. Since starting RedVault, I have transitioned into an Adjunct Associate Professor in the Department of Molecular & Human Genetics and at the Human Genome Sequencing Center (“HGSC”) at the Baylor College of Medicine. Additionally, I am an Adjunct Associate Professor in the Department of Chemistry at Rice University.

5. Prior to my current positions, I was a tenured Associate Professor in the Department of Molecular & Human Genetics and at the HGSC at the Baylor College of Medicine. I have also been a Senior Manager at the HGSC at Baylor College of Medicine. I have also been an Adjunct Associate Professor in the Cell & Molecular Biology Program at the Baylor College of Medicine. Prior to my tenured positions, I held the position of tenure-track Assistant Professor in Molecular and Human Genetics and Senior Manager at the HGSC since November 1999 and the position

of Adjunct Assistant Professor in Chemistry at Rice University since July 2001.

6. In March 2002, I founded LaserGen, Inc. (“LaserGen”), which provides technology and reagents used in, among other things, nucleic acid sequencing methods. From March 2002 to April 2012, I was President and Chief Executive Officer of LaserGen, and from April 2012 to October 2012, I held the position of Chief Technology Officer. I was also a member of the Board of Directors from March 2002 to October 2012. I currently have no working relationship with LaserGen. In March 2016, Agilent Technologies invested \$80M into LaserGen to develop its reversible terminator technology into a clinical diagnostic platform. In March 2018, Agilent Technologies purchased the remaining shares of LaserGen for \$105M.

7. Since 1988, I have performed scientific research in the field of molecular biology. In particular, I have extensive experience with technology development in DNA sequencing methods including genomic DNA isolation, polymerase chain reaction (“PCR”), fragmenting genomic DNA, genomic DNA and complementary DNA (“cDNA”) library construction, bacterial cloning, nucleic acids chemistry, DNA modifying enzymes, polymerase engineering, fluorescent dyes, fluorescence imaging, and data analysis of multi-color images. I have extensive experience in the use and development of methods utilized to detect sequence variation, including single nucleotide variants (“SNVs”) and single

nucleotide polymorphisms (“SNPs”), in all organisms, with particular interest in humans and human immunodeficiency virus (“HIV”).

8. My research has been devoted to developing next-generation sequencing (“NGS”) technologies, developing novel methods to study HIV transmission between individuals, and identifying molecular causes of novel forms of diabetes and their treatment. My colleagues and I have been deeply involved in PCR, DNA fragmentation, library construction, cloning, and the development of sequencing technologies. My industrial experience entails performing analytical chemical testing of inorganic materials (Aerojet-General), organic synthesis and characterization of porous polystyrene beads for applications in high performance liquid chromatography (Bio-Rad), development and applications of a robotic platform in Sanger sequencing (Applied Biosystems), and development and applications of high-throughput DNA sequencing to identify novel gene targets for small molecule screening (Merck Research Laboratories).

9. I received my Bachelor of Science degree in Biochemistry & Biophysics from the University of California, Davis in 1984. Between 1985 and 1988, I completed the required coursework with emphasis in Organic Chemistry as part of the Master of Science program in the department of Chemistry at San Francisco State University. Instead of completing this degree, I pursued a Doctor of Philosophy in 1991 from the Department of Molecular and Human Genetics at

Baylor College of Medicine in Houston, Texas. I received my Ph.D. in 1996 in Molecular and Human Genetics.

10. From 2000 to the present, I have given lectures to graduate and medical students in the Molecular Methods course at the Baylor College of Medicine. The lectures are titled, cDNA and Genomic Libraries, First-generation Sequencing, and Genotyping, and Next-generation Sequencing. From 2001 to 2003, I also gave a lecture titled Mammalian Genome Analysis in the Mammalian Genomics.

11. I have also presented lectures at a variety of academic and industry conferences. In May 2010, I was an invited speaker at the Next-Generation Sequencing Workshop at Lübeck University in Germany. In October 2010, I was an invited speaker at the Centre de Regulació Genòmica (“CRG”) Symposium, Barcelona Spain. In April 2011, I was keynote speaker at the Next-Generation Sequencing Conference in Boston, MA. In February 2012, I gave a presentation related to the development of a novel NGS technology at the Advances in Genome Biology and Technology Meeting (“AGBT”) in Marco Island, FL. I was also invited to speak at the Copenhagenomics Meeting in Copenhagen, Denmark and the American Society of Microbiology Conference in San Francisco, CA both in June of 2012. In May 2015, I presented a keynote lecture at SelectBio’s Advances in Next Generation Sequencing Meeting. In April 2016, I was invited to speak at the Critical Path to TB Drug Regimens (“CPTR”) Workshop in Washington DC.

12. I have reviewed numerous manuscripts for peer-review publications that present data for many of the techniques described above. I have also authored several extensive reviews on sequence technologies and their applications used for the detection of sequence variation, including SNPs and SNVs. I have also received research grants and fellowships both from government and private agencies to fund my investigations of technology development in Sanger sequencing and NGS methods, development of NGS approaches for HIV forensics, the detection of genomic variation in diabetic cohorts using novel methods, and technology development of a novel diagnostic method involving microRNAs. I have received or have been associated with numerous grants largely focusing on gene and genome sequencing totaling over \$170 million since 1999. The source of these grants has been the National Institutes of Health (“NIH”), the National Institute of Justice (“NIJ”), the United States Department of Agriculture (“USDA”), and private foundations.

13. I have authored 56 peer-reviewed papers, seven book chapters, and am an inventor on 49 issued patents and patent applications. My most recent review papers discuss the emerging technologies and advances in DNA sequencing. The patents relate to various aspects of molecular biology, DNA sequencing, and chemical synthesis. For example, I co-invented the pulsed-multiline excitation (“PME”) method in collaboration with Nobel Laureate Robert F. Curl, which has

resulted in U.S. Patent Nos. 6,995,841; 7,511,811; and 8,089,628. By way of collaboration with my scientific team at LaserGen, I have also co-invented novel nucleotide terminators that have resulted in numerous patents including U.S. Patent Nos. 7,893,227; 7,897,737; 7,964,352; 8,148,503; 8,198,029; 8,361,727; 8,497,360; 8,877,905; 8,889,860; 8,969,535; 9,200,319; 9,399,798; 9,689,035.

14. I have served on review panels for Genome Canada, Canadian Institute for Health Research (“CIHR”), National Aeronautics and Space Administration (“NASA”), and U.S. Department of Energy (“DOE”), as well as various NIH study sections for the National Cancer Institute (“NCI”), National Institute of Biomedical Imaging and Bioengineering (“NIBIB”), National Human Genome Research Institute (“NHGRI”), and National Institute for Allergy and Infectious Diseases (“NIAID”). In March 2018, I became a member of the College of Reviewers for CIHR.

15. From 2003 to 2006, I served on the editorial/advisory boards for Genome Research. From 2006 to 2012, I have served as a scientific organizer for the AGBT Meeting. From 2007 to present, I have served as a scientific advisor for Milestones in DNA Technologies. From 2011 to 2013, I have served on the advisory committee of Genome Canada: Advancing Technology Innovation Through Discovery (“ATID”) projects – Finding of Rare Disease Genes in Canada (“FORGE Canada”) and the Canadian Pediatric Cancer Genome Consortium (“CPCGC”).

16. I belong to the American Association for the Advancement of Science, the American Chemical Society, and the Texas Genetics Society. My professional experience, education, and publications are presented in greater detail in my curriculum vitae, including publications and patents, as well as cases that I have provided expert testimony at deposition or trial in the last four years. (Ex. 1009).

17. As an expert in molecular biology, I am qualified to provide an opinion as to what one of ordinary skill in the art would have understood, known or concluded as of the 2010 time frame or several years prior to that.

V. TECHNICAL BACKGROUND

A. Introduction to the '831 Patent

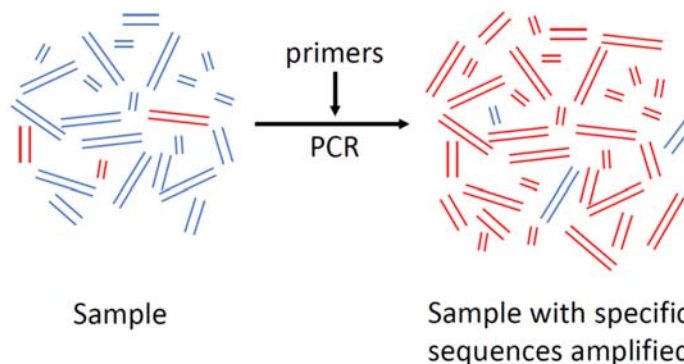
18. The '831 Patent relates to the preparation of sequencing libraries. A sequencing library has a collection of polynucleotide sequences which can be, for example, genomic or cell-free DNA. The polynucleotide sequences are usually taken from some biological sample, such as a blood sample. In many biological samples, there will be a large amount of polynucleotides, including genomic or cell-free DNA. Some of these sequences might be of interest to a medical professional or patient. For example, the presence (or elevated / lowered) amounts of sequences associated with a particular disorder could be used for a diagnosis.

19. To detect sequences of interest from among the (generally) much larger collection of sequences found in a sample in the relevant timeframe (and still today),

a general and multi-step process was used. The first step of the general process was to prepare the sample into a library of sequences. A library preparation method typically has several processing steps that can also include *selectively amplifying* sequences of interest. To selectively amplify sequences of interest, the polymerase chain reaction (PCR) can be used. A more complete introduction to PCR technology is provided in ¶¶23-44, below.

20. PCR uses primer sequences. The primers will bind to sequences in the sample under appropriate conditions, if those sample sequences have reasonably complementary base pairs. Included in a PCR reaction is a DNA polymerase. An

illustration of the PCR process in general is shown at right (created by the Petitioner), in which a sample on the left containing DNA sequences (shown as two

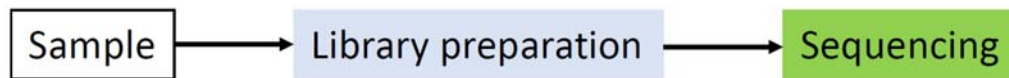


complementary strands) have sequences of interest (shown in red), and sequences that are not of interest (shown in blue). By applying PCR with primer pairs directed to the sequences of interest, a reaction product (right side) is obtained that has the sequences of interest selectively amplified (the size of the samples and number of strands are not meant to be accurate, but only to illustrate the process at a general level). By using multiple rounds of PCR, the sequences of interest can be amplified

to a point where they become dominant sequences in the sample.

21. Using PCR in this fashion, one could amplify target sequence to create a sequencing library. The sequencing library can then be used in a *sequencing analysis* of the amplified target sequences by determining the base-pair order of the polynucleotides in the library. Therefore, the presence, absence and concentration of target sequences in the library can provide information about the original sample, including the disease state(s) of the individual from whom the sample was taken.

22. Two general steps of such a diagnostic process (library preparation and sequencing) are shown below:



23. One example of such a diagnostic process using library preparation and sequencing begins with a sample of maternal blood plasma from a pregnant woman. As was well-known prior to the earliest possible benefit date of the '831 Patent, a pregnant mother's blood contains both her own cell-free DNA and cell-free DNA from the fetus. (Ex. 1006, p. 001)(Ex. 1013, p. 001)(Ex. 1005, 2:29-3:10)(Ex. 1003, ¶0123). One could take, for example, a sample of the maternal plasma and selectively amplify DNA sequences from chromosome 21, thereby allowing one to test for Down syndrome in the fetus. Down syndrome is caused by an extra copy of chromosome 21 (a so-called "trisomy" of chromosome 21). An extra copy of

sequences from chromosome 21 in the blood of a mother without Down syndrome indicates a higher probability Down syndrome in the fetus.

24. The claims of the '831 Patent are directed to the first step of the diagnostic process: library preparation. In general, the claims require preparing a sequencing library using three amplification steps. For example, claim 1 of the '831 Patent is reproduced below, with the three amplifications steps bolded:

“1. A method for preparing a sequencing library from a maternal blood sample, the method comprising:

a. obtaining a maternal blood sample comprising fetal and maternal cell-free DNA;

b. selectively enriching a plurality of non-random polynucleotide sequences of genomic DNA from said fetal and maternal cell-free DNA to generate a library of enriched non-random polynucleotide sequences, wherein said plurality of non-random polynucleotide sequences comprises at least 100 different non-random polynucleotide sequences selected from a chromosome tested for being aneuploid, said enriching comprising:

(i) **a first amplification step** to generate a plurality of first reaction products, said amplification comprising at least 100 first primers configured to amplify at least 100 different non-random polynucleotide sequences;

(ii) **a second amplification step** to generate a second reaction product, said amplification comprising a second set of primers comprising sequences contained in the first reaction products; and

(iii) **a third amplification step** to generate a third reaction

product comprising said library of enriched non-random polynucleotide sequences, said amplification comprising a third set of primers comprising sequences contained in the second reaction products;

wherein at least one primer of at least one of the second and third sets of primers includes a sequence configured to be added to the different non-random polynucleotide sequences to permit the enriched non-random polynucleotide sequences of the library to anneal to a same sequencing primer for the enriched non-random polynucleotide sequences of the library.”

B. DNA Basics

25. The information necessary to reproduce a given organism is found in its genome, which comprises the complete set of an organism’s genes. The genome is comprised of strands of a polymer called deoxyribonucleic acid or “DNA.” Each cell for a given organism typically contains one copy of the genome, called genomic DNA.

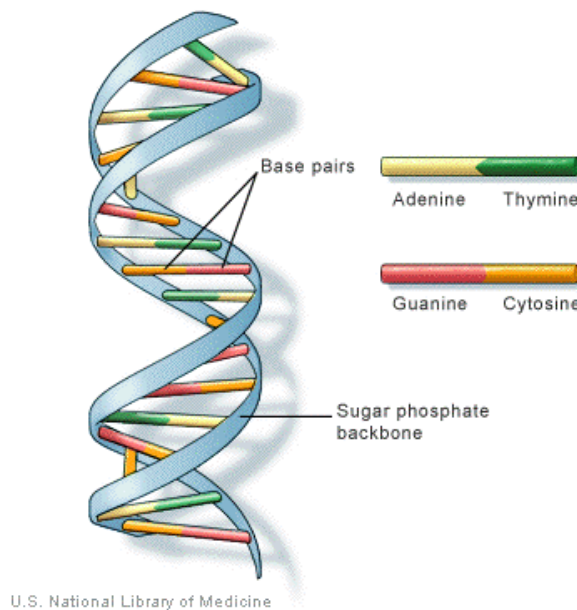
26. DNA strands are polymers of building blocks called nucleotides. A nucleotide is made up of three chemical components or groups: the nucleobase (commonly referred to as a base), the sugar, and one or more phosphate groups. For DNA, the specific sugar group is a 2’-deoxyribose. For ribonucleic acid (“RNA,”) the specific sugar group is a ribose. In addition to this difference, DNA contains the base thymine (“T”) while RNA contains the base uracil (“U”). Using an enzyme

called a polymerase, nucleotides that are added during DNA synthesis are called 2'-deoxyribonucleoside-5'-triphosphates or "dNTPs," and those added during RNA synthesis are called ribonucleotide-5'-triphosphates or "NTPs" or sometimes "rNTPs."

27. A polymerase that synthesizes DNA is called a DNA polymerase and one that synthesizes RNA is called a RNA polymerase. Polymerases can possess other activities in addition to synthesizing nucleic acids. For example, a polymerase can remove nucleotides from one or the other end of nucleic acids. Polymerases that remove nucleotides from the 5'-end of a nucleic acid has a 5'-exonuclease activity. Polymerases that remove nucleotides from the 3'-end of a nucleic acid has a 3'-exonuclease activity.

28. The DNA structure can be characterized as a ladder. The two opposing legs of the DNA ladder are based on a repeated pattern of a sugar group and a phosphate group, called the DNA backbone. The chemical variance occurs in the rungs of the DNA ladder—i.e., the four different bases: adenine ("A"), cytosine ("C"), guanine ("G"), and thymine ("T")—which form hydrogen bonds according to specific base-pairing rules. "A" and "T" can form stable hydrogen bonds, and "C" and "G" can form stable hydrogen bonds. Said in another way, "A" does not pair with "G" and "C" does not pair with "T." For RNA, "U" is substituted for "T" whereby "A" and "U" can form stable hydrogen bonds.

29. The formation of base-pairs between nucleotides on the two opposite strands causes the two strands to coil around each other to form a double helix structure, first described by James Watson and Francis Crick in 1953. Bases of nucleotides that follow these base-pairing rules are also called Watson-Crick base-pairs (“bp”). An example of a double helix is shown below.



30. According to the base-pairing rules, stretches of nucleotides in one strand can form hydrogen bonds only with a complementary stretch of nucleotides in the other strand. Two single strands of DNA that come together by way of hydrogen bonding between stretches of complementary (or substantially complementary) nucleotides are said to be hybridized or annealed to one another. Because of the specificity of base-pairing, under most environmental conditions, a strand of DNA will have a specific stable complementary sequence.

31. This complementarity is critical for the heritability of genetic information. Because of the specificity of base-pairing, DNA polymerase can create or synthesize a complementary sequence from a single strand of a template sequence. As such, each strand of a double-stranded sequence can act as a template for the other. Cells are able to replicate DNA based on this property, and thus, pass exact copies of their genome to their progeny.

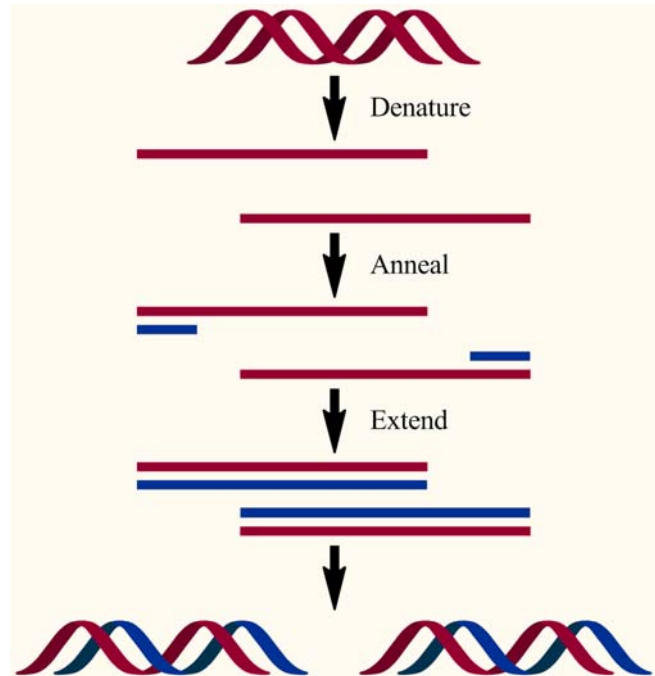
C. Amplification and detection

32. A universal technique for replicating or amplifying nucleic acids in a laboratory setting is the polymerase chain reaction, or just “PCR.” This method was invented by Dr. Kary Mullis in the 1980s. PCR is used to specifically amplify a small amount of nucleic acid molecules, generating thousands to millions of copies of the target nucleic acid. (Ex. 1018)(“Saiki 1985”)(Ex. 1019)(“Saiki 1988”).

33. Generally speaking, PCR involves the repeated denaturation and replication of DNA sequences in vitro (i.e., outside of an organism). DNA polymerase copies or synthesizes the complementary strand from a single-stranded template. For this enzymatic reaction to occur, a partially double-stranded section of DNA is required. Typically, a primer hybridizes to a complementary region of a single-stranded template. Dr. Mullis and his co-inventors used short oligonucleotide primers complementary to the 3'-ends of the complementary strands of a sequence of interest. Because of the base-pairing rules, primer sequences can be designed to

target specific nucleic acid sequences.

DNA polymerase initiates synthesis of the nascent strand from the hybridized primers in a 5'-to-3' direction to create double-stranded DNA. Two primers are used for each template strand in PCR that define the targeted sequence of interest. The general concept of PCR is illustrated by the diagram at right.



34. The first step of a PCR reaction is to melt or denature double-stranded DNA to yield single-stranded DNA. This typically is done by raising the reaction temperature to melt or by chemical means to separate double-stranded DNA or fragments into single strands. Following primer hybridization, the polymerase copies the template strand from the primer in a 5'-to-3' direction, making a complementary strand. Assuming optimal reaction conditions, the quantity of the original target sequence of interest, which is flanked by the two primers, will have been essentially doubled in one PCR cycle.

35. Next, the PCR cycle is repeated multiple times. This is accomplished by denaturing the double-stranded template, annealing the primers, and extending the primers with DNA polymerase to synthesize new double-stranded templates. At

each PCR cycle, the target sequence that lies between the primers (including the primer sequences themselves) will be replicated. Theoretically, a target sequence will be amplified exponentially as described by 2^n , where n is the number of cycles. In practice, however, the efficiency of the reaction varies, and decreases in later cycles. Regardless, PCR is able to create large quantities of DNA within a matter of hours.

36. PCR is a powerful tool for detecting human polymorphic variation that is associated with hereditary disease, aneuploidy, and cancer. Many techniques have been described to discriminate between wild-type and mutant alleles. For example, allele-specific PCR, also called competitive oligonucleotide priming (Ex. 1020)(“Gibbs 1989A”) or called amplification refractory mutation system (Ex. 1021)(“Newton”) has been used for detecting polymorphic-specific markers. Multiplex PCR allows for the simultaneous amplification of multiple target regions and has been used to detect coding exon deletion(s) in X-linked disorders (these exons are gene sequences that are transcribed into messenger RNA (mRNA) and translated into one or more proteins). X-linked disorders include Duchenne muscular dystrophy (Ex. 1022)(“Chamberlain”) and Lesch–Nyhan syndrome (Ex. 1023)(“Gibbs 1990”). Absence of specific PCR product(s) is diagnostic of exon deletion(s) in affected males, and ‘half-dosage’ PCR products are diagnostic of carrier mothers. Other detection methods that involve scanning (i.e., methods that

do not involve DNA sequencing) of PCR products for the presence of genetic alterations are summarized by a review by Dr. Markus Grompe. (Ex. 1024)(“Grompe”).

37. PCR can also be used to quantify the amount or the number of molecules of the target sequence in the biological sample. At least four basic methods have been described to quantify PCR-amplified templates: (i) quantitative PCR using an internal control, (ii) quantitative competitive PCR (Ex. 1025)(“Ferre”), (iii) real-time PCR (Ex. 1026)(“Heid”), and (iv) limited or end-point dilution PCR (Ex. 1027)(“Simmonds”)(Ex. 1028)(“Zhang”)(Ex. 1029)(“Lee”); (Ex. 1030)(“Sykes”)(Ex. 1031)(“Brisco”).

38. Real-time PCR using a 5'-exonuclease fluorogenic or TaqMan assay (Ex. 1032)(“Holland”) has been developed to accurately measure the starting amounts of target sequences or identify sequence variants, including SNPs. Unlike gel electrophoresis, real-time PCR has the unique advantage of being performed in a closed-tube system, which can significantly reduce carryover contamination. Using real-time PCR, one can easily monitor and quantitate the accumulation of PCR products during exponential or log phase amplification. The TaqMan assay utilizes dual reporter and quencher fluorescent dyes that are attached to a non-extendible probe sequence (i.e., the 3'-end of the probe is blocked). The reporter and quencher fluorescent dyes are attached at different internal positions or at

opposite ends to the oligonucleotide probe (Ex. 1033)(“Livak”) such that the fluorescent signal is quenched by the well-understood process called fluorescence resonance energy transfer (“FRET”). Quenched fluorescent probes are called fluorogenic probes. During the extension phase of PCR, the 5’-exonuclease activity of Taq DNA polymerase removes the nucleotides or digests the hybridized fluorogenic probe, which releases the reporter signal. The released reporter signal is measured during each cycle. In addition to quantifying nucleic acids, real-time PCR has broad utility for the identification of sequence variants, including SNPs.

39. The limiting or end-point dilution PCR method is based on diluting and distributing target sequences into individual reaction wells, such that the majority of wells has either zero or just one target sequence. The distribution of target sequences is based on Poisson statistics, which enables the mean number of target sequences per reaction well to be determined. Following the PCR amplification step, the reaction wells are analyzed to determine which reaction wells contain positive PCR products. The method has more recently been coined digital PCR. (Ex. 1034)(“Vogelstein”).

40. DNA sequencing includes several methods and technologies that are used to determine the order of nucleotide bases (A, C, G and T) in a given DNA fragment. DNA sequencing methods can generally be characterized as either first-generation sequencing, which include Sanger sequencing or next-generation

sequencing (“NGS”). In broad terms, NGS technologies are distinguishable from first-generation sequencing technologies in that they produce substantially larger volumes of sequence information cheaply. I have reviewed many of the platforms using NGS technologies. (Ex.1035)(“Metzker 2010”).

41. NGS technologies include a number of methods that are grouped broadly as template preparation, sequencing and imaging, and data analysis. NGS extends this process across millions of reactions in a massively parallel fashion, rather than being limited to one or a few nucleic acid fragments. Since their introduction, NGS platforms have allowed for high-throughput sequencing of nucleic acids on a genome-wide scale and highly multiplexed gene-specific sequencing.

42. The first NGS platform to achieve widespread commercial use was 454 Life Sciences Corporation’s sequencer, also known as Genome Sequencer 20 (“GS20”). (Ex. 1036)(“Marguiles”). The GS20 sequencer first became commercially available in 2005 and produced average read-lengths of ~100 bases. In 2007, 454 Life Sciences released its second instrument, called the GS FLX®, producing average read-lengths of ~250 bases. (Ex. 1037)(“Droege”). In that same year, 454 Life Sciences was acquired by Roche. Subsequently, the Roche/454 GS FLX® Titanium and GS FLX+ instruments have been released to produce longer average read-lengths.

43. The Roche/454 GS20, GS FLX® GS FLX® Titanium, and GS FLX+ sequencers all use a technology known as pyrosequencing, which is a method for detecting a single nucleotide incorporation event by a DNA polymerase as a bioluminescence signal. Specifically, nucleotide incorporation causes the release of a small molecule called pyrophosphate, which initiates a series of enzymatic reactions that produce the bioluminescence signal. Use of this technique typically requires shearing of the double-stranded genomic DNA into smaller pieces of DNA that can be sequenced, although PCR amplicons have been successfully used as templates. The Roche/454 sequencers then uses a bead-based immobilization strategy for DNA preparation. To create genomic libraries, the DNA is sheared into fragments of 300-800 base pairs, and two short pieces of additional DNA (called A/B adaptors) are attached to the ends of the fragmented genomic DNA. The A/B adaptors (or A/B primers) can also be attached to the 5'-ends of targeting primers to include these sequences in the final amplicons. A/B adaptors each consist of a known DNA sequences and provide priming sites for emulsion PCR and subsequently sequencing of the DNA fragments. The method, called emulsion PCR, is then used to copy individual DNA fragments onto beads, prior to the sequencing step.

44. Those beads are deposited onto a PicoTiterPlate® (“PTP”) that accommodates only one individual DNA-attached bead per well. Additional beads

coated with enzymes, called enzyme beads, are also added to the wells. Sequencing reagents (buffers, DNA polymerase, and nucleotides) are then flowed across the wells of the PTP. Nucleotides are sequentially added one at a time during a sequencing cycle. Nucleotide incorporation by the DNA polymerase causes release of pyrophosphate, which reacts with the enzyme beads and produces the bioluminescence signal. With the sequential flow of individual nucleotides, the timing of the bioluminescence signal indicates the order in which nucleotides are incorporated, which ultimately translates into the sequence of the DNA template.

45. The Genome Analyzer II (“GAI”) was developed by Solexa and released in late 2006. After the acquisition of Solexa by Illumina in 2007, this massively parallel sequencing platform achieved widespread commercial use. The GAI instrument utilizes a strategy called sequencing by synthesis (“SBS”), otherwise known as cyclic reversible termination (“CRT”), which was developed as an alternative to the Sanger sequencing method. To create genomic libraries, the DNA is sheared into smaller fragments, and short adaptors are attached or ligated to both ends of the DNA fragment. Bentley describes that the ligated products, which contain the adaptor sequences, are then PCR amplified to include additional sequences used in subsequent amplification and sequencing steps. (Ex. 1008)(“Bentley”). Solid-phase or bridge amplification is then used to make numerous copies of the DNA template. Following amplification, clusters are formed

on the flow cell that contain copies of the single original DNA template fragment. The result is that millions of template clusters are formed.

46. Following bridge amplification, the flow cells are prepared for the SBS or CRT sequencing approach. Then, a universal sequencing primer is added to the flow cell to hybridize to all templates amplified by bridge PCR. In the SBS approach, all four nucleotides contain a unique fluorescent label. Each nucleotide, however, is modified to act as a reversible terminator, which is an important feature of the technique. In the first step, all four reversible terminators are added simultaneously to the flow cells along with DNA polymerase. The DNA polymerase adds a single fluorescent reversible terminator that corresponds to the template base. The terminating group on the incorporated nucleotide blocks any other nucleotides from subsequently being incorporated. Following incorporation, the remaining unincorporated reversible terminators are washed away. Imaging is then performed to determine the identity of the incorporated nucleotide based on the fluorescent signal. This is followed by a cleavage step, which removes the terminating group and the fluorescent dye on the incorporated nucleotide. The cycle is repeated, and the fluorescent identity for each cluster is read using sequential images. Since 2009, Illumina has released new instruments including multiple versions of HiSeq®, MiSeq®, NextSeq® and NovaSeq® systems.

47. Other NGS platforms exist or have existed in the marketplace, for

example Life Technologies' SOLiD® instrument and Pacific Biosciences' single-molecule real-time or SMRT® instrument. (Ex. 1035). In 2011, Ion Torrent released their Personal Genome Machine or PGM®. The PGM® relies on the method of emulsion PCR to copy individual DNA fragments onto beads, called Ion Spheres®, which are deposited into an Ion Chip for sequencing. Sequencing primers and DNA polymerase are bound to the DNA fragments on the surface of the Ion Spheres, and these are loaded onto the chip in individual sensor wells, so that there is one bead per well. The four natural nucleotides (dATP, TTP, dGTP, dCTP) are dispensed one at a time across the Ion Chip. Sensors in each well detect a change in the pH when hydrogen ions are released by the incorporation of a nucleotide into a new growing primer. With the sequential flow of individual nucleotides, the timing of the pH change indicates the order in which nucleotides are incorporated, which ultimately translates into the sequence of the DNA template. (Ex. 1038)(“Rothberg”). Since 2011, Life Technologies has released the Ion Proton® with higher throughput of sequencing data compared with PGM®. More recently, Oxford Nanopore has released its MinION sequencer, which sequences individual DNA strands that are driven through tiny nanopores. (Ex. 1039)(“Jain”).

D. Creating a cell-free DNA library from maternal for aneuploidy detection was well known in the art

48. Prenatal diagnostics is an indispensable part of modern obstetrics care that targets fetal genetic diseases including chromosomal aneuploidy and single-

gene disorders. (Ex. 1040)(“Lo 2007A”). Definitive prenatal diagnosis requires the analysis of fetal genetic materials using invasive techniques such as amniocentesis or chorionic villus sampling (“CVS”). These invasive procedures carry a finite risk of fetal loss, and many researchers have searched over decades for non-invasive alternatives to sample fetal genetic materials. One early approach was to isolate nucleated fetal cells from maternal blood, though because of the extreme rarity of these cells – basically one cell in each milliliter of maternal blood – these heroic efforts proved intractable for routine usage.

49. Not all DNA or RNA, however, is isolated from cellular materials. In 1997, Dr. Dennis Lo made the remarkable discovery that cell-free fetal DNA could be isolated and characterized from plasma or serum obtained from pregnant mothers. (Ex. 1041)(“Lo 1997”). Lo 1997 describes that the plasma- or serum-based approach might also be applicable to screening for chromosomal aneuploidies, such as Down syndrome. By 2000, Dr. Lo also discovered that cell-free fetal RNA could be isolated and characterized from plasma obtained from pregnant mothers. (Ex. 1042)(“Poon”). Cell-free DNA that isolated from maternal blood is composed of a mixture of both maternal and fetal nucleic acids. By 2007, much had been learned about the biology of cell-free DNA in maternal blood including that cell-free fetal DNA (i) consists of primarily short fragment lengths (the majority shorter than 200 bp), (ii) represents a small subfraction of the total cell-free DNA isolated in maternal

blood, (iii) increases in concentration during gestation, and (iv) comes from normal cell turnover events, such as apoptosis and necrosis, of the placenta, which is the predominant source of fetal DNA in maternal plasma.

50. The discovery of cell-free fetal nucleic acids in maternal plasma launched a new industry of non-invasive prenatal diagnostic approaches. The first analytical methods focused on ways to differentiate fetal DNA from the overwhelming amount of maternal DNA in plasma. This was accomplished by targeting specific paternal chromosomal regions, genes, or genetic variations that were inherited by the fetus from the father and were absent in the mother. These applications included detecting Y chromosome sequences in male fetuses and rhesus blood group antigen D (“RHD”) positive fetuses in the plasma of RHD-negative mothers. Other approaches to detect cell-free fetal nucleic acids focused on markers of the placenta, which is of fetal origin, that either were expressed as a specific DNA methylation pattern or as specific RNA molecules.

51. In 2007, two papers were published contemporaneously that described the use of digital PCR for the detection of chromosomal aneuploidy, specifically related to trisomy 21 or Down syndrome. (Ex. 1043)(“Lo 2007B”)(Ex. 1044)(“Fan 2007”). Unlike previous efforts, Lo 2007B and Fan 2007 both described the use of digital PCR to count the number of positive products from PCR targets that did not rely on differences between fetal and maternal DNA. The challenge with this non-

polymorphic approach was that method must be able to quantitatively measure small differences in an aneuploidy sample compared with a normal sample. As described above, fetal DNA represents only a small fraction (on average, ~10%) of the total cell-free DNA isolated in maternal plasma. In the example where 100 copies of a genomic region derived chromosome 21 were isolated from one milliliter of plasma, 90 copies would be maternal and 10 copies would be fetal. For a trisomy 21 pregnancy, the maternal copies would be the same, but the fetus would contribute 15 copies, whereby 105 copies of total cell-free DNA would be isolated from one milliliter of plasma. In other words, the sensitivity of digital PCR in this example would need to be high enough to correctly characterize 105 copies as those derived from an aneuploidy fetus compared with 100 copies from an euploidy fetus.

52. This non-polymorphic approach relies simply on counting the number of PCR products from a sequence region suspected of having a chromosomal aneuploidy and a second sequence region suspected being normal or euploidy. These results would then be compared to a normal sample, whereby the sample having chromosomal aneuploidy could be identified with statistical significance. The precision of digital PCR to accurately measure the small differences in cell-free copy numbers is directly related to (i) percentage of fetal DNA in the cell-free DNA fraction isolated from maternal plasma and (ii) the number of PCR reactions performed for a given assay. For example, Lo 2007B estimated that it would require

7,680 digital PCR reactions to correctly classify trisomy 21 samples in 97% of cases that contained a fetal fraction of 25% from cell-free DNA isolated from maternal plasma. With lower fetal fractions, such as 10%, a much higher number of reaction samples would need to be performed to maintain the level of precision estimated by Lo2007B.

53. As described above, NGS technologies produces large numbers of sequence read data. In 2008, the same research groups led by Dr. Dennis Lo and Dr. Stephen Quake described the application of NGS to chromosomal aneuploidy detection in pregnant mothers. (Ex. 1013)(“Chiu”)(Ex. 1006)(“Fan 2008”). NGS provides much deeper data sampling than that of digital PCR. Both groups described generating several million sequence reads from the cell-free DNA isolated from maternal plasma and mapping or aligning those reads to the human reference genome. The number of mapped sequence reads were then counted to determine the over- and underrepresentation of each chromosome contributed by the fetus. Of the 28 cases analyzed by Chiu, 14 trisomy 21 (Down syndrome) fetuses and 14 euploidy fetuses were correctly identified. Of the 18 cases analyzed by Fan 2008, nine trisomy 21, two trisomy 18 (Edwards syndrome), one trisomy 13 (Patau syndrome) fetuses and six euploidy fetuses were correctly identified.

54. To perform NGS, sequencing libraries were often created from samples using PCR amplification. PCR-based library preparation was well-known and

widely-used by the time the first application leading to the '831 Patent was filed. (Ex. 1012, pp. 001-002)(Ex. 1006, p. 003, right column)(Ex. 1007, p. 002, right column)(Ex. 1014, p. 001-002)(Ex. 1003, ¶0160-0161). By that time, in fact, standard kits to prepare sequencing libraries had been available for at least around two years. (Ex. 1014, p. 001)(Ex. 1005, 3:32-4:2)(Ex. 1003, ¶00179)(Ex. 1007, p. 002, right column). These kits, like the recited claims, employed PCR amplification and utilized primers with sample specific barcodes and sequences to which the sequencing primer could anneal. The kits were to be used with next-generation sequencers which rendered use of such sequences a necessity for fast and cheap sequencing.

55. Next generation sequencers could sequence millions of DNA molecules in a single run and thereby made feasible sequencing DNA from different individuals at the same time. To distinguish samples from different individuals, DNA from a particular individual was “tagged” or “indexed” such that each individual sample had a separate tag, index or barcode. Thus, a “tagged” or “indexed” library of that individual’s DNA fragments could be created and the “tag” or “index” could be used to associate a particular DNA with an individual. The advantage of “indexing” was that DNA from different individuals could be pooled and sequenced together in a single reaction.

56. Not only was the concept of creating an indexed DNA sequencing

library routine and commonplace, the concept of using cell-free DNA from maternal blood to detect chromosomal aneuploidy, such as trisomy 21, the underlying cause of Down syndrome was equally well-known in the art. (Ex. 1003, ¶0122)(Ex. 1005, 1:31-2:5)(Ex. 1006, Abstract). Cell-free DNA in maternal DNA was discovered more than a decade before the priority date and triggered an immediate interest in developing non-invasive prenatal diagnostics. This was because fetal abnormalities could be tested using maternal blood, rather than having to undergo invasive ultrasound procedure with its attendant risk.

57. Soon after the discovery of cell-free DNA, scientists were analyzing the cell-free DNA for fetal abnormalities including aneuploidies. Some of the more common aneuploidies being tested included trisomy (where an individual has three copies of a particular chromosome) of chromosomes 13 (Patau syndrome), 18 (Edwards syndrome) and 21 (Down syndrome). A common way to test for aneuploidy was to sequence the chromosome of interest and use statistical analysis to determine whether the amount of sequences from that chromosome were unusually high or low. (Ex. 1006, p. 001)(Ex. 1013, p. 001).

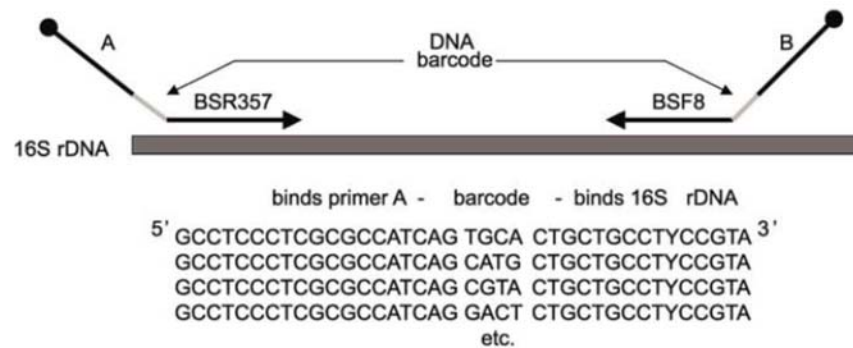
E. Use of indexed DNA library for multiplexed next generation sequencing was wide spread in the art

58. As described above, NGS technologies produce substantially larger volumes of sequence information cheaply in a single run. There are many sample types that can be analyzed using NGS technologies including, but not limited to,

human and other mammalian genomes and transcriptomes, targeted-enrichment of exons or chromosomal regions of these genomes, lower eukaryotic and prokaryotic genomes, and metagenomic samples. In many cases, the NGS instrument throughput far exceeds the sequencing needs of a single sample. To overcome this inefficiency, samples are multiplexed, which means that multiple samples are analyzed by NGS technologies in a single run. This is accomplished by two strategies: (i) physical separation of multiple samples within the Roche/454 PTP or Illumina 8-channel flow cell and/or (ii) molecular barcodes that associate individual DNA fragments with a given sample. For example, samples obtained from a given microbial source have been widely analyzed by the Roche/454 pyrosequencing instruments using barcoded PCR amplicons of 16S rRNA sequences. (Ex. 1045)(“Binladen”); (Ex. 1046)(“Huber”); (Ex. 1047)(McKenna”); (Ex. 1048)(“Hamady 2008”); (Ex. 1049)(“Andersson”); (Ex. 1050)(“Fierer”); (Ex. 1051)(“Dethlefsen”); and (Ex. 1052)(“Hamady 2009”).

59. In addition to the term barcode, these molecular codes have been referred to as tags, multiplex identifiers, and indexes. A barcode consists of a short DNA sequence. The number of nucleotides in a given barcode sequence can range from 3-12 bases and are empirically designed to minimize artifacts due to non-equal representation of particular barcode sequence in the sequencing data or to conform to error profiles of the sequencing method. (Ex. 1053)(“Parameswaran”).

60. Barcoded primers consist of three regions that serves three functions. For example, for the Roche/454 pyrosequencing method, barcode sequences are generally inserted between the A/B primers, used for emulsion PCR and sequencing processes, and the target specific primer region (i.e., 16S rDNA). The barcoded primers are simply made using automated oligonucleotide synthesis machines. An example of McKenna's barcoded primers are illustrated below.



(Ex. 1047, p. 0004, Figure 1B).

61. McKenna's barcoded primers have the general structure of Primer A–Barcode–16S rDNA primer. Primer A, which is common to all barcoded primers of the set, serves the function to prime the emulsion PCR and sequencing steps of the Roche/454 system. The Barcode, which is specific for each barcoded primers of the set (i.e., “TGCA,” “CATG,” etc.), serves the function to uniquely tag a given sample that can be later identified by sequence analysis. 16S rDNA primer, which is also

common to all barcoded primers of the set, serves the function to prime target specific 16S rDNA amplification of each sample during the PCR process.

62. As of January 2010, Illumina's GA sequencer was the most commonly used next-generation sequencer. As I explain earlier, the workflow of Illumina's GA sequencer generally consisted of four steps: DNA library preparation, cluster generation, sequencing-by-synthesis on the GA instrument, and data analysis. (Ex. 1008, p. 001-002)(Ex. 1012 at 001-002, Figure 1). The GA instrument was capable of sequencing a sample from an individual or multiple samples from many different individuals.

63. For example, Bentley used the GA analyzer to analyze an individual human genome. (Ex. 1008, p. 001). To create the DNA library, human genomic DNA was sheared into different fragments. (*Id.*). Two adaptor sequences were ligated to both ends of the DNA fragments. (*Id.*, p. 008). These adaptor-ligated fragments were amplified using two PCR primers targeting the two adaptor sequences, respectively. (*Id.*, pp. 008-009). These PCR primers "include the sequences that anneal to the complementary oligonucleotides bound to the flowcell surface, plus the sequencing primer sites." (*Id.*, p. 009) As shown in the graphic below (created by me), for the paired-read experiments, the two adaptor sequences contain sequences (highlighted in yellow and teal, respectively), which hybridize to their respective two PCR primers and the sequencing primers. (*Id.*) Because all of

the amplified DNA fragments have the same sequencing primer sites, they can all be sequenced using the same pair of sequencing primers.

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Adaptor 1:                               5'ACACTCTTTCCCTACACGACGCTCTTCCGATCxT
PCR Primer 1: 5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCxT
Rd1 Sequencing Primer:                   5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'
=====
Adaptor 2:                               5'-phosphate-GATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
PCR Primer 2: 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCxT
Rd2 Sequencing Primer:                   5'-CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT-3'

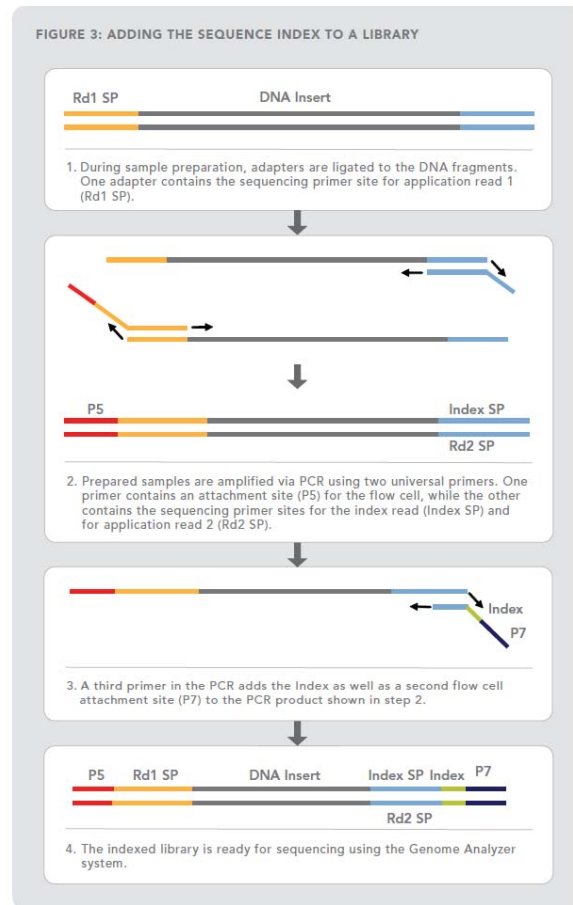
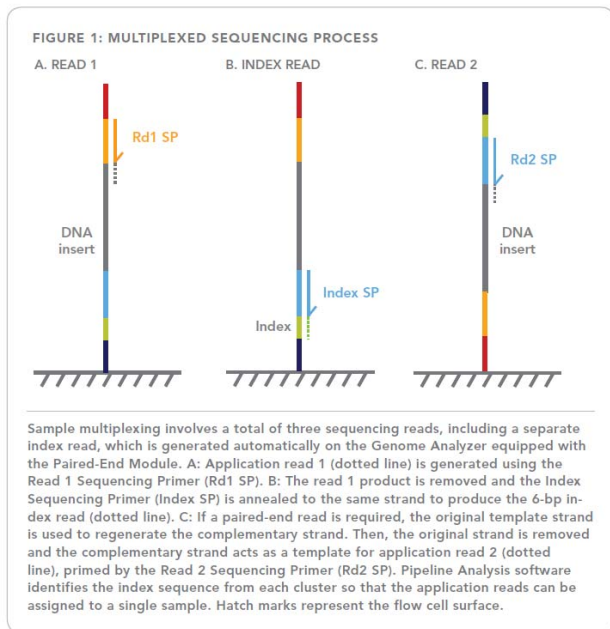
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64. In addition, Quail describes improvements to Illumina’s sequencing system, and discusses the use of adaptors “which essentially consist of the sequences to which the sequence primers hybridize during the sequencing reaction.” (Ex. 1012, p. 002)(“Quail”). It also discusses introducing these adaptors during a PCR amplification step. (*Id.*, p. 003)(“The PCR step introduces into the adapter-ligated template molecules the oligonucleotide sequences required for hybridization to the flowcell surface.”). As with Bentley, because the DNA fragments in Quail have the same sequencing primer sites (“the sequences to which the sequence primers hybridize”), they can all be sequenced using the same pair of sequencing primers.

65. Moreover, as of January 2010, Illumina was selling library preparation kits for multiplex sequencing on its GA sequencer. (Exs. 1014, 1016). To perform multiplexed sequencing on the GA sequencer, a DNA library from each sample is

typically prepared as follows. (Ex. 1014, pp. 002-003, Figure 3)(Ex. 1016). First, two adaptor sequences are ligated to both ends of the DNA fragments in each sample. The ligated products are then amplified using two universal primers targeting those adaptor sequences and are tagged with a unique “index” sequence using a third PCR primer. (*Id.*). After library preparation, multiple libraries are pooled and sequenced simultaneously on the Genome Analyzer.

66. As shown below (Exhibit 1014, Figs. 1 and 3), these adaptors serve several functions. The adaptors contain sequences that hybridize to (i) universal primers for PCR amplification and (ii) their respective sequencing primers for sequencing the target DNA fragments. Thus, they are also called “sequencing adaptors.” One of the adaptors further contains sequences that hybridize to (iii) a third PCR primer to add the index sequence and (iv) a third sequencing primer for sequencing the index. (Ex. 1016, p. 003)(“The adapters contain sequences that correspond to the two surface-bound amplification primers on the flow cells used in the Cluster Station. One of the also contains the index sequence.”)(*id.*, Figs. 1 and 2 (workflows), 014 (adaptors)). Thus, the amplified DNA fragments can all be sequenced using the same sequencing primers on the Genome Analyzer.



67. Craig (Ex. 1056) described a generalized framework for multiplex resequencing of targeted genome regions on the Illumina GA and GAIi instruments. Here, 46 HapMap individuals, previously characterized by the Encyclopedia of DNA Elements (“ENCODE”) project, were evaluated by the targeted resequencing method. Here, Craig amplified the 46 HapMap individuals by whole genome amplification followed by two long-range amplification steps using PCR that 24 regions across the human genome. For each HapMap individual, the 5-kb amplicons for the 14 regions were then pooled together in equal portions and digested with DNase I enzyme to produce fragments in the size range of 200-300 bp. Following

additional library steps, a unique indexed-adaptor sequences was ligated to each HapMap individual library sample by an enzyme called DNA ligase. As described above in this declaration, indexed sequences were routinely added by PCR as well. For example, Illumina described adding index sequences to its adaptor-ligated DNA fragments using a PCR enrichment step. Following additional library steps, each HapMap sample was pooled into a single tube and subjected to a PCR enrichment step using Illumina compatible primers. The pooled indexed-library of 46 HapMap individuals were then sequenced using either an Illumina GA or GAI instrument. Craig reported that >90% of genetic variants were discoverable using their targeted multiplex sequencing approach.

F. By 2010, skilled artisans were focused on creating target-specific DNA libraries

68. As described in my 2010 review of the NGS field, “[d]espite the substantial cost reductions associated with NGS technologies in comparison with the automated Sanger method, whole-genome sequencing is still an expensive endeavor. An interim solution to this problem may be to use NGS platforms to target specific regions of interest.”

69. The concept of targeting specific regions of the genome is well established, with PCR being the most widely used method, albeit on a small scale. A strategy based on microdroplet PCR technology was developed in 2009 to simultaneously amplification of 3,976 products. Here, a microfluidic device creates

aqueous picoliter volume droplets of targeting forward and reverse primers in an oil solution. Primer droplets targeting different regions merge with separate picoliter droplets containing fragmented barcoded genomic DNA and associated PCR reagents, which are then thermal cycled in a single tube. The authors reported an 84% capture efficiency with 90% of the targeted bases showing uniform coverage using the microdroplet PCR method sequenced with either Roche/454 or Illumina/Solexa platform. (Ex. 1054)(“Tewhey”).

VI. BASIS OF MY OPINION

70. I understand that the '831 Patent (Ex. 1001) was filed on April 2, 2015 and claims an earliest priority to January 23, 2010, which is the filing date of U.S. Provisional Serial No. 61/297,755 (the “'755 Provisional,” Ex. 1055). I have not seen any evidence to suggest that any claim of the '831 Patent is entitled to the benefit of an earlier priority date. I am not aware of any claim by the Patent Owner to an earlier priority date that would change any of my opinions as set forth herein. For my analysis below, I have considered January 23, 2010 as the relevant date.

71. In preparing this Declaration, I have reviewed the '831 Patent (Ex. 1001), its file history (Ex. 1010) and the '755 Provisional (Ex. 1055), and considered each of the documents cited herein, in light of general knowledge in the art before January 23, 2010.

72. In formulating my opinions, I have relied upon my 30+ years of

education, training, knowledge, and experience in the relevant art. But at all times, I have attempted to understand and interpret the claims of the Patents-in-Suit from the point of view of the person of ordinary skill in the art as of the early January, 2010 time period.

73. I understand that for the purposes of an IPR, claims are to be given their broadest reasonable interpretation (“BRI”) in light of the specification of the ’831 Patent.

VII. THE ’831 PATENT

74. I understand that the ’831 Patent (Ex. 1001) resulted from the ’854 Application, filed on April 2, 2015, which claims earliest priority to the ’755 Provisional filed on January 23, 2010 and is entitled “methods of fetal abnormality detection.” (Ex. 1055).

75. I have been informed by counsel for Petitioner that claims 1 and 14 are independent claims, which set forth the elements of the invention and do not rely upon or refer to any other claims in the patent.

76. I have been informed by counsel for Petitioner that claims 2-10, 12-13, 15-22 and 24 are dependent claims, which contain all of the features of the claim from which they depend but also contain additional features or limitations. In this case, claims 2-10 and 12-13 are dependent from claim 1 since they refer directly to claim 1, while claims 15-22 and 24 are dependent from claim 14 since they refer

directly to claim 14.

VIII. RELEVANT LAW

77. I am not a lawyer, but I understand the following concerning the applicable law:

A. Anticipation

78. I understand that a patent claim is anticipated when a single piece of prior art discloses every element of the claimed invention, either expressly or inherently, arranged in the same way as in the claim. For inherent anticipation to be found, it is required that the missing descriptive material is necessarily present in the prior art. I understand that, for the purpose of an *Inter Partes* review, prior art that anticipates a claim can include both patents and printed publications from anywhere in the world.

B. Obviousness

79. I understand that a claim in an issued patent can be unpatentable if it is obvious. Unlike anticipation, obviousness does not require that every element of the claim be in a single prior art reference. Instead, it is possible for claim elements to be described in different prior art references, so long as there is motivation or sufficient reasoning to combine the references and a reasonable expectation of success. The prior art references must also be “analogous art,” meaning that they are either from the same field of endeavor as the ’831 Patent, regardless of the problem addressed, or reasonably pertinent to the particular problem with which the

inventor is involved.

80. I understand that a claim is unpatentable for obviousness if the differences 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 alleged invention was made to a person having ordinary skill in the art to which said subject matter pertains.

81. I understand, therefore, that when evaluating obviousness, one must consider obviousness of the claim “as a whole.” This consideration must be from the perspective of one of ordinary skill in the relevant art, and that such perspective must be considered as of the “time the invention was made.”

82. The level of ordinary skill in the art is discussed in ¶79 below.

83. The relevant time frame for obviousness, the “time the invention was made,” is discussed in ¶91, below.

84. I understand that in considering the obviousness of a claim, one must consider the so-called *Graham* factors, including the following four things. These include the scope and content of the prior art, the level of ordinary skill in the art at the relevant time, the differences between the prior art and the claim, and any “secondary considerations.”

85. I understand that “secondary considerations” include real-world evidence that can tend to make a conclusion of obviousness either more probable or

less probable. For example, the commercial success of a product embodying a claim of the patent could provide evidence tending to show that the claimed invention is not obvious. In order to understand the strength of the evidence, one would want to know whether the commercial success is traceable to a certain aspect of the claim not disclosed in a single prior art reference (i.e., whether there is a causal “nexus” to the claim language). One would also want to know how the market reacted to disclosure of the invention, and whether commercial success might be traceable to things other than innovation, for example the market power of the seller, an advertising campaign, or the existence of a complex system having many features beyond the claims that might be desirable to a consumer. One would also want to know how the product compared to similar products not embodying the claim. I understand that commercial success evidence should be reasonably commensurate with the scope of the claim, but that it is not necessary for a commercial product to embody everything that would fall within the scope of the claim.

86. Other kinds of secondary considerations are possible. For example, evidence that the relevant field had a long-established, unsolved problem or need that was later provided by the claimed invention could be indicative of non-obviousness. Evidence that others had tried but failed to make an aspect of the claim might indicate that the art lacked the requisite skill to do so. Evidence of copying of the patent owner’s products before the patent was published might also indicate that

its approach to solving a particular problem was not obvious. Evidence that the art recognized the value of products embodying a claim, for example, by praising the named inventors' work, might tend to show that the claim was non-obvious.

87. I further understand that prior art references can be combined where there is an express or implied rationale to do so. Such a rationale might include an expected advantage to be obtained or might be implied under the circumstances. For example, a claim is likely obvious if design needs or market pressures existing in the prior art make it natural for one or more known components to be combined, where each component continues to function in the expected manner when combined (i.e., when there are no unpredictable results). A claim is also likely unpatentable where it is the combination of a known base system with a known technique that can be applied to the base system without an unpredictable result. In these cases, the combination must be within the capabilities of one of ordinary skill in the art.

88. I understand that when considering obviousness, one must not refer to teachings in the specification of the patent itself. One can, however, refer to portions of the specification admitted to being prior art, including the "BACKGROUND" section. Furthermore, a lack of discussion in the patent specification concerning how to implement a disclosed technique can support an inference that the ability to implement the technique was within the ordinary skill in the prior art.

89. I also understand that, to combine or modify a reference in a way that

can be called obvious, one of ordinary skill would have to have a reasonable expectation of success. I understand that the reasonable expectation of success does not mean certainty or a guarantee of success, but rather only that it is a “reasonable” expectation. The reasonable expectation of success is required for what is recited in the claims but is not required for concepts not recited in the claims.

IX. LEVEL OF ORDINARY SKILL IN THE ART

90. In my opinion, the relevant art was that of molecular biology. I have considered the level of ordinary skill based on my experience in the field, and the educational level of the inventor; the type of problems encountered in the art; prior art solutions to those problems; rapidity with which innovations are made; sophistication of the technology; and educational level of active workers in the field. I understand that not all such factors may be present in every case, and one or more of these or other factors may predominate in this case. In the present case, I understand that both inventors were Ph.D.-level molecular biologists, and that in 2010, Dr. Chuu had approximately 15 years of experience, while Dr. Rava had almost 30 years of experience. Based on this and on my experience in working with others in the field, I believe that the ordinary artisan in the relevant timeframe was highly skilled compared to many other industries and would have had a Ph.D. in molecular biology or a related field and approximately five years of experience working with or researching genetic testing, DNA amplification, and DNA

sequencing.

X. RELEVANT TIMEFRAME FOR DETERMINING OBVIOUSNESS

91. I understand that obviousness must be evaluated “at the time of the invention.” From the cover page of the ’831 Patent, the ’755 Provisional was filed in the United States on January 23, 2010. For the purpose of this declaration, I will analyze obviousness in the time frame immediately prior to this date, although my testimony is usually applicable to a longer period of time before January 23, 2010. My testimony is directed to this timeframe, unless otherwise indicated, even if I do not always use a past tense.

XI. CLAIM CONSTRUCTION

A. Claims 1, 6, 7, 14, 18 and 9—“Aneuploid”

92. I have reviewed the construction of the term “aneuploid” in the petition for *Inter Partes* review, and I agree based on the specification at Ex. 1001, 13:32-46 that “aneuploid” should be defined as “having an abnormal number of chromosome(s), or parts of a chromosome.”

I. GROUNDS OF UNPATENTABILITY

Ground 1. Claims 1-7, 10, 14-19, and 22 are obvious over Fluidigm in view of the knowledge of one of ordinary skill

93. It is my opinion that claims 1-7, 10, 14-19, and 22 of the ’831 Patent would have been obvious to one of ordinary skill in the art in the relevant timeframe over U.S. Pat. App. Publication 2010/0120038 (“**Fluidigm**”) in view of the

knowledge of a person of ordinary skill, as reflected in, for example:

- Ex. 1006, Fan, et al., “Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood,” Vol. 105:16266–16271 PNAS (October 21, 2008)(“**Fan 2008**”);
- Ex. 1008, Bentley, et al., “Accurate whole human genome sequencing using reversible terminator chemistry,” Nature, Vol 456:53-59 (November 2008)(“**Bentley**”);
- Ex. 1012, Quail, et al., “A large genome center’s improvements to the Illumina sequencing system,” Nature Methods, Vol. 5:1005-1010 (Dec. 2008)(“**Quail**”);
- Ex. 1013, Chiu, et al. “Noninvasive Prenatal Diagnosis of Fetal Chromosomal Aneuploidy by Massively Parallel Genomic Sequencing of DNA in Maternal Plasma,” PNAS Vol. 105:20458-20563 (Dec. 23, 2008)(“**Chiu**”);
- Ex. 1014, “Multiplexed Sequencing with the Illumina Genome Analyzer System”, Illumina, 2008 (“**Illumina Datasheet**”).

Overview of Fluidigm

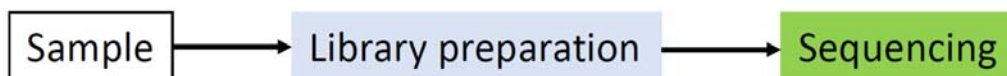
94. The Fluidigm publication discusses methods for improving preparation of DNA libraries, and in particular libraries to be used for later sequencing. Such sequencing libraries are typically amplified target DNA sequences that can have adaptors and index sequences added. Fluidigm discusses library preparation, for

example, near the beginning of its disclosure:

“The present invention relates generally to the area of high-throughput assays for detection of particular target nucleic acids. In particular, the invention relates to **methods for increasing the number of samples and/or targets** that can be analyzed in a single assay.”

(Ex. 1003, ¶0002)(Emphasis added)(Ex. 1004, ¶0002). I understand that the phrase “methods for increasing the number of samples and/or targets that can be analyzed” to be steps of library preparation. The libraries prepared can be used for “assays,” as Fluidigm states, which would include sequencing to determine the existence or amount of particular DNA sequences. For example, Fluidigm states: “[i]f desired, **tagged target nucleotide sequences generated as described herein** may be analyzed by **DNA sequencing.**” (Ex. 1003, ¶0160)(Emphases added)(Ex. 1004, ¶0135).

95. In this way, Fluidigm discloses teaches preparation of a DNA sequence library, followed by sequencing of that library, as shown in the following diagram (drawn by the Petitioner):



96. Fluidigm has several methods to create sequencing libraries. These

methods involve various amplification and tagging steps. One method (the so-called “Modular Approach” is very close to anticipating the claims, as I understand anticipation (see ¶77, above). The “Modular Approach” is discussed mainly under the heading “Detection of Multiple Target Nucleic Acids—Modular Approach” beginning in paragraph 0115 of Fluidigm, although disclosure throughout Fluidigm modifies the Fluidigm embodiments where indicated. The Modular Approach uses three targeted amplification steps on which the steps of independent claims 1 and 14 read. (Ex. 1003, ¶¶0119-0120)(Ex. 1004, ¶¶0094-0095).

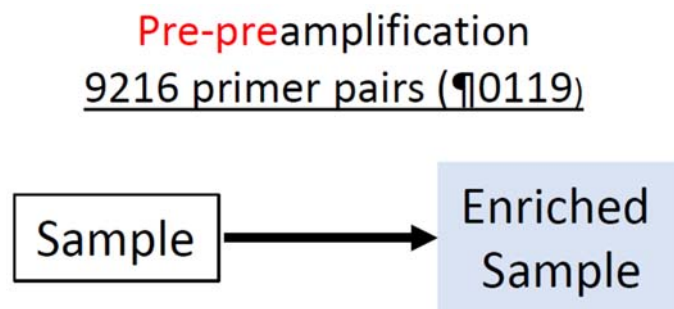
97. The Modular Approach of Fluidigm begins with a sample. The sample can be a sample of maternal blood containing fetal DNA being tested for aneuploidy. (Ex. 1003, ¶¶0122, 0175)(Ex. 1004, ¶¶0097, 0150). It would have obvious to one of ordinary skill in the art the obtaining a maternal blood sample would contain both maternal and fetal cell-free DNA based on the teachings of Lo 1997 (Ex. 1041), Lo 2007A (Ex. 1040), Lo 2007B (Ex. 1043), Fan 2007 (Ex. 1044), Chiu (Ex. 1013), and Fan 2008 (Ex. 1006), for example.

98. The first step of the Modular Approach is to use a “**pre-pre-amplification**” step. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The pre-pre-amplification step uses PCR amplification with 9,216 primer pairs, to amplify 9,216 target sequences. For example, Fluidigm discloses:

“To increase target nucleic acid concentration prior to

encoding, an optional pre-preamplification reaction can be carried out before the encoding preamplification reaction. The pre-preamplification can be carried out in multiplex [sic: multiplex]. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture.”

(Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The method thus far can be depicted as follows:

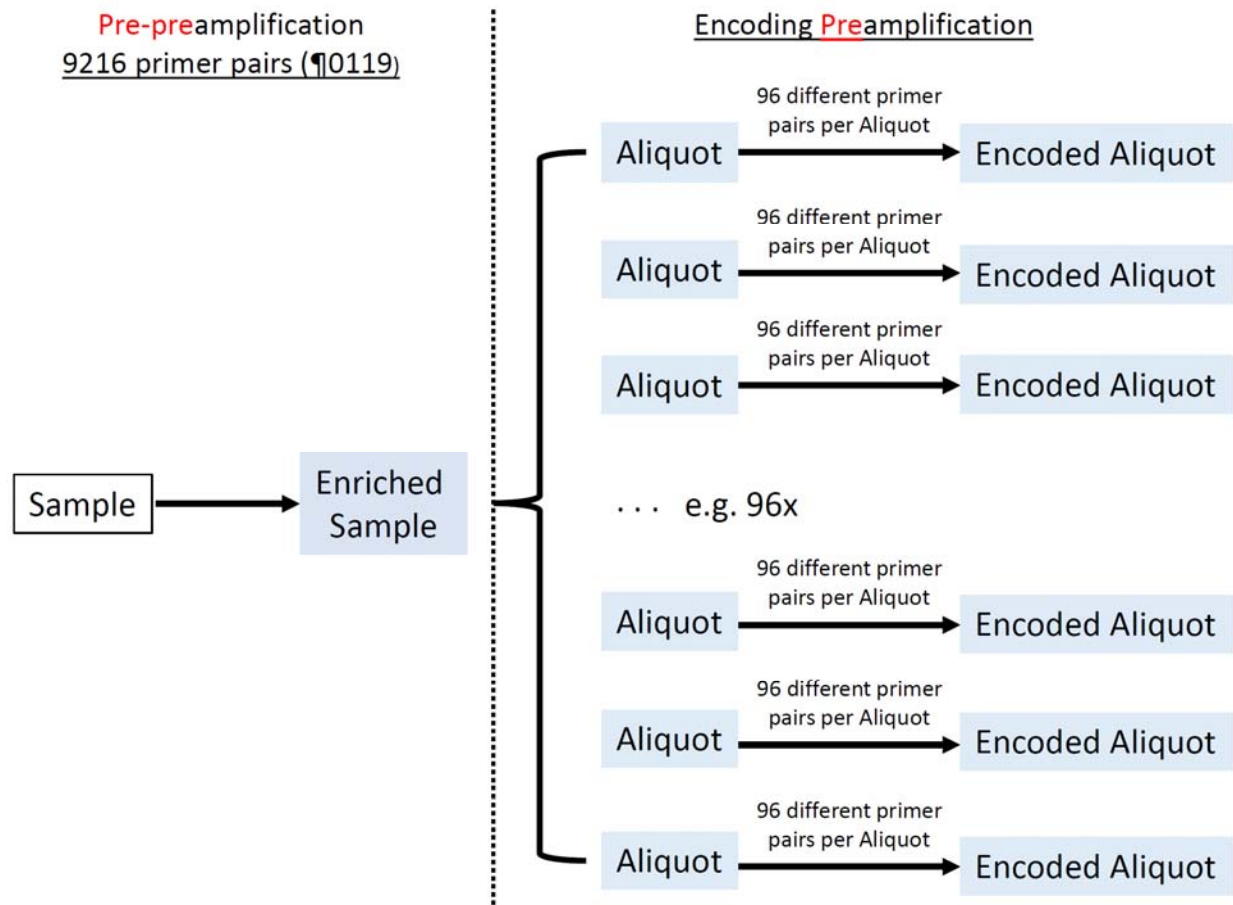


99. The second amplification step in the Fluidigm Modular Approach is the “encoding pre-amplification” step. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). In this step, the output of the first step is divided into different aliquots (in Fluidigm, the example of 96 aliquots is used). (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The 96 aliquots are then each subjected to PCR amplification, using 96 different primer pairs in each aliquot. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The 96 primer pairs per aliquot can be used to introduce “nucleotide tag pairs.” (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The nucleotide tag pairs, which are also known as “bar codes” or “encoding sequences”, are sequences whose purpose can be to identify the DNA sequences in

the sample, or to identify different samples. (Ex. 1003, ¶¶0064, 0094, 0115)(Ex. 1004, ¶¶0005-0008, 0071, 0077-0078, 0082). Fluidigm describes the “encoding pre-amplification” step immediately after describing the “pre-pre-amplification step”, as follows:

“The **pre-pre**amplification can be carried out in multiplex [sic: multiplex]. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture. **This mixture can then be divided into R=96 aliquots and each aliquot subjected to an encoding preamplification reaction** on a microfluidic device, using T=96 different primer pairs that add 96 different nucleotide tag pairs to the target nucleotide sequences in each of the 96 aliquots.”

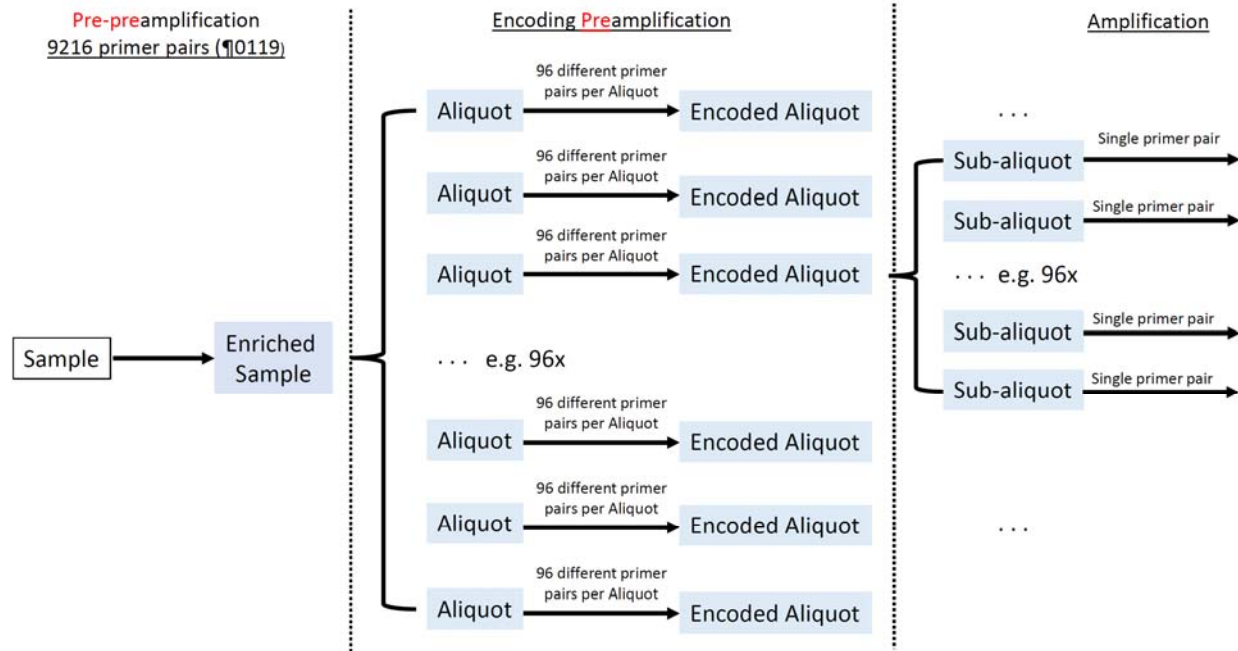
(Ex. 1003, ¶0119)(Emphases added)(Ex. 1004, ¶0094). After the “encoding pre-amplification” step, the Fluidigm Modular Approach can be depicted as follows (illustration by Petitioner):



100. Following the second amplification (*i.e.* the “encoding preamplification”) step, each of the separate aliquots containing tagged DNA proceeds to separate chambers in a microfluidic device. (Ex. 1003, §0120)(Ex. 1004, §0095). There, each aliquot will be subdivided 96 times. Each sub-aliquot is subjected to a third amplification using a single PCR primer pair. (Ex. 1003, §0120)(Ex. 1004, §0095). The single primer used with each subaliquot is specific to the combination of sequence tags used in the “encoding preamplification” step. (Ex. 1003, §0120)(Ex. 1004, §0095). For example, Fluidigm discloses:

“After the encoding preamplification reaction, amplification can be carried out in separate chambers of a microfluidic device. For example, each of the 96 aliquots produced upon encoding preamplification can be loaded into individual sample lines of a matrix-type micro fluidic device, and each of 96 different tag-specific primer combinations can be loaded into individual assay columns. Each different of the 96 primer combination can amplify a different target nucleic acid in each of the 96 aliquots. The resulting 9216 reaction chambers (sub-aliquots) can then be subjected to amplification, followed by detection of amplification product(s)....”

(Ex. 1003, ¶0120)(Emphasis added)(Ex. 1004, ¶0095). I understand this passage to mean that each subaliquot is targeting a different sequence. However, the primers are targeted to specific tag combinations introduced in the encoding amplification. By targeting tag combinations, fewer primers overall are needed for the third amplification step. By physically separating subaliquots, the tag sequences can be re-used, and there is an increased chance of having a single, specific PCR product with minimal to no non-specific products being formed (only one primer pair should be present with each sub-aliquot). With the addition of this amplification step, the Fluidigm Modular Approach can be depicted as follows (illustration by Petitioner):



101. Although Fluidigm teaches that each encoded aliquot is subdivided, the diagram above shows only a single subdivision to save space. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The output of the entire Modular Approach includes 9,216 separate sub-aliquots with 9,216 enriched, tagged DNA sequences to create a sequencing library. (Ex. 1003, ¶¶0160, 0177)(Ex. 1004, ¶¶0135, 0152).

102. The three amplification steps described above in the Modular Approach line up with the three amplification steps of independent claims 1 and 14 of the '831 Patent as follows:

'831 claim step	Corresponding Fluidigm Step
1 st amplification step	" <i>Pre-preamplification</i> " of multiple targets in a sample using target specific primers
2 nd amplification step	" <i>Encoding preamplification</i> " of Step 1 products using tagged primers
3 rd amplification step	" <i>Amplification</i> " of Step 2 products using tag-specific primers

Rationale (Motivation) Supporting Obviousness

103. As mentioned above, Fluidigm's disclosure is close to anticipating the claims. Fluidigm discloses using cell-free fetal DNA from a maternal blood sample in the three-step selective enrichment of claims 1 and 14, using more than 100 target polynucleotide sequences (in fact, 9,216) sequences. To the extent the standard for anticipation would not be met, the differences were minor and would be obvious.

104. One possible difference rests in the fact that the description of the maternal blood samples, aneuploidy detection, and the use of the library for sequencing are not found directly within the paragraphs that nominally relate to the Modular Approach (¶¶0115-0121) but are found elsewhere in Fluidigm. I will discuss these specific provisions that are found elsewhere below in more detail. However, at a high level, it is clear from the Fluidigm disclosure that there are certain statements in Fluidigm concerning blood samples, aneuploidy detection, and the use of the library for sequencing (e.g.) that are broadly applicable to each of the disclosed methods, and specifically applicable to the Modular Approach.

105. Independent claims 1 and 14 of the '831 Patent also require using "sequences selected from a chromosome tested for being aneuploid." Fluidigm expressly suggests the selection of sequences from a chromosome tested for being aneuploidy. For example, Fluidigm expressly teaches that the detection of target

sequence, which obviously need to be selected, are applicable to identifying the presence of aneuploidy:

“The methods of the invention are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids in a nucleic acid sample. Thus, for example, these methods are applicable to identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy.”

(Ex. 1003, ¶0175)(Emphases added)(Ex. 1004, ¶0150). Aneuploidy detection was also being conducted regularly in the course of pre-natal care in the relevant time frame. Because aneuploidy detection is the detection of an abnormal number of chromosomes (e.g., three copies of chromosome 21 when testing for Down syndrome), it would have been obvious to target sequences from a specific chromosome for which aneuploidy was suspected.

106. Also, the last part of independent claim 1 (but not claim 14) requires:

“wherein at least one primer of at least one of the second and third sets of primers includes a sequence configured to be added to the different non-random polynucleotide sequences to permit the enriched non-random polynucleotide sequences of the library to anneal to a same sequencing primer...”

(Ex. 1001, 63:66-64:42). Fluidigm teaches that its embodiments used for encoding

(including the Modular Embodiment) can introduce adaptor sequences. (Ex. 1003, ¶0160-161)(Ex. 1004, ¶¶0135-0136). Certain kinds of adaptor sequences were well-known to be used with NGS sequencing systems of the time. For example, Fluidigm teaches substituting methods for tagging nucleotide sequences for ligation, which was normally used to add sequencing adaptors. (Ex. 1003, ¶0160)(Ex. 1004, ¶0135). Sequencing adaptors are adaptors to which sequencing primers are used, for example, in the Solexa Genome Analyzer system. Note that the company Solexa was acquired by Illumina, and its Genome Analyzer later became the Illumina Genome Analyzer. The use of Solexa sequencing is expressly taught by Fluidigm. (Ex. 1003, ¶0161)(Ex. 1004, ¶0136). Based on Fluidigm's suggestion to use Solexa sequencing, and to substitute tagging methods for ligation to add adaptor sequences, it would have been obvious for one of ordinary skill in the art to have used one of the PCR-based amplification steps in Fluidigm's Modular Approach to add sequencing adaptors that included a same sequencing primer to the library molecules. The various amplification steps of Fluidigm's Modular Approach can be used to introduce an arbitrary tag sequence (within reasonable limits), and it would have been obvious to include a sequencing adaptor that included a same sequencing primer next to the tag sequences.

107. I will further explain the motivation supporting obviousness in the discussion relating to specific claim elements, below.

Graham Factors

108. I discuss the so-called *Graham* factors (referred to in ¶84, above) in the next few paragraphs, ¶¶109-112.

109. As discussed above in ¶90, the level of ordinary skill was high. The ordinary artisan in the relevant timeframe would have had a Ph.D. in molecular biology or a related field and approximately five years of experience working with or researching genetic testing, DNA amplification, and DNA sequencing.

110. The scope and content of the prior art are discussed throughout my declaration concerning each of the grounds.

111. The differences between the prior art and the claims are discussed in the “Overview of the Combination” and in the claim mapping, below.

112. I am not aware of any secondary considerations that would make an inference of non-obviousness more likely.

Reasonable Expectation of Success

113. It is my opinion that one of skill in the art in the relevant timeframe would have had a reasonable expectation of success in applying Fluidigm in a manner that meets the challenged claims of the '831 Patent.

114. As discussed above, by the relevant timeframe, the PCR method, upon which the three amplification steps of Fluidigm’s Modular Approach are described, was well-understood and predictable. In particular, one of ordinary skill in the art

would have been able to use the PCR reaction to target particular DNA sequences and would have been able to create primer pairs configured to add arbitrary nucleotide sequences (like “tags” and “adaptors”) to such targeted sequences. I note that the ’831 Patent itself does not provide anything new with respect to PCR amplification or the actual means by which one would introduce nucleotide tags or sequencing adaptors but is rather directed to the general idea of using PCR amplification in three steps while introducing tags and adaptors.

115. One of ordinary skill would have reasonably expected success in using Fluidigm’s Modular Approach to test samples for aneuploidy of a particular chromosome. As discussed above, in the relevant timeframe, next-generation sequencing was in widespread use. In fact, 454 Life Sciences Corporation’s sequencer, known as Genome Sequencer 20, was commercially in use by 2005. Likewise, Solexa’s next generation sequencer was released in late 2006 and achieved widespread commercial use by 2007. Barcoding or sample tagging was equally prevalent. Further, a kit for making a sequencing library for use with Solexa’s sequencer was commercially available in 2008, underscoring the routine nature of sequencing library preparation. Thus, one of ordinary skill would have expected success in being able to sequence cell-free DNA found in maternal blood.

116. It was further well-known by 2008 that one could isolate, amplify and sequence cell-free genomic DNA from maternal blood to detect aneuploidy,

including fetal trisomy 21. (Ex. 1006, Abstract)(Ex. 1013, Abstract)(Ex. 1003, ¶00175)(Ex. 1005, 25:10-20). As such, one of ordinary skill would have had a reasonable expectation of success in using cell-free DNA from maternal blood in a multi-step PCR method (such as Fluidigm's). One of ordinary skill would have expected reasonable success in identifying sequences from particular chromosomes, because the human genome had already been sequenced by the relevant timeframe. (Ex. 1057)("IHGSC"). Cell-free genomic DNA from maternal blood had also previously been successfully amplified and sequenced to detect aneuploidy, including fetal trisomy 21, trisomy 18 and trisomy 13. (Ex. 1006, Abstract)(Ex. 1013, Abstract). This typically involved amplifying DNA from a suspected chromosome and a reference chromosome and detecting whether levels of sequences of the suspect chromosome were elevated. (Ex. 1013, p. 001, right column). Given the success in using cell-free DNA to perform amplification and sequencing, one of ordinary skill would have had a reasonable expectation of success to employ cell-free DNA from maternal blood in the Fluidigm method with the goal of testing for chromosomal aneuploidy.

Fluidigm is Analogous Art

117. It is my opinion that Fluidigm and the other prior art cited in this Ground would be considered "analogous art" because it is in the same field as the '831 Patent (Fluidigm concerns DNA sequencing library preparation), and/or the

subject matter of the prior art would have been reasonably pertinent to the problems facing the named inventors.

Claim Mapping

118. This section maps the challenged claims to the relevant disclosures of Fluidigm, where the claim text appears in bold-italics, and the relevant mapping follows the claim text.

Claim 1

“1. A method for preparing a sequencing library from a maternal blood sample, the method comprising:”

119. Fluidigm states that its methods beginning with a sample. (Ex. 1003, ¶0116)(Ex. 1004, ¶0091). Fluidigm states that DNA “samples” of fetal DNA can come from **maternal blood**:

“Sample Preparation [¶] DNA or RNA useful in the methods described herein can be extracted and/ or amplified from any source.... For example, **samples of fetal DNA can be obtained from** an embryo (e.g., from one or a few embryonic or fetal cells) or from **maternal blood.**”

(Ex. 1003, ¶0122)(Emphases added)(Ex. 1004, ¶0097). Paragraph 0122 of Fluidigm obviously applies to the “Modular Approach,” because ¶0122 applies to “the methods described herein” which should be read as meaning “in the Fluidigm disclosure.” Furthermore, it makes technical sense that the Modular Approach would begin with such a sample.

120. Fluidigm describes using the methods to prepare a sequencing library, as in ¶¶0160-0161. For example, Fluidigm discloses that:

“If desired, **tagged target nucleotide sequences generated as described herein may be analyzed by DNA sequencing.** Many current DNA sequencing techniques rely on ‘sequencing by synthesis.’ These techniques entail **library creation**, massively parallel PCR amplification of library molecules, and sequencing. Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors.”

(Ex. 1003, ¶0160 see also ¶0177)(Emphases added)(Ex. 1004, ¶¶0135, 0152). As shown in the quote above, Fluidigm suggests analyzing the “nucleotide sequences generated as described herein” using DNA sequencing. Because the “Modular Approach” is “described herein” (*i.e.* in the Fluidigm disclosure) and because the Modular Approach is appropriate for producing a sequencing library, one of ordinary skill would have found it obvious that ¶0160 to applies to the Modular Approach.

121. Thus, one of skill in the art in the relevant timeframe, reading Fluidigm, would have found it obvious that Fluidigm’s Modular Approach was useful for preparing a sequencing library from a maternal blood sample.

“a. obtaining a maternal blood sample comprising fetal and maternal cell-free DNA;”

122. *See* immediately above. Fluidigm in ¶0122 describes using “samples of fetal DNA...from maternal blood.” (Ex. 1003, ¶0122)(Ex. 1004, ¶¶0097). This fetal DNA would have been understood in the relevant timeframe to include cell-free fetal and maternal DNA, and certainly cell-free fetal DNA was a focus at that time. (Ex. 1006, Abstract)(Ex. 1013, Abstract)(Ex. 1005, 5:29-6:8). For example, Fluidigm distinguishes between DNA obtained from cells and DNA obtained from bodily fluids, such as blood:

“Nucleic acids can be extracted or amplified **from cells, bodily fluids (e.g., blood, a blood fraction, urine, etc.),** or tissue samples by any of a variety of standard techniques.”

(Ex. 1003, ¶0122)(Emphasis added)(Ex. 1004, ¶0097). Fluidigm also distinguishes between fetal cells and fetal DNA in maternal blood:

“samples of fetal DNA can be obtained from an embryo (e.g., from one or a few embryonic or **fetal cells) or from maternal blood.**”

(Ex. 1003, ¶0122)(Emphasis added)(Ex. 1004, ¶0097). Fluidigm further indicates that a sample can simply be blood plasma, which is cell-free. (Ex. 1003, ¶0122)(Ex. 1004, ¶0097)(Ex. 1006, p. 001)(Ex. 1013, p. 001). Lo 2007A taught that maternal blood contains cell-free or extracellular fetal nucleic acids: “Since 1997, the progress of this field has been accelerated by **the unexpected finding of extracellular fetal nucleic acids in maternal plasma.**” (Ex. 1040, Abstract)(Emphasis added). These

disclosures would have indicated to one of ordinary skill that Fluidigm considered “blood” and “a blood fractions” to be a source separate from “cells,” (i.e., extracellular) and thus that Fluidigm contemplated using “cell-free” fetal and maternal DNA.

“b. selectively enriching a plurality of non-random polynucleotide sequences of genomic DNA from said fetal and maternal cell-free DNA to generate a library of enriched non-random polynucleotide sequences,”

123. As described above beginning in ¶¶94-106, Fluidigm teaches a process of selectively enriching of a plurality of non-random polynucleotide sequences of genomic DNA from said fetal and maternal cell-free DNA, to generate a library of those sequences. The Modular Approach of Fluidigm uses three targeted amplification steps, which are described above and in each of the three steps (i) – (iii), below.

124. The three steps (detailed below) lead to “selective enriching” because the PCR primers described in the Fluidigm Modular Approach anneal to, and thus cause amplification only of, sequences that contain complementary base pairs.

125. The “plurality of non-random polynucleotide sequences” required in claim 1 of the ’831 Patent are the sequences to which the primers anneal. These sequences are non-random, because they are effectively selected by the primers used, which Fluidigm describes as “target-specific primers.” (Ex. 1003, ¶0119)(Ex.

1004, ¶0094). There are 9,216 pairs of “target-specific primers” in the pre-amplification step of the Modular Approach, resulting in a plurality (e.g., 9,216) of targeted sequences. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094)

126. Fluidigm further teaches that the targeted sequences can be of genomic DNA. Regarding the targeted nucleic acids (polynucleotide sequences), Fluidigm states:

“Target Nucleic Acids [¶0124] **Any target nucleic acid** that can be tagged in an encoding reaction of the invention (described herein) can be detected using the methods of the invention....[¶0125] The targets can include, for example, nucleic acids associated with pathogens, such as viruses, bacteria, protozoa, or fungi; RNAs, e.g., those for which over- or under-expression is indicative of disease, those that are expressed in a tissue- or developmental-specific manner; or those that are induced by particular stimuli; **genomic DNA, which can be analyzed for specific polymorphisms (such as SNPs), alleles, or haplotypes, e.g., in genotyping. Of particular interest are genomic DNAs that are altered (e.g., amplified, deleted, and/or mutated) in genetic diseases or other pathologies; sequences that are associated with desirable or undesirable traits; and/or sequences that uniquely identify an individual (e.g., in forensic or paternity determinations).**”

(Ex. 1003, ¶¶0124-0125)(Emphases added)(Ex. 1004, ¶0094-0095). One of skill in

the art would have found it obvious that the disclosures in ¶¶0124 and 0125 of Fluidigm applies to the Modular Approach, because ¶0124 expressly relates the disclosure to an “encoding reaction of the invention (described herein),” meaning an invention described in Fluidigm. Paragraph 0124 states that sequences that “can be tagged in an encoding reaction” can be used. An encoding reaction (i.e., the “encoding preamplification”) is used in the Fluidigm Modular Approach, as described above in ¶99, and below under claim element (ii). This likewise indicates that ¶¶0124-0125 of Fluidigm are applicable to the Modular Approach. Finally, it was well-known in the relevant timeframe that fetal and maternal DNA from maternal blood included cell-free genomic DNA.

127. Fluidigm also discloses that the Modular Approach can be used to generate a library of enriched non-random polynucleotide sequences. The formation of a sequencing library is discussed above in ¶¶120-121, under the mapping preamble of claim 1. In short, Fluidigm states in ¶0160 that the methods “described herein” (*i.e.* described in Fluidigm) can be used to prepare a library for later sequencing. (Ex. 1003, ¶0160)(Ex. 1004, ¶0135). As further discussed immediately above, the library is a library of polynucleotide sequences that are enriched and non-random.

“wherein said plurality of non-random polynucleotide sequences comprises at least 100 different non-random polynucleotide sequences selected from a chromosome tested for being aneuploid, said enriching comprising:”

128. The Modular Approach of Fluidigm ultimately results in 9,216 different non-random polynucleotide sequences in the library, which is at least 100 different sequences. (Ex. 1003, ¶¶0119 and ¶0116)(Ex. 1004, ¶0094).

129. Fluidigm makes clear that the sequences are both different and non-random, by stating that “target specific primers for 9216 different target nucleic acids can be employed in one mixture.” (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The “nucleic acid” is cell-free genomic DNA, and a polynucleotide sequence. (Ex. 1003, ¶¶0042-0043)(Ex. 1004, ¶0094). Note that Fluidigm states that “[t]he term ‘target nucleic acids’ is used herein to refer to particular nucleic acids to be detected in the methods of the invention.” (Ex. 1003, ¶0048)(Emphasis added)(Ex. 1004, ¶0034). Thus, the use of the term “target-specific primers” indicates that the sequences are non-random. The same 9,216 sequences are retained throughout the entire Modular Approach.

130. Fluidigm also teaches that the methods can be used to create a library for aneuploidy detection. Fluidigm discloses that:

“The methods of the invention are applicable to any technique aimed at **detecting the** presence or **amount of one or more target nucleic acids** in a nucleic acid sample. Thus, for

example, **these methods are applicable to identifying the presence of** particular polymorphisms (such as SNPs), alleles, or haplotypes, or **chromosomal abnormalities**, such as amplifications, deletions, or **aneuploidy**.”

(Ex. 1003, ¶0175)(Emphases added)(Ex. 1004, ¶0150). One of ordinary skill would have found it obvious to use the Modular Approach of Fluidigm to test for aneuploidy, because of the express suggestion quoted above. The suggestion applies to “the methods of the invention”, *i.e.* the methods in Fluidigm, including the Modular Approach.

131. It would have been obvious based on Fluidigm to test sequences relating to a single chromosome. Fluidigm expressly suggests testing for aneuploidy, which is an abnormal number of chromosome(s), or parts of a chromosome. For example, an extra copy of chromosome 21 resulted in the condition trisomy 21 (also known as “Down syndrome”). When testing for aneuploidy (as expressly suggested by Fluidigm), it would have been obvious to target sequences on the chromosome suspected of aneuploidy to determine. This would have allowed one of ordinary skill to count the sequences present, to determine if there was an elevated level of sequences from the chromosome suspected of trisomy. Fluidigm expressly teaches, for example, that “the presence or **amount** of one or more target nucleic acids” can lead to a detection of aneuploidy. (Ex. 1003, ¶¶0175)(Emphasis added)(Ex. 1004, ¶0150). The detection of the

amount of a target nucleic acid on a chromosome (as compared to a reference, e.g. a chromosome not being tested for aneuploidy) would obviously have been a way to test for aneuploidy, which is an abnormal number of a particular chromosome. For example, it would have been obvious that an extra copy of chromosome 21 in the fetal genome would have led to elevated levels of sequences from chromosome 21. It would further have been obvious that using more sequences on chromosome 21 would have been more likely to produce a confident diagnosis.

132. Such counting was a well-known technique in the relevant timeframe. For example, Chiu taught detecting trisomy 21 (“T21”) by testing a large number of sequences on chromosome 21 (“chr21”) and looking for elevated levels of chromosome 21 sequences in maternal blood. Chiu explains:

“[F]etal T21 is diagnosed by **detecting the small increment in the total amount of the chr21** gene locus contributed by the trisomic chr21 in the fetus as **compared with a gene locus on a reference chromosome**. The proportional increment in chr21 sequences is expectedly small because fetal DNA contributes only a minor fraction of DNA in **maternal plasma** (8). To reliably detect the small increase, **a large absolute number of chr21 and reference chromosome sequences** of the loci targeted by the digital PCR assays **need to be analyzed....**”

(Ex. 1013, p. 001, right column)(Emphases added).

133. Likewise, Fan 2008 taught that “[b]y counting the number of sequence tags mapped to each chromosome, the over- or underrepresentation of any chromosome in maternal plasma DNA contributed by an aneuploid fetus can be detected.” (Ex. 1006, p. 001, right column).

134. Moreover, targeting larger numbers of sequences on the same chromosome would obviously have increased the chance of detecting an abnormal number of copies of a chromosome in a statistically reliable way (e.g., based on the use of large numbers). For example, Chiu disclosed that:

“To reliably detect the small increase, a large absolute number of chr21 and reference chromosome sequences of the loci targeted by the digital PCR assays need to be analyzed....”

(Ex. 1013, p. 001, right column). One of ordinary skill would have understood this to mean that by testing additional sequences on the same chromosome, the ability to more reliably detect aneuploidy would have been increased.

“(i) a first amplification step to generate a plurality of first reaction products, said amplification comprising at least 100 first primers configured to amplify at least 100 different non-random polynucleotide sequences;”

135. As discussed above in ¶98, the claimed first amplification step in the Modular Approach is the optional “pre-pre-amplification” step. In the pre-pre-amplification step, the sample is selectively enriched by PCR amplification, using

9,216 first primer pairs. Fluidigm discloses:

“To increase target nucleic acid concentration prior to encoding, an optional pre-preamplification reaction can be carried out before the encoding preamplification reaction. The pre-preamplification can be carried out in multiplex [sic: multiplex]. For example, **target-specific primers for 9216 different target nucleic acids** can be employed in one mixture.”

(Ex. 1003, ¶0119)(Emphasis added)(Ex. 1004, ¶0094)

136. The pre-pre-amplification step “increase[s] target nucleic acid concentration.” (Ex. 1003, ¶0119)(Ex. 1004, ¶0094), and thus provides a plurality of first reaction products, in the form of copies of the target sequences.

137. The pre-pre-amplification amplifies 9,216 “different target nucleic acids.” (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). As explained directly above, “different target nucleic acids” means that the nucleic acids (polynucleotide sequences) are both different and non-random.

“(ii) a second amplification step to generate a second reaction product, said amplification comprising a second set of primers comprising sequences contained in the first reaction products;”

138. As discussed above in ¶¶99-100, the Fluidigm Modular Approach uses a second amplification step with a second set of primers. The Modular Approach performs an “encoding pre-amplification” step. (Ex. 1003, ¶0119)(Ex. 1004,

¶0094). The “encoding pre-amplification” step divides the enriched sample produced by the pre-pre-amplification step into a series of different aliquots. In this particular case, the number chosen is 96 aliquots. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The 96 aliquots then each undergo a PCR amplification using, for example, 96 primer pairs in each aliquot. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). The 96 primer pairs per aliquot can contain “nucleotide tag pairs.” (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). These nucleotide tag pairs (also known as “bar codes” or “encoding sequences”) are sequences whose purpose is to identify samples from which the DNA sequences were taken (for example). (Ex. 1003, ¶¶0005, 0064, 0094, 0115)(Ex. 1004, ¶¶0005-0008, 0071, 0077-0078, 0082, 0090). Fluidigm describes the “encoding pre-amplification” step immediately after describing the “pre-pre-amplification step,” as follows:

“The pre-preamplification can be carried out in multiplex [sic: multiplex]. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture. **This mixture can then be divided into R=96 aliquots and each aliquot subjected to an encoding preamplification reaction** on a microfluidic device, using **T=96 different primer pairs** that add 96 different nucleotide tag pairs to the target nucleotide sequences in each of the 96 aliquots.”

(Ex. 1003, ¶0119)(Emphases added)(Ex. 1004, ¶0094).

139. The “encoding preamplification” step uses a second set of primers,

where the second set of primers comprises sequences contained in the first reaction products. This is because the pre-pre-amplification step amplifies the target sequences for the entire Modular Approach. The encoding amplification step then targets these same sequences, which have been divided into 96 aliquots. Fluidigm discloses that:

“To increase target nucleic acid concentration prior to encoding, an optional pre-preamplification reaction can be carried out before the encoding preamplification reaction. The pre-preamplification can be carried out in multiplex. For example, **target-specific primers for 9216 different target nucleic acids** can be employed in one mixture. This mixture can then be divided into R=96 aliquots and each aliquot subjected to an encoding preamplification reaction on a microfluidic device, using T=96 different primer pairs that add 96 different nucleotide tag pairs to **the target nucleotide sequences** in each of the 96 aliquots.”

(Ex. 1003, ¶0119)(Emphases added)(Ex. 1004, ¶0094). Because the same sequences are being targeted, portions of the primers in the second amplification step (the “encoding amplification”) must be complementary to at least portions of the target sequences that were the first reaction product. Because a primer sequence is complementary to one polynucleotide sequence in a DNA molecule, the primer must comprise at least a portion of the complementary sequence on the other strand of a DNA molecule. Thus, the second set of primers “compris[es] sequences contained

in the first reaction products.”

140. Furthermore, Fluidigm expressly states that “[t]o increase specificity, the primers employed for preamplification can be nested relative to primers employed for pre-preamplification.” (Ex. 1003, ¶¶0119)(Ex. 1004, ¶¶0094). Fluidigm discloses that:

“A second primer pair is ‘nested’ relative to a first primer pair if the first primer pair is employed to amplify a first amplification product and then the second primer pair is employed to amplify a target nucleotide sequence within the first amplification product. Nesting can be used increase the specificity of the amplification reaction.”

(Ex. 1003, ¶¶0058)(Emphasis added)(Ex. 1004, ¶¶0044). This further demonstrates that the second set of primers compris[es] sequences contained in the first reaction products.

141. The second amplification step produces a second reaction product, by amplifying the target nucleic acid sequences and adding encoding sequences to them. (Ex. 1003, ¶¶0119, 0116)(Ex. 1004, ¶¶0094).

“and (iii) a third amplification step to generate a third reaction product comprising said library of enriched non-random polynucleotide sequences, said amplification comprising a third set of primers comprising sequences contained in the second reaction products;”

142. As described above in ¶¶100-101, Fluidigm describes a third

amplification step that follows the “encoding preamplification” step. In the third (“amplification”) step, the (*e.g.* 96) separate aliquots containing tagged DNA can be added to separate chambers in a microfluidic device. (Ex. 1003, ¶0120)(Ex. 1004, ¶0095). From there, each aliquot will be *subdivided* 96 times, and each sub-aliquot is subjected to amplification using a single PCR primer pair specific to the sequence tag combinations employed in the “encoding preamplification.” (Ex. 1003, ¶0120)(Ex. 1004, ¶0095). Fluidigm discloses that:

“After the encoding preamplification reaction, amplification can be carried out in separate chambers of a microfluidic device. For example, each of the 96 aliquots produced upon encoding preamplification can be loaded into individual sample lines of a matrix-type micro fluidic device, and **each of 96 different tag-specific primer combinations can be loaded into individual assay columns.** Each different of the 96 primer combination can amplify a different target nucleic acid in each of the 96 aliquots. **The resulting 9216 reaction chambers (sub-aliquots) can then be subjected to amplification,** followed by detection of amplification product(s)....”

(Ex. 1003, ¶0120)(Emphases added)(Ex. 1004, ¶0095). As noted in the bolded language above, the primers used in the amplification step are “tag-specific primer combinations.” (Ex. 1003, ¶0120)(Ex. 1004, ¶0095). This means that the primers anneal to portions of the encoding tags that were included in the previous encoding

amplification step. To do so, these primers must be complementary to portions of the tag sequences that are the second amplification product. Because a primer is complementary to one polynucleotide sequence in a DNA molecule, the primer must be comprised at least a portion of the complementary sequence on the other strand of a DNA molecule. Thus, the third set of primers “comprising sequences contained in the second reaction products.”

143. The output of the amplification step is 9,216 aliquots, each with a specific, enriched target (*i.e.* non-random) sequence. This is the third amplification product comprising said library of enriched non-random polynucleotide sequences.

“wherein at least one primer of at least one of the second and third sets of primers includes a sequence configured to be added to the different non-random polynucleotide sequences to permit the enriched non-random polynucleotide sequences of the library to anneal to a same sequencing primer for the enriched non-random polynucleotide sequences of the library.”

144. Fluidigm also renders obvious using the primers of the second or third amplification step to add a sequence that anneals to a sequencing primer. Fluidigm describes that the various library preparation methods it discloses can be used to prepare sequencing libraries to be used in later sequencing. (Ex. 1003, ¶¶0160-161, 0177)(Ex. 1004, ¶¶0135-0136, 0152). To facilitate the use in later sequencing, Fluidigm teaches adding adaptors through the tagging amplification steps. Fluidigm states that:

“If desired, tagged target nucleotide sequences generated as described herein may be analyzed by DNA sequencing. Many current DNA sequencing techniques rely on ‘sequencing by synthesis.’ These techniques entail library creation, massively parallel PCR amplification of library molecules, and sequencing. Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random- sequence inserts. The above-described methods for tagging nucleotide sequences can be substituted for ligation, to introduce adaptor sequences.”

(Ex. 1003, ¶0160)(Emphases added)(Ex. 1004, ¶0135).

145. Fluidigm also teaches that “Solexa” sequencing as an example of sequencing that can be used with the Fluidigm methods:

“In particular embodiments, the number of library DNA molecules produced in the massively parallel PCR step is low enough that the chance of two molecules associating with the same substrate, e.g. the same bead (in 454 DNA sequencing) or the same surface patch (in **Solexa** DNA sequencing) is low, but high enough so that the yield of amplified sequences is sufficient to provide a high throughput in automated sequencing.”

(Ex. 1003, ¶0161)(Emphasis added)(Ex. 1004, ¶0136). This is relevant because Solexa sequencing in the relevant timeframe was well-known to use a “**same sequencing primer**” (called a “universal primer for sequencing” in Bentley). (Ex. 1008, p. 001). In Solexa sequencing, two sequencing adaptors are added to library molecules, which are then amplified by bridge PCR. The bound templates from the library are made single stranded. A single same or universal sequencing primer is then annealed to each template having respective sequencing adaptor. This approach has the advantage of only requiring one single, same sequencing primer for each library molecule. (Ex. 1008, p. 001).

146. It would have been obvious to use the primers in the amplification (second or third) steps of Fluidigm to introduce sequencing adaptors into the library sequences of Fluidigm’s Modular approach. This is because Fluidigm states that its methods can be used for sequencing analysis, and expressly states that Solexa sequencing can be used. In Solexa sequencing, it is necessary to introduce an adaptor to the library sequences prior to sequencing. When sequencing occurs, a same sequencing primer anneals to the adaptor. (Ex. 1008, p. 001).

147. Fluidigm also suggests that the adaptors otherwise introduced by ligation could be added using the tagging nucleotide sequences. (Ex. 1003, ¶0160)(Ex. 1004, ¶0135). In Solexa sequencing, the sequencing adaptors were typically introduced by ligation. (Ex. 1008, p. 001)(Ex. 1012, Fig. 1). As noted

above, Fluidigm teaches substituting its encoding methods (including the Modular Approach) for such ligation, specifically to add adaptors. (Ex. 1003, ¶0160)(Ex. 1004, ¶0135). It would have been obvious that the adaptors could be introduced by using PCR primers that contain one or more adaptor sequences. It was well-known in the relevant timeframe that PCR primers could be used to introduce arbitrary sequences of reasonable length. For example, Fluidigm teaches using PCR primers in the “encoding amplification” step to introduce DNA sequence tags (Ex. 1003, ¶0119)(Ex. 1004, ¶0094), which are arbitrary sequences used simply to identify different samples. (Ex. 1003, ¶¶0101, 0064, 0094)(Ex. 1004, ¶¶0080, 0050, 0082). This conclusion is reinforced by the fact that Fluidigm teaches that encoding PCR and encoding ligation can both be used to add a tag sequence. In Fluidigm’s words:

“Nucleotide tags can be added, for example, by an **‘encoding PCR’** in which the at least **one primer comprises a target-specific portion and a nucleotide tag** located on the 5’ end of the target-specific portion, and **a second primer that comprises only a target-specific portion or a target-specific portion and a nucleotide tag** located on the 5’ end of the target-specific portion. For illustrative examples of PCR protocols applicable to encoding PCR, see pending WO Application US03/37808 as well as U.S. Pat. No. 6,605,451. **Nucleotide tags can also be added by an ‘encoding ligation’ reaction** that can comprise a ligation reaction in which at least one primer comprises a target-specific portion and nucleotide

tag located on the 5' end of the target-specific portion, and a second primer that comprises a target-specific portion only or a target-specific portion and a nucleotide tag located on the 5' end of the target specific portion.”

(Ex. 1003, ¶¶0062)(Emphases added)(Ex. 1004, ¶¶0048).

148. Fluidigm also teaches that tag sequences introduced by PCR can be functional. For example, Fluidigm teaches that tag sequences can anneal to a later-employed PCR primer. (Ex. 1003, ¶¶0086, 0092, 0108, 0119)(Ex. 1004, ¶¶0074, 0085, 0094). Because one can introduce arbitrary functional sequences using PCR primers (known to be an alternative to ligation), it would have been obvious to introduce a sequencing adaptor sequence to perform Solexa sequencing, as expressly suggested by Fluidigm.

149. Furthermore, it would have been obvious to introduce sequencing adaptors to include a same sequencing primer in the third (“amplification step”) because the primers used in this step can anneal to the encoding tags introduced in the “encoding pre-amplification step,” thus requiring fewer primers overall.

150. In this way it would have been obvious to have at least one primer of at least one of the second and third sets of primers (*i.e.* the primers of the [third] amplification step of Fluidigm’s Modular Approach) to include a sequence (*i.e.* a sequencing adaptor for Solexa sequencing) configured to be added to the different non-random polynucleotide sequences (because the adaptors will be added in the

Modular Approach's [third] amplification step) to permit the enriched non-random polynucleotide sequences of the library to anneal to a same sequencing primer (*i.e.* the Solexa sequencing primer) for the enriched non-random polynucleotide sequences of the library (*i.e.* the output of Fluidigm's Modular Approach).

Claim 2

“2. The method of claim 1, wherein at least one primer of at least one of the second and third sets of primers includes a sequence configured to be added to the different non-random polynucleotide sequences to add an index to the enriched non-random polynucleotide sequences of the library, the index being indicative of the maternal blood sample from which the library was generated.”

151. In my opinion, claim 2 of the '831 Patent would also have been obvious in view of Fluidigm. For example, Fluidigm teaches that samples can be mixed, and when mixed, can use sample-specific nucleotide tags to identify the origin of the sample (*e.g.*, the person from whom the sample was taken). (Ex. 1003, ¶¶0002, 0005, 0062, 0094, 0115)(Ex. 1004, ¶¶0005-0008, 0071, 0077-0078, 0082).

152. Specifically, Fluidigm teaches an “encoding amplification” step that adds a nucleotide tag or index using a second set of primers, as discussed above in ¶¶99 and 138-141. This constitutes at least one primer of at least one of the second set of primers including a nucleotide tag or index sequence configured to be added to the different non-random polynucleotide sequences.

153. It would have been obvious to use a primer in the second step of Fluidigm's Modular Approach to add an index to the enriched non-random

polynucleotide sequences of the library, the index being indicative of the maternal blood sample from which the library was generated.

154. Fluidigm teaches that samples from different individuals can be mixed, and before they are mixed, the samples should be tagged to identify the origin of the sample:

“In certain embodiments, the method entails providing **S samples that will be mixed together** prior to assay, where S is an integer greater than 1. Each of these samples can be **separately subjected to an encoding reaction** that produces a set of T tagged target nucleotide sequences, **each tagged target nucleotide sequence comprising a sample specific nucleotide tag** and a target nucleotide sequence.”

(Ex. 1003, ¶0005)(Emphases added)(Ex. 1004, ¶0005).

155. Fluidigm also defines a “sample-specific nucleotide tag” as follows:

“The term ‘sample-specific’ nucleotide tag is used herein to refer to **a nucleotide tag that encodes the identity of the sample** of the target nucleotide sequence to which the tag is, or becomes, linked in an encoding reaction.”

(Ex. 1003, ¶0064)(Emphasis added)(Ex. 1004, ¶0050). In the context of the present Ground, the tag is an index that is indicative of the maternal blood sample from which the library was generated.

156. In my opinion, it would have been obvious to add a sample-specific

nucleotide tag using the second set of primers. These primers were already intended to add tags (*see* discussion in ¶¶138-141, above). Based on Fluidigm’s teaching to mix samples and track them using a sample-specific nucleotide tag, it would have been obvious to do so. One of ordinary skill would also have had a reasonable expectation of success, because (as discussed above in ¶147), it was well-known that one could add arbitrary sequences of reasonable length using PCR primers.

Claim 3

“3. The method of claim 1, wherein at least one primer of the third set of primers includes a sequence configured to be added to the different non-random polynucleotide sequences to add an index to the enriched non-random polynucleotide sequences of the library, the index being indicative of the maternal blood sample from which the library was generated.”

157. In my opinion, claim 3 of the ’831 Patent would also have been obvious in view of Fluidigm, for largely the same reasons discussed above under claim 2, ¶¶151-156. Fluidigm teaches mixing samples and using a sample-specific nucleotide tag to track the sample’s origin. (Ex. 1003, ¶0002, 0094)(Ex. 1004, 0082). The sample-specific nucleotide tags could be added by PCR, including during the third (amplification) step explained above in ¶¶142-143 and 100-101. It would have been obvious to one of ordinary skill in the relevant timeframe to add the sample-specific nucleotide tag prior to mixing samples. It would further have been apparent that this could happen during any of the three PCR-based amplification steps, including the third (“amplification”) step. Thus, it would have

been obvious to use at least one primer of the third set of primers having a sample-specific nucleotide sequence (*i.e.* a sequence configured to be added to the different non-random polynucleotide sequences to add an index to the enriched non-random polynucleotide sequences of the library). This would have had the added advantage of requiring fewer primers, because the primers could target tags added in the second step. (Ex. 1003, ¶0119)(Ex. 1004, ¶0094). This is because the sample-specific nucleotide sequence “encodes the identity of the sample” (Ex. 1003, ¶0064)(Ex. 1004, ¶0050), it is indicative of the maternal blood sample from which the library was generated.

Claim 4

“4. The method of claim 1, wherein primers of the second set of primers contain universal sequences such that nucleic acids of the second reaction product contain common universal sequences added to the different non-random polynucleotide sequences, and wherein the third set of primers are configured to hybridize at least in part to the common universal sequences.”

158. In my opinion, claim 4 of the '831 Patent would also have been obvious in view of Fluidigm. For example, Fluidigm teaches that each aliquot in the encoding amplification step is amplified using primers (*i.e.* primers of the second set of primers) that introduce tags (*i.e.* the common universal sequences) to which primers in the third set of primers (*i.e.* primers in the amplification step) are configured to hybridize. Fluidigm discloses that:

“Each of the R aliquots can be separately subjected to an

encoding reaction that produces a set of T tagged target nucleotide sequences, wherein T is the number of target nucleic acids to be detected in each aliquot, T being an integer greater than one (e.g., 96). Each tagged target nucleotide sequence includes a first nucleotide tag 5' of a target nucleotide sequence, a target nucleotide sequence, and a second nucleotide tag 3' of the target nucleotide sequence. The combination of nucleotide tags in each of said T tagged target nucleotide sequences is unique for every tagged target nucleotide sequence in each aliquot. However, **the same set of first and second nucleotide tag combinations is used in the encoding reaction in each of the aliquots.**”

(Ex. 1003, ¶0116)(Emphasis added)(Ex. 1004, ¶0091).

159. This approach allows Fluidigm to re-use tag sequences. As Fluidigm states that:

“Within each module, the sets of tag pairs differ from one another, but same set of tag pairs is used for each module.”

(Ex. 1003, ¶0115)(Ex. 1004, ¶0090).

160. For this reason, tag combinations present in one aliquot are re-used in other aliquots:

“[I]n certain embodiments, the combination of nucleotide tags in each of said T tagged target nucleotide sequences is present in a tagged target nucleotide sequence in each of the other aliquots, although each tag combination can be attached to a

different target nucleotide sequence.”

(Ex. 1003, ¶0116)(Ex. 1004, ¶0091). Because the same tag-pair will be present in each of the 96 aliquots and attached to different target sequences, the various tag pairs are common universal sequences.

161. The third set of primers hybridizes to these sequences, because the third set of primers (in the “amplification step”) are tag-specific primers, as Fluidigm explains in ¶0120. (Ex. 1003, ¶0120)(“96 different **tag-specific primer combinations** can be loaded into individual assay columns”)(Emphasis added)(Ex. 1004, ¶0095). This means that the third set of primers are configured to hybridize to the tags introduced in the second step (the “encoding amplification”).

Claim 5

“5. The method of claim 4, wherein at least one primer of the third set of primers includes a sequence configured to be added to the different non-random polynucleotide sequences to add an index to the enriched non-random polynucleotide sequences of the library, the index being indicative of the maternal blood sample from which the library was generated.”

162. In my opinion, claim 5 of the ’831 Patent would also have been obvious in view of Fluidigm. Claim 5 is obvious over Fluidigm for the same reasons given for claim 3, as discussed in ¶157. The requirement of intervening claim 4 does not alter the analysis presented under claim 3.

Claim 6

“6. The method of claim 1, wherein said plurality of non-random polynucleotide sequences comprises at least 300 different non-random polynucleotide sequences selected from the chromosome tested for being aneuploid.”

163. In my opinion, claim 6 of the '831 Patent would also have been obvious in view of Fluidigm. As explained above in ¶¶131-134, it would have been obvious to test a specific chromosome for being aneuploid based on Fluidigm's suggestion to use the “amount or presence” of target sequences to test for aneuploidy, and the known successes in performing similar analyses. (Ex. 1003, ¶0175)(Ex. 1004, ¶0150)(Ex. 1006, p. 001, right column)(Ex. 1013, p. 001, right column). Because Fluidigm's modular approach uses 9,216 sequences, it would have been obvious to have at least 300 such sequences come from the chromosome being tested for aneuploidy, in order to determine whether the amount of such sequences was abnormally high, for example in the case of Down syndrome. It would have been obvious that testing more sequences would result in a higher chance to correctly detect aneuploidy. Chromosomes are typically very long in length compared to those polynucleotide templates that are sequenced with next generation sequencers. Targeting larger numbers of sequences on a chromosome would obviously have increased the chance of detecting an abnormal number of copies of a chromosome in a statistically reliable way. For example, Chiu in 2008 stated that:

“To reliably detect the small increase, a large absolute number of chr21 and reference chromosome sequences of the loci

targeted by the digital PCR assays need to be analyzed....”

(Ex. 1013, p. 001, right column). One of ordinary skill would have understood this to mean that by testing additional sequences on the same chromosome, the ability to more reliably detect aneuploidy would have been increased.

Claim 7

“7. The method of claim 1, wherein said plurality of non-random polynucleotide sequences comprises at least 500 different non-random polynucleotide sequences selected from the chromosome tested for being aneuploid.”

164. In my opinion, claim 7 of the '831 Patent would also have been obvious in view of Fluidigm. For the same reasons explained above for claim 6 in ¶162, it would have been obvious to have at least 500 of such sequences from the chromosome being tested for being aneuploidy.

Claim 10

“10. The method of claim 1, wherein said selectively enriching comprises performing PCR.”

165. In my opinion, claim 10 of the '831 Patent would also have been obvious in view of Fluidigm. The “selectively enriching” of claim 1 comprises steps b(i), (ii) and (iii). Each of these steps use primers, which indicates the use of PCR. (Ex. 1003, ¶¶0119-0120)(Ex. 1004, ¶¶0094-0095).

166. Fluidigm also teaches that the “encoding amplification” step (the second amplification) of the Modular Approach can use PCR. Fluidigm states that:

“As used herein, the term ‘**encoding reaction**’ refers to reaction in which at least one nucleotide tag is added to a target nucleotide sequence. Nucleotide tags can be added, for example, by an ‘**encoding PCR**’ in which the at least one primer comprises a target-specific portion and a nucleotide tag located on the 5’ end of the target-specific portion, and a second primer that comprises only a target-specific portion or a target-specific portion and a nucleotide tag located on the 5’ end of the target-specific portion.”

(Ex. 1003, ¶0062)(Ex. 1004, ¶0048).

Claim 14

167. In my opinion, claim 14 of the ’831 Patent would also have been obvious in view of Fluidigm.

“14. A method for preparing a sequencing library from a maternal blood sample, the method comprising:”

168. The preamble of claim 14 of the ’831 Patent is disclosed in the same manner as the preamble of claim 1, as discussed in ¶¶119-121.

“a. obtaining a maternal blood sample comprising fetal and maternal cell-free DNA;”

169. Element a. of claim 14 of the ’831 Patent is disclosed in the same manner as Element a. of claim 1, as discussed in ¶122

“b. selectively enriching a plurality of non-random polynucleotide sequences of genomic DNA from said fetal and maternal cell-free DNA to generate a library of enriched non-random polynucleotide sequences,”

170. Element b. of claim 14 of the '831 Patent is disclosed in the same manner as Element b. of claim 1, as discussed in ¶¶124-134.

“wherein said plurality of non-random polynucleotide sequences comprises at least 100 different non-random polynucleotide sequences selected from a chromosome tested for being aneuploid, said enriching comprising:”

171. Element b. of claim 14 of the '831 Patent is disclosed in the same manner as Element b. of claim 1, as discussed in ¶¶124-134.

“(i) amplifying said plurality of non-random polynucleotide sequences from said maternal and fetal genomic DNA using a first pair of primers,”

172. Element b(i). of claim 14 of the '831 Patent is disclosed in the same manner as Element b(i). of claim 1, as discussed in ¶¶135-137.

“wherein said plurality of non-random polynucleotide sequences comprises at least 100 different non-random polynucleotide sequences selected from a chromosome tested for being aneuploid;”

173. Element b(i). of claim 14 of the '831 Patent is disclosed in the same manner as Element b(i). of claim 1, as discussed in ¶¶124-137.

“(ii) amplifying the product of (i) with a second set of primers;”

174. Element b(ii). of claim 14 of the '831 Patent is disclosed in the same manner as Element b(ii). of claim 1, as discussed in ¶¶138-141, noting that this claim element is broader than the corresponding element of claim 1.

“(iii) amplifying the product of (ii) with a third set of primers;”

175. Element b(iii). of claim 14 of the '831 Patent is disclosed in the same manner as Element b(iii). of claim 1, as discussed in ¶¶142-143, noting that this claim element is broader than the corresponding element of claim 1.

“and wherein one of said second or third sets of primers includes an indexing sequence.”

176. This element of claim 14 of the '831 Patent is disclosed in the same manner as claim 2, as discussed in ¶¶151-156, noting that this claim element is broader than both claim 2 and distinct from the last “wherein” clause of claim 1.

Claim 15

“15. The method of claim 14, wherein said third set of primers includes the indexing sequence.”

177. In my opinion, claim 15 of the '831 Patent would also have been obvious in view of Fluidigm. Claim 15 is obvious over Fluidigm for the same reasons given for claim 3, as discussed in ¶157. The dependency from claim 14 does not alter the analysis presented under claim 3.

Claim 16

“16. The method of claim 14, wherein said indexing sequence distinguishes polynucleotides in the maternal blood sample from polynucleotides in a different sample.”

178. In my opinion, claim 16 of the '831 Patent would also have been obvious in view of Fluidigm. As discussed above under claim 2 (§§151-156), Fluidigm renders obvious using “sample-specific” DNA tags. (Ex. 1003, ¶0075)(Ex. 1004, ¶0061). Sample-specific tags “encode[] the identity of the sample of the target nucleotide sequence to which the tag is, or becomes, linked in an encoding reaction.” (Ex. 1003, ¶0064)(Ex. 1004, ¶0050). Because the tag “encodes the identity” of a sample, and is attached to particular sequence from that sample, the tag obviously distinguishes sequences in different samples. The tags are obviously different, because when samples are pooled in a multiplexed run (*see* ¶149, below), the tags must have distinct sequences in order to be able to separate distinguish sequence reads from different samples.

Claim 17

“17. The method of claim 14, wherein said cell-free DNA is from a plurality of different individuals, and wherein said indexing sequence identifies a maternal blood sample from each of said plurality of different individuals.”

179. In my opinion, claim 17 of the '831 Patent would also have been obvious in view of Fluidigm. It would specifically have been obvious to use cell-free DNA from a plurality of individuals, where each individual's sequences are

identified with an index sequence. Fluidigm expressly discloses that:

“In various embodiments, methods are provided for detecting a plurality of target nucleic acids (i.e., T target nucleic acids, where T is an integer greater than one) in a plurality of samples. In certain embodiments, the method entails providing **S samples that will be mixed together** (i.e., pooled) prior to assay, where S is an integer greater than 1. Each of these samples is separately subjected to an encoding reaction that produces a set of T tagged target nucleotide sequences, wherein **each nucleotide tag encodes information about the identity and/or sample source of a particular target nucleic acid**. For each of these S samples (i.e., each of the samples to be pooled), the tagged target nucleotide sequences are mixed to form an assay mixture.”

(Ex. 1004, ¶¶0005, 0086)(Emphases added)(Ex. 1004, ¶¶0005-0008, 0071, 0077-0078, 0082). In the Fluidigm Modular Approach, it would have been obvious to combine samples after the encoding amplification step described above (i.e. after adding sample-specific tags), because if added earlier one would not be able to use the tags to distinguish the samples.

Claim 18

“18. The method of claim 14, wherein said plurality of non-random polynucleotide sequences comprises at least 300 different non-random polynucleotide sequences selected from the chromosome tested for being aneuploid.”

180. In my opinion, claim 18 of the '831 Patent would also have been obvious in view of Fluidigm. For the same reasons explained above for claim 6 in ¶162, it would have been obvious to have at least 300 of such sequences from the chromosome being tested for being aneuploidy. The change in dependency to claim 14 does not affect the analysis.

Claim 19

“19. The method of claim 14, wherein said plurality of non-random polynucleotide sequences comprises at least 500 different non-random polynucleotide sequences selected from the chromosome tested for being aneuploid.”

181. In my opinion, claim 19 of the '831 Patent would also have been obvious in view of Fluidigm. For the same reasons explained above for claim 6 in ¶162, it would have been obvious to have at least 500 of such sequences from the chromosome being tested for being aneuploidy. The change in dependency to claim 14 does not affect the analysis.

Claim 22

“22. The method of claim 14, wherein said selectively enriching comprises performing PCR.”

182. In my opinion, claim 22 of the '831 Patent would also have been obvious in view of Fluidigm. For the same reasons explained above for claim 10 in ¶¶165-166, Fluidigm discloses and renders obvious the use of PCR, because each of its three amplification steps in the Modular Approach use primers, and the “encoding

amplification” step is expressly PCR.

Ground 2. Claims 1-10, 13-22 and 24 are obvious as in Ground 1 in further view of Sequenom.

183. It is further my opinion that claims 1-10, 13-22 and 24 of the '831 Patent would have been obvious over Fluidigm and the knowledge of one of ordinary skill, as applied above in Ground 1, in view of WO2009/032781A2 (“Sequenom”)(Ex. 1005).

Overview of the Combination

184. In my analysis for Ground 2, I am using Fluidigm and the knowledge of one of ordinary skill in the same manner as discussed under Ground 1 (¶¶93-182), and the discussion from Ground 1 is applies to Ground 2 in the same way. Sequenom provides additional support for obviousness of the claims challenged in Ground 1, and teaches subject matter directly related to dependent claims 8-9, 13, 20-21 and 24. For these dependent claims, there are two distinct sets: The first set of claims, claims 8, 9, 20 and 21, recite limitations relating to the length of the “plurality of non-random polynucleotide sequences.” Fluidigm does not teach the length of the sequences expressly. However, Fluidigm does suggest targeting fetal DNA in maternal blood samples. Sequenom discloses that fetal DNA in maternal blood samples was known to be of a length the meets the limitations of claims 8, 9, 20 and 21. Sequenom further teaches targeting sequences that are between 10 and 500 base

pairs and 50 and 150 base pairs in length.

185. The second set of claims, claims 13 and 24, recites that the “chromosome tested is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y.” While Fluidigm expressly discloses testing for chromosomal aneuploidy, Fluidigm does not disclose particular chromosomes to test. Sequenom is cited because it expressly identifies trisomy 13, 18 and 21 as types of chromosomal aneuploidy that are desirably diagnosed using fetal DNA found in maternal blood.

Rationale for the Combination

186. As I discuss above in ¶¶93-182, it is my opinion that claims 1-7, 10, 14-19, 22 of the '831 Patent would have been obvious over Fluidigm. Sequenom supports my opinion of obviousness, because it demonstrates the well-known use of cell-free DNA from maternal plasma (Ex. 1005, 1:31-2:5, 5:5-13, 8:2-5, 10:26-11:20) for pre-natal diagnostic testing for chromosomal aneuploidy. (Ex. 1005, 10:26-11:20, 24:23-26:27). For example, Sequenom states that:

“Thus the present invention in part features methods of **detecting abnormalities in a fetus by detecting fetal DNA in a biological sample obtained from a mother....**Exemplary diseases that may be diagnosed include, for example, preeclampsia, preterm labor, hyperemesis gravidarum, ectopic pregnancy, **fetal chromosomal aneuploidy (such as trisomy 18, 21, or 13)**, and intrauterine growth retardation.”

(Ex. 1005, 25:10-20)(Emphases added). This supports the motivation (already expressly present in Fluidigm) to use Fluidigm's methods of library preparation on cell-free fetal and maternal DNA to target sequences on a chromosome being tested for aneuploidy.

187. Sequenom also provides specific motivation for dependent claims 8-9, 13, 20-21 and 24, which will be discussed under each claim below.

Graham Factors

188. The four Graham factors (*see* ¶84) are the same as discussed under Ground 1, except that the scope and content of the prior art and differences between the prior art as relevant to claims challenged in Ground 2 are discussed in Ground 2.

Reasonable Expectation of Success

189. In my opinion, by the relevant timeframe one of ordinary skill would have expected reasonable success in combining Sequenom with Fluidigm. The discussion under Ground 1 in ¶¶113-117 is also relevant here. In the combination, Sequenom is cited primarily as disclosing the knowledge of one of ordinary skill concerning the length characteristics of cell-free fetal DNA in maternal blood, the nature of certain types of aneuploidy (*e.g.* trisomy 21), and the desire to test for them. One of ordinary skill would have been able to target DNA sequences of certain lengths by introducing specific forward and reverse primers separated by a region of interest as discussed by Sequenom.

Sequenom is Analogous Art

190. Sequenom is analogous art because it is in the same field as the '831 Patent (DNA sequencing library preparation), and its methods of library preparation would have been reasonably pertinent to the problems facing the named inventors.

Claim Mapping

Claims 1-7, 10, 14-19, and 22

191. Claims 1-7, 10, 14-19, and 22 of the '831 Patent are mapped as discussed in Ground 1, above, ¶¶118-182.

Claims 8-9 and 20-21

192. I have reviewed claims 8 and 20 of the '831 Patent, which appear to be of the same scope except that claim 8 depends from claim 1, while claim 20 depends from claim 14. Because the difference in dependency makes no difference to the analysis, I will analyze claims 8 and 20 together.

“8 [20]. The method of claim 1 [14], wherein each of said plurality of non-random polynucleotide sequences is from 10 to 500 nucleotide bases in length.”

193. As discussed above in ¶¶119-122, Fluidigm renders obvious using cell-free fetal DNA in maternal blood samples. Furthermore, Sequenom teaches the use of cell-free fetal DNA in a maternal plasma sample to test for aneuploidy (¶156, above).

194. As noted in above (¶¶184-156), Sequenom teaches that fetal DNA in

maternal blood samples:

“is relatively small (**approximately 500 base pairs or less**), whereas the majority of circulatory, extracellular maternal DNA in maternal plasma is greater than approximately 500 base pairs.”

(Ex. 1005, 2:1-4)(Emphasis added). Thus, it would have been unlikely to target sequences longer than 500 base pairs to achieve success PCR products when targeting both fetal and maternal cell-free DNA.

195. Sequenom also teaches that it is preferable when testing fetal DNA to target small sequences. (Ex. 1005, 2:2-12). For example, Sequenom teaches that:

“Methods of the present invention allow for the selective enrichment of any **nucleic acid less than a given size** based on the placement of the outside primers. For example, outside primers designed to anneal to a non-target nucleic acid 500 base pair apart often will allow for the **preferential amplification of target nucleic acid less than 500 base pair**. In one embodiment of the invention, methods provided herein are used to preferentially amplify nucleic acid within the range of about 25 bases to about 10,000 bases from a sample comprising a background of longer nucleic 20 acid. In certain embodiments, **the target nucleic acid is at least about 75 base pairs**, but less than about 1200 base pairs. **In some embodiments, the target nucleic acid is less than 500 base pairs.**”

(Ex. 1005, 3:14-21)(Emphases added).

196. Sequenom further teaches using inside primers to target sequences having smaller lengths (that fall within the claimed ranges):

“In some embodiments of the invention, the inside primers flank a locus of interest. In certain embodiments, the pair of inside primers are less than **10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1 000, 1500** or more base pairs apart.”

(Ex. 1005, 3:22-25)(Emphasis added – bold: claims 8 and 20; bold underlined: claims 9 and 21). Using inside primers in this manner will result in sequences of the targeted lengths. In one example, Sequenom teaches using inside primers to target a sequence of 120 base pairs. (Ex. 1005, 30:6, et seq.). Such use of inside primers to target sequences of a particular length was well-known in the art and could have been adapted with a reasonable expectation of success in Fluidigm’s Modular Approach (within the 9,216 primer pairs).

197. It would have been obvious in view of Sequenom to have each of the plurality of non-random polynucleotide sequences in Fluidigm be from 10 to 500 nucleotide bases in length. Specifically, by suggesting targeting fetal DNA in maternal blood, Fluidigm is already suggesting targeting short (generally under 500 base pair) sequences. Furthermore, Sequenom teaches that it is advantageous to target short sequences. (Ex. 1005, 2:2-12). By adopting Sequenom’s specific suggestion of using inside primers 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200,

250, 300, 350, 400, 450, or 500 base pairs apart, the combination would meet claims 8 and 20.

Claims 9 and 21

198. Claims 9 and 21 of the '831 Patent are identical except for their dependencies. Claims 9 and 21 restrict the range of base-pair lengths in claims 8 and 20, respectively, to 50 to 150 base pairs. In my opinion, the combination of Fluidigm and Sequenom would render claims 9 and 21 obvious for the same reasons discussed in ¶¶192-197 with respect to claims 8 and 20, above.

Claims 13 and 24

199. In my opinion, claims 13 and 24 of the '831 Patent would also have been obvious in view of Fluidigm and Sequenom. Claims 13 and 24 are identical, except that claim 13 depends from claim 1, while claim 24 depends from claim 14. Because the difference in dependency makes no difference to the analysis, claims 13 and 24 are addressed together.

“13 [24]. The method of claim 1 [14], wherein said chromosome tested is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y.”

200. As discussed above in ¶¶113-116, 119-122 and 130-134, Fluidigm teaches that its methods can be applied to fetal DNA in maternal blood and can be used to detect chromosomal aneuploidy. Sequenom expressly identifies trisomy 13,

18 and 21 (the latter being associated with Down syndrome) as types of chromosomal aneuploidy that can be detected by examining cell-free fetal DNA in maternal blood. (Ex. 1005, 2:10-21, 9:9-18). Sequenom states that, for example:

“[T]he present invention in part features methods of **detecting abnormalities in a fetus by detecting fetal DNA in a biological sample obtained from a mother**. The methods according to the present invention in part provide for detecting fetal DNA in a maternal sample by preferentially amplifying fetal DNA in a background of maternal DNA based on DNA characteristics (e.g., size). See Chan et al. Clin Chem. 2004 Jan;50(1):88-92; and Li et al. Clin Chem. 2004 Jun;50(6):1002-11. Employing such methods, fetal DNA can be predictive of a genetic anomaly or genetic-based disease. These methods are applicable to any and all pregnancy-associated conditions for which nucleic acid changes, mutations or other characteristics (e.g., methylation state) are associated with a disease state. **Exemplary diseases that may be diagnosed include**, for example, preeclampsia, preterm labor, hyperemesis gravidarum, ectopic pregnancy, **fetal chromosomal aneuploidy (such as trisomy 18, 21, or 13)**, and intrauterine growth retardation.”

(Ex. 1005, 25:10-20)(Emphases added). Sequenom specifically teaches using cell-free fetal and maternal DNA from maternal plasma (which is the liquid and cell-free component of blood). (Ex. 1005, 1:31-2:5, 5:5-13, 5:29-6:8, 8:2-5, 10:26-11:20).

DNA in maternal plasma belongs to both the mother and the fetus and is cell-free.

201. Furthermore, in the relevant timeframe, it was well-known that trisomy 21 (also called Down syndrome) was routinely tested in pregnant women. (Ex. 1006, p. 001)(Ex. 1013, p. 001). Accordingly, one of ordinary skill would have been motivated to test for at least trisomy 21 using the method taught in Fluidigm based on the disclosure by Sequenom and the knowledge in the art.

Ground 3. Claim 12 is obvious as in Ground 1 in further view of Harismendy

202. In my opinion Claim 12 of the '831 Patent would have been obvious over Fluidigm and the knowledge of one of ordinary skill, as presented in ¶¶93-182, above, in further view of Harismendy. (Ex. 1007)(“Harismendy”).

Overview of Claim 12

203. Claim 12 recites:

“The method of claim 1, wherein said non-random polynucleotide sequences comprise sequences that are sequenced at a rate of greater than 5-fold than other sequences on the chromosome.”

204. Claim 12 appears to relate to the '831 Patent's discussion of “hotspots.” According to the '831 Patent, a hotspot is a portion of the genome that, when sequenced, appears at a higher frequency (*i.e.* number of unique sequence reads) than other sequences. The '831 Patent describes that:

“Selecting Sequences Based on ‘Hotspots’ [¶] Sequencing data

can be analyzed to identify polynucleotide sequences to be selectively enriched. **Some polynucleotide sequences** from a sample comprising nucleic acids (e.g., genomic DNA) **can be sequenced at a higher frequency** than other polynucleotide sequences. These sequences may be more likely to be enriched by, for example, amplification methods.”

(Ex. 1001, 6:59-65)(Emphases added). The ’831 Patent suggests targeting these high-frequency regions (“hotspots”) to reduce the cost of the process:

“Identifying and enriching these polynucleotide sequences can reduce the number of nucleic acids that need to be analyzed to determine the presence or absence of fetal aneuploidy. This enrichment can reduce the cost of aneuploidy determination.”

(Ex. 1001, 6:65-7:3)

205. The ’831 Patent does not, however, explain how to identify hotspot regions, but rather merely states that frequency rate data can be obtained from an unidentified database:

“In one embodiment, the non-random sequences to be selectively enriched are identified based on the number of times they are sequenced in a database of sequence information, independent of the rate of sequencing of a second set of sequences.”

(Ex. 1001, 8:16-20; 7:10-22).

206. The sequencing rate is thus the so-called “coverage” of a DNA sequence in a database. (Ex. 1001, 3:34-36)(“Sequences for enrichment can be

chosen on the basis of being in a ‘hotspot,’ a region of relatively high sequence coverage.”) The term “coverage” means the number of unique sequence reads at a given location (base pair) on a chromosome. The ’831 Patent “hotspot” strategy thus essentially entails looking in a database that is not described at all to determine the coverage of various sequences and selecting those sequences with high coverage.

207. Claim 12 also does not require any particular strategy for selecting sequences. In claim 12, “sequences” (*i.e.* two or more sequences) among the at least 100 sequences required by claim 1 must be “sequenced at a rate of greater than 5-fold than other sequences” (*i.e.* two other sequences) on the same chromosome.

Overview of the Combination

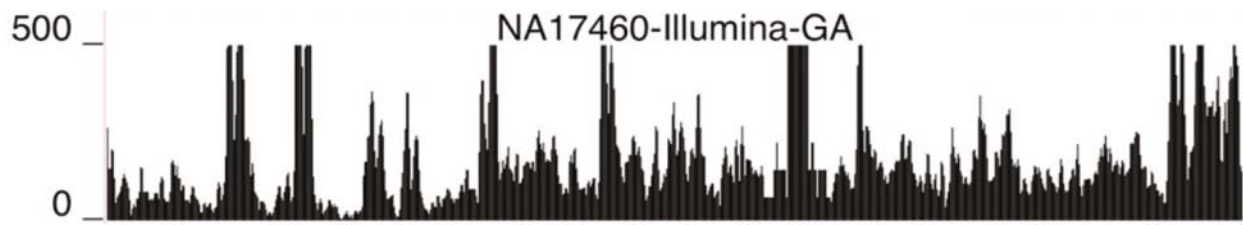
208. In my opinion, Harismendy demonstrates that it would have been obvious to target portions of the genome that sequence at a five-fold greater rate than other sequences on the same chromosome. Harismendy studied and compared different NGS platforms. With each of the platforms, Harismendy observed the hotspot phenomenon:

“Interestingly, each NGS technology has a unique reproducible pattern of non-uniform sequence coverage: sequences with high or low coverage in one sample typically had high or low coverage in the other three samples (Fig. 3).”

(Ex. 1007, p. 5)(Emphasis added).

209. In Figure 2 of Harismendy, there is shown the variation of coverage

(fold) for various different sequencing platforms across a 100-kb portion of chromosome 3. (Ex. 1007, p. 4, Fig. 2, caption). A portion of Fig. 2, reproduced below, shows non-uniform sequence coverage for an approximately 17-kb region of chromosome 3 when using the Illumina Genome Analyzer (“GA”) system:



(Ex. 1007, p. 4, Fig. 2). In the Figure, the y-axis is the “fold” coverage scale, while the x-axis reflects a position on the chromosome. (Ex. 1007, p. 4, Fig. 2, caption). As can be seen in the full figure 2, the fold varies across the gene from near-zero to well into the thousands (in the portions shown above, several peaks are off the top of the scale).

210. I note that similar variations have been observed by others in connection with other chromosomes. For example, Fan, *et al.* (Ex. 1006), in the process of testing for trisomy 21, “observed a nonuniform distribution of sequence tags across each chromosome. This pattern of intrachromosomal variation was common among all samples, including randomly sheared genomic DNA, indicating that the observed variation was most probably due to sequencing artifacts.” (Ex. 1006, p. 001).

Rationale for the Combination

211. In my opinion, it would have been obvious to target high-coverage regions of a chromosome in Fluidigm's method based on Harismendy.

212. Harismendy notes that each NGS platform tested has its own pattern of coverage. (Ex. 1007, p. 5). Fluidigm teaches using its methods to prepare libraries for later Solexa sequencing. "Solexa" refers to a company that was later purchased by Illumina. After the purchase, Illumina began selling Solexa's Genome Analyzer product. (Ex. 1014).

213. Harismendy teaches that the Illumina GA sequencer performs best when the sequence coverage is high, and worst at low coverage:

"[T]he performance of the NGS technologies at low sequence coverage is correlated with per-base sequence coverage uniformity; the **Illumina GA**, which has the highest coverage variability, **performs the worst at lower coverage...**"

(Ex. 1007, p. 8, right column)(Emphases added). Harismendy also reports that:

"At high sequence coverage all NGS platforms have excellent variant calling accuracy (>95%) as assessed by the detection of known SNP variants."

(Ex. 1007, p. 9, left column, top)(Emphasis added)

214. To increase the accuracy of the Illumina GA sequencer, it would have been obvious in my opinion to target regions of a chromosome with high sequence

coverage. To do this, one of ordinary skill would have obviously chosen at least two of (and preferably all of) Fluidigm's 9,216 primer pairs to anneal to sequences on a chromosome that at higher coverage than other sequences. Furthermore, because the coverage varies by thousands across a chromosome (as indicated in Harismendy—see Fig. 3), it would have been obvious to have at least two sequences that are sequenced at a rate of greater than 5-fold than other sequences on the chromosome. (Ex. 1007, pp. 003-004)(e.g., “For all three NGS technologies there is greater than a hundred-fold variation in the per base sequence coverage depth (Figure 2).”

215. Finally, selecting similarly high-coverage sequences would have lowered the variation in coverage, thus reducing the total amount of sequence generation required. (Ex. 1007, p. 9, left column bottom)(“[T]he uniformity of per-base sequence coverage must be improved to reduce the total amount of sequence generation required....”).

Graham Factors

216. The *Graham* factors (see ¶84, above) are the same as discussed under Ground 1, except that the scope and content of the prior art and differences between the prior art as relevant to claims challenged in Ground 3 are discussed in Ground 3.

Reasonable Expectation of Success

217. One of ordinary skill would have expected reasonable success in

combining Harismendy with Fluidigm. In the combination, Harismendy is cited primarily as describing the knowledge of one of ordinary skill concerning the non-uniform coverage with some regions having >5-fold coverage. Selecting these sequences would simply have been a matter of obtaining the available coverage data or sequencing portions of the chromosomes to be tested, both of which were well within ordinary skill in the relevant timeframe.

Harismendy is Analogous Art

218. Harismendy is analogous art at least because it is directed to DNA sequencing, and thus reasonably pertinent to problems faced by the '831 Patent named inventors (DNA sequencing library preparation).

Claim Mapping

219. As discussed above, it was obvious to target, as part of Fluidigm's Modular Approach using 9,216 target sequences, at least two sequences that are sequenced at a rate of greater than 5-fold than other sequences on the chromosome based on Harismendy. The combination of Harismendy with Fluidigm meets the requirement of claim 12 of the '831 Patent.

Ground 4. Claims 12 are obvious as in Ground 2 in further view of Harismendy

Ground 4 is the same as Ground 3 but based on Ground 2 instead of Ground 1. The discussion under Ground 3 applies in the same way to Ground 2 as to Ground

1. As in Ground 2, Sequenom provides additional support for obviousness.

XII. OATH

220. This declaration and my opinions herein are made to the best of my knowledge and understanding, and based on the material available to me, at the time of signing this declaration. I declare under penalty of perjury under the laws of the United States of America that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. §1001) and may jeopardize the validity of the application or any patent issuing thereon.

Date: June 29, 2018

Signed: 