

**UNITED STATES PATENT AND TRADEMARK OFFICE**

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**BEFORE THE PATENT TRIAL AND APPEAL BOARD**

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TEMPUS AI, INC.,

Petitioner,

v.

GUARDANT HEALTH, INC.,

Patent Owner.

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Case IPR2025-01435

U.S. Patent 10,793,916

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**DECLARATION OF MICHAEL L. METZKER, PH.D.  
IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW  
OF CLAIMS 13-30 OF U.S. PATENT 10,793,916**

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I, Michael L. Metzker, hereby declare as follows.

1. I am over the age of eighteen (18) and otherwise competent to make this declaration.

2. I have been retained as an expert witness on behalf of Tempus AI, Inc. (“Tempus”) in connection with the above-captioned requested *inter partes* review (“IPR”).

### **I. OVERVIEW AND SUMMARY OF OPINIONS**

3. I understand that the petition for *inter partes* review involves Claims 13-30 of the ‘916 Patent<sup>1</sup>, which issued on October 6, 2020.<sup>2</sup> I am informed by counsel and understand that the ‘916 Patent is assigned to Guardant Health, Inc. (“Guardant”).

4. I am informed by counsel and understand that the ‘916 Patent resulted from U.S. Patent Application No. 16/575,128 (“the ‘128 Application”), filed on September 18, 2019, naming AmirAli Talasaz.<sup>3</sup> I am further informed by counsel and understand that the ‘916 Patent claims priority to Provisional Applications: 61/845,987, filed on Jul. 13, 2013; 61/793,997, filed on Mar. 15, 2013; 61/704,400,

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<sup>1</sup> See Ex. 1001, Talasaz, *Systems and methods to detect rare mutations and copy number variation*, U.S. Patent 10,793,916 (2020) (the “‘916 Patent”).

<sup>2</sup> *Id.*

<sup>3</sup> *Id.*

filed on Sep. 21, 2012; 61/696,734, filed on Sep. 4, 2012.

5. I am informed by counsel and understand that the claimed priority date for the '916 Patent is no earlier than September 4, 2012, the filing date of the '734 Provisional,<sup>4</sup> and I refer to this date throughout this Declaration.

6. It is my opinion that Claims 13-30 of the '916 Patent are unpatentable as obvious in view of the prior art. My opinion is based on the following grounds:

<b>Ground</b>	<b>Description</b>
<b>Ground 1</b>	Claims 13-30 are rendered obvious under 35 U.S.C. § 103 by the '188 Patent (Schmitt) in view of Forshew (2012) and the '202 Patent Pub. (Porreca)
<b>Ground 2</b>	Claims 13-30 are rendered obvious under 35 U.S.C. § 103 by the '188 Patent (Schmitt) in view of Forshew (2012) and the '331 Patent Pub. (Sacko)

## II. MY BACKGROUND AND QUALIFICATIONS

7. I am currently Founder, President, and Chief Executive Officer of RedVault Biosciences, LP (“RedVault”), which I founded in April 2013. RedVault is a biotechnology company committed to creating innovative technologies to advance genomic medicine. Before taking my position at RedVault, I was a tenured and adjunct Associate Professor in both the Department of Molecular & Human Genetics and at the Human Genome Sequencing Center (“HGSC”) at Baylor College

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<sup>4</sup> *Id.*

of Medicine (“BCM”). I have also been an adjunct Associate Professor in the Cell & Molecular Biology Program at BCM and adjunct Assistant and Associate Professor in Chemistry at Rice University. At BCM, I worked as part of the team at the HGSC that contributed to the Human Genome Project.

8. From September 2022 to September 2023, I was also Co-Founder and Chief Technical Officer of 454 Bio, Inc. 454 Bio is developing a portable DNA sequencer, a customizable device for decentralized, on-site sequencing.

9. 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. From 2016 to 2018, Agilent Technologies acquired LaserGen for \$185M to convert its reversible terminator technology into a clinical diagnostic platform. I currently have no working relationship with LaserGen.

10. Since 1988, I have performed scientific research in the fields of molecular biology and chemical synthesis of nucleoside and nucleotide inhibitors. 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 also 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 a particular focus in humans and human immunodeficiency virus (“HIV”).

11. My research has been devoted in large part 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).

12. I received my Bachelor of Science degree in Biochemistry &

Biophysics from the University of California, Davis in 1984. Between 1985 and 1988, I enrolled in a Master of Science program in the department of Chemistry at San Francisco State University. Although I completed the required coursework for a Master of Science degree with an emphasis in Organic Chemistry, I elected not to complete this degree. Instead, in 1991, I enrolled in a doctoral program in the Department of Molecular and Human Genetics at Baylor College of Medicine in Houston, Texas. In 1996, I received my Ph.D. in Molecular and Human Genetics.

13. From 2000 to 2018, I gave lectures on sequencing technologies to graduate and medical students as part of the *Molecular Methods* course at BCM. These lectures were 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* course at BCM.

14. I have also spoken at a variety of academic and industry conferences in the fields of molecular biology and DNA sequencing, including the *Next-Generation Sequencing* Workshop at Lübeck University in Germany; the *Centre de Regulació Genòmica* (“CRG”) Symposium, Barcelona, Spain; the *Next-Generation Sequencing* Conference in Boston, MA, the *Advances in Genome Biology and Technology Meeting* (“AGBT”) in Marco Island, FL; the *Copenhagenomics* Meeting in Copenhagen, Denmark; the *American Society of Microbiology* Conference in San

Francisco, CA; the SelectBio's *Advances in Next Generation Sequencing* Meeting; and the *Critical Path to TB Drug Regimens* ("CPTR") Workshop in Washington, DC.

15. I have authored 58 peer-reviewed papers and seven book chapters. Several of my review papers discuss emerging technologies and advances in DNA sequencing, including detection of sequence variations. I have written book chapters on topics related to polymerase chain reaction ("PCR") and DNA sequencing. I have also reviewed numerous manuscripts for peer-review publications in the fields of molecular biology and DNA sequencing, including manuscripts applying the sequencing techniques described *supra*.

16. I have also conducted extensive research in the fields of molecular biology and DNA sequencing. My research has investigated and advanced fundamental techniques such as Sanger sequencing and NGS methods, chemical synthesis of nucleoside and nucleotide inhibitors, Sanger sequencing and NGS approaches for HIV forensics, and detection of genomic variation. Some of this research involved developing automated Sanger sequencing assays to measure mixed nucleotide ratios in DNA sequences of interest. In addition, my colleagues and I developed a novel diagnostic platform using intracellular and extracellular microRNAs for detection of various analytes. Like cell-free DNA, extracellular microRNAs are small nucleic acids that can be used as biomarkers for diagnostic

purposes. My research has been funded by the National Institutes of Health (“NIH”), National Institute of Justice (“NIJ”), United States Department of Agriculture (“USDA”), and private foundations. Since 1999, I have received or have been associated with numerous grants largely focusing on gene and genome sequencing totaling over \$170 million.

17. I am an inventor on 57 U.S. and European issued patents and patent applications directed towards molecular biology, DNA sequencing, and chemical synthesis. For example, I coinvented labelled and unlabeled cleavable terminating groups and methods for DNA sequencing described in U.S. Patent No. 8,148,503; 9,200,319 and 10,041,115. I also coinvented methods for the use of BODIPY fluorophore-labeled DNA for dye-primer sequencing described in U.S. Patent No. 5,861,287 and oligonucleotides labelled with BODIPY fluorophore compounds described in U.S. Patent No. 5,994,063. Further, I coinvented the pulsed-multiline excitation (“PME”) method in collaboration with the late Nobel Laureate Robert F. Curl, Ph.D., which 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 also coinvented novel nucleotide terminators, which 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; 10,041,115; and 11,001,886.

18. I have served on review panels for Genome Canada, Canadian Institute for Health Research (“CIHR”), National Aeronautics and Space Administration (“NASA”), and the 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.

19. From 2003 to 2006, I served on the editorial/advisory board for the journal *Genome Research*. From 2006 to 2012, I served as a scientific organizer for the AGBT Meeting. From 2011 to 2013, I 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”).

20. I have also served as an expert witness in several criminal trials in the United States involving the transmission of human immunodeficiency virus (“HIV”) between individuals. The criminal case involving Richard Schmidt was portrayed in an episode titled *Shot of Vengeance* on Forensic Files in 2003. The criminal case involving Philippe Padieu was reported on ABC News 20/20 by Elizabeth Vargas and on Oprah, both in 2009, and helped inspired an episode titled *Quickie* on Law

& Order: Special Victims Unit in 2010.

21. 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 (Ex. 1004), including publications and patents authored in the previous five years, as well as cases in which I provided expert testimony at deposition or trial.

22. Accordingly, I am an expert in molecular biology and sequencing technologies, including nucleic acid library preparation, Sanger sequencing and next-generation sequencing, assay development and detection and analysis of labeled nucleotides, and have been since before September 4, 2012. For that reason, I am qualified to provide an opinion as to what one of ordinary skill in the art would have understood, known, or concluded before September 4, 2012.

23. I am being compensated for my time in connection with this IPR at my standard consulting rate, which is \$750 per hour. My compensation does not depend in any way on the opinions I express or the outcome of this IPR.

### **III. MATERIALS AND INFORMATION CONSIDERED**

24. In formulating my opinions, I have relied upon my 30+ years of education, training, knowledge, and experience in the relevant art. In formulating

my opinions, I have also considered the viewpoint of one of ordinary skill in the art (“POSA”)<sup>5</sup> prior to September 4, 2012.

25. In preparing this Declaration, I have reviewed the ’916 Patent, the file history of the ’916 Patent, and the materials cited herein, including the following exhibits, in light of the general knowledge in the art before September 4, 2012. The materials cited herein are publications that were typically read and reasonably relied upon by one of ordinary skill in this field, including the bibliographic information presented therein.

<b>Exhibit</b>	<b>Description</b>
1001	Talasaz, <i>Systems and methods to detect rare mutations and copy number variation</i> , U.S. Patent No. 10,793,916 (2020) (the “’916 Patent”) <sup>916 Patent</sup>
1002	Prosecution history for the ’916 Patent (“’916 Patent File History”) <sup>916 Patent</sup>
1003	Declaration of Michael Metzker, Ph.D.
1004	Curriculum Vitae of Michael Metzker, Ph.D.
1005	Schmitt <i>et al.</i> , <i>Method of lowering the error rate of massively parallel DNA sequencing using duplex consensus sequencing</i> , U.S. Patent No. 9,752,188 (2017) (the “’188 Patent (Schmitt)”)

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<sup>5</sup> Throughout this Report, I also refer to one of ordinary skill in the art as a POSA.

1006	Vogelstein <i>et al.</i> , <i>Safe sequencing system</i> , PCT Publication No. WO 2012/142213 A2 (2012) (the “PCT ’213 Appl. (Vogelstein)”)
1007	Fodor <i>et al.</i> , <i>Digital counting of individual molecules by stochastic attachment of diverse labels</i> , U.S. Patent Application Publication No. 2011/0160078 (2011) (the “’078 Patent Appl. (Fodor)”) (“Fodor”)
1008	Hendricks, <i>Enzymatic ligation of nucleic acids</i> , PCT International Publication No. WO 2012/099832 A2 (2012) (the “PCT ’832 Appl. (Hendricks)”)
1009	Diehn <i>et al.</i> , <i>Identification and use of circulating tumor markers</i> , U.S. Patent Application Publication No. 2014/0296081 (2014) (the “’081 Patent Appl. Diehn”)
1010	Forsheew <i>et al.</i> , <i>Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA</i> , <i>Science Translational Medicine</i> 4:136ra68, pp. 1–12 (2012) (“Forsheew (2012)”)
1011	Hicks <i>et al.</i> , <i>Varietal counting of nucleic acids for obtaining genome copy number information</i> , U.S. Patent 9,404,156 (2016) [hereinafter “’156 Patent (Hicks)”]
1012	Shiroguchi <i>et al.</i> , <i>Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes</i> , <i>Proceedings of the National Academy of Sciences of the United States</i> 109:1347–1352 (2012) [hereinafter “Shiroguchi (2012)”]
1013	Saxonov, <i>Methods and compositions for nucleic acid analysis</i> , U.S. Patent Application Publication No. 2012/0316074 (2012) (the “’074 Patent Appl. (Saxonov)”)

1014	Schwarzenbach <i>et al.</i> , <i>Cell-free nucleic acids as biomarkers in cancer patients</i> , NATURE REVIEWS CANCER 11:426–437 (2011) (“Schwarzenbach”).
1015	Human genome variation, fact sheet, National Human Genome Research Institute (NHGRI)
1016	<i>Guardant Health, Inc. v. Foundation Medicine</i> , IPR2017-01448, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 9,340,830
1017	Metzker, <i>Sequencing technologies — the next generation</i> , NATURE REVIEWS GENETICS 11:31 –46 (2010) (“Metzker2010”)
1018	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-00816, Paper 3 – Petition for Inter Partes Review of U.S. Patent No. 10,760,127
1019	Metzker & Caskey, <i>Polymerase Chain Reaction</i> , In ENCYCLOPEDIA OF MEDICAL DEVICES AND INSTRUMENTATION, Second Edition, Volume 5 (2006) (“Metzker2006”).
1020	Mamanova <i>et al.</i> , <i>Target-enrichment strategies for next-generation sequencing</i> , NATURE METHODS 7:111–118 (2010) (“Mamanova”)
1021	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-00450, Paper 3 – Petition for Inter Partes Review of U.S. Patent No. 10,689,699
1022	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-01388, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 10,689,699

1023	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-00816, Paper 3 – Petition for Inter Partes Review of U.S. Patent No. 10,760,127
1024	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-00935, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 10,287,631
1025	U.S. Provisional Patent Application No. 61/600535 (“Diehn Provisional”)
1026	<i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , IPR2022-01400, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 11,149,306
1027	<i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , IPR2022-01400, Paper 41 – Final Written Decision
1028	<i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , IPR2022-01400, Paper 9 – Institution Decision
1029	Li et al., <i>Structure-independent and quantitative ligation of single-stranded DNA</i> , Analytical Biochemistry (2005)
1030	Chee, <i>Nucleic acid constructs and methods of use</i> , U.S. Patent 9,085,798 (2015) (the “’798 Patent (Chee)”)
1031	Thomas et al., <i>Sensitive mutation detection in heterogeneous cancer specimens by massively parallel picoliter reactor sequencing</i> , NATURE MEDICINE 12:852–855 (2006)
1032	Buckingham, <i>Chromosomal structure and chromosomal mutations</i> , In MOLECULAR DIAGNOSTIC FUNDAMENTAL, METHODS, & CLINICAL APPLICATIONS, Eds. Buckingham & Flaws, F.A. Davis Company, Chapter 8, pp. 155–172 (2007)

1033	Gemayel <i>et al.</i> , <i>Variable tandem repeats accelerate evolution of coding and regulatory sequences</i> , ANNUALS REVIEW OF GENETICS 44:445–477 (2010)
1034	Tóth <i>et al.</i> , <i>Microsatellites in different eukaryotic genomes: Survey and analysis</i> , GENOME RESEARCH 10:967–981 (2000)
1035	Laghi <i>et al.</i> , <i>Differences and evolution of the methods for the assessment of microsatellite instability</i> , ONCOGENE 27:6313–6321 (2008)
1036	Richard & Pâques, <i>Mini- and microsatellite expansions: the recombination connection</i> , EMBO REPORTS 1:122–126 (2000)
1037	Hastings <i>et al.</i> , <i>Mechanisms of change in gene copy number</i> , NATURE REVIEWS GENETICS 10:551–564 (2009)
1038	Hiatt <i>et al.</i> , <i>Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation</i> , GENOME RESEARCH 23:843–854 (2013)
1039	Somatic & germline mutations, <a href="https://my.clevelandclinic.org/health/body/23067-somatic--germline-mutations">https://my.clevelandclinic.org/health/body/23067-somatic--germline-mutations</a> (last visited July 30, 2025)
1040	Gene changes and cancer, <a href="https://www.cancer.org/cancer/understanding-cancer/genes-and-cancer/gene-changes.html">https://www.cancer.org/cancer/understanding-cancer/genes-and-cancer/gene-changes.html</a> (last visited July 30, 2025)
1041	International Human Genome Sequencing Consortium, <i>Initial sequencing and analysis of the human genome</i> , NATURE 409:860–921 (2001)
1042	International Human Genome Sequencing Consortium, <i>Finishing the euchromatic sequence of the human genome</i> , NATURE 431:931–945 (2004)

1043	Forbes, et al., <i>COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer</i> , NUCLEIC ACIDS RESEARCH 39:D945–D950 (2011)
1044	<i>Genomic Data Commons Data Portal</i> , <a href="https://portal.gdc.cancer.gov/">https://portal.gdc.cancer.gov/</a> ((last visited July 30, 2025))
1045	Metzker, <i>Emerging technologies in DNA sequencing</i> , GENOME RESEARCH 15:1767–1776 (2005)
1046	Rothberg <i>et al.</i> , <i>An integrated semiconductor device enabling non-optical genome sequencing</i> , NATURE 475:348-352 (2011)
1047	Jain <i>et al.</i> , <i>Improved data analysis for the MinION nanopore sequencer</i> , NATURE METHODS 12:351-356 (2015)
1048	Kinde <i>et al.</i> , <i>Detection and quantification of rare mutations with massively parallel sequencing</i> , PNAS (June 7, 2011) (“Kinde”)
1049	Turner <i>et al.</i> , <i>Methods for genomic partitioning</i> , ANNUAL REVIEW OF GENOMICS AND HUMAN GENETICS 10:263-284 (2009)
1050	Edwards & Gibbs, <i>Multiplex PCR: Advantages, development, and applications</i> , GENOME RESEARCH 3:S65-S75 (1994)
1051	Sharma <i>et al.</i> , <i>(TG/CA)<sub>n</sub> repeats in human gene families: abundance and selective patterns of distribution according to function and gene length</i> , BMC GENOMICS 6:83 pp. 1–12 (2005) (“Sharma (2005)”)
1052	Gnirke <i>et al.</i> , <i>Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing</i> , NATURE BIOTECHNOLOGY 27:182–189 (2009) (“Gnirke (2009)”).

1053	Meyerson <i>et al.</i> , <i>Advances in understanding cancer genomes through second-generation sequencing</i> , NATURE REVIEWS GENETICS 11:685–696 (2010) (“Meyerson (2010)”)
1054	Preston <i>et al.</i> , <i>Innovation at Illumina: The road to the \$600 human genome</i> , NATURE PORTFOLIO (2023) at <a href="https://www.nature.com/articles/d42473-021-00030-9">https://www.nature.com/articles/d42473-021-00030-9</a> (“Preston (2023)”).
1055	Bentley <i>et al.</i> , <i>Accurate whole human genome sequencing using reversible terminator chemistry</i> , NATURE 456:53–59 (2008) (“Bentley (2008)”)
1056	Kircher <i>et al.</i> , <i>Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform</i> , NUCLEIC ACIDS RESEARCH 40:e3 pp. 1–8 (2012) (“Kircher 2012”)
1057	Pierce <i>et al.</i> , <i>A unique and universal molecular barcode array</i> , NATURE METHODS 3:601–603 (2006) (“Pierce (2006)”)
1058	Schmitt <i>et al.</i> , <i>Detection of ultra-rare mutations by next-generation sequencing</i> , PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES 109: 14508-14513 (2012) (“Schmitt (2012)”)
1059	Glenn, <i>Field guide to next-generation DNA sequencers</i> , MOLECULAR ECOLOGY RESOURCES 11:759-769 (2011) (“Glenn (2011)”)
1060	Cock <i>et al.</i> , <i>The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants</i> , NUCLEIC ACIDS RESEARCH 38:1767–1771 (2010) (“Cock (2010)”)
1061	CASAVA 1.8: enhanced variant calling in whole-genome resequencing data (2011) (“CASAVA User Guide”)

1062	Li et al., Mapping short DNA sequencing reads and calling variants using mapping quality scores, <i>Genome Research</i> 18:1851–1858 (2008) (“Li (2008)”)
1063	Li & Durbin, Fast and accurate short read alignment with Burrows–Wheeler transform, <i>Bioinformatics</i> 25:1754–1760 (2009) (“Li & Durbin (2009)”)
1064	Li & Durbin, Fast and accurate long-read alignment with Burrows–Wheeler transform, <i>Bioinformatics</i> 26: 589–595 (2010) (“Li & Durbin 2010”)
1065	Langmead et al., Ultrafast and memory-efficient alignment of short DNA sequences to the human genome, <i>Genome Biology</i> 10:R25.1–R25.10 (2009) (“Langmead (2009)”)
1066	Li et al., <i>The sequence alignment/map format and SAMtools</i> , <i>BIOINFORMATICS</i> 25:2078–2079 (2009) (“Li (2009)”)
1067	DePristo et al., <i>A framework for variation discovery and genotyping using next-generation DNA sequencing data</i> , <i>NATURE GENETICS</i> 43:491–498 (2011) and <i>Online Methods</i> (collectively, “DePristo (2011)”)
1068	McKenna et al., <i>The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data</i> , <i>GENOME RESEARCH</i> 20:1297-1303 (2010) (“McKenna (2010)”)
1069	<a href="https://github.com/broadinstitute/gatk/releases">https://github.com/broadinstitute/gatk/releases</a> (last visited Aug 11, 2025) (“GATK Updates”)
1070	Koboldt et al., <i>VarScan: variant detection in massively parallel sequencing of individual and pooled samples</i> , <i>BIOINFORMATICS</i> 25:2283–2285 (2009) (“Koboldt (2009)”)

1071	Koboldt <i>et al.</i> , <i>VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing</i> , GENOME RESEARCH 22:568–576 (2012) (“Koboldt (2012)”)
1072	Cibulskis <i>et al.</i> , <i>Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples</i> , NATURE BIOTECHNOLOGY 31:213–219 (2013) (“Cibulskis (2013)”)
1073	U.S. Patent No. 9,840,743
1074	U.S. Patent No. 9,834,822
1075	Porreca <i>et al.</i> , <i>Methods and compositions for evaluating genetic markers</i> , U.S. Patent Publication No. 2012/0165202 A1 (the “202 Patent Pub. (Porreca)”)
1076	Sacko <i>et al.</i> , <i>Method for assaying nucleic acids by fluorescence</i> , U.S. Patent Application Publication No. US 2010/0264331 (2010) (the “331 Patent Pub. (Sacko)”)
1077	Van Lier <i>et al.</i> , <i>A review on the molecular diagnostics of Lynch syndrome: a central role for the pathology laboratory</i> , Journal of Cellular and Molecular Medicine 14:181–197 (2010) (“van Lier (2010)”)
1078	Boland & Goel, <i>Microsatellite instability in colorectal cancer</i> , Gastroenterology, 138:2073–2087 (2010) (“Boland (2010)”)
1079	Boland <i>et al.</i> , <i>A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer</i> , Cancer Research 58:5248–5257 (1998) (“Boland (1998)”)
1080	Sinicropea & Sargent, <i>Clinical implications of microsatellite instability in sporadic colon cancers</i> , Current Opinion in Oncology 21:369–373 (2009) (“Sinicropea (2009)”)

1081	Li, <i>Mechanisms and functions of DNA mismatch repair</i> , Cell Research 18:85–98 (2008) (“Li (2008b)”)
1082	U.S. Provisional Application 61/613,413 (“Schmitt '413 Provisional”)
1083	Foundation Medicine, Inc. v. Guardant Health, Inc., IPR2019-00652, Paper 47 – Final Written Decision (“822FWD”)
1084	Foundation Medicine, Inc. v. Guardant Health, Inc., IPR2019-00652, Paper 54 – Termination
1085	Foundation Medicine, Inc. v. Guardant Health, Inc., IPR2019-00652, Paper 12 – Decision Granting Institution of <i>Inter Partes Review</i>

#### IV. UNDERSTANDING OF THE LAW

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

##### A. Prior Art

27. I have been informed by counsel and that the law provides certain categories of information, known as prior art, that may be used to render patent claims anticipated or obvious.

28. I have been asked to assume that the relevant time for my analysis of the pertinent art is on or before September 4, 2012, the earliest claimed priority date for the '916 Patent.

##### B. Level of Ordinary Skill in the Art

29. I understand that the assessment of the patentability of the claims of the

'916 Patent must be undertaken from the perspective of one of ordinary skill in the art as of the earliest claimed priority date of the '916 Patent, which I have been informed by counsel is September 4, 2012. I was not asked to provide an opinion as to whether the '916 Patent is entitled to that priority date, but reserve the right to do so in the future.

30. I have been informed by counsel and understand that the following factors are relevant to determining the level of ordinary skill in the art: (i) type of problems encountered in the art, (ii) prior art solutions to those problems, (iii) rapidity with which innovations are made, (iv) sophistication of the technology, and (v) educational level of active workers in the field.

31. I understand that one of ordinary skill in the art is assumed to be aware of all pertinent prior art, and that the skilled artisan has the ability to understand the technology, draw inferences, and make modest adaptations or advances. One of ordinary skill in the art is thus a person of ordinary creativity, not an automaton, and uses common sense.

**C. Claim Construction**

32. I understand that the first step in comparing prior art to patent claims is to properly construe the claims to determine claim scope and meaning. I have been informed by counsel and understand that in IPR proceedings, claim terms are presumed to take on the ordinary and customary meaning that they would have to

one of ordinary skill in the art in the context of the entire disclosure of the specification and prosecution history. I also understand that if an inventor acts as his or her own lexicographer, the definition must be set forth in the specification with reasonable clarity, deliberateness, and precision.

**D. Obviousness**

33. I have been informed by counsel and understand that a patent claim will be rendered obvious and therefore unpatentable 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 invention was made to one of ordinary skill in the pertinent art. For the purposes of obviousness, I understand that one of ordinary skill in the art may rely on a single prior art reference or multiple references in combination.

34. I have been informed by counsel and understand that the following factors are considered when determining whether a patent claim would have been obvious to one of ordinary skill in the art: *(i)* the scope and content of the prior art, *(ii)* the differences between the prior art and the claims, *(iii)* the level of ordinary skill in the pertinent art, and *(iv)* the existence of secondary considerations of nonobviousness. These secondary considerations, which I understand are also called “objective indicia” may include factors such as: *(i)* the invention’s satisfaction of a long-felt unmet need in the art; *(ii)* unexpected results of the invention; *(iii)*

skepticism of the invention by experts; (iv) commercial success of an embodiment of the invention; and (v) praise by others for the invention. I have also been informed by counsel and understand that there must be a nexus or connection between the evidence that is the basis for an asserted secondary consideration and the scope of the claimed invention.

35. I have been informed by counsel and understand that a claimed invention can be rendered obvious when, for example, there is some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill in the art to modify the prior art reference or to combine prior art reference disclosure to arrive at the claimed invention. In other words, even if one reference does not show the whole of the invention, if it would have been obvious to one of ordinary skill in the art at the relevant time to add the missing pieces to the invention (for example as a matter of standard practice or application of a well-known principle in the field), then a single reference can render a claim invalid even if it does not show the whole invention. Moreover, a combination of two or more prior art references can render a claim invalid as obvious whether or not there is an explicit suggestion in one of the references to combine the two references, if as a matter of skill or practice in the field, it would be routine to do so. Further, I understand that obviousness does not require absolute predictability. Only a reasonable expectation that the beneficial result will be achieved by the modifications or combination is

necessary to show obviousness. I also understand that a patent may be invalid as obvious to try if the options that one of ordinary skill would have encountered were finite, small, or easily traversed, and one of ordinary skill in the art would have had a reason to select the route that produced the claimed invention. I understand that this motivation from the art can be explicit or implicit using a skilled artisan's common sense.

**V. QUALIFICATIONS AND KNOWLEDGE OF ONE OF ORDINARY SKILL IN THE ART**

36. Based on my knowledge and expertise, one of ordinary skill in the art at the time of the alleged invention of the '916 Patent would have had a Ph.D. in bioinformatics, genetics, molecular biology or a related field, and at least five years of research in an academic or industry setting, including at least two to three years of research experience in the field of cancer genomics. One of ordinary skill in the art would have had knowledge of DNA sequencing, including next generation sequencing (NGS) and related sequencing methods, and related sample preparation techniques; bioinformatics methods and tools for analyzing sequence reads and mapping sequence reads onto genomes; and methods for identifying genetic variants in a sample.

**VI. CLAIM CONSTRUCTION**

37. I have been informed by counsel and understand that the first step in

comparing prior art to patent claims is to properly construe the claims to determine claim scope and meaning. I also understand that in IPR proceedings, claim terms are presumed to take on the ordinary and customary meaning that they would have to one of ordinary skill in the art in the context of the entire disclosure of the specification and prosecution history. I further understand that if an inventor acts as his or her own lexicographer, the definition must be set forth in the specification with reasonable clarity, deliberateness, and precision.

38. To the extent Patent Owner proposes and/or relies on specific constructions for any term in this proceeding, I reserve the right to supplement and modify my opinions.

## **VII. TECHNICAL BACKGROUND**

39. The challenged claims of the '916 Patent are directed to methods for detecting genetic variations in microsatellite regions in a sample of cell-free DNA ("cfDNA"). The challenged claims recite several technical terms and techniques to do so, including tagging cell-free nucleic acid molecules from a sample with molecular barcodes (attached by ligation), amplifying the tagged molecules, sequencing the tagged, amplified molecules, and then analyzing the sequence reads for microsatellite changes in certain regions to detect genetic variation in those regions. The tagged barcodes are used in the traditional manner to group sequence reads and collapse them into consensus sequences. The final step involves

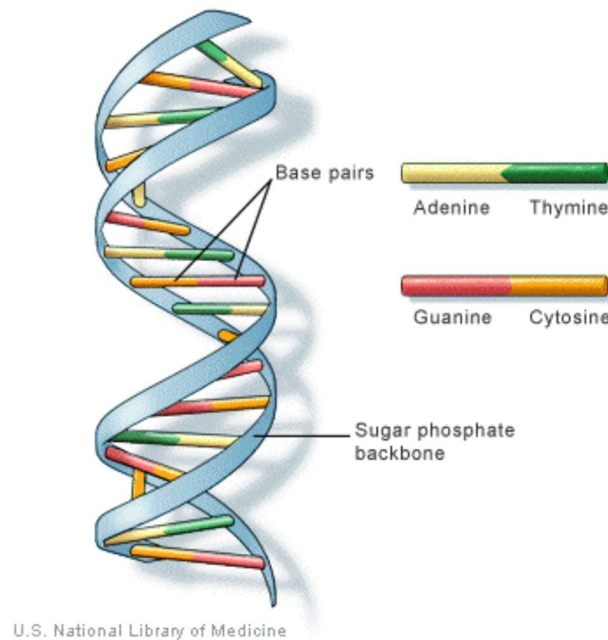
determining a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions, thereby detecting the genetic variation in the microsatellite regions. To better understand the appropriate context of the technology at issue, a brief background primer is provided *infra*.

**A. DNA Basics**

40. DNA is made of four different nucleotides or bases, each represented by a different letter: adenine (A), thymine (T), cytosine (C) and guanine (G). The order of these letters (*i.e.*, the DNA sequence) encodes the information that instructs cells when and how to perform certain functions.

41. The four nucleic acid bases form stable 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” only pairs with “T” and does not pair with “G” or “C.” Likewise, “C” only pairs with “G” and does not pair with “A” or “T.”

42. 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, as shown *infra*.



43. 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 nucleic acids that come together by way of hydrogen bonding between stretches of complementary (or substantially complementary) nucleotides are said to be hybridized to one another. Because of the specificity of base-pairing, under most environmental conditions, a strand of DNA will have a specific complementary sequence.

### **B. Human genetics**

44. DNA is typically contained within the nucleus of a cell and is packed into structures called *chromosomes*. The complete set of DNA found in the nucleus is referred to as a “genome” and is called *genomic DNA* or “gDNA.” The *haploid* human genome contains about 3 billion nucleotides that are distributed among 23

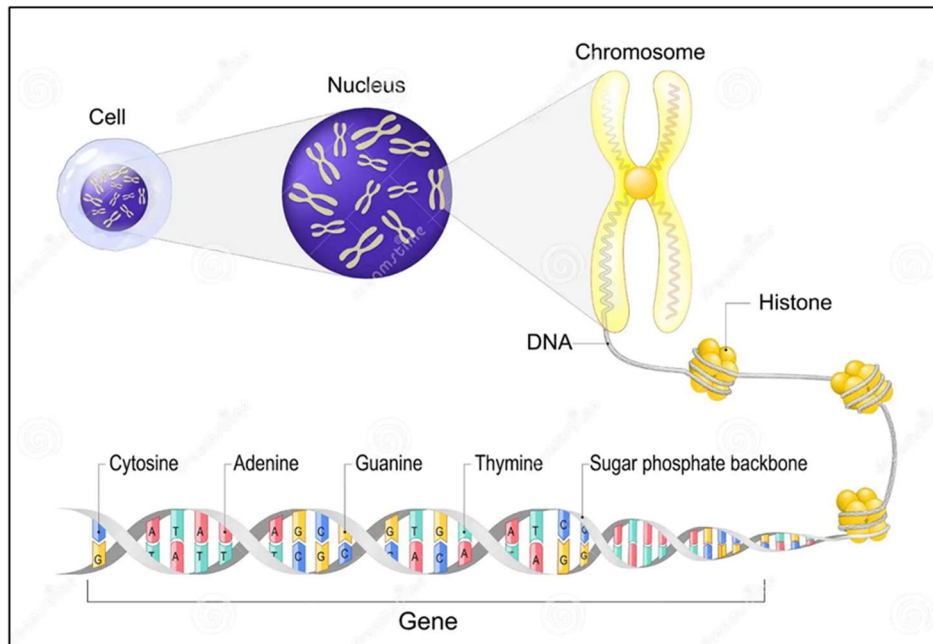
chromosomes, and the *diploid* human genome contains about 6 billion nucleotides that are distributed among 46 chromosomes. Said another way, a typical human genome contains 46 chromosomes—44 (22 pairs) autosomes and two (one pair) sex chromosomes (*i.e.*, X and Y).<sup>6</sup> One set of each chromosome pair is inherited from a person's mother and the other from a person's father. In people, almost every cell in the body contains a complete copy of the genome in the nucleus of a cell, organized as chromosomes,<sup>7</sup> as illustrated in the figure *infra*.<sup>8</sup>

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<sup>6</sup> See Ex. 1032 (Buckingham (2007) at 156.

<sup>7</sup> *Id.* Excluded are reproductive cells, called germ cells, which only have one genome copy (*i.e.*, the haploid genome), as well as cells that do not contain a nucleus (*e.g.*, red blood cells), and therefore do not contain genomic DNA.

<sup>8</sup> Image obtained from <http://www.cell-anatomy-nucleus-chromosomes-close-up-dna-molecule-histone-sugar-phosphate-backbone-guanine-cytosine-thymine-adenine-247771836.webp/> (last visited July 25, 2025).



### C. Cell-free DNA

45. As shown *supra*, DNA is typically contained within the nucleus of a cell and is considered *cellular DNA*. In addition, DNA can exist as *extracellular DNA*, also called *cell-free DNA*, and refers to short fragments of DNA (generally 140-170 bp in length) found in blood.<sup>9</sup> This is because cells typically shed their DNA into the bloodstream through certain biological process associated with cell death (*e.g.*, necrosis or apoptosis) or through active secretion.<sup>10</sup>

### D. Genetic Variants & Disease

46. Genetic variants can be associated with cancer.<sup>11</sup> As I explain *infra*,

<sup>9</sup> See Ex. 1010 (Forsheaw (2012)) at 1.

<sup>10</sup> See Ex. 1014 (Schwarzenbach (2011)).

<sup>11</sup> See Ex. 1031 (Thomas (2006)) at Abstract.

such variants may be classified by the type of alteration or cell type in which the variant occurs. I described both types in the section *infra* as well as how genomic variants are identified.

*i. Types of genetic variants*

47. For example, the smallest genetic variants are single nucleotide variants (SNVs). Each SNV reflects a difference in a single nucleotide (or letter) at a particular genomic position. For a given SNV, the DNA letter at that genomic position might be, for example, a “C” in one person but a “T” in another person.<sup>12</sup> Another type of variant is single nucleotide polymorphisms (SNPs), which also refers to a difference in a single nucleotide (or letter) at a particular genomic position. However, to be considered a SNP, a variant is typically present in at least 1% of the human population.<sup>13</sup> As such, an SNV is a more general term that includes both relatively common (such as SNPs) and rare single-nucleotide differences. For simplicity, all single-nucleotide differences can be referred to as SNVs, regardless of their relative frequency.<sup>14</sup>

48. Another class of genetic variants are insertions and deletions, often

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<sup>12</sup> See Ex. 1015 (NHGRI variation fact sheet) at 4.

<sup>13</sup> *Id.*; see also Ex. 1032 (Buckingham (2007)) at 156.

<sup>14</sup> See Ex. 1015 (NHGRI variation fact sheet) at 4.

called *indels*.<sup>15</sup> A genomic insertion results when one or more nucleotides (usually fewer than 50 base-pairs) are inserted into a DNA sequence (*i.e.*, extra nucleotides found in the genome). A genomic deletion, on the other hand, results when one or more nucleotides (usually fewer than 50 base-pairs) are deleted from a DNA sequence (*i.e.*, missing nucleotides found in the genome). Indels are not as common as SNVs but nonetheless can have a large impact on health and disease. The most common type of indel variants are tandem repeats, which are also called *microsatellites*, which I will address separately below.

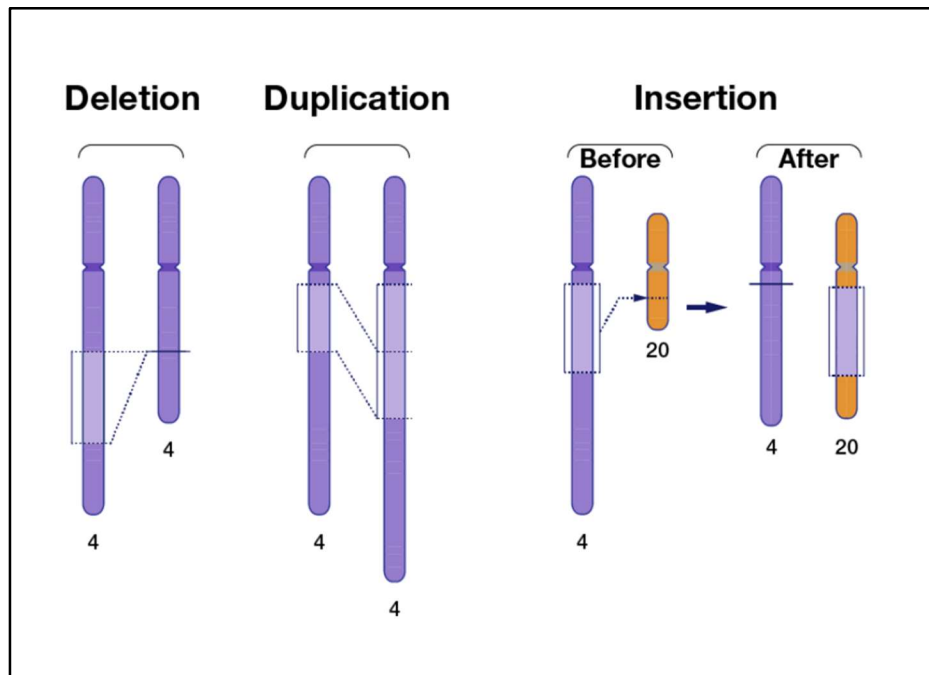
49. Larger indels (*i.e.*, typically > 50 base-pairs) form yet another class of genetic variants called *structural variants*. Tandem repeats that contain more than 50 base-pairs are considered structural variants. Large tandem repeats account for nearly 50% of structural variants in the human genome. When structural variants reflect differences in the total number of base-pairs involved, these are called *copy-number variants* or (CNVs).<sup>16</sup> Several examples of copy number variations, such as large deletions, duplications, and insertions are illustrated *infra*.<sup>17</sup>

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<sup>15</sup> *Id.*

<sup>16</sup> See Ex. 1037 (Hastings (2009)) at Abstract.

<sup>17</sup> Figure adapted from Ex. 1015 (NHGRI variation fact sheet) at 6.



ii. *Cell type: how genomic variants are acquired*

50. Genetic variants or mutations may be inherited, called *germline* variants.<sup>18</sup> Germline variants are first present in reproductive cells (e.g., egg or sperm cells) of a parent, and are then passed from parent to their offspring. Germline transmission typically results in the genetic variant being present in all cells of the offspring.<sup>19</sup>

51. Genetic variants or mutations may also be acquired during a person's lifetime in non-reproductive cells, called *somatic* variants.<sup>20</sup> Somatic variants are

<sup>18</sup> See Ex. 1038 (Hiatt (2013) at 843.

<sup>19</sup> See Ex. 1039 (Cleveland Clinic webpage) at 2–3.

<sup>20</sup> *Id.*; see Ex. 1040 (American Cancer Society webpage) at 4.

not heritable and accumulate over a person’s lifetime, often due to environmental factors or errors in copying the genome during cell division. Unlike germline mutations, however, somatic variants are not present in all of the cells in a person’s body. Rather, somatic variants are only present in the original cell in which the mutation first occurred and then copied and transmitted through cell division to their daughter cells. Some somatic variants can lead to the development of diseases, such as cancer. Unlike germline variants, somatic variants do not typically occur in reproductive cells (*e.g.*, egg or sperm cells) and therefore are not passed on to future generations.<sup>21</sup>

*iii. How genetic variants are identified*

52. Comparing the genome sequence derived from a cell of one person against the genome sequence derived from a cell of another person or a reference human genome<sup>22</sup> will generally reveal far more similarities to each other than differences. For example, two people’s genomes are generally more than 99% identical to one another because “on average, a single-nucleotide difference exists

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<sup>21</sup> *Id.*

<sup>22</sup> A “reference” human genome sequence is an established, well-accepted sequence of a human genome. I was part of the effort, called the *Human Genome Project*, that decoded the first human reference genome. *See* Ex. 1041 (IHGSC (2001)); *see also* Ex. 1042 (IHGSC (2004)).

between two people's genomes once every 1,300 nucleotides or so."<sup>23</sup> The differences among human genomes are sometimes called "variants," "variations," "polymorphisms," or "mutations." A person's set of genetic variants is part of what makes them unique<sup>24</sup> and may contribute to an individual's risk for developing diseases, such as cancer.

53. Cataloging those mutations that are associated with cancer and other human disease has long been an area of intense study, dating back decades. Public databases of such genetic variants have long existed.<sup>25</sup> A number of different types of genetic variants have been identified that are associated with cancer, such as SNVs, MSIs, and CNVs, as described *supra*.

**E. Microsatellite Instability (MSI)**

54. I address microsatellites separately here because the '916 Patent refers to these variants in particular. However, as I described *supra*, microsatellite variants are the most common type of indel variants. Microsatellites, also called *simple sequence repeats* (SSRs) or *simple tandem repeats* (STRs) are tandemly repeated tracts of DNA composed of 1-9 base-pair units.<sup>26</sup> Because of their variability,

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<sup>23</sup> See Ex. 1015, (NHGRI variation fact sheet) at 3.

<sup>24</sup> *Id.* at 4.

<sup>25</sup> See e.g., Ex. 1043 (Forbes (2011)); see also Ex. 1044 ("GDC data portal").

<sup>26</sup> See Ex. 1033 (Gemayel (2010)) at 446; see also Ex. 1034 (Tóth (2000)) at 967.

tandem repeats are also called *variable number of tandem repeats* (VNTRs) and are highly variable among people.<sup>27</sup> One example of a tandem repeat or microsatellite is a (CA)*n* repeat, where *n* can range from a few repeats to over 20 *dinucleotide repeat* units or just *dinucleotide repeats*.<sup>28</sup> Tandem repeats can also contain *trinucleotide repeats* (e.g., (CAG)*n*), *tetranucleotide repeats* (e.g., (GATA)*n*),<sup>29</sup> and so forth. Microsatellite instability (MSI) occurs in tandem repeats from the accumulation of uncorrected insertion/deletion of repetitive DNA tracts in cancer patients with deficient mismatch repair (MMR) systems<sup>30</sup> and/or aberrant recombination events that can occur between repetitive DNA tracts.<sup>31</sup>

55. In other words, MSI refers to a form of genomic instability that occurs when the DNA mismatch repair (MMR) system is impaired.<sup>32</sup> The MMR system normally functions to identify and correct base-pairing errors that arise during routine DNA replication. When MMR is defective, these replication errors persist, particularly in regions of repetitive DNA known as microsatellites. The result is that

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<sup>27</sup> See Ex. 1033 (Gemayel (2010)) at 446.

<sup>28</sup> See Ex. 1051 (Sharma (2005)) at 4.

<sup>29</sup> See Ex. 1034 (Tóth (2000)) at 970–976; *see also* Ex. 1032 (Buckingham (2007)) at 234.

<sup>30</sup> See Ex. 1035 (Laghi (2008)) at Abstract.

<sup>31</sup> See Ex. 1036 (Richard (2000)) at Abstract.

<sup>32</sup> See, e.g., Ex. 1035 (Laghi (2008)).

the number of repeated bases in the newly synthesized DNA differs from the original sequence—either through the addition of repeats or the loss of repeats, events known respectively as insertions and deletions (indels), as described *supra*.

56. For example, in a normal genome, a microsatellite locus might consist of eight consecutive adenine (A) bases. If MMR function is defective, DNA replication at this locus can lead to changes in repeat length due to uncorrected slippage errors by the DNA polymerase. In the illustrated example,<sup>33</sup> the microsatellite becomes shorter by four nucleotides—an instance of a deletion mutation within the repeat tract.

57. Genetic differences such as the loss of four base pairs in a gene can have significant biological consequences, depending on the genomic location and functional relevance of the altered sequence. The relationship between MSI and colorectal cancer has been recognized and investigated for decades. MSI have been associated with other cancers including small cell lung cancer<sup>34</sup> and head and neck cancer.<sup>35</sup> Historical studies have documented the discovery of microsatellite loci associated with colorectal cancer throughout the 1990s and 2000s, as shown in the

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<sup>33</sup> See Ex. 1077 (van Lier (2010)) at 184.

<sup>34</sup> See Chen *et al.*, *Microsatellite alterations in plasma DNA of small cell lung cancer patients*, NATURE MEDICINE 2:1033–1035 (1996) (“Chen (1996)”)

<sup>35</sup> See Nawroz *et al.*, *Microsatellite alternations in serum DNA of head and neck cancer patients*, NATURE MEDICINE 2:1035-1037 (1996) (“Nawroz (1996)”)

accompanying timeline.<sup>36</sup>

58. In 1997, the National Cancer Institute (NCI) issued formal guidelines to standardize the detection and classification of MSI in tumor samples, providing a framework for research and clinical diagnostics. These guidelines included the following: “The form of genomic instability associated with defective DNA mismatch repair in tumors is to be called microsatellite instability (MSI). (b) A panel of five microsatellites has been validated and is recommended as a reference panel for future research in the field. Tumors may be characterized on the basis of: high-frequency MSI (MSI-H), if two or more of the five markers show instability (i.e., have insertion/deletion mutations), and low-frequency MSI (MSI-L), if only one of the five markers shows instability.”<sup>37</sup>

59. By 1997—and in fact earlier—it was well established that microsatellite length changes had direct clinical relevance for cancer diagnosis and management. It was also recognized that such insertions and deletions within microsatellite regions are a hallmark consequence of defective MMR activity.

60. Years before 2012, MSI detection from cell-free DNA (cfDNA) in blood was being employed as a biomarker in clinical cancer testing. For example,

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<sup>36</sup> See Ex. 1078 (Boland (June 2010)) at 23.

<sup>37</sup> See Ex. 1079 (Boland (1998)) at 1.

the '331 Patent Pub. (Sacko) explains that “microsatellite mutations and instabilities detected in the free genomic DNA of the serum suggest that it could be a new potential marker, with considerable specificity for monitoring tumors.”<sup>38</sup> This statement reflects the recognition that MSI in cfDNA could serve as a noninvasive and highly specific method for tracking tumor presence and progression. By 2012, detection MSI from cfDNA in blood was used as a biomarker in clinical testing for cancer.<sup>39</sup>

**F. Next generation sequencing (NGS)**

61. DNA sequence technologies are important because they can help diagnose disease by identifying genetic variants. The Human Genome Project begun in October 1990 as a joint effort of the NIH and DOE, with the ultimate goal of determining the complete sequence of the three billion nucleotides that make up the human genome.<sup>40</sup> I was part of the project that was responsible for sequencing the first human genome at Baylor College of Medicine. It took approximately 13 years to complete the sequence of the first human genome. While the sequencing technologies I described *infra* can produce entire genomes faster and cheaper than

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<sup>38</sup> See Ex. 1076 (Sacko), [0005].

<sup>39</sup> See Ex. 1080 (Sinicrope) at 1.

<sup>40</sup> I note this is the haploid state, and as diploid organism, the human genome is twice this size.

the first human genome, doctors or researchers who suspect certain genetic variants that may cause a particular disease will only focus on those parts of the genome that may contain such variants, called *targeted* approaches.

62. DNA sequencing includes several methods and technologies that are used to determine the order of nucleotide bases (that is, A, C, G, and T) in a DNA fragment. Modern methods involve inputting many polynucleotide fragments at once into an automated device called a *sequencer*. After performing a series of chemical and enzymatic reactions to determine the base at each position in the sequences, the sequencer outputs ordered lists of those polynucleotide bases in the form of *sequence reads*. By December 2013, sequencing techniques were routinely and conventionally used for disease detection and other diagnostic methods,<sup>41</sup> and its workflows were well-known to practitioners.

63. NGS technologies can produce substantially larger volumes of sequence information (*e.g.*, millions or even billions of sequence reads) at an affordable cost. For example today a human genome can be sequenced for around \$100.<sup>42</sup> *Next-generation sequencing* (NGS) platforms have allowed for high-

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<sup>41</sup> See, *e.g.*, Ex. 1017 (Metzker (2010)).

<sup>42</sup> See Ex. 1054 (Preston (2023)). In 2023, the authors wrote “[t]oday, a human genome can be sequenced for \$600, with some predicting that the \$100 genome is not far behind.”

throughput sequencing of nucleic acids both on a genome-wide scale and in a targeted, highly multiplexed manner. The most widely used NGS sequencing platform is the Illumina sequencing-by-synthesis (SBS) platform,<sup>43</sup> which has been in use since the 2006. NGS can generate single-end sequence reads from one end of the DNA fragment or paired-end sequence reads from both ends of the DNA fragment.

64. In general, DNA sequencing methods can be characterized as either *first-generation sequencing*, which include Sanger sequencing,<sup>44</sup> or NGS, discussed *infra*. In broad terms, next-generation sequencing 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 next-generation sequencing technologies.<sup>45</sup> I may rely on figures and information from this article.

65. There have been a number of NGS platforms that have been released commercially since 2005, including those sold by 454 Life Sciences Corporation—the GS20 and GS FLX® instruments,<sup>46</sup> Life Technologies—the SOLiD®

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<sup>43</sup> See, e.g., Ex. 1017 (Metzker (2010)) at 34.

<sup>44</sup> See Ex. 1045 (Metzker (2005)) at 1768–1770.

<sup>45</sup> See, e.g., Ex. 1017 (Metzker (2010)).

<sup>46</sup> *Id.* at 39, Figures 1a, 3c, 3d.

instrument,<sup>47</sup> and Pacific Biosciences—the single-molecule real-time or SMRT® instrument.<sup>48</sup> Other NGS systems that have been commercialized include the Ion Torrent—the Personal Genome Machine (“PGM®”)<sup>49</sup> and Ion Proton® instruments and Oxford Nanopore—the MinION sequencer.<sup>50</sup> The Genome Analyzer (“GA”) I sequencer was developed by Solexa and released in late 2006. The GA II sequencer was developed after the acquisition of Solexa by Illumina in 2007. Since 2009, Illumina has released new instruments including multiple versions of HiSeq®, MiSeq®, NextSeq® and NovaSeq® systems.

**G. Amplification and target enrichment of multiple loci**

*i. Polymerase chain reaction (PCR) amplification*

66. Sequencing is generally not performed on the DNA fragments as they are originally collected from a sample. Prior to sequencing, the fragments are generally modified and/or amplified to create multiple copies of each DNA fragment. This is typically necessary to generate sufficient copies for the sequencing process to produce usable data.

67. A universal technique for replicating or amplifying nucleic acids in a

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<sup>47</sup> *Id.* at 36-39 at Figures 1a, 3a, 3b.

<sup>48</sup> *Id.* at 39 at Figures 1e, 4.

<sup>49</sup> *See* Ex. 1046 (Rothberg (2011)).

<sup>50</sup> *See* Ex. 1047 (Jain (2015)).

laboratory setting is the *polymerase chain reaction* (PCR). This method was invented by Dr. Kary Mullis in the 1980s.<sup>51</sup> PCR is used to specifically amplify a small amount of nucleic acid molecules, generating thousands to millions of copies of the target nucleic acid.

68. Generally speaking, PCR involves repeated denaturation and replication of DNA sequences *in vitro* (i.e., outside of an organism). The enzyme *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 colleagues used short oligonucleotide primers complementary to the 3'-ends of the 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: a *forward* primer will hybridize to the 3'-end of a single-stranded template DNA. DNA polymerase binds to this partially double-stranded complex and extends the primer by copying of sequence of the single-stranded

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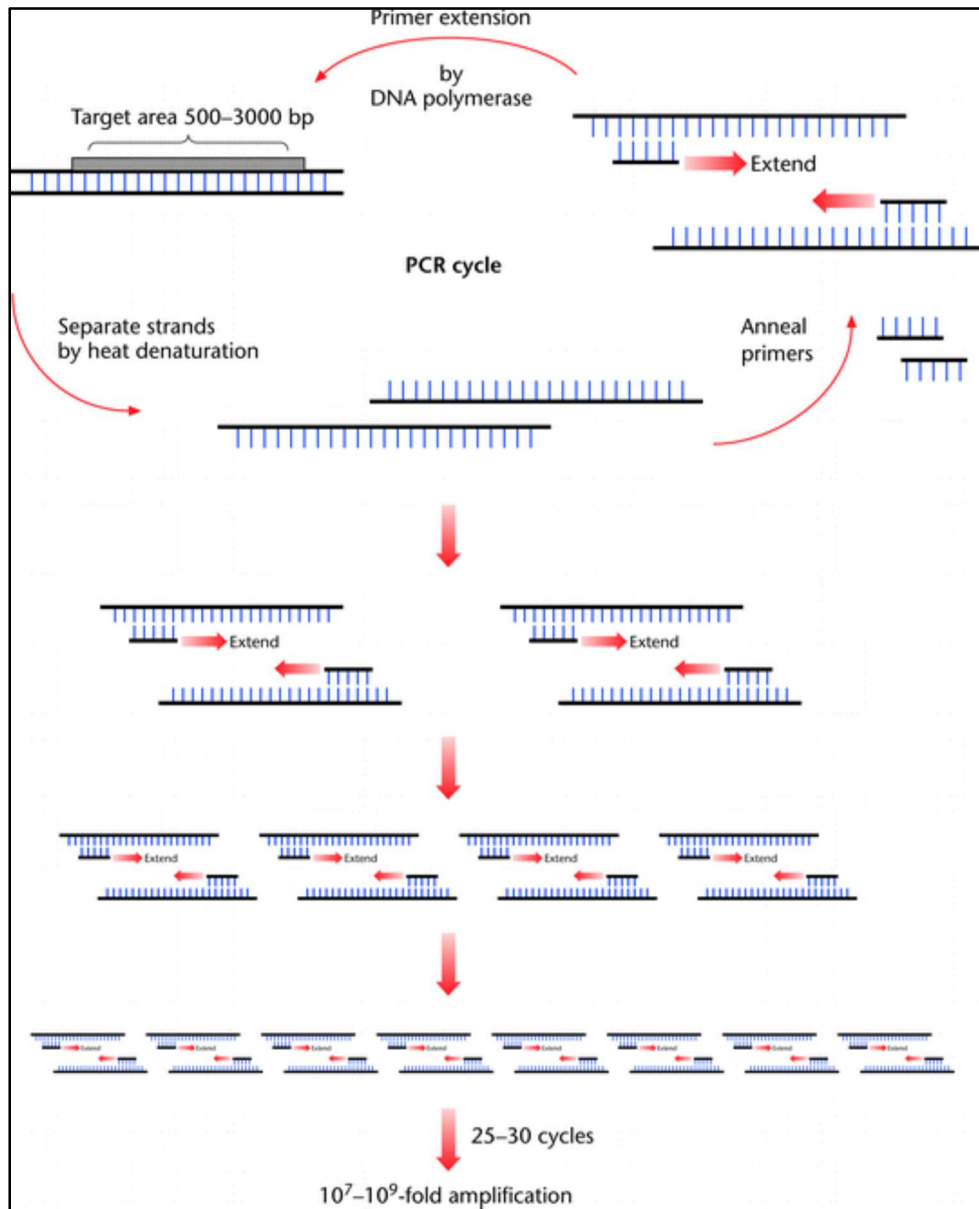
<sup>51</sup> See Ex. 1019 (Metzker (2006) at 380.

template in the 5'-to-3' direction. Primer extension will build a complementary strand of DNA.<sup>52</sup> Also in the same cycle, a *reverse primer* will hybridize to the 3'-end of the other single stranded template DNA. Similarly, DNA polymerase binds to this partially double-stranded complex and extends the primer by copying of sequence of the single-stranded template in the 5'-to-3' direction. Primer extension will build another complementary strand of DNA. The general concept of PCR is illustrated by the figure *infra*.<sup>53</sup>

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<sup>52</sup> See Ex. 1019 (Metzker (2006)) at 380.

<sup>53</sup> *Id.* at Figure 1.



69. Amplification can be *universal*—amplifying all molecules present in a sample equally—or *targeted*—amplifying just those that are of interest. The latter approach is a process referred to as *target enrichment*. By using specially designed primers to preferentially amplify sequences of interest over other background sequences that are present, downstream sequencing resources can be used efficiently

and cost effectively. After this process, a sample of interest is said to be enriched.

Several target enrichment methods have been described that target multiple loci in a single reaction tube to increase the proportion of specific target nucleic acid sequences from a biological sample,<sup>54</sup> as described *infra*.

*ii. Multiplex PCR*

70. Multiplex PCR refers to amplifying multiple different target sequences in a target DNA using different target-specific primer pairs in the same reaction tube or volume.<sup>55</sup> Multiple different targets can also be amplified in separate PCR reactions, called single-plex or uniplex reactions, though this is generally more time consuming and labor-intensive.<sup>56</sup>

71. In targeted multiplex PCR, target-specific primers operate in the same manner as in single-plex PCR, whereby primer pairs for each target region undergo the PCR cycle, as illustrated in the figure *supra*. Rather than having only one set of primers (a forward primer and a reverse primer) in a reaction as in single-plex PCR, however, multiplex PCR requires at least two or more sets of target-specific primer pairs, and can include dozens of different primer pairs in the same reaction.<sup>57</sup> The

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<sup>54</sup> See Ex. 1020 (Mamanova (2010)) at 111.

<sup>55</sup> *Id.* at Figure 1 legend; see also Ex. 1049 (Turner (2009a)) at 266.

<sup>56</sup> See Ex. 1050 (Edwards (1994)) at S66.

<sup>57</sup> See Ex. 1049 (Turner (2009a)) at 269.

mixture of many target-specific primer pairs with different sequences allows targeting of many sequences located within the template DNA, and results in amplification of a plurality of specific targets.<sup>58</sup>

72. A challenge with PCR, particularly multiplex PCR, is the formation of unwanted amplification products called, *primer dimers*.<sup>59</sup> Primer dimers form when target-specific primers hybridize to one another rather than the target sequence and typically result due to primers in the reaction being in a relatively high concentration compared to the template DNA.<sup>60</sup> The presence of multiple primers in multiplex PCR tends to increase the propensity of primer dimer formation, which has the potential to obscure downstream sequencing by increasing the amount of nonspecific background noise.<sup>61</sup>

*iii. Hybrid capture*

73. Several hybridization-based capture methods have been developed to target genomic sequences of interest, most of which can be employed after nucleic acid extraction and library preparation.<sup>62</sup> One such solution-based hybridization

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<sup>58</sup> See Ex. 1019 (Metzker (2006)) at 384.

<sup>59</sup> See Ex. 1050 (Edwards (1994)) at S69.

<sup>60</sup> *Id.*

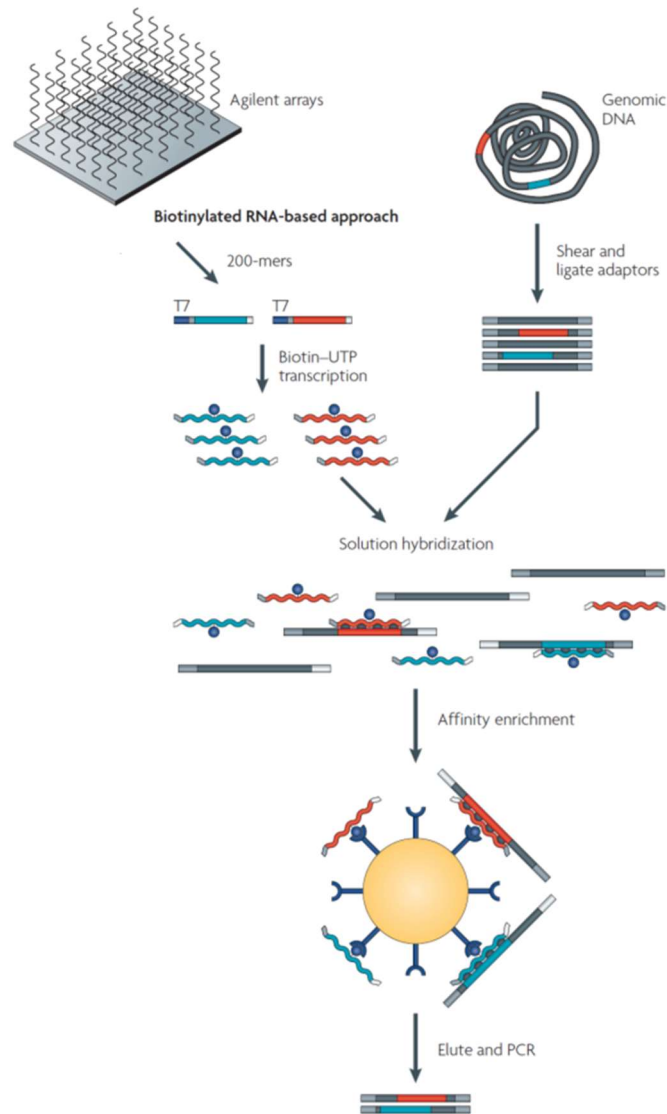
<sup>61</sup> *Id.*

<sup>62</sup> See Ex. 1017 (Metzker 2010)) at 41, Figure 5.

method employs the use of RNA “baits.” Generally, DNA fragments are first ligated with adaptor sequences to create adaptor-ligated libraries, as described *infra*. These libraries are denatured, for example, by exposure to heat. The denatured adaptor-ligated products are then hybridized with single-stranded, biotinylated RNA baits that are specific to the region(s) of interest. The resulting double-stranded, hybridized duplexes are captured by streptavidin-coated magnetic beads. A magnetic field is applied, capturing the bead-DNA complexes. This is illustrated in the figure *infra*.<sup>63</sup>

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<sup>63</sup> *Id.* at Figure 5c.



Adaptor-ligated DNA fragments that are not targeted, and therefore not captured by RNA baits, are then washed away, and the remaining enriched adaptor-ligated DNA is eluted and PCR amplified to create a capture hybrid enriched library that can be sequenced by NGS technologies. The combination of genome enrichment methods, such as hybrid capture, and next generation sequencing technologies have facilitated an “. . . increase in the efficiency and resolution of detection of each of the principle

types of somatic cancer genome alterations, including nucleotide substitutions, small insertions and deletions, copy number alterations, chromosomal rearrangements and microbial infections.”<sup>64</sup>

#### **H. Library preparation, Including Tagging With Barcodes**

74. Before NGS, the nucleotide fragments of interest are typically combined with relatively short pieces of DNA called *adaptors*<sup>65</sup> that serve a variety of functions. This modification step is referred to as *tagging*. These adaptors can be added through the amplification process discussed above (*e.g.*, by PCR)<sup>66</sup> or alternatively through direct attachment (*e.g.*, by a process called *ligation*).<sup>67</sup>

75. In Illumina sequencing, these tagged adaptors always included known primer sequences necessary for the sequencing process itself. The adaptors also frequently included unique or non-unique identifier tags which could, for example, serve to distinguish original DNA fragments and their resulting sequence reads from one another. Such identifiers, which had long been used in other molecular biology techniques (such as array hybridization) before their use in NGS, are typically

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<sup>64</sup> See Ex. 1053 (Meyerson (2010) at Abstract.

<sup>65</sup> “Adaptors” have also been spelled as “adapters.” Both spellings have been used in many of the documents cited in this Declaration, and their descriptions therein should be considered to have identical functions.

<sup>66</sup> See Ex. 1010 (Forsheew (2012), Supplementary Methods at 3.

<sup>67</sup> See Ex. 1055 (Bentley (2008) at Figure 1; *see also* E. 1056 (Kircher (2012) at 2.

referred to as barcodes.<sup>68</sup>

76. Barcodes can be unique or non-unique. In the former case, there are more barcodes than fragments of interest—consider using 1000 barcodes to label 500 DNA fragment of interest. In the latter case, there are less barcodes than fragments. Unique tagging can still be achieved, however, by considering the barcodes together with some portion of the sample fragments themselves (*e.g.*, a few nucleotides).

77. Tagging with adaptors can be of one DNA strand (at one or both ends), or in the case of double-stranded DNA fragments, both strands (again at one or both ends). This latter approach is called “duplex tagging,”<sup>69</sup> and was also well known by December 28, 2013.

### **I. Sequence reads**

78. The output of sequencing using NGS methods is typically a large dataset of DNA sequences. These sequences are commonly referred to as *sequence reads*. Each read represents the order of the nucleotide bases that was detected by the sequencer. Typically, the length of a sequence read is governed by the type of NGS platform used. For example, by 2012, Illumina sequencers produced 150 base

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<sup>68</sup> See Ex. 1057 (Pierce (2006)).

<sup>69</sup> See Ex. 1056 Kircher (2012)); *see also* Ex. 1058 (Schmitt (2012)).

reads, Ion Torrent sequencers produced 400 base reads, and PacBio sequencers produced ~1,000 base reads.<sup>70</sup> The data are typically stored in standardized file formats (*e.g.*, FASTQ file)<sup>71</sup> and can be further analyzed to determine the order of nucleotides in the original sample, identify variants, and perform other downstream analyses, as described *infra*.

## **J. Mapping/Alignment**

79. After sequence reads are generated, they are usually compared to a reference DNA sequence to know where in the genome the sample DNA fragments came from. This bioinformatic process is called *mapping* or *read alignment*. There are multiple alignment tools that have been developed to map sequence reads to a reference genome. For example, such alignment tools include programs called ELAND,<sup>72</sup> MAQ,<sup>73</sup> BWA,<sup>74</sup> and Bowtie,<sup>75</sup> just to name a few. Once sequence reads are mapped reads to the reference sequences, they are formatted into a common alignment format, called Sequence Alignment/Map (SAM) format, which provides

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<sup>70</sup> See Ex. 1059 (Glenn (2011)) at Table 2.

<sup>71</sup> See Ex. 1060 (Cock (2010)).

<sup>72</sup> See Ex. 1055 (Bentley (2008)) at 54; *see also* Ex. 1061 (CASAVA 1.8 User Guide) at 6.

<sup>73</sup> See Ex. 1062 (Li (2008)).

<sup>74</sup> See Ex. 1063 (Li & Durbin (2009)); *see also* Ex. 1064 (Li & Durbin (2010)).

<sup>75</sup> See Ex. 1065 (Langmead (2009)).

a well-defined interface between alignment and downstream analysis.<sup>76</sup> A companion format, called a Binary Alignment/Map (BAM) format or BAM file was developed that represents the same information as a SAM file, but in a compressed memory format.<sup>77</sup>

80. Processing such an enormous volume of NGS sequence data require intensive computer efforts. For example, mapping tens to hundreds of millions of sequence reads using alignment algorithms to create BAM files requires a significant computer infrastructure utilizing multiple centralized processing units (CPUs).<sup>78</sup> In the case of human DNA, such reference sequences have long been made available by the research community as public resources. In 2010, the Broad Institute created the Genome Analysis Toolkit (GATK) that provided a structured programming framework to bridge the development gap that existed between NGS read production and genetic variant analysis, the framework of which was based on Google's programming philosophy of MapReduce.<sup>79</sup> Development of the GATK engine has been an indispensable platform that is constantly being improved and optimized,

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<sup>76</sup> See Ex. 1066 (Li (2009)).

<sup>77</sup> *Id.*

<sup>78</sup> See Ex. 1067 (DePristo (2011)) at 491 (“Mapping reads to the reference genome is a first critical computational challenge . . .”) (citations omitted).

<sup>79</sup> See Ex. 1068 (McKenna (2010)) at 1297.

even today for “correctness, stability, and CPU and memory efficiency.”<sup>80</sup>

81. Mapping sequence reads to a reference sequence facilitates the identification of variants, a process referred to as *variant calling* because a DNA fragment can be identified as containing a variant when its sequence is different from the reference at one or more nucleotide positions. There were also a variety of variants callers, such as VarScan,<sup>81</sup> VarScan2,<sup>82</sup> and MuTect,<sup>83</sup> just to name a few. For example, these variant callers could analyze a BAM file of a sequence read to identify an “A” at a position where the reference sequence identifies a “T,” which would characterize this genetic variant as an SNV. The output of variant callers is provided in another standardized format, called the *variant call format* (VCF).<sup>84</sup>

82. However, sequence read data created by NGS was not error-free,<sup>85</sup> mapping to a single sequence read alone (or even mapping a handful of reads) is

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<sup>80</sup> *Id.*; see also Ex. 1069 (GATK Updates).

<sup>81</sup> See Ex. 1070 (Koboldt (2009)).

<sup>82</sup> See Ex. 1071 (Koboldt (2012)).

<sup>83</sup> See Ex. 1072 (Cibulskis (2013)).

<sup>84</sup> See Danecek *et al.*, *The variant call format and VCFtools*, *Bioinformatics* 27:2156–2158 (2011) (“Danecek (2011)”).

<sup>85</sup> Even at 99.9% accuracy (called Q30 by practitioners), there will be an error—a base called incorrectly—at 1 out of every 1000 bases. When billions of bases are being called in a single sequencing run, there will be a significant number of errors spread across the resulting sequence reads; see also Ex. 1017 (Metzker (2010)) at 34; Ex. 1048 (Kinde (2011)); Ex. 1058 (Schmitt (2012)).

generally not sufficient to know whether a difference between a given sequence read and a reference sequence is, in fact, a true mutation in the original sample. That is, in the example *supra*, the “A” in the sequence read could not reflect a variant at all but rather could represent a *sequencing error*.<sup>86</sup> Sequence redundancy, using multiple sequence reads in an assembly or alignment is well established to reduce errors in individual sequence reads by generating a *consensus sequence*.<sup>87</sup>

### **K. Generating Consensus Sequences**

83. Early on, practitioners recognized the need for high quality sequencing reads to accurately call variants, particularly those that are rare and thus not expected to be widely present in subject DNA. Thus, while efforts continue to improve the sequencing process itself to increase its fidelity, researchers also developed other techniques to increase the accuracy of detecting rare genetic variants.

84. Once such technique that relies on the barcodes discussed earlier is known as *consensus sequences*.<sup>88</sup> It is undisputed that consensus sequencing was well-known in the art by December 28, 2013. At a high level, consensus sequencing

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<sup>86</sup> See Ex. 1048 (Kinde (2011)); Ex. 1005 (Schmitt (2012)).

<sup>87</sup> See, e.g., Schatz *et al.*, *Assembly of large genomes using second-generation sequencing*, *Genome Research* 20:1165–1173 (2010) (“Schatz (2010)”) at 1167 (“These assemblers also attempt to correct sequencing errors by using overlapping reads to confirm each other.”).

<sup>88</sup> See Ex. 1048 (Kinde (2011)); Ex. 1005 (Schmitt (2012)).

first involves bioinformatically “grouping” together similarly-tagged sequence reads (those having the same barcode identifier) into sets of reads called “families.” For each family, those grouped sequence reads are then “collapsed” into a single, accepted consensus sequence based on the sequence that appears most frequently (e.g., a majority rule). This sequence is expected to be the most accurate because sequence reads with sequencing errors at a given position will generally be present at a very low frequency.

85. For example, while one sequence in a family may have an incorrect base at a particular position due to an error, it will be expected to be out-represented in that family by other sequences without an error at that position.

86. Consensus sequencing thus results in increased practitioner confidence in the variant identification, leading to better and more actionable data. It has wide application and can be used to detect all manner of variants, including those discussed above, with improved accuracy..

## **VIII. THE '916 PATENT**

### **A. Overview**

87. The two challenged independent claims of the '916 Patent, Claims 13 and 30, recite sequencing methodologies coupled with straightforward analytical steps that were well-known before September 2012. Specifically, the claimed methods involve tagging cell-free nucleic acid molecules from a sample with

molecular barcodes (applied via ligation), amplifying the tagged molecules, sequencing the resulting tagged, amplified molecules, and then evaluating the sequence reads for microsatellite changes in defined regions to detect genetic variation. While the claims do not explicitly reference using consensus sequences, the tagged barcodes are employed in the conventional fashion to group sequence reads and collapse them into consensus sequences. The concluding step entails determining “a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions.”

88. The '916 Patent specification does not assert the invention of novel tagging, amplification, sequencing, grouping/collapsing, or quantification methodologies. Rather, it acknowledges that the techniques required to implement the claimed methods were well-known and routine in the art at the relevant time. For example, the '916 Patent specification admits that many processes and techniques were known before September 2012.<sup>89</sup>

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<sup>89</sup> Ex. 1001 ('916 Patent) at 1:49-51 (“[c]ell free DNA (‘cfDNA’) has been known in the art for decades and may contain genetic aberrations associated with a particular disease.”); 36:10-12 (“cell free polynucleotides may be isolated and extracted using a variety of techniques known in the art” including by “commercially available kits”); 38:31–36 (“assignment of unique or non-unique identifiers, or molecular barcodes in reactions of this disclosure may follow methods and systems described by, for example,” prior-art patents and patent applications); 38:56–59 (“PCR for sequencing may be performed using any means, including but not limited to use of

89. With respect to the recited “polymorphic forms,” the ’916 Patent broadly states that such forms “include but are not limited to” a variety of known genetic variations, although the challenged claims are limited to one specific type—“microsatellite changes.”<sup>90</sup> As I described *supra*, microsatellite changes—including the utility of detecting such changes—was well-known by the relevant time. Likewise, the patent describes the claimed “quantitative measure” in non-restrictive terms, encompassing “for example, number, count, frequency (whether relative, inferred or absolute).”<sup>91</sup> The ’916 Patent neither purports to invent nor recites any novel technique for quantitative measurement.

90. Even if the specification does not expressly concede this point, each of the claimed steps was already well-known in the art and would have been obvious to apply for the purpose of MSI detection. As further detailed *infra*, the dependent claims merely add conventional sequencing operations and standard quantitative

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commercial kits provided by Nugen (WGA kit), Life Technologies, Affymetrix, Promega, Qiagen and the like”); 30:22–25 (“subsequent sequencing of cell free polynucleotides” may be done “by techniques known in the art”); 49:26-27 (“sequencing may be performed using any nucleic acid sequencing platforms known in the art.”); 35:37–40 (as to mapping/aligning sequences, “sequences can be interrogated using the genome brow[s]er available” online); and 46:8–10 (“[c]onsensus sequences can be generated from families of sequence reads by any method known in the art.”).

<sup>90</sup> *Id.* at 9:19-24.

<sup>91</sup> *Id.* at 35:32-34.

analyses.

**B. Prosecution History**

91. The '916 application was filed on September 18, 2019, and its prosecution was accorded accelerated "Track One" status under 37 C.F.R. § 1.102(e). The first Notice of Allowability issued on June 29, 2020—less than ten months from the filing date.<sup>92</sup>

92. On October 22, 2019, the applicant submitted a Notice of Concurrent Proceedings identifying Petitions for Inter Partes Review of U.S. Patent Nos. 9,840,743 and 9,834,822 (IPR2019-00634 and IPR2019-00652).<sup>93</sup> I am further informed by counsel and understand that U.S. Patent Nos. 9,840,743 and 9,834,822 also claim priority to the '128 Application and the same list of provisional applications that the '916 Patent claims priority to.<sup>94</sup> I note however that, while the '916 application stated that it was part of the same family as the '743 patent, there is no mention that the '822 patent is also related to this family.<sup>95</sup>

93. On February 14, 2020, the Examiner issued an Office Action rejecting all pending claims for nonstatutory obviousness-type double patenting over claims

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<sup>92</sup> See Ex. 1002 ('916 Patent File History) Ex. 1002504.

<sup>93</sup> *Id.* at 223.

<sup>94</sup> See Ex. 1001, at 1; Ex. 1073(U.S. Patent 9,840,743); Ex. 1074(U.S. Patent 9,834,822).

<sup>95</sup> See Ex. 1001, at 1.

1–33 of U.S. Patent No. 9,902,992 in view of Sacko et al. (US 2010/0264331), and similarly over claims of U.S. Patent No. 10,494,678 as well as claims of five additional co-pending applications.<sup>96</sup>

94. For each rejection, the Examiner stated, “[t]he [patented/copending] claims are generic with respect to the instant claims . . . while the instant claims require the species ‘microsatellite changes’. Sacko et al. discloses that cell-free nucleic acids from blood of cancer patients are associated with microsatellite mutations and instabilities (see paragraph 0005). One of ordinary skill in the art considering the [patented/copending] methods would have been motivated to modify them by applying them to microsatellite changes because Sacko et al. disclosed that microsatellite changes were known to occur in cell-free nucleic acids from blood of cancer patients.”<sup>97</sup>

95. In response to these rejections, the applicant did not attempt to distinguish the claims of the ’916 Patent from those of its family members, but instead filed a Terminal Disclaimer on May 4, 2020.<sup>98</sup> The applicant did not contest the Examiner’s findings. Although the applicant submitted amendments, these were not directed to overcoming the Examiner’s obviousness-type double patenting

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<sup>96</sup> Ex. 1002 at 441-449.

<sup>97</sup> *Id.* at 445-448.

<sup>98</sup> *Id.* at 459.

rejection, but rather were made “to better clarify the claimed subject matter and/or correct for antecedent basis or dependency purposes.”<sup>99</sup> The Examiner subsequently issued a Notice of Allowability on June 29, 2020.<sup>100</sup>

96. In the ensuing prosecution, the applicant likewise made no amendments or arguments aimed at distinguishing the ’916 claims. On July 13, 2020, the applicant submitted additional amendments “for clarity and/or antecedent basis purposes,” after which an issue notification issued on August 19, 2020.<sup>101</sup>

97. On August 28, 2020, the applicant petitioned to withdraw the application from issue under 37 C.F.R. § 1.313(c)(2) and filed an IDS citing the Final Written Decisions in IPR2019-00634 and IPR2019-00652 (822FWD).<sup>102</sup> I will address the 822FWD separately below. Guardant provided no explanation for the citations, offering no discussion of the decisions or their relevance. The petition was granted the same day.<sup>103</sup>

98. On September 3, 2020, the Examiner issued a corrected Notice of Allowability, which did not substantively address either cited Final Written

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<sup>99</sup> *Id.* at 452-457, 476.

<sup>100</sup> *Id.* at 504-505.

<sup>101</sup> *Id.* at 507-515.

<sup>102</sup> *Id.* at 539-549.

<sup>103</sup> *Id.*

Decision.<sup>104</sup> The '916 Patent issued on October 6, 2020—more than two years before the Federal Circuit appeal of the 822FWD was resolved.<sup>105</sup>

## **IX. THE '822 IPR FINAL WRITTEN DECISION**

99. The '822 IPR Final Written Decision was issued by the Board on August 18, 2020.<sup>106</sup> The Board found that Claims 1–11, 13, 17–20 of the '822 Patent were obvious based on '188 Patent (Schmitt), the '188 Patent (Schmitt) (2012)<sup>107</sup>, and Fan (2008)<sup>108</sup> or Forshew (2012).<sup>109</sup>

100. Claim 12 of the '822 Patent which was not found obvious, recites the following limitation “The method of claim 1, wherein the population of polynucleotides is tagged with n different unique identifiers, wherein n is no more than  $100 \cdot z$ , wherein z is a mean of an expected number of duplicate molecules having the same start and stop positions in the sample.”<sup>110</sup> I note that none of the claims challenged for the '916 recite this limitation.

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<sup>104</sup> *Id.* at 551.

<sup>105</sup> Ex. 1001 at 1.

<sup>106</sup> Ex. 1083 (822FWD) at cover page.

<sup>107</sup> *See* Ex. 1058 (Schmitt (2012)).

<sup>108</sup> Fan *et al.*, *Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood*, Proceedings of the National Academy of Sciences of the United States 105:16266–16271 (2008) (“Fan (2008)”).

<sup>109</sup> *See* Ex. 1010 (Forshew (2012)); *see also* Ex. 1083 (822FWD) at 67.

<sup>110</sup> *Id.* at 46.

101. The Board found that the following limitations were taught by '188 Patent (Schmitt) alone or in various combination with Forshew (2012), Fan (2008), and/or the '188 Patent (Schmitt) (2012):

- “converting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides,”<sup>111</sup>
- “each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.”<sup>112</sup>
- “[ ] amplifying the tagged polynucleotides and sequencing the amplified progeny.”<sup>113</sup>
- “determining the frequency of bases called at the locus from among the families”<sup>114</sup>
- “further comprising detecting, at one or more loci, at least one single nucleotide variant, at least one gene fusion, and at least one copy number variant.”<sup>115</sup>
- “selectively enriching regions from a genome or transcriptome of the subject prior to sequencing.”<sup>116</sup>
- “mapping the sequence reads to a reference sequence.”<sup>117</sup>

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<sup>111</sup> *Id.* at 29-31.

<sup>112</sup> *Id.* at 29-31.

<sup>113</sup> *Id.* at 31-32.

<sup>114</sup> *Id.* at 31-32.

<sup>115</sup> *Id.* at 43.

<sup>116</sup> *Id.* at 42-43.

<sup>117</sup> *Id.* at 31-32.

- “grouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide.”<sup>118</sup>
- “at each genetic locus of a plurality of genetic loci in the one or more reference sequences, collapsing sequence reads in each family to yield a base call for each family at the genetic locus.”<sup>119</sup>

102. In addition, the Board found that Fan (2008) and/or Forshew (2012) teaches “providing a population of cell-free (“cfDNA”) molecules obtained from a bodily sample from a subject.”<sup>120</sup>

103. Finally, the Board previously found that ’188 Patent (Schmitt) and Forshew (2012) would have been combined by one of ordinary skill in the art.<sup>121</sup>

104. I am informed by counsel and understand that Guardant either did not challenge these findings on appeal to the Federal Circuit or did not succeed in its challenge. I understand that the Federal Circuit remanded the case to the Board based on its determinations with respect to secondary considerations. I also understand that the appeal was heard on February 8, 2023, and decided on May 5,

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<sup>118</sup> *Id.*

<sup>119</sup> *Id.*

<sup>120</sup> *Id.* at 29.

<sup>121</sup> *See id.* at 49. I note the Board found that “an ordinarily skilled artisan would have been motivated to use Schmitt’s DCS method to detect low-frequency genetic mutations in the cfDNA samples disclosed in Fan or Forshew.”

2023, after the '916 Patent issued.<sup>122</sup> I am informed by counsel and understand that the parties settled thereafter, before the Board was able to render a subsequent ruling.

## **X. OVERVIEW OF THE PRIOR ART**

### **A. The '188 Patent (Schmitt)**

105. The '188 Patent (Schmitt) issued from U.S. Application No. 14/386,800 (the "'800 application"), which is the national stage of PCT/US2013/032665, filed March 15, 2013. The '800 application claims the benefit of U.S. Provisional Application No. 61/613,413, filed March 20, 2012 ("'413 Provisional").<sup>123</sup>

106. I understand that the '800 Application claims priority to the '413 Provisional and thus has a priority date of March 20, 2012.

107. I understand that the '188 Patent (Schmitt) was relied on in IPR2019-00652, which was an IPR of the '822 Patent that claims the same priority date as the '916 Patent.<sup>124</sup> I further understand that, after the Board instituted that IPR, the same Patent Owner did not contest that the '188 Patent (Schmitt) was entitled to the March 20, 2012 priority date.<sup>125</sup> The '188 Patent (Schmitt) was thus available to one of

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<sup>122</sup> *Guardant Health, Inc. v. Vidal*, 2023 WL 3262962, at \*2-3 (Fed. Cir. May 5, 2023).

<sup>123</sup> Ex. 1082 (U.S. Provisional Application 61/613,413 (Schmitt '413 Provisional))

<sup>124</sup> Ex. 1083 (822FWD); EX1074 ('822 Patent).

<sup>125</sup> Ex. 1083 (822FWD).

ordinary skill in the art before September 4, 2012.

108. The '188 Patent (Schmitt) discloses a DNA sequencing error-correction approach termed Duplex Consensus Sequencing (“DCS”).<sup>126</sup> In DCS, each strand of a double-stranded DNA fragment is independently tagged and sequenced.<sup>127</sup> Because both complementary strands are analyzed, nucleotide changes present at the same position in each strand are identified as putative “true mutations”.<sup>128</sup> Mutations observed in both strands are distinguished from sequencing or PCR errors, which typically occur in only one strand.<sup>129</sup>

109. In DCS, sample DNA fragments are labeled with molecular barcodes, which may be unique or non-unique, to enable identification of individual molecules. Figure 1 illustrates the process: ligation of adaptors to the target DNA followed by PCR amplification. The adaptors are “single molecule identifier” (SMI) adaptor molecules designed for use in sequencing double-stranded nucleic acids.<sup>130</sup> Each SMI adaptor contains (i) an SMI sequence or tag—a degenerate or semi-

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<sup>126</sup> Ex. 1005 ('188 Patent (Schmitt)) at Abstract, *see also id.* at 3:10-40.

<sup>127</sup> *Id.*

<sup>128</sup> *Id.*

<sup>129</sup> *Id.*

<sup>130</sup> *See id.* at 2:66–3:1.

degenerate  $n$ -mer<sup>131</sup>—and (ii) an SMI ligation adaptor that permits ligation to the DNA fragment.<sup>132</sup>

110. The '188 Patent (Schmitt)'s DCS method comprises: ligation of a target nucleic acid to one or more SMI adaptor molecules; amplification to generate amplified products; and sequencing of the amplified products.<sup>133</sup>

111. In one example, the SMI sequence is described as “a unique, double-stranded, complementary  $n$ -mer random tag” such that each DNA fragment is uniquely labeled with two distinct SMI sequences.<sup>134</sup> The  $n$ -mer length is selected to yield a sufficient number of unique tags for labeling sheared DNA fragments.<sup>135</sup> The '188 Patent (Schmitt) describes an example of a “nucleotide  $n$ -mer sequence which is 12 nucleotides in length” that, once ligated to each end of the target DNA fragment, “results in the generation of up to  $4^{24}$  (i.e.,  $2.8 \times 10^{14}$ ) distinct tag sequences.<sup>136</sup>

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<sup>131</sup> An “ $n$ -mer” refers to a short nucleotide sequence comprising “ $n$ ” number of nucleotides.

<sup>132</sup> *See id.* at 3:1–9; *see also id.* at 5:57–59, 6:46–47.

<sup>133</sup> *Id.* at 3:12–20.

<sup>134</sup> *Id.* at 3:47–53.

<sup>135</sup> *Id.* 6:59–63 (“nucleotide  $n$ -mer sequences may be any suitable length to produce a sufficiently large number of unique tags to label a set of sheared DNA fragments from a segment of DNA.”)

<sup>136</sup> *Id.* 6:66–7:5.

112. Another of the '188 Patent (Schmitt)'s examples, termed the "hybrid method," uses an SMI sequence comprising a shorter *n*-mer tag (e.g., 1–4 or more degenerate or semi-degenerate bases).<sup>137</sup> These shorter tags are not unique but function as unique molecular identifiers when combined with sequence information from the sheared ends of the target fragment.<sup>138</sup>

113. For error correction, reads with identical SMI tag sets are grouped into paired families, each corresponding to the consensus sequence of an original double-stranded fragment.<sup>139</sup> Figure 3 depicts the grouping process and the determination of mutations within consensus sequences. Mutation identification requires comparison to a reference wild-type sequence. Variants detected in only one strand or in a small subset of family members are classified as sequencing or PCR errors.<sup>140</sup> True mutations are present in both strands and in all members of a family pair.<sup>141</sup> DCS is described as enabling quantitative detection of DNA damage, and the ability to infer such damage is noted as a potentially useful biomarker for applications including cancer risk assessment, cancer metabolic state, and mutator phenotypes

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<sup>137</sup> *Id.* 9:9–13.

<sup>138</sup> *Id.*

<sup>139</sup> *Id.* at 4:4–10.

<sup>140</sup> *Id.*, 4:10–18.

<sup>141</sup> *Id.*, 4:18–20.

associated with defective damage repair.<sup>142</sup>

114. The '188 Patent (Schmitt)'s reference to detecting "mutator phenotype related to defective damage repair" would inform one of ordinary skill in the art that DCS could have been used to identify microsatellite instability (MSI), because MSI is a well-known manifestation of a mutator phenotype caused by mismatch repair deficiency. Microsatellites—short tandem repeats—are prone to insertion–deletion mutations under such conditions. By applying DCS to these loci, uniquely tagging and sequencing both strands of each DNA molecule, and generating strand-specific consensus sequences for comparison to a wild-type reference, repeat length changes present in both strands can be confirmed as true mutations. Quantifying the proportion of molecules with these confirmed alterations yields a direct measure of MSI, placing MSI detection and quantification squarely within the biomarker applications described in the '188 Patent (Schmitt).

115. The '188 Patent (Schmitt) was cited to the Examiner during prosecution via an Information Disclosure Statement (IDS) submitted by the Patent Owner on October 18, 2019, as one of 702 listed references.<sup>143</sup> I am informed by counsel and understand that the record does not indicate that the '188 Patent (Schmitt) was relied

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<sup>142</sup> *Id.*, 15:42-51.

<sup>143</sup> Ex. 1002 at 139.

upon as the basis for any rejection or that it was substantively discussed during prosecution.

116. In IPR2019-00652, the '188 Patent (Schmitt) served as the primary reference in the Petition challenging the related '822 Patent, which, like the '916 Patent, claims priority to the same parent application. In its Final Written Decision, the Board relied on the '188 Patent (Schmitt) in combination with three additional references to find claims of the '916 Patent unpatentable.<sup>144</sup>

**B. Forshew (2012)**

117. Forshew (2012) constitutes prior art to the '916 Patent under 35 U.S.C. § 102(a)(1). Forshew (2012) was thus available to one of ordinary skill in the art before September 4, 2012.

118. Forshew describes a “liquid biopsy” method for detecting rare cancer-associated mutations in circulating, cell-free DNA (cfDNA).<sup>145</sup> Forshew (2012) describes the extraction of cell-free DNA (cfDNA) from plasma samples of cancer patients for mutation analysis. The study notes that “[p]lasma of cancer patients contains cell-free tumor DNA”<sup>146</sup> and reports that “[c]irculating DNA was extracted

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<sup>144</sup> Ex. 1083 at 67.

<sup>145</sup> Ex. 1010, Abstract.

<sup>146</sup> Ex. 1010, 1

from between 0.85 and 2.2 ml of plasma”<sup>147</sup>. The approach involves targeting genomic DNA fragments from selected regions, amplifying them, attaching sample-specific barcodes, sequencing, and aligning the resulting reads to a reference sequence to identify mutations<sup>148</sup>.

119. This method, termed tagged-amplicon deep sequencing (“TAm-Seq”), is defined by ForsheW (2012) as: “We describe a tool for noninvasive mutation analysis on the basis of tagged-amplicon deep sequencing (TAm-Seq), which allows amplification and deep sequencing of genomic regions spanning thousands of bases from as little as individual copies of fragmented DNA. We applied this technique for detection of both abundant and rare mutations in circulating DNA from blood plasma of ovarian and breast cancer patients.”<sup>149</sup>

120. As described in ForsheW (2012), TAm-Seq can be applied for longitudinal monitoring of mutation frequencies in serial patient samples and for comparing mutation frequencies in a given sample to those in a reference sample<sup>150</sup>. The method is demonstrated for identifying *p53* and *EGFR* mutations in cfDNA<sup>151</sup>.

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<sup>147</sup> *Id.* at 10

<sup>148</sup> *Id.* at Fig. 1, Supplement 1–4

<sup>149</sup> *Id.* at 1-2.

<sup>150</sup> *Id.* at 8–9, Fig. 4

<sup>151</sup> *Id.*

121. I understand that ForsheW (2012) was cited to the Examiner during prosecution as part of an Information Disclosure Statement (IDS) submitted October 18, 2019, in which it appeared among 702 listed references<sup>152</sup>. I am informed that the record does not indicate that ForsheW (2012) was relied upon in any rejection or substantively discussed during prosecution<sup>153</sup>.

122. In its Final Written Decision for IPR2019-00652, the Board relied on ForsheW (2012) in combination with the '188 Patent (Schmitt) to find claims 1–11, 13, and 17–20 of the related '822 patent unpatentable<sup>154</sup>.

**C. The '202 Patent Pub. (Porreca)**

123. The '202 Patent Pub. (Porreca) was filed April 30, 2010 and qualifies as prior art to the '916 Patent under 35 U.S.C. § 102(a)(1) and (2). The '202 Patent Pub. (Porreca) was thus available to one of ordinary skill in the art before September 4, 2012.

124. During prosecution of the '916 Patent, '202 Patent Pub. (Porreca) was cited to the USPTO in an Information Disclosure Statement (IDS) submitted on October 18, 2019, as one of 702 listed references.<sup>155</sup>

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<sup>152</sup> Ex. 1002 at 170 listing ForsheW as reference 18.

<sup>153</sup> *See generally* Ex. 1002

<sup>154</sup> Ex. 1083 at 67.

<sup>155</sup> Ex. 1002 at 146.

125. The '202 Patent Pub. (Porreca) describes methods for improving “multiplex analysis of genomic loci.”<sup>156</sup> The '202 Patent Pub. (Porreca) teaches that such methods apply to “any application where reduction of bias, e.g., associated with genomic isolation, amplification, sequencing, is important.”<sup>157</sup>

126. The '202 Patent Pub. (Porreca) contemplates using NGS methods “to make quantitative measurements (including genotype calling),” and that “these methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids.”<sup>158</sup>

127. The '202 Patent Pub. (Porreca) describes “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats”<sup>159</sup>, including detection in the context of “cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26).”<sup>160</sup> Referring to microsatellites specifically, the '202

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<sup>156</sup> See Ex. 1075 ('202 Patent Pub. (Porreca)) at Abstract; see also *id.* at ¶ [0002] (“methods and compositions for determining genotypes in patient samples.”).

<sup>157</sup> *Id.* at ¶ [0025] (referring to “detection of mutations in maternally-circulating fetal DNA”).

<sup>158</sup> *Id.* at ¶ [0136] ); see also *id.*, ¶ [0175] (counting based on “the number of different barcodes that are present”).

<sup>159</sup> *Id.* at ¶ [0157].

<sup>160</sup> *Id.* at ¶ [0160].

Patent Pub. (Porreca) describes that “[g]enomic regions that contain nucleic acid sequence repeats are often the site of genetic instability due to the amplification or contraction of the number of sequence repeats (e.g., the insertion or deletion of one or more units of the repeated sequence).”<sup>161</sup>

128. The ’202 Patent Pub. (Porreca) describes the clinical utility of genotyping assays, where molecular data obtained from patient-derived samples, including tumor tissue or circulating tumor DNA, directly inform disease characterization and personalized treatment strategies. For instance, the ’202 Patent Pub. (Porreca) further states that “a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject)”<sup>162</sup>. It also teaches that “genetic information from a tumor or circulating tumor cells is used to determine prognosis and guide selection of appropriate drugs/treatments.”<sup>163</sup>

**D. The ’331 Patent Pub. (Sacko)**

129. The ’331 Patent Pub. (Sacko) was published October 21, 2010, and qualifies as prior art to the ’916 Patent under 35 U.S.C. § 102(b). The ’331 Patent

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<sup>161</sup> *Id.* at ¶[0158].

<sup>162</sup> *Id.* at ¶[0017].

<sup>163</sup> *Id.* at ¶[0017]

Pub. (Sacko) was thus available to one of ordinary skill in the art before September 4, 2012.

130. The '331 Patent Pub. (Sacko) reports that “DNA extracted from the plasma of cancer patients usually has tumoral DNA characteristics [citing Anker P. et al., 1999] such as strand instability, the presence of specific oncogenes, tumor suppressor genes and microsatellite alterations”<sup>164</sup>. In other words, the '331 Patent Pub. (Sacko) describes that cell-free DNA (cfDNA) obtained from cancer patients' plasma retains genetic and structural abnormalities present in tumor DNA, making it a suitable analyte for detecting tumor-associated genomic changes, including microsatellite instability (MSI).<sup>165</sup>

131. The '331 Patent Pub. (Sacko) recognized that MSI signatures in cfDNA could be employed as noninvasive biomarkers for tracking tumor presence, burden, and progression over time: “[t]he microsatellite mutations and instabilities detected in the free genomic DNA of the serum suggest that it could be a new potential marker, with considerable specificity for monitoring tumors”<sup>166</sup>

132. The '331 Patent Pub. (Sacko) explains that microsatellite instabilities

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<sup>164</sup> Ex. 1076 at ¶[0005] (citing 1997 reference); *see also id.*, ¶[0003] (stating that “[the existence of cell free nucleic circulating in the blood has been known for a number of years,” and citing 1947 and 1966 references).

<sup>165</sup> *Id.*

<sup>166</sup> *Id.*

could serve as a “new potential marker” and could be used to “complement the analysis of markers commonly used to monitor patients suffering from cancers or undergoing chemotherapy”<sup>167</sup>.

133. The ’331 Patent Pub. (Sacko) was cited by the Examiner in an obviousness-type double-patenting rejection over other patents and applications in the same family. In particular, the Examiner found that “One of ordinary skill in the art considering the patented methods would have been motivated to modify them by applying them to microsatellite changes because the ’331 Patent Pub. (Sacko) et al. disclosed that microsatellite changes were known to occur in cell-free nucleic acids from blood of cancer patients”<sup>168</sup>.

## **XI. OPINIONS REGARDING THE VALIDITY OF THE ’916 PATENT CLAIMS**

### **A. Ground I: Claims 13-30 of the ’916 Patent were obvious over the ’188 Patent (Schmitt) in view of Forshew (2012) and the ’202 Patent Pub. (Porreca)**

134. For the reasons I explain below, Claims 13-30 of the ’916 Patent are obvious based on the teachings of the ’188 Patent (Schmitt) in view of Forshew (2012) and The ’202 Patent Pub. (Porreca).

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<sup>167</sup> *Id.* at ¶[0006]

<sup>168</sup> Ex. 1002 (’916 Patent File History) at 445.

### 1. Motivation to Combine

135. In my opinion, one of ordinary skill in the art would have been motivated to combine the teachings of the '188 Patent (Schmitt), Forshew (2012), and The '202 Patent Pub. (Porreca). Specifically, the combination of Schmitt's duplex consensus sequencing ("DCS") method with Forshew (2012)'s methods for sequencing and analyzing cell-free DNA ("cfDNA") would have been apparent to a skilled artisan and carried a reasonable expectation of success. The Board has previously concluded that one of ordinary skill in the art would have combined the '188 Patent (Schmitt) and Forshew (2012) for this purpose, underscoring the technical compatibility and complementary nature of these methods.<sup>169</sup>

136. As I described *supra*, the '188 Patent (Schmitt) describes analytical methods for detecting genetic variants in DNA, using platforms compatible with NGS and digital quantification to identify low-frequency variants.<sup>170</sup> The '188 Patent (Schmitt)'s DCS approach "allows for a quantitative detection of sites of DNA damage" and that "the ability to indirectly infer that damage is present on the DNA could be useful biomarker" for "cancer risk, cancer metabolic state, [and]

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<sup>169</sup> See Ex. 108(822FWD), p. 49.

<sup>170</sup> Ex. 1005 ('188 Patent (Schmitt) at Abstract, 1:28-55, 3:10-40, 16:4-57; 19:8-25:51.

mutator phenotype related to defective damage repair”<sup>171</sup>. One of ordinary skill in the art would have understood the ’188 Patent (Schmitt)’s reference to the phrase “mutator phenotype related to defective damage repair” encompasses defects in the DNA mismatch repair (MMR) pathway. As I described *supra*, defective MMR was known to cause an increase in the frequency of insertions and deletions in microsatellite regions, *i.e.*, MSIs.<sup>172</sup> Given the capability of DCS to achieve base-level resolution and quantitative error suppression, one of ordinary skill in the art would have recognized that it would be applicable and useful for detecting and quantifying the occurrence of variants in microsatellite regions.<sup>173</sup>

137. For at least these reasons, one of ordinary skill in the art would have understood the ’188 Patent (Schmitt)’s teaching about “defective damage repair” refers to defective MMR and observation of increased insertions and deletions in microsatellite regions. One of ordinary skill in the art would have known that the ’188 Patent (Schmitt) taught the use of DCS to quantify sites of DNA damage and to infer a “mutator phenotype related to defective damage repair,” taught the detection and quantification of MSIs.

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<sup>171</sup> Ex. 1005 (’188 Patent (Schmitt) at 15:41-51.

<sup>172</sup> *See* Ex. 1081 (Li (2008b)) at 93 (“defects in MMR confer a mutator phenotype” in simple repeat sequences), *see also supra* at § VII.D.1.

<sup>173</sup> Ex. 1005 (’188 Patent (Schmitt) at 3:44-62; 4:4-29.

138. Forshew (2012), like the '188 Patent (Schmitt) and the '916 Patent, is directed to methods for detecting genetic mutations in nucleic acid samples and is therefore analogous art. Similar to the '188 Patent (Schmitt), Forshew (2012) employs NGS to detect “both abundant and rare mutations in circulating DNA from blood plasma”<sup>174</sup>. A key advantage of Forshew (2012)’s approach is its ability to monitor mutation frequencies in an individual by repeatedly sequencing cfDNA from serial plasma samples<sup>175</sup>. Forshew (2012) also recognizes the risk of amplification errors during PCR and specifically addresses this challenge by implementing “[d]uplicate sequencing of each sample . . . to avoid false positives stemming from PCR errors”<sup>176</sup>. This emphasis on error suppression aligns closely with the teachings of Schmitt’s DCS method.

139. In my opinion, one of ordinary skill in the art considering the teachings of Forshew (2012) for screening cfDNA, would have also looked to the '188 Patent (Schmitt)’s DCS teachings for the same reasons.<sup>177</sup> While the '188 Patent (Schmitt) describes DCS’s application to prenatal screening for fetal aneuploidy, which

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<sup>174</sup> Ex. 1010 (Forshew (2012)) at 1-2.

<sup>175</sup> *Id.* at 9.

<sup>176</sup> *Id.*

<sup>177</sup> *Id.* at Abstract, 1–2; *see also* Ex. 1005 ('188 Patent (Schmitt)) at Abstract, 1:28–55.

similarly relies on on a type of cfDNA called fetal cfDNA, the '188 Patent (Schmitt) also expressly refers to “early detection of cancer.”<sup>178</sup> One of ordinary skill in the art applying Forshew (2012)’s teachings for screening for cancer mutations in cfDNA in patient plasma, would have found it obvious to look to the '188 Patent (Schmitt)’s DCS method to reduce amplification/sequencing errors and to improve detection of rare genetic mutations.<sup>179</sup>

140. One of ordinary skill in the art applying Forshew (2012)’s teachings with the objective to detect cfDNA genetic mutations with high accuracy and sensitivity would have been motivated to use Schmitt’s improved DCS method, which the '188 Patent (Schmitt) teaches “reduces or eliminates artifactual mutations arising from DNA damage, PCR errors, and sequencing errors,” and “allows rare variants in heterogeneous populations to be detected with unprecedented sensitivity.”<sup>180</sup> I understand that the Board previously found that one of ordinary skill in the art “reading Schmitt would have understood that Schmitt’s DCS method was a reasonable substitute for the deep-sequencing and error-correction techniques used in the prior art—such as the [NGS] techniques Fan and Forshew used for

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<sup>178</sup> Ex. 1005 ('188 Patent (Schmitt)) at 1:42–45.

<sup>179</sup> Ex. 1010, 1 (“[s]ensitive methods for detecting cancer mutations in plasma may find use in early detection screening, prognosis, monitoring tumor dynamics over time, or detection of minimal residual disease”).

<sup>180</sup> Ex. 1005 ('188 Patent (Schmitt)) at 2:56–60.

prenatal cfDNA screening and for tumor cfDNA screening, respectively.”<sup>181</sup>

141. Porreca, like the '188 Patent (Schmitt) and Forshew (2012), addresses the detection of genetic mutations in nucleic acid samples and is therefore analogous art. The '202 Patent Pub. (Porreca) contemplates the use of NGS methods for quantitative, multiplexed analysis of multiple genetic loci in patient samples<sup>182</sup>, including sequencing of amplicons with sample-specific barcodes. Like the '188 Patent (Schmitt), the '202 Patent Pub. (Porreca) teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats”<sup>183</sup> for purposes of diagnosis and to guide selection of appropriate drugs/treatment.<sup>184</sup> With respect to microsatellites, the '202 Patent Pub. (Porreca) explains that genetic instability may manifest as “cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci.”<sup>185</sup> One of ordinary skill in the art would recognize that integrating the '202 Patent Pub. (Porreca)'s MSI detection framework with the '188 Patent (Schmitt)'s high-fidelity

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<sup>181</sup> See Ex. 1083 (822FWD) at p.55.

<sup>182</sup> See Ex. 1075 ('202 Patent Pub. (Porreca)) at Abstract, ¶¶ [0002], [0017], [0136], [0175] (referring to NGS of amplicons comprising barcodes).

<sup>183</sup> See Ex. 1075 at ¶[0157].

<sup>184</sup> *Id.* at ¶[0017]

<sup>185</sup> Ex. 1075 at ¶[0160]

DCS and Forshew (2012)'s cfDNA sequencing pipeline would yield a powerful approach for sensitive and quantitative MSI detection in minimally invasive plasma or blood samples from cancer patients.

142. One of ordinary skill in the art would have further been motivated to combine the '188 Patent (Schmitt), Forshew (2012) and the '202 Patent Pub. (Porreca) in light of the known clinical relevance of MSI as a biomarker for cancer diagnosis, prognosis, and therapy selection.<sup>186</sup> As of September 2012 (and earlier), the application of cfDNA for non-invasive cancer detection was well-known, and one of ordinary skill in the art would have understood that using MSI detection approaches to cfDNA could be used to detecting disease, including cancer, including as taught by The '202 Patent Pub. (Porreca). In my opinion, one of ordinary skill in the art would have reasonably expected that combining the teachings of the '188 Patent (Schmitt), Forshew (2012) and/or the '202 Patent Pub. (Porreca) would provide a reliable and quantitative measure of microsatellite changes in cfDNA without the need for undue experimentation.

143. As I described *supra*, each of these references used known techniques and conventional tools—thus their combination would have been a predictable use of these techniques and tools to address a long-recognized problem. For instance, the

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<sup>186</sup> See Ex. 1005('188 Patent (Schmitt)) at 15:42-51.

'188 Patent (Schmitt) and the '202 Patent Pub. (Porreca) each rely on routine molecular biology and sequencing methods that were widely used at the time. The '188 Patent (Schmitt) describes the sample preparation, sequencing, and computational tools to analyze nucleic acid samples, such as cfDNA,<sup>187</sup> and the '202 Patent Pub. (Porreca) provides the direction and teaching to interpret and quantify microsatellite variations. Given these teachings, one of ordinary skill in the art would have recognized that applying Porreca's MSI detection teachings to cfDNA using the '188 Patent (Schmitt)'s methodology and techniques would provide qualitative MSI status and a quantitative measure of microsatellite changes, resulting in improved clinical resolution. Such a combination would have been obvious to and readily accomplished by one of ordinary skill in the art.

## **2. Reasonable Expectation of Success**

144. In my opinion, one of ordinary skill in the art would have held a reasonable expectation of success in combining the '188 Patent (Schmitt)'s and Forshew (2012)'s teachings to detect rare mutations in cfDNA, thereby arriving at the claimed inventions. The Board has already determined that "an ordinarily skilled artisan . . . would have had a reasonable expectation of success in using Schmitt's

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<sup>187</sup> *Id.* at 7:1–45; *see also id.* at 11:8–30

DCS method to detect genetic mutations in cfDNA”,<sup>188</sup> noting that “all necessary techniques for carrying out the claimed method steps of the [related] ’822 patent were known and required only ordinary skill to perform.”<sup>189</sup> The ’916 Patent similarly recites sample preparation, sequencing, and analytical steps that were routine as of the relevant date, all of which are disclosed in the ’188 Patent (Schmitt).

145. The ’188 Patent (Schmitt) teaches that “the DCS approach can be generalized to nearly any sequencing platform” and highlights its “compatibility . . . with existing sequencing workflows”<sup>190</sup>. The Board, in its Final Written Decision on the related ’822 Patent (priority date September 4, 2012), recognized that the ’188 Patent (Schmitt) advises a skilled artisan to apply DCS broadly as a general error-correction method for NGS.<sup>191</sup> The Board further observed that the ’822 patent demonstrates that the technical steps required—such as isolating cfDNA, barcoding, amplifying, sequencing, and computationally analyzing reads—were already routine in the art.<sup>192</sup> This alignment between the ’188 Patent (Schmitt)’s generalized

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<sup>188</sup> See Ex. 1083 (822FWD) at p.56.

<sup>189</sup> *Id.* at p.57

<sup>190</sup> See Ex. 1005 (’188 Patent (Schmitt)) at 18:44–61.

<sup>191</sup> See Ex. 1083 (822FWD) at p.58.

<sup>192</sup> *Id.* at p.56 (“Schmitt suggests to the skilled artisan to use the DCS method as a general technique for next generation sequencing and error correction, and the ’822 patent evinces that the technical details for isolating, barcoding, amplifying,

platform compatibility and established laboratory workflows would have reinforced one of ordinary skill in the art's reasonable expectation of success.

146. As with the '822 Patent, one of ordinary skill in the art would have reasonably expected success in extending the combination of the '188 Patent (Schmitt) and ForsheW (2012) to include Porreca, thereby enabling quantitative measurement of microsatellite changes in cfDNA samples. By September 2012 (if not earlier), the integration of cfDNA analysis with established NGS platforms and bioinformatics pipelines for mutation detection was commonplace,<sup>193</sup> and explicitly taught by the '188 Patent (Schmitt)<sup>194</sup> in conjunction with ForsheW (2012). The '202 Patent Pub. (Porreca) provided complementary teachings for detecting alterations in short tandem repeat regions, including microsatellite instability.<sup>195</sup> The '188 Patent (Schmitt) and ForsheW (2012) to include '202 Patent Pub. (Porreca) all references relied upon routine molecular biology and sequencing tools already familiar to the skilled artisan, without requiring novel reagents, chemistries, or unconventional instrumentation.

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sequencing, and analyzing cfDNA molecules were known to be routine in the art. ***These factors persuade us that an ordinarily skilled artisan would have had a reasonable expectation of success.***”(emphasis added).

<sup>193</sup> See *supra* at § VII.

<sup>194</sup> See Ex. 1005 '188 Patent (Schmitt) at 6:25–45; 10:1–15

<sup>195</sup> See Ex. 1075 ('202 Patent Pub. (Porreca)) at ¶¶ [0157]–[0160]

147. The proposed combination represents a predictable application of well-established techniques to address a recognized diagnostic challenge, using only conventional laboratory tools. For example, the '188 Patent (Schmitt), Forshew (2012), and the '202 Patent Pub. (Porreca) employ standard molecular biology workflows and sequencing methodologies common in the field at the time. One of ordinary skill in the art would have understood that microsatellite loci—being short, repetitive DNA sequences—are readily detectable using the sequencing and analysis methods taught by the '188 Patent (Schmitt), and that alterations in regions containing nucleic acid repeats, as described by Porreca, could be quantitatively assessed through the same DCS techniques. Given that the '188 Patent (Schmitt), Forshew (2012), and '202 Patent Pub. (Porreca) and their combination applies known detection concepts and techniques to a known analyte (cfDNA) and the long-known target of microsatellite regions, the integration would have been an obvious and technically straightforward step for one of ordinary skill in the art without the need for undue experimentation.

### **3. Independent Claim 13**

- a) 13(pre): “A method for detecting a genetic variation in one or more microsatellite regions in a sample of cell-free nucleic acid molecules from a subject having a cancer, the method comprising:”

148. In my opinion, the '188 Patent (Schmitt) either alone or in combination

with Forshew (2012) and the '202 Patent Pub. (Porreca) teaches the preamble of Claim 13 of the '916 Patent to the extent the preamble is limiting.

149. For example, the '188 Patent (Schmitt) describes a tag-based sequencing error-correction strategy known as *Duplex Consensus Sequencing* (“DCS”), which assigns unique molecular identifiers to both strands of each DNA molecule. By requiring concordant sequencing of both strands, DCS markedly reduces errors introduced during amplification or sequencing, thereby “allow[ing] rare variants . . . to be detected with unprecedented sensitivity.”<sup>196</sup> The '188 Patent (Schmitt) further explains that DCS “allows for a quantitative detection of sites of DNA damage” and that “the ability to indirectly infer that damage is present on the DNA could be a useful biomarker” for applications including “cancer risk, cancer metabolic state, [and] mutator phenotype related to defective damage repair”<sup>197</sup>. One of ordinary skill in the art would have understood that “defective damage repair” encompasses defective mismatch repair (“MMR”), a condition known to cause elevated rates of insertions and deletions within microsatellite regions. Accordingly, in my opinion, the '188 Patent (Schmitt) alone teaches an approach capable of generating a quantitative measure of microsatellite variation.

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<sup>196</sup> See Ex. 1005 ('188 Patent (Schmitt)) at 2:56-62; see also *id.* at 16:4-57..

<sup>197</sup> *Id.* at 15:42-51.

150. One of ordinary skill in the art would have found it obvious to integrate the '188 Patent (Schmitt)'s DCS method with the cfDNA sequencing workflow taught by Forshew (2012). For example, Forshew (2012) describes applying "deep sequencing" to cell-free tumor DNA in order to detect low-frequency mutations, noting that such an approach "may find use in early detection screening, prognosis, monitoring tumor dynamics over time, or detection of minimal residual disease."<sup>198</sup> Forshew (2012) explains that cfDNA can be extracted from the plasma of cancer patients for direct mutation analysis,<sup>199</sup> and provides specific examples in which between 0.85 and 2.2 mL of plasma yielded sufficient cfDNA for sequencing.<sup>200</sup> Forshew (2012) reports the successful identification of cancer-associated mutations directly from plasma cfDNA<sup>201</sup>. Combining Forshew (2012)'s clinical cfDNA framework with the '188 Patent (Schmitt)'s high-fidelity DCS method would have been an obvious way to improve the accuracy and sensitivity of mutation detection in these samples.

151. In my opinion, if the combination of the '188 Patent (Schmitt) and Forshew (2012) did not teach "detecting a genetic variation in one or more

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<sup>198</sup> Ex. 1010 (Forshew (2012)) at 1 (citation omitted).

<sup>199</sup> *Id.*

<sup>200</sup> *Id.* at 10.

<sup>201</sup> *Id.* at 4, Tables 1–2

microsatellite regions in a sample of cell-free nucleic acid molecules from a subject having a cancer,” (though they do) one of ordinary skill in the art would have been led to that application by the ’202 Patent Pub. (Porreca). For example, the ’202 Patent Pub. (Porreca) teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats”<sup>202</sup> and identifies “cancer” as a condition “associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26).”<sup>203</sup> One of ordinary skill in the art, knowing the clinical significance of MSI, would have recognized that integrating Porreca’s MSI detection teachings with the ’188 Patent (Schmitt)’s DCS and Forshew (2012)’s cfDNA methodology would enable highly sensitive, quantitative analysis of microsatellite alterations in a minimally invasive sample.

152. Thus, in my opinion, the ’188 Patent (Schmitt) in combination with Forshew (2012) and the ’202 Patent Pub. (Porreca) renders obvious this preamble.

- b) 13(a): “(a) ligating molecular barcodes from a set of molecular barcodes having 2 to 1,000,000 different molecular barcode sequences to a plurality of the cell-free nucleic acid molecules from the sample to produce tagged parent polynucleotides;”

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<sup>202</sup> Ex. 1075 (’202 Patent Pub. (Porreca)) at ¶[0157]

<sup>203</sup> *Id.* at ¶[0160].

153. In my opinion, the ‘188 Patent (Schmitt), either alone or in combination with ForsheW (2012), teaches element 13(a).

154. As the Board determined in the 822 Final Written Decision, the combined disclosures of the ‘188 Patent (Schmitt) and ForsheW (2012) describe “converting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides,” wherein “each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.”<sup>204</sup> This refers to ligating barcodes—short stretches of defined nucleotides—to each sample DNA fragment so that individual molecules can be tracked and error-corrected after sequencing.

155. The ‘916 Patent itself acknowledges that the concept of attaching “barcodes” to DNA fragments—short, defined sequences used to uniquely identify molecules—was known in the art as early as 2000.<sup>205</sup> The ‘188 Patent (Schmitt) provides a concrete implementation of this principle, teaching the attachment of barcodes incorporated into “SMI-containing adaptors” to DNA fragments.<sup>206</sup> In the DCS workflow, SMI adaptor molecules comprise both an “SMI sequence (or ‘tag’)

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<sup>204</sup> Ex. 1083 (822FWD) at 29-31.

<sup>205</sup> Ex. 1001(‘916 Patent) at 38:31-36.

<sup>206</sup> See Ex. 1005 (‘188 Patent (Schmitt)) at 2:66–3:9, 3:10–20, Fig. 1, Fig. 4

of nucleotides” and an SMI ligation adaptor, which are ligated to the ends of the target parent polynucleotides.<sup>207</sup> The ’188 Patent (Schmitt) teaches that the SMI tags “may be between approximately 3 to 20 nucleotides in length.”<sup>208</sup> Because the possible number of unique barcode sequences increases exponentially with tag length—according to the formula  $4^n$  for a tag of  $n$  nucleotides—a 4-mer tag, as expressly disclosed by the ’188 Patent (Schmitt)<sup>209</sup>, yields  $4^4 = 256$  possible unique sequences.

156. The ’188 Patent (Schmitt) further describes a DCS “hybrid method” for tagging DNA fragments, in which the adaptors carry “a combination of sheared ends and a shorter  $n$ -mer tag (such as 1 or 2 or 3 or 4 or more degenerate or semi-degenerate bases)”.<sup>210</sup> Because degenerate positions in a tag can represent more than one possible nucleotide, such designs can yield barcode sets ranging in size from 2 to as many as 1,000,000 possible unique sequences.

157. Taken together, the ’188 Patent (Schmitt) teaches ligating molecular barcodes—drawn from a set containing between 2 and 1,000,000 possible unique sequences—to a plurality of nucleic acid molecules from a sample, thereby

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<sup>207</sup> *Id.* at 3:1–9, 5:57–59, 6:46–51, 7:38–41.

<sup>208</sup> *Id.* at 6:63–64.

<sup>209</sup> *Id.* at 4:30–54; *see also id.* at 9:9–13

<sup>210</sup> *Id.* at 9:9–11.

producing uniquely tagged parent polynucleotides. And, to the extent that Patent Owner argues the '188 Patent (Schmitt) does not explicitly state that these polynucleotides are cfDNA, Forshew (2012) fills that gap by expressly teaching the extraction and analysis of cfDNA from patient plasma, as described above for the preamble of Claim 13 of the '916 Patent.<sup>211</sup> The combined teachings of these references would have been readily available for one of ordinary skill in the art to apply the '188 Patent (Schmitt)'s barcoding strategy directly to cfDNA molecules.

- c) 13(b): “(b) amplifying a plurality of the tagged parent polynucleotides to produce amplified tagged progeny polynucleotides;”
- d) 13(c): “(c) sequencing a plurality of the amplified tagged progeny polynucleotides to produce a set of sequencing reads; and”

158. In my opinion, the '188 Patent (Schmitt) teaches elements 13(b) and 13(c).

159. I understand that the Board has previously determined that “Schmitt, either alone or in combination with Schmitt 2012, discloses [] amplifying the tagged polynucleotides and sequencing the amplified progeny.”<sup>212</sup> This describes the well-

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<sup>211</sup> See Ex. 1083 (822FWD) at 29. (“Step (a) of claim 1 recites “providing a population of cell-free DNA (‘cfDNA’) molecules obtained from a bodily sample from a subject.” *We agree with Petitioner that Fan and Forshew teach this limitation.*”) (emphasis added, citation omitted).

<sup>212</sup> *Id.* at 31-32, *citing* Ex. 1005 ('188 Patent (Schmitt)) at 3:10–20 and 21:55–57.

established process of taking barcoded DNA fragments, making multiple identical copies through polymerase chain reaction (PCR) or similar amplification methods, and then reading the nucleotide sequences of those amplified products using a high-throughput sequencing platform, such as NGS.

160. The '188 Patent (Schmitt)'s DCS method explicitly sets out a workflow comprising: “ligating a double-stranded target nucleic acid molecule to at least one SMI adaptor molecule to form a double-stranded SMI–target nucleic acid complex; amplifying the double-stranded SMI–target nucleic acid complex, resulting in a set of amplified SMI–target nucleic acid products; and sequencing the amplified SMI–target nucleic acid products”<sup>213</sup>. One of ordinary skill in the art would have recognized this description of a stepwise process in which unique sequence tags are attached to both strands of each DNA molecule via ligation, followed by amplification of those tagged molecules, and finally sequencing these tagged molecules to determine their nucleotide sequence—allowing for high-fidelity detection of rare genetic variants.

- e) 13(d): “(d) determining, from among a plurality of sequencing reads in the set of sequencing reads, a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions based at least on sequence information of the

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<sup>213</sup> See Ex. 1005 ('188 Patent (Schmitt)) at 3:10–20 (emphasis added); see also *id.* at 21:55–57.

molecular barcodes, thereby detecting the genetic variation in the one or more microsatellite regions.”

161. In my opinion, the '188 Patent (Schmitt) alone or with Forshew (2012) and the '202 Patent Pub. (Porreca) teaches “determining, from among a plurality of sequencing reads in the set of sequencing reads, a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions based at least on sequence information of the molecular barcodes, thereby detecting the genetic variation in the one or more microsatellite regions.”

162. First, the '188 Patent (Schmitt)'s DCS process determines a quantitative measure of all classes of mutations—including microsatellite changes—by distinguishing true biological variants from technical artifacts. Specifically, the '188 Patent (Schmitt) describes confirming a “true” mutation by: (i) identifying a sequence difference between complementary DNA strands; (ii) comparing that difference to the double-stranded consensus sequence, which has been error-corrected; and (iii) confirming the mutation only if it appears on both complementary strands and in all members of the corresponding barcode-defined “family.”<sup>214</sup> This requirement removes mutations caused by PCR or sequencing errors, ensuring that

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<sup>214</sup> *Id.* at 3:31-40; *see also id.*, 16:37-57.

the quantification reflects genuine or “true” genetic variation<sup>215</sup>.

163. The ’188 Patent (Schmitt) also discloses that the barcodes are used to generate consensus sequences and therefore teaches that identification of mutations—including in microsatellite regions—in the DCS process is based at least on the sequence information of the barcodes. The ’188 Patent (Schmitt) explains for its DCS method “a family of molecules is obtained that arose from a single DNA molecule; members of the same PCR ‘family’ are then grouped together by virtue of having a common (i.e., the same) SMI tag sequence.”<sup>216</sup>

164. For “quantitative measure,” as the ’916 Patent explains, measuring the frequency is a “quantitative measure.”<sup>217</sup> The ’188 Patent (Schmitt) teaches that the DCS method allows quantification of true mutations by detecting “the presence of a true mutation (as opposed to a PCR error or other artifactual mutation) in a target nucleic acid sequence.”<sup>218</sup> I understand that the Board found that the ’188 Patent (Schmitt) teaches “determining the frequency of bases called at the locus from among the families” and “further comprising detecting, at one or more loci, at least

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<sup>215</sup> *Id.* at 16:43–47

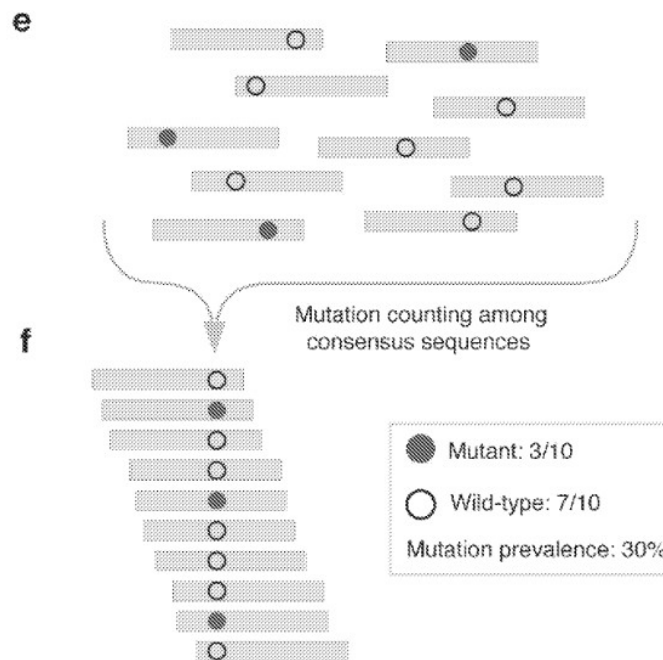
<sup>216</sup> *Id.* at 21:55-61; *see also id.* at 20:39-64 (“[r]eads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read”).

<sup>217</sup> *See* Ex. 1001 (’916 Patent) at 35:32-34.

<sup>218</sup> *See* Ex. 1005 (’188 Patent (Schmitt)) at 16:43–47.

one single nucleotide variant, at least one gene fusion, and at least one copy number variant.”<sup>219</sup>

165. Figure 3 of the '188 Patent (Schmitt) illustrates how sequence tags (SMIs) enable the grouping of reads into consensus families—here, ten total consensus sequences—followed by quantification of observed mutations, in this example three confirmed variants. The '188 Patent (Schmitt) refers to this as “[m]utation counting,” in the figure illustrated *infra*:



166. One of ordinary skill in the art would have recognized that the '188 Patent (Schmitt)'s quantification of “true mutations” inherently extends to mutations

<sup>219</sup> Ex. 1083, pp.31-32 and 43, *citing* Ex. 1005Ex. 1005 at 4:25-29, Figure 3, 62:49-51.

in microsatellite regions. Because microsatellite loci are abundant throughout the genome, DNA fragments from these regions are expected to be sufficiently representative of the DNA sample, including those examined in the '188 Patent (Schmitt). There is no technical limitation in the DCS workflow that would exclude repetitive sequences from analysis, meaning that the same barcoding, consensus-building, and quantification steps could be directly applied to microsatellite-containing fragments.

167. The '188 Patent (Schmitt) also directs one of ordinary skill in the art toward analyzing mutations in microsatellite regions by noting that DCS “allows for a quantitative detection of sites of DNA damage” and that such detection can serve as a biomarker for “cancer risk, cancer metabolic state, [and] mutator phenotype related to defective damage repair.”<sup>220</sup> One of ordinary skill in the art would have understood that “defective damage repair” encompasses defects in the DNA mismatch repair (MMR) pathway, which are known to cause increased insertions and deletions specifically within microsatellite loci. Accordingly, on its own, the '188 Patent (Schmitt) teaches determining quantitative measures of microsatellite changes using the DCS method.

168. If the '188 Patent (Schmitt) were not found to expressly teach

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<sup>220</sup> Ex. 1005 ('188 Patent (Schmitt)) at 15:42-51 (teachings of “quantitative detection of sites of DNA damage”).

quantification of microsatellite changes, one of ordinary skill in the art would still find it obvious to apply the DCS method to such regions in light of Forshew (2012) and/or the '202 Patent Pub. (Porreca). For example, Forshew (2012) describes identifying “cancer mutations present in circulating [cell-free] DNA” by targeting selected genomic regions,<sup>221</sup> and one of ordinary skill in the art would have classified microsatellite loci as such “regions of interest” given the established link between microsatellite instability (MSI) and cancer or elevated cancer risk.

169. Likewise, the '202 Patent Pub. (Porreca) teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats,”<sup>222</sup> specifically noting that MSI—characterized by altered repeat lengths at loci such as BAT-25 and BAT-26—is associated with cancer.<sup>223</sup> As taught by the '202 Patent Pub. (Porreca), detecting MSI is quantitative because “an increase or decrease in genomic copy number of nucleic acid repeats at one or more microsatellite loci.”<sup>224</sup> These teachings together would prompt one of ordinary skill in the art to apply the '188 Patent (Schmitt)’s high-fidelity DCS process to

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<sup>221</sup> Ex. 1010 (Forshew (2012)) at Abstract

<sup>222</sup> See Ex. 1075 ('202 Patent Pub. (Porreca)) at [0157].

<sup>223</sup> *Id.* at ¶[0160]

<sup>224</sup> *Id.*; see also *id.* at ¶[0136] (“quantitative measurements” made by “utilize[ing] the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids”).

microsatellite-containing cfDNA fragments to generate a quantitative measure of MSI in ctDNA.

170. As described *supra*, this supports my opinion that the '188 Patent (Schmitt) alone, or in combination with ForsheW (2012) and/or the '202 Patent Pub. (Porreca), teaches 13(d).

**4. Claim 14: “The method of claim 13, wherein the genetic variation in the one or more microsatellite regions comprises an insertion or deletion (indel).”**

171. In my opinion, the '188 Patent (Schmitt) teaches Claim 14. As I explained in my Technical Background, it was well established before September 2012 that insertions and deletions within microsatellite regions—collectively termed microsatellite instability (MSI)—were a hallmark of certain cancers. The '188 Patent (Schmitt) teaches that its Duplex Consensus Sequencing (DCS) method can confirm “the presence of a true mutation (as opposed to a PCR error or other artifactual mutation) in a target nucleic acid sequence.”<sup>225</sup> In other words, DCS provides a way to distinguish genuine biological variation from noise introduced during DNA amplification or sequencing. The '188 Patent (Schmitt) further explains that DCS “allows for a quantitative detection of sites of DNA damage” and that such detection may serve as a biomarker for “cancer risk, cancer metabolic state,

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<sup>225</sup> Ex. 1005 ('188 Patent (Schmitt)) at 16:43–47.

[and] mutator phenotype related to defective damage repair,”<sup>226</sup> thereby directly linking its error-corrected sequencing approach to detecting and quantifying microsatellite instabilities.

172. In my opinion, one of ordinary skill in the art would recognize that the ’188 Patent (Schmitt)’s disclosure of a “mutator phenotype related to defective damage repair” is a reference to the phenotype observed when the DNA mismatch repair (MMR) pathway is impaired. Such MMR defects are well known to cause elevated rates of insertions and deletions specifically within microsatellite loci, producing the MSI.

173. Furthermore, it would have been obvious to one of ordinary skill in the art to integrate the ’188 Patent (Schmitt)’s DCS method with Porreca’s “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats.”<sup>227</sup> The ’202 Patent Pub. (Porreca) explicitly connects such insertion/deletion detection to “cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci.”<sup>228</sup> From this, one of ordinary skill in the art would understand that MSIs—i.e., insertion/deletion variations within

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<sup>226</sup> *Id.* at 15:42-51.

<sup>227</sup> Ex. 1075 (’202 Patent Pub. (Porreca)) at ¶[0157].

<sup>228</sup> *Id.* at ¶[0160].

microsatellite regions—are particularly relevant cancer-associated mutations. Accordingly, applying the '188 Patent (Schmitt)'s DCS methodology to circulating tumor DNA (ctDNA) for the purpose of MSI detection would have been a logical and technically straightforward extension of the combined teachings.

**5. Claim 15: “The method of claim 13, wherein the sample is a bodily fluid sample selected from the group consisting of blood, plasma, and serum.”**

174. In my opinion, Claim 15 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings alone or in combination with Forsheew (2012).

175. One of ordinary skill in the art would recognize that the '188 Patent (Schmitt) teaches methods for analyzing bodily fluid samples—specifically blood, plasma, or serum—that contain circulating tumor DNA (ctDNA). The '188 Patent (Schmitt) explains that the application of “deep sequencing” in clinical settings was already well known and in active development for uses such as “prenatal screening for fetal aneuploidy [9, 10], early detection of cancer [11] and monitoring its response to therapy [12, 13] with nucleic acid-based serum biomarkers.”<sup>229</sup> In other words, the '188 Patent (Schmitt) situates its methods within established, clinically relevant sequencing workflows that analyze nucleic acids circulating in blood,

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<sup>229</sup> Ex. 1005 ('188 Patent (Schmitt)) at 1:41-45.

plasma, or serum.

176. It also would have been obvious to one of ordinary skill in the art to combine the '188 Patent (Schmitt) with Forshew (2012). Forshew (2012) teaches methods for extracting cell-free DNA (cfDNA) from plasma samples for the purpose of identifying mutations in cancer patients. For example, Forshew (2012) notes that “[p]lasma of cancer patients contains cell-free tumor DNA”<sup>230</sup> and specifies that “[c]irculating DNA was extracted from between 0.85 and 2.2 ml of plasma”<sup>231</sup>. As reported in Table S6, Forshew (2012) obtained cfDNA yields ranging from 0.9 to 19.7 ng from such plasma samples.<sup>232</sup> I am informed that the Board has previously agreed that Forshew (2012) teaches “providing a population of cell-free DNA (‘cfDNA’) molecules obtained from a bodily sample from a subject.”<sup>233</sup> One of ordinary skill in the art would have understood that the “bodily sample” in this context refers to blood, plasma, or serum—bodily fluids known to contain cfDNA, including ctDNA in cancer patients.

**6. Claim 16: “ The method of claim 13, wherein a molecular barcode from the set of molecular barcodes is attached on**

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<sup>230</sup> Ex. 1010 (Forshew (2012)) at 1.

<sup>231</sup> *Id.* at 10.

<sup>232</sup> *Id.* at 32, Table S6

<sup>233</sup> Ex. 1083 (822FWD) at 29.

**both ends of a molecule of the plurality of the cell-free nucleic acid molecules.”**

177. In my opinion, Claim 16 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings.

178. The '188 Patent (Schmitt) describes embodiments in which “the SMI adaptor molecules are ligated to both ends of a target nucleic acid molecule, and then this complex is used according to the methods described below.”<sup>234</sup> One of ordinary skill in the art would have understood that this configuration is used to attach each end of the nucleic acid molecule with a sequence identifier, thereby enabling highly accurate error correction and read pairing. Accordingly, in view of the '188 Patent (Schmitt), it would have been obvious to one of ordinary skill in the art to attach a molecular barcode from the set of molecular barcodes to both ends of a molecule in the plurality of cell-free nucleic acid molecules.

179. Moreover, to the extent the '188 Patent (Schmitt) alone does not explicitly disclose this limitation, one of ordinary skill in the art would have found it obvious to incorporate Porreca's teachings. The '202 Patent Pub. (Porreca) explains that “the skilled artisan will appreciate that as part of a MIP library

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<sup>234</sup> Ex. 1005 ('188 Patent (Schmitt)) at 7:38-42. I understand and am informed that, in the district court litigation Guardant has taken the position that this element is met when at least one barcode is attached to each end of a molecule. For purposes of this declaration, I apply that understanding, but reserve the right to supplement my opinions.

preparation process, adapters may be ligated onto the ends of the molecules of interest.”<sup>235</sup> One of ordinary skill in the art would have known that adapter ligation at both ends is a well-known step to facilitate downstream amplification, sequencing, and bioinformatic reconstruction of the original molecule, further reinforcing the compatibility of Porreca’s approach with the ’188 Patent (Schmitt)’s DCS framework.

**7. Claim 17: “The method of claim 13, wherein the molecular barcodes from the set of molecular barcodes have 2 to 1,000 different molecular barcode sequences.”**

180. In my opinion, Claim 17 would have been obvious to one of ordinary skill in the art based on the ’188 Patent (Schmitt)’s teachings. *See* Claim 4 above.

181. As explained above, the ’188 Patent (Schmitt) provides explicit examples of 4-mer tags, each composed of four nucleotide positions, which yield a set of 4<sup>4</sup> (i.e., 256) unique molecular barcode sequences.<sup>236</sup> In addition, the ’188 Patent (Schmitt) describes a “hybrid method” in which the adaptor contains a shorter n-mer tag—such as 1, 2, 3, or 4 or more degenerate or semi-degenerate bases<sup>237</sup>—which would generate barcode sets ranging in complexity from 2 to approximately 1,000 possible sequences, depending on the number and degeneracy of positions.

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<sup>235</sup> Ex. 1075 (’202 Patent Pub. (Porreca)) at ¶[0149].

<sup>236</sup> *See* Ex. 1005 (’188 Patent (Schmitt)) at 4:30–54; *id.* at 9:9–13

<sup>237</sup> *Id.* at 9:9–11.

**8. Claim 18: “The method of claim 13, wherein the molecular barcodes are ligated to the plurality of the cell-free nucleic acid molecules by blunt-end ligation or sticky-end ligation.”**

182. In my opinion, Claim 18 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings alone or in combination with the teachings of Forsheew (2012).

183. The '188 Patent (Schmitt) describes that the SMI adaptor—which contains the SMI sequence (i.e., the molecular barcode)—is attached to the target nucleic acid fragment through a ligation reaction, and that adaptors may be ligated to both ends of the parent polynucleotide molecule.<sup>238</sup> The '188 Patent (Schmitt) further specifies that “[t]he SMI ligation adaptor may be any suitable ligation adaptor that is complementary to a ligation adaptor added to a double-stranded target nucleic acid sequence including, but not limited to a T-overhang, an A-overhang, a CG overhang [i.e., sticky ends], a blunt end, or any other ligatable sequence.”<sup>239</sup> To one of ordinary skill in the art, this means the '188 Patent (Schmitt) expressly teaches barcode attachment through either sticky-end ligation or blunt-end ligation.

**9. Claim 19: “The method of claim 13, further comprising selectively enriching at least a portion of the amplified tagged**

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<sup>238</sup> Ex. 1005 ('188 Patent (Schmitt)) at 3:1-6.

<sup>239</sup> *Id.* at 7:58-62.

**progeny polynucleotides for target regions associated with cancer prior to the sequencing.”**

184. In my opinion, Claim 19 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings alone or in combination with the teachings of ForsheW (2012).

185. The Board determined that the combination of the '188 Patent (Schmitt) and ForsheW (2012) teaches “selectively enriching regions from a genome or transcriptome of the subject prior to sequencing.”<sup>240</sup> One of ordinary skill in the art would recognize “selectively enriching” as a known molecular biology approach for isolating defined genomic regions of interest before downstream analysis—most commonly achieved through hybridization-based capture or targeted amplification—to focus sequencing on loci relevant to the biological or clinical question.

186. As described earlier, the '188 Patent (Schmitt) teaches generating amplified, tagged progeny polynucleotides and then applying a targeted selection step before sequencing. In Example 1, the '188 Patent (Schmitt) reports that “[t]arget capture was performed with the Agilent SureSelect system.”<sup>241</sup> This approach employed 120-nucleotide “capture baits” designed to hybridize to specific

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<sup>240</sup> Ex. 1083 (822FWD) at 29.

<sup>241</sup> Ex. 1005 ('188 Patent (Schmitt)) at 20:23-28, 22:50-67.

sequences within a defined 758 kb genomic region.<sup>242</sup> In this context, “target capture” refers to a hybrid capture process, in which complementary oligonucleotide probes selectively bind the desired DNA fragments. The ’188 Patent (Schmitt) further notes that “selection and capture may be accomplished by any selection by hybridization method,”<sup>243</sup> indicating flexibility in the capture chemistry for enrichment of specific genomic targets.

187. One of ordinary skill in the art would have found it obvious to combine the ’188 Patent (Schmitt)’s selective enrichment of amplified, tagged progeny with Forshew (2012)’s targeted amplification strategy to focus on genomic regions implicated in cancer. Forshew (2012) teaches that “[p]rimers were designed to amplify regions of interest in overlapping short amplicons (table S1),”<sup>244</sup> and further explains: “We designed a set of 48 primer pairs to amplify 5995 bases of genomic sequence covering coding regions (exons and exon junctions) of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1) by overlapping short amplicons (Fig. 1A).”<sup>245</sup> These loci are well-recognized in the field as oncogenes or tumor suppressor genes frequently altered in cancer. Forshew

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<sup>242</sup> *Id.* at 20:23-29.

<sup>243</sup> *Id.* at 22:60-61.

<sup>244</sup> Ex. 1010 (Forshew (2012)) at 2.

<sup>245</sup> *Id.* at 3

(2012) applied this targeted approach to cfDNA extracted from plasma, directly identifying mutations present in cancer patients.<sup>246</sup> One of ordinary skill in the art would have recognized that pairing the '188 Patent (Schmitt)'s teachings with Forsheo (2012)'s cancer gene-specific targeting would provide a sensitive method for detecting clinically significant mutations in cfDNA.

**10. Claim 20: “The method of claim 13, further comprising filtering out sequencing reads from among the set of sequencing reads that fail to meet a quality threshold.”**

188. In my opinion, Claim 20 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings.

189. The '916 Patent itself acknowledges that “[m]ethods of reducing noise and/or distortion from a sequencing process are known” and that such approaches “include, for example, filtering sequences, e.g., requiring them to meet a quality threshold, or reducing GC bias.”<sup>247</sup> This admission shows that computational filtering based on base quality or sequence composition was routinely applied to improve the fidelity of downstream analyses.

190. The '188 Patent (Schmitt) reports using “[a] standard sequencing approach with quality filtering for a Phred score of 30 . . . .”<sup>248</sup> One of ordinary skill

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<sup>246</sup> *Id.* at 4, Tables 1-2.

<sup>247</sup> Ex. 1001 ('916 Patent) at 32:27–31

<sup>248</sup> Ex. 1005 ('188 Patent (Schmitt)) at 24:66–67

in the art would have recognized that the Phred score is a widely adopted metric for estimating the accuracy of base calls in DNA sequencing, where a score of 30 corresponds to a 99.9% probability that the base is called correctly.

191. The '188 Patent (Schmitt) likewise discloses sequence filtering as part of the DCS workflow, stating that “the paired target nucleic acid strands can be filtered to remove nucleotide positions where the sequences seen on both of the paired partner DNA strands are not complementary.”<sup>249</sup> This step ensures that only positions showing biologically plausible Watson–Crick complementarity between paired strands are retained for consensus building, thereby excluding potential sequencing or PCR artifacts. The '188 Patent (Schmitt) further explains that “[o]nly DNA positions that yield the same DNA sequence in a specified proportion of the PCR duplicates in a family, such as 90% of the duplicates in one embodiment, are used to create the PCR consensus sequence.”<sup>250</sup>

192. Accordingly, in view of the '188 Patent (Schmitt)'s teachings, one of ordinary skill in the art would have found it obvious to implement a quality-based filtering step—to reduce sequencing noise and increase confidence in mutation detection.

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<sup>249</sup> *Id.* at 16:40-44.

<sup>250</sup> Ex. 1005 ('188 Patent (Schmitt)) at 21:61-64.

**11. Claim 21: “The method of claim 13, further comprising mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence.”**

193. In my opinion, Claim 21 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings alone or in combination with Forshew (2012)'s teachings.

194. The Board has recognized that the '188 Patent (Schmitt) teaches “mapping the sequence reads to a reference sequence.”<sup>251</sup> The '188 Patent (Schmitt) describes this explicitly: “[r]eads were aligned to the human genome with the Burrows Wheeler Aligner (BWA),”<sup>252</sup> and further notes use of the “hg19” human reference genome,<sup>253</sup> a widely adopted version of the human genome assembly in use at the time. One of ordinary skill in the art would have understood that this alignment process—using BWA to compare each sequencing read to a known reference genome—constitutes mapping sequence reads to a reference sequence.

195. Forshew (2012) likewise teaches mapping sequencing reads to a reference sequence as a step before downstream grouping and analysis. For example, Forshew (2012) reports that “[s]equencing reads were aligned to the hg19

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<sup>251</sup> Ex. 1083 (822FWD) at p.32, citing Ex. 1005 ('188 Patent (Schmitt)) at 20:39-64, 23:10-14, 24:33-37.

<sup>252</sup> Ex. 1005 ('188 Patent (Schmitt)) at 20:39–64.

<sup>253</sup> *Id.* at 23:10–14.

reference genome using ELAND (Illumina) and subsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations.”<sup>254</sup>

196. As discussed above, one of ordinary skill in the art would have found it obvious to integrate Forshew (2012)’s teachings with the ’188 Patent (Schmitt)’s DCS methodology, as both rely on standard, well-established bioinformatics approaches for aligning reads to a reference genome prior to error correction and mutation quantification.

- 12. Claim 22: “ The method of claim 21, further comprising grouping a subset of sequencing reads into families based on sequence information of the molecular barcodes and (1) a start base position of a given sequencing read from among the subset of sequencing reads at which the given sequencing read is determined to start mapping to the reference sequence or (2) a stop base position of the given sequencing read at which the given sequencing read is determined to stop mapping to the reference sequence.”**

197. In my opinion, Claim 22 would have been obvious to one of ordinary skill in the art based on the ’188 Patent (Schmitt)’s teachings.

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<sup>254</sup> Ex. 1010 (Forshew (2012)) at SI 19; *see also id.* SI 4 (“Each set of reads was aligned independently to the hg19 reference genome using bwa-short in the single-end mode. Using expected genomic positions, each set of aligned reads was separated further into its constituent amplicons. A pileup was generated for each amplicon using samtools v1.12a. Using a base quality and a mapping quality cut-off of 30, observed frequencies of non-reference alleles for every sequenced locus across all amplicons and barcodes were calculated.”)

198. The '188 Patent (Schmitt) describes that, in the DCS workflow, sequencing reads are grouped into families based on SMIs: “Following tagging with a double-stranded SMI and PCR amplification, a family of molecules is obtained that arose from a single DNA molecule; members of the same PCR ‘family’ are then grouped together by virtue of having a common (i.e., the same) SMI tag sequence.”<sup>255</sup> The '188 Patent (Schmitt) further clarifies that “[r]eads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”<sup>256</sup> One of ordinary skill in the art would have recognized that this describes grouping sequence reads that originate from the same tagged parent polynucleotide—identified by their matching barcode—into discrete families, which is essential for downstream consensus generation and error correction.

199. The '188 Patent (Schmitt) also teaches an alternative “hybrid” approach in which grouping incorporates both the SMI tag sequence and information about the DNA fragment’s shear points: “[c]ombining information regarding the shear

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<sup>255</sup> Ex. 1083 (822FWD) at 32.

<sup>256</sup> *Id.*

points of DNA with the SMI tag sequence.”<sup>257</sup> One of ordinary skill in the art would have understood this to mean grouping reads not only by their identical barcode but also by their start and stop positions—sequences derived from each end of the original sheared fragment—thereby increasing confidence that grouped reads originate from the same physical DNA molecule.

200. In this way, the ’188 Patent (Schmitt) discloses grouping strategies that combine two orthogonal identifiers: (1) the SMI barcode and (2) the start and stop positions of the fragment, which correspond to the sequences at its sheared ends. One of ordinary skill in the art would have appreciated that incorporating start/stop positional data provides an additional discriminatory parameter beyond the barcode alone, thereby refining family assignments and reducing the likelihood of misgrouping reads from different template molecules.

201. Consistently, the Board has previously found that the ’188 Patent (Schmitt) teaches “grouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads

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<sup>257</sup> Ex. 1005 (’188 Patent (Schmitt)) at 17:61-18:2; *see also id.* at 9:9-14, 17:41-61; *see also* Ex. 1083 (822FWD) at 39 (referring to the ’188 Patent (Schmitt)’s disclosure of combining “sequence information from the sheared ends with the short n-mer tag” to achieve a “unique molecular identifier”).

amplified from the same tagged parent polynucleotide”<sup>258</sup>, confirming that the method integrates both barcode and positional information to accurately reconstruct original template molecules. The Board also found that “library generation, shearing (or not shearing), and ligating adaptors to DNA fragments were well known and routine steps in the art before the earliest priority data for the ’822 patent.”<sup>259</sup> .

**13. Claim 23: “The method of claim 22, further comprising, for a plurality of the families, collapsing sequencing reads within a given family of the plurality of the families to generate consensus sequences.”**

202. In my opinion, Claim 23 would have been obvious to one of ordinary skill in the art based on the ’188 Patent (Schmitt)’s teachings.

203. The ’188 Patent (Schmitt) describes that, within the DCS framework, sequencing reads sharing an identical SMI tag are first grouped into families and then “collapsed to generate a consensus read.”<sup>260</sup> One of ordinary skill in the art would have understood that the ’188 Patent (Schmitt)’s reference to a “consensus read” refers to a consensus sequence. Further, the ’188 Patent (Schmitt) explains that to generate a consensus sequence, “[s]equencing positions were discounted if

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<sup>258</sup> Ex. 1083 (822FWD) at 32.

<sup>259</sup> *Id.* at 41.

<sup>260</sup> Ex. 1005 (’188 Patent (Schmitt)) at 20:50-52 (“Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”).

the consensus group covering that position consisted of fewer than 3 members or if fewer than 90% of the sequences at that position in the consensus group had the identical sequence.”<sup>261</sup> One of ordinary skill in the art would have understood that this process yields a high-confidence consensus base call for each family, limited to positions with adequate depth and agreement to minimize sequencing or amplification artifacts. Likewise, the Board previously found that the ’188 Patent (Schmitt) teaches “at each genetic locus of a plurality of genetic loci in the one or more reference sequences, collapsing sequence reads in each family to yield a base call for each family at the genetic locus.”<sup>262</sup>

**14. Claim 24: “The method of claim 23, further comprising identifying one or more consensus sequence having the genetic variation as compared to the reference sequence.”**

204. In my opinion, Claim 24 would have been obvious to one of ordinary skill in the art based on the ’188 Patent (Schmitt)’s teachings alone or in combination with the teachings of Forshew (2012).

205. The ’188 Patent (Schmitt) explains that the DCS method quantitatively distinguishes true mutations—those present in the original DNA molecule—from PCR artifacts or sequencing errors, describing this as detecting “the presence of a

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<sup>261</sup> *Id.* at 20:53-56.

<sup>262</sup> Ex. 1083 (822FWD) at 32.

true mutation (as opposed to a PCR error or other artifactual mutation) in a target nucleic acid sequence.”<sup>263</sup> The ’188 Patent (Schmitt) further links this capability to its use as a biomarker, stating that “the ability to indirectly infer that damage is present on the DNA could be [a] useful biomarker,” with applications including “cancer risk, cancer metabolic state, [and] mutator phenotype related to defective damage repair.”<sup>264</sup> One of ordinary skill in the art would recognize that the ’188 Patent (Schmitt)’s reference to “damage” in this context encompasses the identification of consensus sequences containing genetic variations when compared to a reference sequence.

206. Forshew (2012) likewise describes applying “deep sequencing” to cell-free tumor DNA in order to detect low-frequency mutations, directly identifying such mutations in plasma from cancer patients.<sup>265</sup> Forshew (2012)’s workflow explicitly involves comparing sequencing reads to a reference genome: “Sequencing reads were aligned to the hg19 reference genome using ELAND (Illumina) and subsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations.”<sup>266</sup>

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<sup>263</sup> Ex. 1005 (’188 Patent (Schmitt)) at 16:43–47.

<sup>264</sup> *Id.* at 15:42–51

<sup>265</sup> Ex. 1010 (Forshew (2012)) at 4, Tables 1-2.

<sup>266</sup> *Id.* at SI 19

207. The '202 Patent Pub. (Porreca), in turn, teaches detecting genetic variation in microsatellite regions through comparison to a reference measurement, explaining that “a capture efficiency that is different from a reference capture efficiency is indicative of the presence, in the biological sample, of a target nucleic acid having an abnormal length.”<sup>267</sup>

208. In view of these disclosures, Claim 24 would have been obvious from the combination of the '188 Patent (Schmitt)'s DCS method with ForsheW (2012)'s cfDNA sequencing approach and Porreca's MSI detection framework.

**15. Claim 25: “The method of claim 13, wherein the sample of cell-free nucleic acid molecules comprises 1 nanogram (ng) to 100 ng of cell-free nucleic acid molecules.”**

209. In my opinion, Claim 25 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings alone or in combination with the teachings of ForsheW (2012) or the '202 Patent Pub. (Porreca).

210. ForsheW (2012) reports isolating cfDNA from plasma volumes ranging between 0.85 mL and 2.2 mL.<sup>268</sup> Table S6 indicates that the resulting cfDNA yields sequenced from various patient samples spanned from 0.9 ng to 19.7 ng.<sup>269</sup>

211. The '202 Patent Pub. (Porreca) likewise discloses that the amount of

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<sup>267</sup> Ex. 1075 ('202 Patent Pub. (Porreca)) at ¶[0047].

<sup>268</sup> Ex. 1010 (ForsheW (2012)) at 10.

<sup>269</sup> *Id.* at Table S6.

genomic nucleic acid analyzed per subject may range from as little as 1 ng up to 10 micrograms (e.g., 500 ng to 5 micrograms), while also noting that both lower quantities (<1 ng) and higher quantities (>10 micrograms, including 10–50 micrograms or 50–100 micrograms or more) can be employed.<sup>270</sup>

212. Thus, one of ordinary skill in the art would recognize that the cfDNA amounts described in ForsheW (2012) and the nucleic acid input ranges disclosed in the '202 Patent Pub. (Porreca) teaches the claimed range of 1 to 100 ng of cell-free nucleic acid molecules in the sample.

**16. Claim 26: “The method of claim 13, wherein the cell-free nucleic acid molecules comprise cell-free deoxyribonucleic acid (cfDNA) molecules.”**

213. In my opinion, Claim 26 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings alone or in combination with the teachings of ForsheW (2012).

214. The Board has previously determined that ForsheW (2012) discloses “providing a population of cell-free ('cfDNA') molecules obtained from a bodily sample from a subject.”<sup>271</sup>

215. ForsheW (2012) details a sequencing workflow known as “tagged-

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<sup>270</sup> Ex. 1075 ('202 Patent Pub. (Porreca)) at ¶[0192].

<sup>271</sup> Ex. 1083 (822FWD) at 29.

amplicon deep sequencing (TAm-Seq),” which involves targeted amplification followed by deep sequencing of predefined genomic regions corresponding to cancer-associated genes.<sup>272</sup> Beyond its application to DNA from solid tumor specimens, Forshew (2012) applied TAm-Seq to cfDNA obtained from plasma of cancer patients, thereby enabling the non-invasive detection of tumor-associated mutations directly from blood-derived material.<sup>273</sup> Specifically, Forshew (2012) describes extracting cfDNA from plasma samples<sup>274</sup> and provides quantitative data in Table S6 showing estimated cfDNA yields sequenced from multiple patients.

**17. Claim 27: “The method of claim 13, further comprising determining a confidence score based on the genetic variation detected in the one or more microsatellite regions.”**

216. In my opinion, Claim 27 would have been obvious to one of ordinary skill in the art based on the ’188 Patent (Schmitt)’s teachings alone or in combination with the teachings of the ’202 Patent Pub. (Porreca).

217. The ’916 Patent acknowledges that, at the relevant time, “known statistics of typical variances at reported positions in non-disease reference sequences” were available and could be leveraged to calculate a confidence score

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<sup>272</sup> Ex. 1010 (Forshew (2012) at Abstract; *see also id.*, 3, Table S1

<sup>273</sup> *Id.* at 4, Tables 1–2

<sup>274</sup> *Id.* at 10.

for a detected mutation.<sup>275</sup> One of ordinary skill in the art would have recognized that such known statistical baselines could likewise be applied to variations observed in microsatellite regions to generate a corresponding confidence score.

218. The '188 Patent (Schmitt) describes that its DCS method enables quantification of true mutations—defined as mutations present in both strands of a DNA duplex and distinguished from PCR or sequencing artifacts—by “detecting the presence of a true mutation (as opposed to a PCR error or other artifactual mutation) in a target nucleic acid sequence.”<sup>276</sup> Because the '188 Patent (Schmitt) emphasizes the necessity of verifying that a mutation is “true,” one of ordinary skill in the art would have been motivated to assign a confidence score to each mutation call generated by DCS to quantitatively assess its reliability. The '188 Patent (Schmitt) further provides a framework for such evaluation, disclosing that “the background (artifactual) error frequency of DCS may be calculated as: (probability of error on one strand)(*probability of error on other strand*)(probability that both errors are complementary).”<sup>277</sup> One of ordinary skill in the art would have recognized that a confidence score could be derived by comparing the observed mutation signal against this calculated background error rate.

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<sup>275</sup> Ex. 1001 ('916 Patent) at 51:5-8.

<sup>276</sup> Ex. 1005 ('188 Patent (Schmitt)) at 16:43–47.

<sup>277</sup> *Id.* at 28:34-39.

219. Even if the '188 Patent (Schmitt) does not explicitly recite “determining a confidence score” for a detected variation, it would have been obvious to integrate the '188 Patent (Schmitt)'s DCS-based mutation detection with the '202 Patent Pub. (Porreca)'s disclosure of “determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.”<sup>278</sup> One of ordinary skill in the art would have been motivated to make this combination because assigning a statistical confidence score provides a quantitative means to assess the certainty of a mutation call and thus directly informs the reliability of the detected genetic variation.

**18. Claim 28: “The method of claim 13, further comprising determining a treatment regimen for the subject based on the quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions.”**

220. In my opinion, Claim 28 would have been obvious to one of ordinary skill in the art based on the '188 Patent (Schmitt)'s teachings alone or in combination with the teachings of Forshew (2012) and the '202 Patent Pub. (Porreca).

221. The '916 Patent acknowledges that, at the relevant time, it was well understood in the field that genetic alterations present in cfDNA could serve as biomarkers for early cancer detection and for monitoring disease progression,

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<sup>278</sup> Ex. 1075 ('202 Patent Pub. (Porreca)) at Claim 16.

including informing treatment strategies and ongoing disease management.<sup>279</sup>

222. The '188 Patent (Schmitt) teaches that the DCS method confers “the ability to indirectly infer that damage is present on the DNA,” which can be a valuable biomarker “for cancer risk, cancer metabolic state, [and] mutator phenotype related to defective damage repair.”<sup>280</sup> One of ordinary skill in the art would have understood that defective damage repair encompasses defective DNA mismatch repair, which is linked to microsatellite instability (MSI). At the relevant time, it was already recognized in the clinical community that MSI testing could provide prognostic value in colorectal cancer (CRC) patients and could guide therapeutic decisions.<sup>281</sup>

223. Also, the '202 Patent Pub. (Porreca) discloses “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats”<sup>282</sup> and specifically identifies the clinical relevance of such changes in the context of “cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats

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<sup>279</sup> Ex. 1001('916 Patent) at 1:39-51.

<sup>280</sup> Ex. 1005 ('188 Patent (Schmitt)) at 15:42–51

<sup>281</sup> Ex. 1080 (Sinicrope (2009)) at 3 (“patients with sporadic MSI-H colon cancers did not derive benefit from 5-FU-based chemotherapy in contrast to those with non-MSI tumors”).

<sup>282</sup> Ex. 1075 ('202 Patent Pub. (Porreca)) at ¶[0157]

at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26).”<sup>283</sup> Importantly, the ’202 Patent Pub. (Porreca) also teaches that detection of such insertions or deletions—MSIs included—can inform treatment selection. For example, the ’202 Patent Pub. (Porreca) states that “a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci...[and] genetic information from a tumor or circulating tumor cells is used to determine prognosis and guide selection of appropriate drugs/treatments.”<sup>284</sup> Given these teachings, one of ordinary skill in the art would have found it obvious to combine the ’188 Patent (Schmitt)’s DCS method and Forshew (2012)’s cfDNA analysis with Porreca’s MSI detection framework for purposes of developing a treatment regimen for a subject based on a quantitative measure of polymorphic forms—such as microsatellite changes—in one or more microsatellite regions.

**19. Claim 29: “The method of claim 28, further comprising administering the treatment regimen to the subject.”**

224. In my opinion, the ’188 Patent (Schmitt) in view of the ’202 Patent Pub. (Porreca) teaches Claim 29. As described *supra*, Schmitt’s teachings in combination

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<sup>283</sup> *Id.* at ¶[0160]

<sup>284</sup> *Id.* at ¶[0017].

with the '202 Patent Pub. (Porreca) teach determining a treatment regimen.<sup>285</sup> The '202 Patent Pub. (Porreca) further teaches using the detected genetic variation for administering patient treatment: “genetic information from a tumor or circulating tumor cells is used to determine prognosis and *guide selection of appropriate drugs/treatments*.”<sup>286</sup> Thus, it would be obvious to apply the teachings of the '202 Patent Pub. (Porreca) to determine a treatment regimen, then administer that treatment.

## 20. Independent Claim 30

a) 30(pre)

225. See 13(pre) and 26 above.

b) 30(a)

226. See 13(a) and Claim 16 above.

c) 30(b)

227. See 13(b) above.

d) 30(c)

228. See 13(c) above.

e) 30(d)

229. See Claims 22-23 above.

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<sup>285</sup> See Claim 28.

<sup>286</sup> See Ex. 1075 ('202 Patent Pub. (Porreca)) at ¶ [0017] (emphasis added).

f) 30(e)

230. *See* 13(d) above.

**B. Ground 2: Claims 13-30 of the '916 Patent were obvious over the '188 Patent (Schmitt) in view of Forshew (2012) and the '331 Patent Pub. (Sacko)**

231. For the reasons I explain below, Claims 13-30 of the '916 Patent are obvious based on the teachings of the '188 Patent (Schmitt) in view of Forshew (2012) and the '331 Patent Pub. (Sacko).

232. As I explained for Ground 1, the '188 Patent (Schmitt)—either alone or in combination with Forshew (2012) and Porreca—discloses limitations 13[pre], 13[d], 26, 28 and 29.<sup>287</sup> If, however, the Patent Owner contends that the '188 Patent (Schmitt), with or without Forshew (2012) and Porreca, fails to disclose aspects of those limitations that specifically address detecting microsatellite changes in cfDNA, the '331 Patent Pub. (Sacko) provides such disclosure. The '331 Patent Pub. (Sacko) teaches detection of microsatellite changes in cfDNA, and one of ordinary skill in the art would have found it obvious to incorporate the '331 Patent Pub. (Sacko)'s teachings into the '188 Patent (Schmitt)–Forshew (2012) framework, thus supplying an alternative basis for concluding that claims 13, 26, 28, and 29 are obvious.

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<sup>287</sup> *See* XI.A.

233. The '916 prosecution history further supports my opinion regarding the teachings of the '331 Patent Pub. (Sacko). During prosecution, the Examiner rejected the '916 Patent claims on the ground of obviousness-type double patenting over other patents and applications in the same family in view of the '331 Patent Pub. (Sacko). In responding via terminal disclaimer, the Patent Owner did not contest the Examiner's specific findings that: (1) "patent claims are generic with respect to the [916] claims, reciting [various genres] while the instant claims require the species 'microsatellite changes'"; (2) the '331 Patent Pub. (Sacko) disclosed "that cell-free nucleic acids from blood of cancer patients are associated with microsatellite mutations and instabilities"; and (3) "[o]ne of ordinary skill in the art considering the patented methods would have been motivated to modify [the detection steps] by applying them to microsatellite changes because the '331 Patent Pub. (Sacko) et al. disclosed that microsatellite changes were known to occur in cell-free nucleic acids from blood of cancer patients."<sup>288</sup>

### **1. Motivation to Combine**

234. As I explained previously, one of ordinary skill in the art would have been motivated to combine the '188 Patent (Schmitt) and Forshew (2012). In addition, in my opinion, one of ordinary skill in the art would have been motivated

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<sup>288</sup> Ex. 1002 ('916 Patent File History) at 445.

to combine the teachings of the '188 Patent (Schmitt) and Forshew (2012) with the '331 Patent Pub. (Sacko).

235. The '188 Patent (Schmitt)'s Duplex Consensus Sequencing (DCS) method “allows for a quantitative detection of sites of DNA damage” and teaches that such detection “could be [a] useful biomarker” for applications including “cancer risk, cancer metabolic state, [and] mutator phenotype related to defective damage repair.”<sup>289</sup> One of ordinary skill in the art would understand that the '188 Patent (Schmitt)'s reference to quantifying sites of DNA damage and inferring a “mutator phenotype related to defective damage repair” encompasses the detection and quantification of microsatellite instabilities (MSIs). *See* Discussion in XI.A.1.

236. To the extent that, the '188 Patent (Schmitt) does not teach the detection and quantification of MSIs, the '331 Patent Pub. (Sacko) provides that teaching. The '331 Patent Pub. (Sacko) states that “DNA extracted from the plasma of cancer patients usually has tumoral DNA characteristics. . . .such as microsatellite alterations.”<sup>290</sup> One of ordinary skill in the art aiming to improve the detection and quantification of genomic instability in a minimally invasive sample type—such as cfDNA for diagnostic or therapeutic purposes—would have found it obvious to

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<sup>289</sup> Ex. 1005 ('188 Patent (Schmitt)) at 15:42-51.

<sup>290</sup> Ex. 1076 ('331 Patent Pub. (Sacko)) at ¶ [0005].

integrate the '331 Patent Pub. (Sacko)'s teachings on the clinical relevance of MSIs in ctDNA from blood plasma into the high-sensitivity cfDNA analysis frameworks described by the '188 Patent (Schmitt) and ForsheW (2012), both of which are capable of detecting low-prevalence mutations.

237. This motivation would have been reinforced by the '331 Patent Pub. (Sacko)'s emphasis on the importance of sensitivity in cfDNA measurements,<sup>291</sup> as such sensitivity enables a reliable and quantitative measurement of microsatellite changes in ctDNA. One of ordinary skill in the art would have been further motivated by the growing recognition—described in the '331 Patent Pub. (Sacko) and other sources—that MSI is an informative biomarker for cancer diagnosis and therapy selection.<sup>292</sup> Given that the use of cfDNA for non-invasive cancer detection and monitoring was well-established, one of ordinary skill in the art would have appreciated that applying MSI detection techniques to cfDNA, alone or alongside other variant detection methods, could provide a highly sensitive and clinically valuable tool. Moreover, the '331 Patent Pub. (Sacko) qualifies as analogous art to the '188 Patent (Schmitt), ForsheW (2012), and the '916 Patent—as cited by the Examiner during prosecution—because it is likewise directed to identifying genetic

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<sup>291</sup> *See Id.* at ¶[0013].

<sup>292</sup> *Id.* at ¶[0005]; *see also* Ex. 1005 ('188 Patent (Schmitt)) at 1:26–35.

mutations indicative of disease states, including cancer.

## **2. Reasonable Expectation of Success**

238. As I explained above, one of ordinary skill in the art would have had a reasonable expectation of success in combining the teachings of the '188 Patent (Schmitt) and ForsheW (2012).

239. One of ordinary skill in the art would likewise have had a reasonable expectation of success in combining the '188 Patent (Schmitt) and ForsheW (2012) with the '331 Patent Pub. (Sacko) to determine a quantitative measure of microsatellite changes in a cfDNA sample. At the relevant time, the use of cfDNA in conjunction with established NGS and bioinformatics techniques for detecting genetic alterations was both well-known and expressly taught by the '188 Patent (Schmitt) and ForsheW (2012). *See* Discussion in XI.A.2. The '331 Patent Pub. (Sacko) further documented that microsatellite alterations—specifically insertions and deletions—are present in ctDNA obtained from blood plasma.<sup>293</sup>

240. One of ordinary skill in the art would have recognized that microsatellite loci—short, repetitive DNA sequences—are readily detectable using the sequencing and analysis methodologies taught by the '188 Patent (Schmitt). The '331 Patent Pub. (Sacko)'s description of MSI in cfDNA would be understood as

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<sup>293</sup> Ex. 1076 ('331 Patent Pub. (Sacko)) at ¶[0005].

directly amenable to quantitative assessment through these same NGS-based techniques. Because this combination applies established detection principles to a known analyte (cfDNA) and a well-characterized target (microsatellites), one of ordinary skill in the art would have had a high degree of confidence in obtaining the desired result without undue experimentation.

241. The combination represents a predictable application of established techniques to address a recognized problem using conventional tools. Each of the '331 Patent Pub. (Sacko), Forshew (2012), and the '188 Patent (Schmitt) relies on standard molecular biology and sequencing methodologies that were in routine use in the field. The '188 Patent (Schmitt) supplies the sample preparation, sequencing, and computational framework for cfDNA analysis,<sup>294</sup> while the '331 Patent Pub. (Sacko) provides the clinical rationale and methodological basis for interpreting microsatellite variations in cfDNA. One of ordinary skill in the art would have understood that Sacko's teaching of MSI presence in cfDNA could be quantified using the '188 Patent (Schmitt)'s analytical approach, in combination with Forshew (2012) as discussed, to provide not only a qualitative MSI status but also a quantitative measure of microsatellite change—enhancing clinical resolution. This combination would therefore have been obvious and fully within the capabilities of

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<sup>294</sup> Ex. 1005 ('188 Patent (Schmitt)) at 7:1–45; 11:8–30.

a skilled artisan, without requiring undue experimentation.

### **3. Independent Claim 13**

- a) 13(pre): “A method for detecting a genetic variation in one or more microsatellite regions in a sample of cell-free nucleic acid molecules from a subject having a cancer, the method comprising:”

242. For the reasons I explained previously, the preamble of Claim 13 would be obvious to one of ordinary skill in the art based on the teachings of the '188 Patent (Schmitt) and Forshew (2012), to the extent this preamble is limiting. *See* Section XI.A.3.a.

243. To the extent the '188 Patent (Schmitt), in combination with Forshew (2012), does not itself teach “detecting a genetic variation in one or more microsatellite regions,” one of ordinary skill in the art would have found it obvious to do so in view of the '331 Patent Pub. (Sacko)’s teachings. For example, the '331 Patent Pub. (Sacko) explains that “microsatellite mutations and instabilities detected in the free genomic DNA of the [plasma] serum suggest that it could be a new potential marker, with considerable specificity for monitoring tumors.”<sup>295</sup> The clinical significance of evaluating microsatellite changes in a subject with cancer was well established. The '188 Patent (Schmitt) on the other hand teaches that quantifying DNA defects enables MSI detection. In light of the '331 Patent Pub.

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<sup>295</sup> Ex. 1076 ('331 Patent Pub. (Sacko)) at ¶[0005].

(Sacko)'s disclosure that cfDNA can be used to screen for "microsatellite mutations and instabilities," one of ordinary skill in the art would have been motivated to combine the '331 Patent Pub. (Sacko) with the '188 Patent (Schmitt) and ForsheW (2012) to implement a method for detecting a genetic variation in one or more microsatellite regions in a sample of cell-free nucleic acid molecules from a subject having cancer.

b) 13(d): "(d) determining, from among a plurality of sequencing reads in the set of sequencing reads, a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions based at least on sequence information of the molecular barcodes, thereby detecting the genetic variation in the one or more microsatellite regions."

244. For the reasons I explained above, the '188 Patent (Schmitt) alone or combined with the teachings of ForsheW (2012) teaches this limitation. *See* 13[d] in Section XI.A.

245. To the extent the '188 Patent (Schmitt), alone or in combination with ForsheW (2012), does not teach this limitation, in my opinion it would have been obvious to incorporate the '331 Patent Pub. (Sacko)'s teachings. The '331 Patent Pub. (Sacko) informs that cancer mutations of particular interest include MSIs present in ctDNA.<sup>296</sup> ("microsatellite mutations and instabilities detected in the free

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<sup>296</sup> Ex. 1076 ('331 Patent Pub. (Sacko)) at ¶[0005].

genomic DNA of the serum suggest that it could be a new potential marker, with considerable specificity for monitoring tumors”). Based on the ’331 Patent Pub. (Sacko), one of ordinary skill in the art would have understood that the MSIs—i.e., the genetic variation within one or more microsatellite regions—detected in circulating tumor DNA are precisely the type of mutations that could be analyzed using the ’188 Patent (Schmitt)’s DCS methods.

**4. Claim 26: “The method of claim 13, wherein the cell-free nucleic acid molecules comprise cell-free deoxyribonucleic acid (cfDNA) molecules.”**

246. For the reasons I explained above, the ’188 Patent (Schmitt) alone or combined with the teachings of Forshew (2012) teaches Claim 26. To the extent the ’188 Patent (Schmitt), alone or in combination with Forshew (2012), does not teach Claim 26, in my opinion it would have been obvious to incorporate the ’331 Patent Pub. (Sacko)’s teachings.

247. The ’331 Patent Pub. (Sacko) reports that “DNA extracted from the plasma of cancer patients usually has tumoral DNA characteristics [] such as strand instability. . . and microsatellite alterations.”<sup>297</sup> The ’331 Patent Pub. (Sacko) further teaches that “[t]he microsatellite mutations and instabilities detected in the free genomic DNA of the serum suggest that it could be a new potential marker, with

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<sup>297</sup> Ex. 1076 (’331 Patent Pub. (Sacko)) at ¶[0005].

considerable specificity for monitoring tumors.”<sup>298</sup> In addition, the ’331 Patent Pub. (Sacko) describes MSIs as a “new potential marker” with clinical applications, including to “complement the analysis of markers commonly used to monitor patients suffering from cancers or undergoing chemotherapy.”<sup>299</sup> Accordingly, it would have been obvious to combine the ’188 Patent (Schmitt) and ForsheW (2012)’s teachings with the ’331 Patent Pub. (Sacko) to achieve a method where the cell-free nucleic acid molecules are cfDNA molecules.

**5. Claim 28: “The method of claim 13, further comprising determining a treatment regimen for the subject based on the quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions.”**

248. For the reasons I explained above, the ’188 Patent (Schmitt) alone or combined with the teachings of ForsheW (2012) teaches Claim 28. To the extent the ’188 Patent (Schmitt), alone or in combination with ForsheW (2012), does not teach Claim 28, in my opinion it would have been obvious to incorporate the ’331 Patent Pub. (Sacko)’s teachings.

249. The ’331 Patent Pub. (Sacko) teaches that “[t]he microsatellite mutations and instabilities detected in the free genomic DNA of the serum suggest

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<sup>298</sup> *Id.*

<sup>299</sup> *Id.* at ¶[0006].

that it could be a new potential marker, with considerable specificity for monitoring tumors.”<sup>300</sup> The ’331 Patent Pub. (Sacko) further explains that MSIs could serve as a “new potential marker” that may “complement the analysis of markers commonly used to monitor patients suffering from cancers or undergoing chemotherapy,” which one of ordinary skill in the art would have recognized as readily combinable with the teachings of the ’188 Patent (Schmitt) and Forshew (2012).<sup>301</sup> In my opinion, from this combination, it would have been obvious to one of ordinary skill in the art determine a treatment regime for the subject based on a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions.

**6. Claim 29: “The method of claim 28, further comprising administering the treatment regimen to the subject.”**

250. In my opinion, the ’188 Patent (Schmitt) in view of the ’331 Patent Pub. (Sacko) teaches Claim 29. As explained above, the ’188 Patent (Schmitt)’s teachings in combination with the ’331 Patent Pub. (Sacko) teach determining a treatment regimen. *See* Claim 28. According to the ’331 Patent Pub. (Sacko), when used as a biomarker, MSIs “complement the analysis of markers commonly used to

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<sup>300</sup> Ex. 1076 (’331 Patent Pub. (Sacko)) at ¶[0005].

<sup>301</sup> *Id.* at ¶[0006].

monitor patients suffering from cancers or undergoing chemotherapy.”<sup>302</sup> The ’331 Patent Pub. (Sacko)’s teaching to use MSI markers to monitor ongoing chemotherapy therefore teaches administering a determined treatment.

### 7. Remaining Limitations and Claims

251. In my opinion, as I discussed above for Ground 1, the remaining limitations of claim 13, and Claims 14-25, 27, and 30, are obvious by Ground 2 for the same reasons.

#### C. SECONDARY CONSIDERATIONS CANNOT REBUT THE STRONG SHOWING OF OBVIOUSNESS

252. I have been informed by counsel that long-felt but unsolved needs, failure of others, unexpected results, commercial success, copying, licensing, and praise may constitute secondary considerations of non-obviousness.

253. It is my opinion that there are no secondary considerations supporting non-obviousness here for the reasons discussed *supra*. .


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<sup>302</sup> *Id.*

I confirm that the contents of this Declaration are true to the best of my knowledge and belief insofar as it states facts and that it contains my honest opinions on the matters upon which I have been asked to give them.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Dated: August 15, 2025

  
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Michael Metzker, Ph.D. \_\_\_\_\_