

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

FORESIGHT DIAGNOSTICS INC.,
Petitioner,

v.

PERSONALIS, INC.,
Patent Owner.

Case Nos. IPR2023-00545 and IPR2023-00546
U.S. Patent Nos. 11,299,783 and 10,450,611

DECLARATION OF HENRY MORRICE FURNEAUX, PH.D.

TABLE OF CONTENTS

	Page No.
I. ENGAGEMENT.....	1
II. BACKGROUND AND QUALIFICATIONS.....	2
A. Patents Awarded.....	7
B. Research And Teaching Experience	9
C. Industry Experience.....	12
D. Professional Society Involvement.....	13
III. MATERIALS CONSIDERED	13
IV. LEGAL STANDARDS	14
A. Anticipation	14
B. Nonobviousness.....	14
V. THE PERSON OF ORDINARY SKILL IN THE ART	15
VI. THE GROUNDS CHALLENGING PATENTABILITY OF THE '783 AND '611 PATENTS.....	17
VII. FORESIGHT HAS NOT PROVEN UNPATENTABILITY OF ANY CLAIM FROM THE '783 PATENT	20
A. Overview of the '783 Patent	21
B. GROUND 1: NOVELTY OVER LEY	27
1. Claim 1	28
a. Ley does not teach Limitation 1(a).....	28

TABLE OF CONTENTS
(cont'd.)

	Page No.
i. Claim construction of “variable portion” and “fixed portion”	30
ii. Ley does not teach a variable portion or fixed portion	
31	
b. Limitation 1(b).....	35
c. Limitation 1(c)	37
i. Claim construction of “to identify”	38
ii. Ley does teach limitation 1(c) under the plain meaning of “to identify”	39
d. Limitation 1(d).....	42
i. Claim construction of limitation 1(d)	43
ii. Ley does not teach limitation 1(d)	44
C. GROUND 2: NONOBVIOUSNESS OVER LEY	45
1. Claim 1	45
a. Limitation 1(a)	45
i. Reason 1: Ley counsels against the modification proposed by Dr. Quackenbush	47
ii. Reason 2: Adding more PCR primers would complicate the assay	50

TABLE OF CONTENTS
(cont'd.)

	Page No.
iii. Reason 3: Ley's mutations had unknown clinical utility.....	53
b. Limitation 1(b).....	57
c. Limitation 1(c).....	58
d. Limitation 1(d).....	62
e. No motivation to modify Ley	63
2. Claims 3, 8, 15, and 16	64
3. Claim 22	65
D. GROUND 3: NONOBVIOUSNESS OVER FORSHEW AND DAWSON.....	74
1. Claim 1	75
a. Limitation 1(a).....	75
i. Forshew does not teach the claimed "variable portion"	76
ii. No suggestion for the claimed "variable portion" ..	78
iii. No motivation to combine "variable portion" and "fixed portion"	80
b. Limitation 1(d).....	90
2. Claim 22	95

TABLE OF CONTENTS
(cont'd.)

	Page No.
E. OBJECTIVE INDICIA OF NONOBVIOUSNESS.....	97
1. Nexus.....	98
2. Industry Praise.....	107
3. Skepticism of Experts	108
VIII. FORESIGHT HAS NOT PROVEN UNPATENTABILITY OF ANY CLAIM FROM THE '611 PATENT	109
A. OVERVIEW OF THE '611 PATENT	109
B. GROUND 1: NOVELTY OVER LEY	116
1. Claim 1	116
a. Ley does not teach the required sequencing depth.....	116
b. Ley does not teach 175 kilobases or less.....	119
c. Ley does not teach sequencing “to identify”	123
i. Claim construction of “to identify”	123
ii. Ley does teach limitation 1(e) under the plain meaning of “to identify”	124
2. Claim 5	127
a. Claim construction of “variable portion” and “fixed portion”	128
b. Ley does not teach a variable portion or fixed portion.....	130
3. Claims 10 and 28.....	134

TABLE OF CONTENTS
(cont'd.)

	Page No.
C. GROUND 2: NONOBVIOUSNESS OVER LEY	135
1. Claim 1	135
a. Ley does not suggest the required sequencing depth ...	136
b. Ley does not suggest 175 kilobases or less	138
c. Ley does not suggest sequencing “to identify”	140
d. No motivation to modify Ley	144
2. Claim 5	146
a. Ley counsels against the modification proposed by Dr. Quackenbush	148
b. Adding more PCR primers would complicate the assay	152
c. Ley’s mutations had unknown clinical utility	154
3. Claim 10	157
4. Claim 28	157
D. GROUND 3: NOVELTY OVER NEWMAN	159
1. Claim 1	159
a. Limitation 1(a)	160
b. Limitation 1(d)	160
c. Limitation 1(e): said additional biological sample	164
d. Limitation 1(e): coverage of 175 kilobases or less	165
e. Limitation 1(e): sequencing depth	166

TABLE OF CONTENTS
(cont'd.)

	Page No.
2. Claim 5.....	167
3. Claims 10 and 28.....	169
4. Claim 11.....	170
E. GROUND 4: NONOBVIOUSNESS OVER NEWMAN.....	172
1. Claim 1.....	172
a. Limitation 1(d).....	172
b. Limitation 1(e).....	174
c. Limitation 1(a).....	175
d. Limitation 1(e).....	177
e. No motivation to modify Ley.....	177
2. Claim 5.....	178
3. Claims 10, 11, and 28.....	181
F. OBJECTIVE INDICIA OF NONOBVIOUSNESS.....	181
1. Nexus.....	181
2. Industry Praise.....	191
3. Skepticism of Experts.....	192
IX. CERTIFICATION.....	193

1. I, Henry Morrice Furneaux, Ph.D., make this Declaration in connection with *inter partes* review proceeding no. IPR2023-00545 concerning U.S. Patent No. 11,299,783 (“the ’783 patent”) as well as proceeding no. IPR2023-00546 concerning U.S. Patent No. 10,450,611 (“the ’611 patent”).

2. I previously submitted a declaration in connection with the related *inter partes* review proceeding no. IPR2023-00224 concerning U.S. Patent No. 11,384,394 (“the ’394 patent”) as well as proceeding no. IPR2023-00317 concerning U.S. Patent No. 11,408,033 (“the ’033 patent”).

3. I am over the age of twenty-one (21) and am competent to make this Declaration. I reside at 146 Kenyon Street, Hartford, CT 06105.

4. As discussed herein, the claims of the ’783 patent are not anticipated by Ley EX1012. The claims of the ’783 patent also are nonobvious over (1) Ley EX1012 and (2) the combination of Forshew EX1030 and Dawson EX1070. The claims of the ’611 patent are novel and nonobvious over Ley EX1012, and they are also novel and nonobvious over Newman EX1043.

I. ENGAGEMENT

5. I have been retained by counsel for Personalis, Inc. (“Personalis”) in the above-captioned IPR matters as an independent technical expert to review and evaluate claims of the ’783 Patent and ’611 Patent. My opinions are based on my

skills, knowledge, training, education, and experience in matters of this nature, and my examination of the materials used in preparing this report.

6. I am being compensated on an hourly basis for my work performed in connection with this matter. I have received no additional compensation for my work in this matter, and my compensation does not depend upon the contents of this report, any testimony I may provide, or the ultimate outcome of these matters.

II. BACKGROUND AND QUALIFICATIONS

7. In general, I have knowledge and experience with both the general scientific contexts relevant to these litigation proceedings and specific details of the technologies employed. Based upon my experience, qualifications and expertise, I am qualified as an expert in the fields of nucleic acid biochemistry, the hybridization of DNA to RNA, DNA recombination, the isolation of human genes, the de novo sequencing and assembly of human DNA sequence, the use of PCR and DNA sequencing to identify mutations in human tumor tissue, the manipulation of gene expression by siRNAs and microRNAs, the mechanism of action of enzymes such as DNA ligase and DNA polymerase that are used in molecular biology protocols and the identification of SNPs in noncoding regions of the genome that regulate gene expression via microRNA.

8. I have summarized in this section my educational background, career history, relevant publications, and other relevant qualifications. My full curriculum vitae has been submitted as Exhibit 2010 in this proceeding.

9. I earned a Bachelor of Science in Biochemistry, with honors, from the University of Aberdeen (Scotland, United Kingdom) in 1975. This department was headed by Professor Hamish Keir, a student of Professor J.N Davidson, who wrote the classic textbook “The Nucleic Acids”. Thus, this experience provided a rigorous introduction to nucleic acids.

10. I earned a Ph.D. in Biochemistry, also from the University of Aberdeen, UK in 1978. My thesis work was on the structure and function of poly-ADP ribose, a novel nucleic acid that I discovered had novel effects upon RNA transcription. See Furneaux HM, Pearson CK: The effects of NAD⁺ on RNA synthesis in isolated nuclei from BHK cells. Biochem Soc. Trans, 6:753-755, 1978.

11. In 1978, in a seminar given by Professor Ed Southern, I heard about the exciting hypothesis that mature eukaryotic mRNA might be spliced from a precursor mRNA. This mechanism was unknown and so I accepted a position in Jerry Hurwitz’s lab in New York, to try to establish a system that could recapitulate mRNA splicing in vitro. I was very fortunate to be trained by Dr Hurwitz who not only discovered many of the enzymes used in Molecular biology but also provided

a very rigorous training environment. Indeed, several of Dr. Hurwitz's trainees either won Nobel prizes, became members of the National Academy or attained comparable levels of achievement. We were amongst the first to recapitulate this reaction and the first to show that mRNA splicing required the action of a ribonucleoprotein complex. See Furneaux HM, Perkins KK, Freyer GA, Arenas J, Hurwitz J: Isolation and characterization of two fractions from HeLa cells required for mRNA splicing in vitro. Proc Natl. Acad. Sci, 82: 4351-4355, 1985.

12. From 1984 to 1987, I was a Research Fellow in the Program in Molecular Biology at Sloan Kettering Institute, the experimental research arm of Memorial Sloan Kettering Cancer Center in New York City. From 1987 to 1989, I was promoted to an Assistant Laboratory Member in the Program in Molecular Pharmacology and Therapeutics. In this position, I pioneered the use of molecular biology techniques to address problems in Oncology. For example, my lab was amongst the first to perform PCR analysis on DNA from human Cerebral Spinal Fluid (CSF) to identify a cancer-causing virus. See Henson J, Rosenblum M, Armstrong D, Furneaux H: Amplification of JC virus DNA from brain and cerebrospinal fluid of patients with progressive multifocal eucoencephalopathy. Neurology 41:1967-1971, 1991. My lab also pioneered the recovery of DNA from archival paraffin embedded human tumor slides. My lab showed that this DNA

could be sequenced and we were amongst the first to identify recurrent mutations in the p53 gene, that could be linked to benzopyrene. See Schlegel U, Rosenfeld M, Voldanandt M, Rosenblum M, Furneaux HM: p53 Mutations in primary lung tumors are conserved in brain metastasis. J Neuro-Oncology 14: 93-100, 1992.

13. From 1989 to 1995, I was promoted to Assistant Member and Head of the Laboratory of Molecular Neuro-Oncology at Sloan Kettering. Concurrently, I was also an Assistant Professor of Neuroscience at the Cornell University Graduate School of Medical Sciences (now known as the Weill Cornell Graduate School of Medical Sciences). In this phase of my career, my lab focused on the isolation of the genes that encoded human tumor antigens. This was an innovative project, since the conventional dogma at that time was that the immune system did not recognize tumors. My lab was the first to clone the human paraneoplastic tumor antigen genes, HuD, HuR, LEMS and CDR62. In addition, the cloned tumor antigens provided an ELISA assay for the early detection of lung cancer and neurological disease, which was patented and licensed to Quest Diagnostics. These studies involved cDNA library expression cloning, extensive DNA sequence analysis, gene expression by RT-PCR and an innovative immunohistochemical technique that we devised to directly visualize the expression of tumor antigens and antigen-reactive T cells in human tumor tissue. See Szabo A, Dalmau J, Manley G, Rosenfeld M, Wong E,

Henson J, Posner JB, Furneaux HM: HuD a paraneoplastic encephalomyelitis antigen contains RNA binding domains and is homologous to Elav and Sex-Lethal. Cell 67: 325-333, 1991; Fathallah-Shaykh H, Wolf S, Wong E, Posner JB, Furneaux HM: Cloning of a leucine zipper protein recognized by the sera of patients with antibody-associated paraneoplastic cerebellar degeneration. Proc Nat Acad. Sci, 88:3451-3454, 1991.

14. From 1996 to 2000, I was promoted to Associate Member. Concurrently, I was also an Associate Professor of Neuroscience at the Cornell University Graduate School of Medical Sciences. In this phase, my lab continued to focus on the structure and function of the HuD and HuR tumor antigens and demonstrated their role in regulation of gene expression via their interaction with the AU-rich elements in the 3'UTR of mRNA. See Chung S, Jiang L, Cheng S, and Furneaux HM*. Purification and properties of HuD, a neuronal RNA binding protein. J. Biol. Chem. 271:11518-11524, 1996. It is important to note that mutation of these noncoding elements is a driver of carcinogenesis.

15. In 2000, I accepted the position of Associate Professor of Molecular, Microbial and Structural Biology at the University of Connecticut School of Medicine. Beginning in 2003 and through 2009, I was also the Director of the Molecular Biology and Biochemistry Graduate Program at the University of

Connecticut School of Medicine. In this phase, my lab continued studies on HuR, and HuD. My lab also pursued studies on the structure and function of microRNAs. This included innovative studies on the regulation of gene expression by SNPs present in microRNA target sites in non-coding regions. *See* Jensen KP, Covault J, Conner TS, Tennen H, Kranzler HR, Furneaux HM. A common polymorphism in serotonin receptor 1B mRNA moderates regulation by miR-96 and associates with aggressive human behaviors. *Molecular Psychiatry* 2009 14(4):381-9.

16. In 2009 I left academia to create a company (Clonegene LLC) that could generate income to fund my basic science research. Clonegene is a for-profit company that designs, generates and distributes advanced reagents used in biochemistry research. The income generated has permitted me to pursue, without constraint, novel independent research.

17. I am also concurrently an adjunct professor in the Department of Biology at Capital Community College in Hartford, Connecticut. I have held this position since 2011. This outreach activity allowed me to teach advanced concepts in molecular biology to a community that had traditionally been underexposed to cutting-edge biology.

A. Patents Awarded

18. I am a named co-inventor on at least the following six (6) U.S. Patents:

- U.S. Patent No. 5,603,934 (Antigen recognized by patients with antibody associated paraneoplastic sensory neuronopathy, issued Feb. 1997);
- U.S. Patent No. 5,614,371 (Ri fusion antigen recognized by antibodies associated with paraneoplastic opsoclonus and methods of use thereof, issued Mar. 1997);
- U.S. Patent No. 5,668,013 (Antigen recognized by patients with antibody associated paraneoplastic cerebellar degeneration, DNA encoding same and uses thereof, issued Sept. 1997);
- U.S. Patent No. 5,807,705 (Antigen recognized by patients with antibody associated paraneoplastic sensory neuronopathy, DNA encoding same and uses thereof, issued Sept. 1998);
- U.S. Patent No. 5,925,526 (Antigen recognized by patients with antibody associated cerebellar degeneration, DNA encoding same and uses thereof, issued July 1999); and
- U.S. Patent No. 6,193,948 (Antigen recognized by patients with antibody associated paraneoplastic sensory neuropathy, DNA encoding same and uses thereof, issued Feb. 2001).

B. Research And Teaching Experience

19. Over the course of my research career, I am the recipient of over \$3.75 million in public and outside funding, including from the American Chemical Society, Brain Tumor Society, Burke Prostate Cancer Fund, Connecticut Department of Public Health, National Institute of Health, and National Science Foundation. I am an author or contributor to at least eighty (80) publications in the areas of RNA biochemistry, mRNA stability, enzyme mechanisms, human genomics, RNA therapeutics, immunology, biochemistry, molecular biology, and cell biology as it relates to human diseases and developmental issues. A significant number of these publications are recognized as very innovative in their field. Overall, my publications have earned an h-index citation value of 55 (Google Scholar indicates that an h-index of 20 is good, 40 is outstanding, and 60 is truly exceptional.)

20. Among my editorial work, I am: Associate Editor and member of the editorial board of BMC Biochemistry; and an ad hoc reviewer for the Journal of Biological Chemistry, Molecular and Cellular Biology, European Molecular Biology Organization Journal, Nucleic Acids Research, RNA Journal, and Journal of Clinical Investigation.

21. Among my recent federal study section service, I participated in the U.S. Department of Defense's 2007 Breast Cancer Research Program – Molecular Biology & Genetics Study Section, and 2009 Breast Cancer Research Program – Concept – Molecular Biology & Genetics Study Section; and the National Institute of Health's October 2007 KO8 – KO2 Awards – NHLBI Study Section, February 2008 Oncological Sciences (F09) Study Section, June 2008 Oncological Sciences (F09) Study Section, July 2008 KO8 KO2 Awards – NHLBI Study Section, and June 2009 Oncological Sciences (F09) Study Section.

22. Further, I am co-author or co-reviewer of several book chapters, including:

- Leslie Pick, Henry M. Furneaux & Jerard Hurwitz, Purification and characterization of wheat germ ligase and associated activities, 181 Methods Enzymology, 18-36 (1990).
- Jerome B. Posner & Henry M. Furneaux, Paraneoplastic neurological syndromes, 68 Proc. Ass'n. Rsch. Nervous Mental Disease, 187-219 (1990).
- Henry M. Furneaux, Neuronal nuclear auto antibodies, Type 1 (Hu), in Auto antibodies, Elsevier Science, 551-54 (J.B. Peter and Y. Shoenfeld eds., 1996).

- B. Joseph & Henry M. Furneaux, cDNA Libraries and Neurological Disease, 55(6) Archives Neurology, 785-88 (1998).
- Sung-Suk Chae, Ji-Hye Paik, Jonathan Schubert-Coleman, & Henry Furneaux, Timothy Hla: Application of the RNA Interference (RNAi) Technology to Angiogenesis Research, Methods in Endothelial Cell Biology - Springer Lab, (H. Augustin ed., 2004).

23. Among my educational and teaching experience, I was: Designer and Director of the Molecular Basis of Neurological Disease, a course for MD/PhD students, at the Cornell University Graduate School of Medical Sciences (now known as the Weill Cornell Graduate School of Medical Sciences) (1990-2000); Designer and Director of the Molecular Basis of Disease, a course for PhD and MD/PhD students, at the University of Connecticut Medical School (2001-2009); Course Director of the Molecular Biology and Biochemistry Program Journal Club at the University of Connecticut Medical School (2001-2006); Lecturer in Biochemistry I, a first year biochemistry course for PhD students, at the University of Connecticut Medical School (2002-2006); Lecturer in Organ Systems II, a first year medical school course, at the University of Connecticut Medical School (2003-2006); Lecturer in outreach discovery lectures to encourage research careers for high school students (2003-2009); Co-designer and teacher of The Logic of Biology, a

new discussion course for all incoming first year students to introduce them to experimental logic and contemporary problems in biology, at the University of Connecticut (2006-2009); and Lecturer in HB3, a first year medical school course, including design of a new introductory module that combines the fields of vascular biology, hematology and immunology, at the University of Connecticut Medical School (2007-2009).

24. Among my recent major scholastic administrative tasks, I was: Director of the Graduate Program in Biochemistry and Molecular Biology at the University of Connecticut Medical School (2003-2009); member of the executive committee of the Department of Molecular, Microbial and Structural Biology at the University of Connecticut Medical School (2003-2009); member of the Graduate School Planning Committee at the University of Connecticut Medical School (2004); first year graduate student advisor at the University of Connecticut Medical School (2004-2007); and member of the Scientific Advisory Committee – General Clinical Research Center at the University of Connecticut Medical School (2005-2009).

C. Industry Experience

25. I am currently the Chief Executive Officer of Clonegene LLC (“Clonegene”) in Hartford, Connecticut. I have held this position since 2009.

Clonegene is a for-profit company that designs, generates and distributes quality reagents used in Biochemistry research. These include monoclonal antibodies, RNA binding proteins, enzymes and synthetic mRNA. In particular, I use my skills to design novel research reagents based on my understanding of the needs of contemporary researchers.

D. Professional Society Involvement

26. Among the scientific societies and honors I have earned are: Member of the New York Academy of Science; Member of The Harvey Society; Member of The RNA Society; Holder of the Neil Levitan Chair in Neuro-Oncology at SKI, of the MSKCC (1998-2000); and cited in “Who’s Who in America.”

III. MATERIALS CONSIDERED

27. In forming my opinions, I have reviewed the following documents and materials:

- The '783 and '611 patents;
- The declarations submitted by Dr. John Quackenbush in these proceedings (Exhibit 1020 in IPR2023-00545 and Exhibit 1061 in IPR2023-00546) as well as exhibits cited in the Quackenbush declarations.
- The Board’s institution decisions in these matters; and

- Materials cited in this declaration.

IV. LEGAL STANDARDS

28. I am not a patent attorney. I have been informed of the following legal standards that apply in this matter.

A. Anticipation

29. I have been informed that anticipation (lack of novelty) is established only when it is proven that a single prior art reference discloses each and every limitation of the claimed invention, either explicitly or inherently, as the limitations are arranged in the claim. I have also been informed that a single prior art reference may be deemed to “inherently anticipate” a claim if and only if a limitation that is not expressly stated in the reference is necessarily present in the product or process that the reference describes, not merely probably or possibly present in that product or process.

B. Nonobviousness

30. I understand that a patent claim is invalid as obvious if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious to a person of ordinary skill in the art at the time of the invention. I understand that an obviousness analysis considers the following factors: (a) the scope and content of the prior art; (b) the

differences between the prior art and the claims; (c) the level of ordinary skill in the art; and (d) any objective indicia of non-obviousness (such as unexpected results, skepticism in the field, industry praise, and commercial success). I understand that such objective indicia of nonobviousness must have a connection or “nexus” to the claims. I further understand that where the objective evidence is tied to a specific product that embodies the claimed invention and is coextensive with them, the objective evidence is entitled to a rebuttable presumption of nexus.

31. In connection with obviousness, I understand there should have been some reason that would have led a person of ordinary skill in the art to combine or modify the prior art to obtain the claimed invention. Furthermore, there must have been a reasonable expectation that a person of ordinary skill in the art would have succeeded in obtaining the claimed invention based on the prior art.

V. THE PERSON OF ORDINARY SKILL IN THE ART

32. I understand that the Board has adopted the definition of the person of ordinary skill in the art proposed by Petitioner Foresight in these proceedings. The Board has preliminarily determined that a person of ordinary skill in the art would have had:

- 1) an advanced degree (such as an M.D. or the equivalent of a Ph.D. in a life or physical sciences, engineering, or mathematics discipline),

- 2) an understanding of various sequencing and next generation sequencing methods, sample preparation methods, targeted enrichment methods, bioinformatics methods for interpreting sequencing data, and methods for identifying genetic variants in a sample,
- 3) an understanding of clinical applications of sequencing technology to conditions associated with nucleic acid variation, such as cancer, non-invasive prenatal testing, and transplant rejection, and
- 4) an understanding of cancer biology as it relates to mutations associated with tumor diagnosis, progression, and treatment, as well as awareness of the various types of mutations that can occur in and influence the course of a patient's cancer.

Institution Decision at pages 18-19.¹ I have applied this definition of a person of ordinary skill in the art in forming the opinions described in this declaration.

¹ The citations in this section of the declaration refer to the Institution Decision from IPR2023-00545. I understand that the Board made equivalent findings on the definition of a person of ordinary skill in the art in the Institution Decision from IPR2023-00546.

VI. THE GROUNDS CHALLENGING PATENTABILITY OF THE '783 AND '611 PATENTS

33. I understand that the Board has instituted trial on the following three grounds raised by Foresight in challenging patentability of the claims of the '783 patent:

Ground	'783 patent claims challenged	Type of challenge	Reference(s)
1	1-2, 5-7, 9, 14-15, 17-19, 21, 23-25, 27	Anticipation	Ley (EX1012)
2	1-9, 13-19, 21-29	Obviousness	Ley (EX1012)
3	1-9, 13-19, 21-29	Obviousness	Forsheew (EX1030) & Dawson (EX1070)

34. I understand that the Board has instituted trial on the following four grounds raised by Foresight in challenging patentability of the claims of the '611 patent:

Ground	'611 patent claims challenged	Type of challenge	Reference(s)
1	1-2, 4-5, 9-11, 13, 18, 19, 28-30	Anticipation	Ley (EX1012)
2	1-5, 9-11, 13, 18, 19, 28-30	Obviousness	Ley (EX1012)
3	1-5, 9-11, 13, 18, 19, 28-30	Anticipation	Newman (EX1043)
4	1-5, 9-11, 13, 18, 19, 28-30	Obviousness	Newman (EX1043)

35. The challenges raised by Foresight Diagnostics do not render any of the claims of the '783 and '611 patents unpatentable for reasons described herein.

36. Below is a table that includes a side-by-side comparison of claim 1 from the '783 and '611 patents to show some similarities and differences between the claims:

'783 Patent Claim 1	'611 Patent Claim 1
1. A method for personalized genetic testing, comprising:	1. A method for personalized genetic testing, comprising:
	(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;
	(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set

'783 Patent Claim 1	'611 Patent Claim 1
	of genetic variants, wherein said set of genetic variants are identified with respect to a reference;
(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample,	(c) obtaining a probe set comprising a plurality of nucleic acid probe molecules having said plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in said at least one biological sample;
further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data	
(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;	(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and

'783 Patent Claim 1	'611 Patent Claim 1
(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and	(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).
(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.	

VII. FORESIGHT HAS NOT PROVEN UNPATENTABILITY OF ANY CLAIM FROM THE '783 PATENT

37. This section of my declaration provides an overview of the '783 patent, followed by an explanation of why each of Grounds 1-3 raised by the Petition do not prove anticipation or obviousness of the claims of the '783 patent.

A. Overview of the '783 Patent

38. The '783 patent relates to systems and methods for sample processing and data analysis for use in the diagnosis, monitoring, treatment, and prevention of diseases. For example, the abstract of the '783 patent states:

This disclosure provides systems and methods for sample processing and data analysis. Sample processing may include nucleic acid sample processing and subsequent sequencing. Some or all of a nucleic acid sample may be sequenced to provide sequence information, which may be stored or otherwise maintained in an electronic storage location. The sequence information may be analyzed with the aid of a computer processor, and the analyzed sequence information may be stored in an electronic storage location that may include a pool or collection of sequence information and analyzed sequence information generated from the nucleic acid sample. Methods and systems of the present disclosure can be used, for example, for the analysis of a nucleic acid sample, for producing one or more libraries, and for producing biomedical reports. Methods and systems of the disclosure can aid in the diagnosis, monitoring, treatment, and prevention of one or more diseases and conditions.

EX1001 (the '783 patent) at Abstract.

39. The '783 patent includes “method[s] for personalized genetic testing.”

See EX1001 (the '783 patent) at 72:19, which is the preamble of claim 1.

40. Claim 1 is the only independent claim, and it recites four steps (a)-(d) as shown below:

Claim 1. A method for personalized genetic testing, comprising:

(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample, further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data;

(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;

(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a

sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and

(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.

EX1001 (the '783 patent) at claim 1.

41. The specification of the '783 patent explains that high costs previously limited “clinical adoption of exome-scale sequencing,” and that “[t]his problem is even worse in cancer, where the depth of deoxynucleic acid (DNA) sequencing required can be much higher (e.g., >500×) than that for inherited diseases (e.g., 30-100×).” EX1001 (the '783 patent) at 4:30-32; 4:33-36. And “[w]hile array-based DNA synthesis is now widely used to capture whole exomes, transcriptomes, or application-specific subsets of exomes (e.g., the genes involved with a specific Mendelian disease), a limitation of the field, as recognized herein, is the potential to leverage array synthesis of DNA in a personalized manner.” *Id.* at 4:37-42. Instead, “[t]he field has largely used array-based synthesis to develop standard products which are broadly applicable across a whole set of human patients and/or research subjects.” *Id.* at 4:42-45; *see also id.* at 4:45-47 (“Even where custom array synthesis

is proposed, it is to sequence regions of the genome defined independent of a specific sample.”).

42. The methods claimed by the '783 patent are directed to “personalized genetic testing.” See EX1001 (the '783 patent) at 72:19, which is the preamble of claim 1. The claimed method first requires obtaining a personalized probe set, which can “include probes to a genomic region or a panel comprising multiple genomic regions” and “can be amplification based or capture hybridization-based.” EX1001 (the '783 patent) at 29:17-19. A probe “generally refer[s] to a nucleic acid molecule comprising a single-stranded portion capable of hybridizing to a complementary nucleic acid sequence,” and “can be used for detection or enrichment of [said] nucleic acid molecules.” EX1001 (the '783 patent) at 29:5-9. “A probe may be included in a set, or plurality, of probes.” EX1001 (the '783 patent) at 29:14-15. The claimed probe set includes probes that are complementary to and thereby configured to enrich or amplify the identified genetic variants. EX1001 (the '783 patent) at 29:4-9; *see also generally id.* at Example 7 (describing exemplary methods to synthesize probes). Further, the claimed method requires a personalized probe set comprising nucleic acid probe molecules having oligonucleotide-directed genomic content comprising (i) at least one variable portion from a result of said sequence

data and (ii) at least one fixed portion independent of said result of said sequencing data. *See* EX1001 (the '783 patent) at 72:20-35, which is limitation 1(a).

43. Second, the personalized probe set is then applied to an “additional biological sample” to selectively enrich or amplify sequences comprising the genetic variants. EX1001 (the '783 patent) at 11:3-7. The additional biological sample may be a samples of the same tissue from same subject taken at a later time point, a sample of different tissue from the same subject, or a sample from a biological relative of the subject, for example. *See, e.g.*, EX1001 (the '783 patent) at Example 1; *id.* at 5:4-10. The enriched or amplified sequences of the genetic variants generate a sequence library. EX1001 (the '783 patent) at 42:52-62; *see also id.* at 72:36-40, which is limitation 1(b).

44. Third, the sequence library is sequenced to identify a presence or absence of the genetic variants in the additional biological sample. The sequencing footprint of this step is less than the sequencing footprint of the sequencing data used to generate the personalized probe set in the first step. For example, to test for 500 variants this lower footprint sequencing step may require synthesis of about “175,000 bases.” EX1001 (the '783 patent) at 63:14-17 (“The genomic region captured by each such probe may be at least about 350 bases. Therefore, for at least about 500 sequences, the footprint of this assay may be about 175,000 bases.”).

“Compared to [sequencing] an exome, where the footprint assay is typically at least 35 million bases, this may result in 200x less sequencing.” EX1001 (the '783 patent) at 63:17-20. This allows for a “dramatic reduction” in the number of regions that need to be sequenced and thus allows sequencing to be more affordable for family members. EX1001 (the '783 patent) at 63:20-24. With the reduction in sequencing footprint, the sequencing depth can be greater than the depth of the first sequencing step without being cost prohibitive, which the specification explains as follows:

To increase the confidence of detecting a potentially mosaic variant in the parents, they will need to be sequenced at greater depth than the child. If the assay for additional members of a family pedigree is exome sequencing, it may be cost prohibitive to sequence at that depth. Using method provided herein, the footprint of the assay for incremental family members is much smaller (e.g., 175,000 bases vs 35 million bases, as discussed above). This smaller footprint makes it affordable to sequence the incremental samples at much greater depth, thus improving the sensitivity for detection of mosaic variants in the parents.

EX1001 (the '783 patent) at 64:41-52; *see also id.* at 72:41-47, which is limitation 1(c). I note that the specification states that “[t]he term ‘mosaicism,’ as used herein, generally refers to genetic changes which occur after an embryo has started to develop. These changes will only be found in a fraction of the cells of a human

body.” *Id.* at 30:22-24. In other words, mosaicism refers to genetic changes which occur after the sperm fuses with the egg.

45. Finally, a report is generated which identifies both (i) whether a health condition or disease state is present based on identification of genetic variants tested in the third step from the additional biological sample, and (ii) genetic variants which inform therapy choices or changes in therapy for the patient. This report may vary based on the type of medical conditions being examined. *See, e.g.*, EX1001 (the '783 patent) at 8:36-39 (reports may identify “genetic variants shared by family members of the same family pedigree”); *id.* at 8:40-46 (reports may be “generated based at least in part on a possibility that one or more genetic variants of the subject are mosaic and included in the ectodermal lineage of the subject”); *id.* at 8:47-52 (reports may be generated “in part on a possibility that one or more of the genetic variants are gonadal mosaic in a father of the subject”); *see also id.* EX1001 (the '783 patent) at 72:48-53, which is limitation 1(d).

B. GROUND 1: NOVELTY OVER LEY

46. Ley (EX1012) does not anticipate independent claim 1. Ley further does not anticipate dependent claim 15 for additional reasons discussed below.

1. Claim 1

47. Ley (EX1012) fails to anticipate claim 1 because it does not teach any of the four limitations required by the claim. In particular, Ley does not teach:

- “(i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data” as required by limitation 1(a),
- an “additional biological sample from said subject” as required by limitation 1(b),
- “subjecting said sequencing library to sequencing *to identify* a presence or absence of at least a subset of said set of genetic variants” as required by limitation 1(c), and
- “generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject” as required by limitation 1(d).

a. Ley does not teach Limitation 1(a)

48. Limitation 1(a) recites “obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules

having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample, ***further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data.***²

49. Ley does not teach a personalized probe set comprising nucleic acid probe molecules having oligonucleotide-directed genomic content comprising “(i) ***at least one variable portion*** from a result of said sequence data ***and*** (ii) ***at least one fixed portion*** independent of said result of said sequencing data” as required by limitation 1(a).

² All emphases supplied unless otherwise noted.

i. **Claim construction of “variable portion” and “fixed portion”**

50. I understand that claim terms generally are afforded their plain and ordinary meaning to a person of ordinary skill in the art. I understand, however, that when a specification contains a definition for a term, then that definition governs the meaning of the term.

51. The recited “variable portion” and “fixed portion” do not have a plain meaning to a person of ordinary skill in the art. However, the specification of the '783 patent provides an express definition of “variable portion” and “fixed portion” as follows:

In some applications, it may be desirable for the oligo-directed genomic content of the personalized assay to contain both ***a variable portion (defined based on the genetic characteristics originally determined for the individual subject) and at least one fixed portion (which does not change from one subject to another).***

EX1001 (the '783 patent) at 41:45-50. The variable portion changes from subject to subject because it is “defined based on the genetic characteristics originally determined for the individual subject,” whereas the fixed portion “does not change from one subject to another.” *Id.*; see also *id.* at 66:36-39 (“Thus, as discussed above, the genomic content of a personalized genetic assay may include ***a portion which is variable subject to subject, and another portion which is fixed.***”). The

title of the section describing variable and fixed content is “Combinations of ***Fixed and Variable (personal)*** Genomic Content in the Array-Synthesized Nucleic Acids.” EX1001 (the '783 patent) at 41:40-41. The variable portion is “personal” to the subject, whereas the fixed portion does not change from subject to subject.

52. Limitation 1(a) provides further clarification regarding the “variable portion” and “fixed portion” by stating that the variable portion is “from a result of said sequence data” and the fixed portion must be “independent of said result of said sequencing data.” EX1001 (the '783 patent) at 72:32-35.

53. Based on the definition in the specification and the claim language that provides further clarification on the meaning of “variable portion” and “fixed portion” within the context of the claimed invention, *the “variable portion” recited by limitation 1(a) should be construed to refer to a portion “which changes from one subject to another and results from said sequence data,” and the “fixed portion” should be construed to refer to a portion “which does not change from one subject to another and is independent of said result of said sequencing data.”*

ii. **Ley does not teach a variable portion or fixed portion**

54. Dr. Quackenbush states in his declaration that “Ley does not provide the primer sequences or the exact exons that were analyzed.” EX1020

(Quackenbush Declaration IPR2023-00545) ¶124. Ley does not disclose PCR primer sequences in either the body of the paper (EX1012) or the Supplementary Information (EX1042). In fact, the word “primer” does not appear in Ley (EX1012) or Ley’s Supplementary Information (EX1042).

55. Dr. Quackenbush asserts that Ley (EX1012) discloses PCR primers “for known mutations of the BRCA2 and TP53 genes” that supposedly meet the “fixed portion” of limitation 1(a). EX1020 (Quackenbush Declaration IPR2023-00545) ¶120. Ley reports that the amino acid at position 372 in the BRCA2 protein, derived from one allele, had changed from asparagine to histidine, namely Ley reports N372H. Ley reports that the amino acid at position 72 in the TP53 protein, derived from both alleles, had changed from proline to arginine, namely P72R. See Ley (EX1012) at page 70, Table 3.

56. The asparagine (N) at position 372 of the amino acid sequence is coded by an AAT codon in the reference DNA sequence of the human BRCA2 gene. This is easily determined using simple software that converts the nucleotide sequence provided in the Genbank Record for BRCA2 in Exhibit 1058. The amino acid histidine (H) is coded by either a CAT codon or a CAC codon. See *e.g.*, EX2172 (Lewin) at page 172 (providing DNA codons for amino acids). Thus, the genetic variant reported in Ley’s subject could have had either of these two codons in the

affected allele. Ley does **not disclose** which of these two possible codons were present in the subject's BRCA2 gene. Dr. Quackenbush's assertion that Ley's subject had "known mutations of the BRCA2" gene does not answer whether the mutation in the DNA sequence of the subject's BRCA2 gene changes from one subject to another. Because there are two different potential codons that Ley's subject may have had, the genomic content created by Ley's PCR primers will change from one subject to another. If the genomic content of Ley's PCR primers changes from one subject to another, then they would not meet the "fixed portion" recited by limitation 1(a) because the "fixed portion" must "not change from one subject to another" under the appropriate construction of that term.

57. The proline (P) at position 72 of the amino acid sequence is coded by a CCC codon in the reference DNA sequence of the TP53 gene. This is easily determined using simple software that converts the nucleotide sequence provided in the Genbank Record for TP53 in Exhibit 1059. The amino acid arginine (R) is coded by CGC, CGT, CGA, CGG, AGA, or AGG codons. See *e.g.*, EX2172 (Lewin) at pages 172 (providing DNA codons for amino acids). Thus, Ley's subject could have had any of these six codons in each TP53 allele. Ley does not disclose which of these six possible codons were present in the subject's alleles. Dr. Quackenbush's assertion that Ley's subject had "known mutations" of the TP53 gene does not

answer whether the mutation in the DNA sequence of the subject's TP53 gene changes from one subject to another. Because there are at least six different potential codons that Ley's subject may have had, the genomic content created by Ley's PCR primers could very well change from one subject to another. If the genomic content of Ley's PCR primers changes from one subject to another, then they would not meet the "fixed portion" recited by limitation 1(a) because the "fixed portion" must "not change from one subject to another" under the appropriate construction of that term.

58. If Dr. Quackenbush had asserted that the PCR primers used by Ley to generate amplicons of the subject's BRCA2 and TP53 mutations do not change from one subject to another, then he should have also explained how the PCR primers used by Ley to generate amplicons of the other eight mutations from Ley that he alleges meet the claimed "variable portion," see EX1020 ¶119, do not actually constitute "fixed portions" that do not change from one subject to another. I note that the other eight mutations were located in a patient with "a **typical** acute myeloid leukaemia genome," rather than an atypical genome. EX1012 (Ley) at page 66. Ley explains that specific patient being studied, UPN 933124, was selected because the patient had "[t]**ypical** features of M1 AML" that was "**representative of most patients** with M1 AML." EX1042 (Ley Supplementary Information) at page 1. Ley "required a gene expression profile **typical** of M1 AML with **normal cytogenetics**,

to assure that the sample was ***not an outlier*** for this subtype.” EX1042 (Ley Supplementary Information) at page 1. Because Ley’s patient was selected to be typical and representative of most patients with M1 AML, then the PCR primers used by Ley to generate amplicons of the other eight mutations that Dr. Quackenbush relies upon to supposedly meet the “variable portion” could very well ***not*** change from one subject to another. Ley states that “[a]ll of the PCR-amplified exons from the eight genes containing validated somatic mutations were sequenced in 187 further cases of AML using samples from our discovery and validation sets; no further somatic mutations were detected in these genes (data not shown).” EX1012 (Ley) at page 69. The data from these patients is not provided. There is no information contained within Ley (EX1012) or Ley’s Supplementary Information (EX1042) to know with certainty what the genomic content of Ley’s undisclosed PCR primers was to make a determination if Ley discloses something that meets the claimed “variable portion” and/or “fixed portion.”

b. Limitation 1(b)

59. Limitation 1(b) recites “using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences ***in at least an additional biological sample from said subject***, to generate

a sequencing library.” Thus, limitation 1(b) requires an “additional biological sample from said subject.”

60. Dr. Quackenbush relies on the **same** biological samples from Ley (EX1012) to supposedly meet the “at least one biological sample of said subject” required by limitation 1(a), see EX1020 ¶¶113-114, as well as the “additional biological sample from said subject” required by limitation 1(b), see EX1020 ¶121.

61. First, he relies on the “the primary tumor, skin sample, and relapse tumor sample” from Ley to allegedly meet the “at least one biological sample of said subject” of limitation 1(a). See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶113-114. I note that Dr. Quackenbush also relies upon those same three samples from Ley to meet claim 2. Claim 2 depends from claim 1 and recites that the “at least one biological sample” from limitation 1(a) comprises “a plurality of biological samples.” Dr. Quackenbush relies on “the primary tumor sample, the relapse tumor sample, and the skin biopsy sample” from Ley to allegedly meet the “at least one biological sample” recited by claim 2. See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶132-133. Accordingly, Dr. Quackenbush’s position is clear—he relies on all three samples from Ley to allegedly meet the “at least one biological sample of said subject” of limitation 1(a). See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶113-114, 132-133.

62. Second, he relies on the same “relapse tumor” and “skin biopsy” samples to assert that Ley anticipates the “additional biological sample from said subject” for limitation 1(b). See EX1020 (Quackenbush Declaration IPR2023-00545) ¶121.

63. Because Dr. Quackenbush relies on the relapse tumor and skin biopsy samples to assert that Ley anticipates the “at least one biological sample of said subject” of limitation 1(a), there are no additional samples left in Ley to meet the “additional biological sample from said subject” required for limitation 1(b). Dr. Quackenbush relies on the “relapse tumor” and “skin biopsy” samples to assert that Ley anticipates the “at least one biological sample of said subject” for limitation 1(a), see EX1020 ¶¶113-114, and again relies on the same “relapse tumor” and “skin biopsy” samples to assert that Ley anticipates the “additional biological sample from said subject” for limitation 1(b), see EX1020 ¶121. Thus, Dr. Quackenbush’s anticipation position relies on double-counting Ley’s relapse tumor and skin biopsy samples.

c. Limitation 1(c)

64. Limitation 1(c) requires “subjecting said sequencing library to sequencing *to identify* a presence or absence of at least a subset of said set of genetic

variants.” Ley does not teach limitation 1(c) under the appropriate construction of the term “to identify.”

i. Claim construction of “to identify”

65. I understand that claim terms generally are afforded their plain and ordinary meaning to a person of ordinary skill in the art.

66. I also understand that the Board preliminarily determined that “step 1(c) is silent as to whether a genetic variant is identified de novo in an unknown sample, or identified in a previously tested sample for some validating or confirmatory purpose.” Institution Decision at page 29. I understand that the Board also preliminarily determined that “[i]n showing that 10 variant alleles were present in both primary and relapse tumor samples, Ley Figure 3 satisfies claim step 1(c) by ‘identifying a presence of at least a subset of said set of genetic variants’ in a patient sample.” *Id.*

67. I disagree with the Board’s preliminary determinations because they do not comply with how a person of ordinary skill in the art would have understood the plain meaning of “to identify” as recited by limitation 1(c).

68. The specification does not define “to identify.” I understand that this term should therefore be accorded its plain and ordinary meaning to a person of ordinary skill in the art.

69. The dictionary definition of “identify” is “to establish the identity of.” See EX2170 (American Heritage Dictionary, 4th edition, 2000) at page 871. Thus, a person of ordinary skill in the art would have understood the plain meaning of “to identify” as recited by limitation 1(c) to mean “*to establish the identity of.*”

ii. **Ley does teach limitation 1(c) under the plain meaning of “to identify”**

70. Dr. Quackenbush believes that Ley (EX1012) performs separate steps of identification and validation. Specifically, he states “[o]nce the variants of interest, i.e., the ‘putative variants,’ *were identified, Ley then describes ‘validation* using custom PCR and capillary sequencing’ as well as sequencing of ‘validated somatic mutations’ ‘by Roche/454 sequencing of PCR-generated amplicons made from primary genomic DNA.” EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. I note that the “validation” step referenced by Dr. Quackenbush actually has two separate steps. For the “custom PCR and capillary sequencing” step, Ley confirms the exon in which the mutation is present. For the “Roche/454 sequencing” step, Ley determines the prevalence of the genetic variants by quantifying readcount data. Thus, Ley initially identifies variants of interest, and then separately performs the “validation” referenced by Dr. Quackenbush.

71. Ley's identification step uses sequencing data to establish the identity of somatic mutations in the typical AML genome. See EX1012 (Ley) at pages 68-69 (section of Ley entitled "Identification of somatic mutations in coding sequences"). For this identification, Ley sequenced PCR-generated amplicons to "determine[] that 152 of these variants were false positive (that is, wild type) calls, 14 were inherited SNPs, and eight were somatic mutations in both the original tumor and the relapse sample (Table 2)." EX1012 (Ley) at page 69.

72. The "validation" protocol from Ley that Dr. Quackenbush relies upon to allegedly meet limitation 1(c) is a separate protocol from Ley's identification step. For example, Dr. Quackenbush relies on Ley Figure 3. See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶122-124. Ley's Figure 3 depicts the quantities of genetic variants present in Ley's subject based on readcount data:

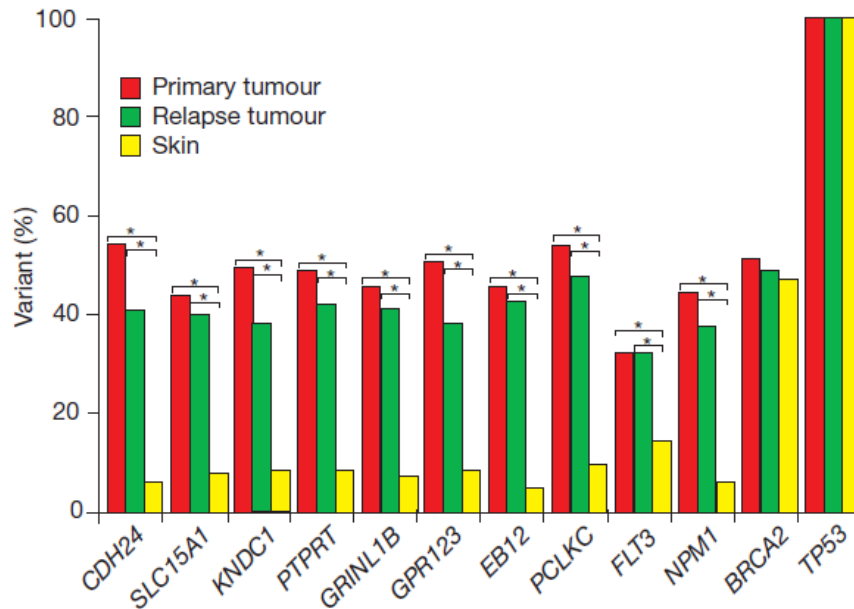


Figure 3 | Summary of Roche/454 FLX readcount data obtained for ten somatic mutations and two validated SNPs in the primary tumour, relapse tumour and skin specimens. The readcount data for the variant alleles in the primary tumour sample and relapse tumour sample are statistically different from that of the skin sample for all mutations ($P < 0.000001$ for all mutations, Fisher's exact test, denoted by a single asterisk in all cases). Note that the normal skin sample was contaminated with leukaemic cells containing the somatic mutations. The patient's white blood cell count was 105,000 (85% blasts) when the skin punch biopsy was obtained.

EX1012 (Ley) at page 69, Figure 3. The mutation frequencies are quantified during the validation protocol by tallying readcounts. The identity of the mutations was established in the proceeding (and separate) protocol described in the section entitled "Identification of somatic mutations in coding sequences" on pages 68-69 of Ley. In fact, to pursue this analysis, it must be the case that the variant is no longer regarded as putative but is already regarded as validated.

73. A person of ordinary skill in the art would not have confused Ley's "validation" protocol with the claimed "to identify" step of limitation 1(c). Rather, a person of ordinary skill in the art would understand that Ley teaches an identification step that is distinct and separate from Ley's "validation" step. This understanding is supported by Dr. Quackenbush's explanation that "[o]nce the variants of interest, i.e., the 'putative variants,' **were identified, Ley then describes 'validation** using custom PCR and capillary sequencing' as well as sequencing of 'validated somatic mutations' 'by Roche/454 sequencing of PCR-generated amplicons made from primary genomic DNA.'" EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. The result of Ley's "validation" step is the quantification of variant frequencies by tallying readcount sequencing data rather than establishing the identity of a presence or absence of variants as claimed by limitation 1(c).

d. Limitation 1(d)

74. Limitation 1(d) requires "generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said biological sample based on said identified at least a subset of (c) and (ii) genetic variants **which inform a therapy choice or a change in therapy for the subject.**" Ley (EX1012) does not teach limitation 1(d) under the appropriate construction of this limitation.

i. **Claim construction of limitation 1(d)**

75. I understand that the Board preliminarily agreed with arguments made by Foresight that limitation 1(d) should not be given patentable weight. See Institution Decision at pages 23-25. I understand that the Board preliminarily determined that “even if step 1(d) were somehow interpreted as further requiring diagnosing a medical condition and/or informing a therapy, we would agree at present with Petitioner [Foresight] that such ‘diagnosing’ or ‘informing’ encompass mere mental steps” that are not entitled to patentable weight. *Id.* at page 24. I further understand that Personalis has not previously addressed the arguments raised by Foresight, and now has the opportunity to address those arguments at the present time.

76. Limitation 1(d) requires “generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said biological sample based on said identified at least a subset of (c) and (ii) genetic variants ***which inform a therapy choice or a change in therapy for the subject.***” Limitation 1(d) requires a specific contemporaneous action (i.e., informing a therapy choice or a change in therapy for the subject) as a result of the information in the report that identifies genetic variants. It is my opinion that limitation 1(d) is not directed to a mere mental step because it requires a therapy choice or a change in therapy to be made for the

subject, and that therapy choice or change in therapy must be informed by the genetic variants identified in the report. Further, there is a functional relationship between the information in the report that identifies genetic variants of limitation 1(d) and the concrete step of informing a therapy choice or a change in a therapy for the subject in limitation 1(d). The functional relationship is that the therapy choice or change in therapy must be informed by the genetic variants identified in the report.

ii. Ley does not teach limitation 1(d)

77. Dr. Quackenbush asserts that Ley Figure 3 “informs a therapy choice or a change (e.g., switching away from conventional therapy, or resuming therapy upon relapse) for the subject.” See EX1020 (Quackenbush Declaration IPR2023-00545) ¶127.

78. Ley (EX1012) does not inform a contemporaneous therapy choice or a change in therapy for the subject because the subject of Ley’s assay **was deceased** at the time when Ley’s assay was performed. The subject of Ley’s assay (UPN 933124) had “**expired** 24 months after her initial diagnosis” and “[i]nformed consent for whole genome sequencing **was subsequently obtained** from her next of kin and family members.” EX1042 (Ley Supplementary Information) at page 2. “**Subsequent to the death of patient 933124**, the WU HSC [Washington University Human Studies Committee] approved an amendment to the protocol that

incorporated language specifically permitting the use of banked DNA for whole genome sequencing, to which her husband consented.” EX1042 (Ley Supplementary Information) at page 3. I am not aware of any therapy choice or change in therapy that is available for the deceased subject of Ley’s assay.

C. GROUND 2: NONOBVIOUSNESS OVER LEY

79. Ley does not render obvious independent claim 1. Ley also does not render obvious dependent claims 3, 8, 15, 16, and 22 for additional reasons discussed in this section.

1. Claim 1

80. Ley does not suggest any of the four limitations required by claim 1. Each of the four limitations are addressed below.

a. Limitation 1(a)

81. Dr. Quackenbush alleges that Ley’s assay included twelve PCR primer pairs: ten PCR primer pairs for “non-synonymous” mutations and two PCR primer pairs for “control” mutations as follows:

Those skilled in the art would know that the creation of “PCR-generated amplicons made from genomic DNA” for sequencing by the Roche/454 platform necessarily involves the use of a probe set, specifically PCR primers directed to the validated somatic mutations. *In Ley, the probe set would thus necessarily include PCR primers that hybridize to the*

10 non-synonymous (variable) mutations identified in the patient's tumor (CDH24, SLC15A1, KNDC1, PTPRT, GRINL1B, GPR123, EB12, PCLKC, FLT3, NPM1) and the two fixed (control) mutations (BRCA2, TP53).

See EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. As discussed above, Ley does not disclose the sequence of any of the PCR primers that Dr. Quackenbush alleges were necessarily included in Ley's assay. *Supra* Section VII.B.1.a.

82. Dr. Quackenbush asserts that a person of ordinary skill in the art would have been motivated to modify Ley's assay (which he asserts already includes twelve undisclosed PCR primer pairs) to include even more PCR primer "probes targeted to [1] genetic variants involved in the pharmacogenomics of cancer drugs, i.e., genetic variants that were known to have companion diagnostics, as well as to [2] tumor-driver mutations that were known in the art" to allegedly meet the "fixed portion" of limitation 1(a). See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶182-187.

83. There are at least three reasons why a person of ordinary skill in the art would not have been motivated to further complicate Ley's assay with even more PCR primer pairs directed to genetic variants involved in the pharmacogenomics of cancer drugs and tumor-driver mutations that were known in the art. Each of the three reasons are discussed below.

i. **Reason 1: Ley counsels against the modification proposed by Dr. Quackenbush**

84. First, Ley teaches “[o]ur results strongly support the notion that hypothesis-driven (for example, candidate gene-based) examination of tumor genomes by PCR-directed or capture-based methods is inherently limited, and will miss key mutations.” EX1012 (Ley) at page 70. Dr. Quackenbush’s obviousness position requires a person of ordinary skill in the art to add more PCR primer pairs to Ley’s assay, where the additional PCR primer pairs would be directed to (1) genetic variants involved in pharmacogenomics of cancer drugs and (2) tumor-driver mutations known in the prior art. See EX1020 (Quackenbush Declaration IPR2023-00545) ¶182. Adding PCR primer pairs directed to genetic variants involved in pharmacogenomics of cancer drugs and tumor-driver mutations known in the prior art are the type of “hypothesis-driven” examination of tumor genomes by PCR-directed methods that Ley describes as inherently limited and that will miss key mutations. Thus, Ley’s own teaching counsels a person of ordinary skill in the art to avoid Dr. Quackenbush’s proposed modification of Ley’s assay.

85. Rather than increase the number of PCR primers used in Ley’s assay, a person of ordinary skill in the art would have instead sought to **decrease** the number of PCR primers for several reasons. I have reviewed the transcript of Dr.

Quackenbush's October 20 deposition for this case. Dr. Quackenbush testified that a person of ordinary skill in the art would have been motivated by four factors when designing assays related to tumors:

- (1) a desire to increase accuracy for an assay, *see* EX2163 (Quackenbush Transcript) at 17:19-20:1, 21:12-23:10;
- (2) a desire to reduce the cost of an assay, *see* EX2163 (Quackenbush Transcript) at 27:15-30:22;
- (3) a desire to simplify an assay, *see* EX2163 (Quackenbush Transcript) at 36:7-21; and
- (4) the desire to simplify the computational analysis for data generated by an assay, *see* EX2163 (Quackenbush Transcript) at 41:18-44:19.

Dr. Quackenbush's proposal to increase the number of PCR primers used by Ley's assay is counterproductive to each of these four factors.

86. Increasing the number of PCR primers would decrease the accuracy of the assay due to amplification issues that are discussed in the very next subsection of this Declaration entitled "Reason 2: Adding more PCR primers would complicate the assay."

87. Increasing the number of PCR primers would have been expected to increase the cost of the assay by requiring the design and synthesis of more PCR

primers (as well as increasing costs due to issues with potential complications associated with adding more PCR primers to an assay that are discussed in the next subsection of this Declaration). Cost is an important driver of assay design because if an assay is too expensive, then a person of ordinary skill in the art would not have the resources to run the assay. Labor costs in performing an assay is most often greater than the reagent costs required to perform an assay. I note that Dr. Quackenbush testified that cost was “really driving some of the science” because “[i]f the assay was simply too expensive, then one could not effectively run this assay” as follows:

Q. Do you feel that reducing sequencing costs was a real consideration for the person of skill in the art? Or is it really just merely a dollar sign? You can always write another grant application to get more dollars and it is not really that much of a consideration.

MR. KRAUSE: Objection, calls for speculation.

THE WITNESS: So, you know, based on your questions, you have never written a grant application, so you don't know how challenging it is to receive one. ***The budgets are fixed and often require balancing many different things.***

In our assays, you know, with a fixed budget, as a laboratory scientist, we have always striven to be as cost efficient as possible, because that typically allows us to collect data on larger numbers of subjects,

which in some applications is, is really driving some of the science that we do.

So, a person of ordinary skill in the art would consider that. *If the assay was simply too expensive, then one could not effectively run this assay.*

EX2163 (Quackenbush Transcript) at 29:6-30:10.

88. Adding PCR primers to Ley's assay would complicate the assay rather than simplify it. There were known complication issues with adding more PCR primers to an assay that are discussed in the very next subsection of this Declaration entitled "Reason 2: Adding more PCR primers would complicate the assay."

89. Also, adding PCR primers to Ley's assay would generate more data, which would increase the amount of computational analysis for the assay. Computation analysis for Ley's assay would include processing the sequencing data as described, for example, in the "Methods Summary" section of Ley. *See, e.g.,* Ley (EX1012) at page 71 (section entitled "Methods Summary").

ii. **Reason 2: Adding more PCR primers would complicate the assay**

90. Second, a person of ordinary skill in the art would not have been motivated to add even more PCR primer pairs to Ley's assay (which Dr. Quackenbush asserts already had twelve PCR primer pairs) because it would

complicate the assay. For example, Mamanova (EX1007) states that “PCR is difficult to multiplex to any useful degree: the simultaneous use of many primer pairs can generate a high level of nonspecific amplification, caused by interaction between the primers, and more-over amplicons can fail to amplify.” EX1007 (Mamanova) at page 112. Mertes (EX1006) teaches that PCR “does not scale easily, in any format” and “any significant extent of parallelized singleplex or multiplex PCR would need to be supported by the use of automated robotics” and the products “need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible)” as follows:

The main downside of the method is that it does not scale easily, in any format, to enable the targeting of very large genome subregions or many DNA samples. To use this method effectively, any significant extent of parallelized singleplex or multiplex PCR would need to be supported by the use of automated robotics, individual PCR amplicons (or multiplex products) need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible), and the amount of DNA material a study requires can be substantial as this requirement grows linearly with the number of utilized PCR reactions.

EX1006 (Mertes) at page 379.

91. As another example, two of Foresight’s founders (Ash Alizadeh and Maximilian Diehn)³ wrote that “technical limitations related to multiplexing of PCR assays have to date limited the number of genomic positions that can be interrogated” and that PCR assays can only use a “small combination of amplicons.” See Bratman, Alizadeh, and Diehn et al., “Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq,” *Expert Rev. Mol. Diagn.* 15:715-719 (2015), EX2164, at page 716. They also wrote that the “[l]imitations of [PCR-based] approaches include interrogation of relatively few genomic loci owing to difficulties related to multiplexing of PCR assays.” See Chaudhuri, Alizadeh, and Diehn et al., “Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA,” *Semin. Radiat. Oncol.* 25:305-312 (2015), EX2165, at pages 307-308. In 2021, Foresight founder Diehn wrote that “PCR-based assays have limited multiplexability.” See Dudley and Diehn, “Detection and Diagnostic Utilization of Cellular and Cell-Free Tumor DNA,” *Annu. Rev. Pathol. Mech. Dis.* 16:199-222 (2021), EX22166, at page 202.

³ Foresight was founded by Ash Alizadeh, Maximilian Diehn, David Kurtz, and Jake Chabon. EX2151.

iii. **Reason 3: Ley's mutations had unknown clinical utility**

92. Third, Dr. Quackenbush cites Ross (EX1014) and Burell (EX1071) to assert that a person of ordinary skill in the art would have been motivated to add to Ley's assay more PCR primer pairs for genetic variants involved in the pharmacogenomics of cancer drugs and tumor-driver mutations known in the prior art. See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶183-186. Ley (EX1012), however, explains that the mutations identified by Ley's assay had unknown clinical utility. For example, Ley explains "until hundreds (or perhaps thousands) of normal genomes and other AML tumours are sequenced, ***the contextual relevance of the mutations found in this genome will be unknown.***" EX1012 (Ley) at page 71. Ley further states that the function of the mutations found by its assay "***is not yet known.***" EX1012 (Ley) at page 66.

93. A person of ordinary skill in the art would not have been motivated to combine PCR primers for Ley's mutations of unknown clinical significance with PCR primers for genetic variants and tumor-driver mutations known in the prior art because Ross (EX1014) teaches to avoid assays that include "abnormalities of unknown clinical importance" as follows:

Given the potential generation of 'actionable' gene sequence abnormalities and abnormalities of unknown clinical importance, the

successful NGS-based clinical service will have a result report that is focused on the abnormalities that can lead directly to the best available therapy, direct a patient to an ongoing clinical trial, or result in some other actionable response from the treating oncologist.

EX1014 (Ross) at page 537. Ross further teaches it is not practical or feasible to test for all mutations of potentially important clinical significance in a given tumor “given the amount of tumor sample, time, and money it would take to perform so many individual tests.” EX1014 (Ross) at page 537.

94. Ross (EX1014) also teaches that “[c]linical applications, particularly in oncology, demand high levels of accuracy, sensitivity, and specificity for sequence-based tests focused on calling out specific genetic alterations.” EX1014 (Ross) at page 535. Foresight’s founders wrote that “only highly sensitive detection techniques are likely to be of clinical use.” See Chaudhuri, Alizadeh, and Diehn et al., “Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA,” *Semin. Radiat. Oncol.* 25:305-312 (2015), EX2165, at page 306. Adding even more PCR primers to the set of twelve PCR primers that Dr. Quackenbush asserts are already used with Ley’s assay would have been expected to raise concerns about reducing the accuracy, sensitivity, and specificity of Ley’s assay due to amplification issues associated with PCR primers discussed above, *see supra* Section VII.C.1.a.ii. A reduction in accuracy, sensitivity,

and specificity is something that Ross teaches a person of ordinary skill in the art to avoid.

95. I note that Ley's assay used the Illumina sequencer, ABI 3730 Sanger sequencer, and the Roche/454 sequencer. *See* EX1012 (Ley) at page 71. Ross (EX1014) teaches that the Illumina sequencer had "low multiplex capability." EX1014 (Ross) at 530-31, Table 3. Ross teaches that Sanger sequencing is limited "to single-gene or hot-spot sequence analysis," *id.* at page 535, and was believed to be "incapable of scaling sufficiently to support the breadth of information required to manage an ever-growing arsenal of targeted anticancer drugs," *id.* at page 528. Ross also teaches the Roche/454 sequencer had "low parallelism." EX1014 (Ross) at pages 530-31, Table 3. Based on at least the foregoing, a person of ordinary skill in the art would not have been motivated to modify Ley's sequencing workflow to add more PCR primers directed to genetic variants involved in the pharmacogenomics of cancer drugs and tumor-driver mutations known in the prior art.

96. As mentioned in the first sentence of the first paragraph of this subsection of this Declaration, Dr. Quackenbush cites Burell (EX1071) to assert that a person of ordinary skill in the art would have been motivated to add to Ley's assay more PCR primer pairs for genetic variants involved in the pharmacogenomics of

cancer drugs and tumor-driver mutations known in the prior art. *See* EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶183-186. Burell (EX1071) is a review paper that discusses genetic heterogeneity in cancer evolution. Burell discusses the problems of genetic heterogeneity without offering a solution. Burell teaches that the key driver mutations are present in a small minority population, which would not be revealed in Ley's assay that was only able to detect allele frequencies at approximately 3% for the "skin" sample. EX1012 (Ley) at 69, Figure 3. Thus, Ley's assay would not have been suitable to address the heterogeneity problems identified in Burell. The Burell reference was co-authored by Charles Swanton. Dr. Swanton wrote elsewhere that "[i]t would therefore be important to assess an equally plausible explanation—that ctDNA analysis may in fact be superior to a single tumor biopsy for resolving the majority of copy number aberrations present in a single tumor." EX2002 (Swanton) at page 7. Thus, Swanton instructs a POSA not to pursue the single tumor biopsy assay (which is the type of assay that Ley discloses). Instead, Swanton states that a superior assay would use "unbiased shotgun MPS approaches to ctDNA analysis" of the plasma to catch all the minor allele variants in heterogenous tumors rather than the targeted PCR approach used by Ley. *Id.* Furthermore, Burell does not discuss PCR primers or teach how to use next generation sequencing instruments.

b. Limitation 1(b)

97. Limitation 1(b) recites “using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences *in at least an additional biological sample from said subject*, to generate a sequencing library.” Thus, limitation 1(b) requires an “additional biological sample from said subject.”

98. As discussed above, Dr. Quackenbush relies on the *same* biological samples from Ley (the skin sample and relapse tumor sample) to meet the “at least one biological sample of said subject” of limitation 1(a), *see* EX1020 ¶¶113-114, as well as the “additional biological sample from said subject” of limitation 1(b), *see* EX1020 ¶121. *Supra* Section VII.B.1.b. Because Dr. Quackenbush relies on the same relapse tumor and skin biopsy samples to meet the “at least one biological sample of said subject” of limitation 1(a), there are no additional samples left in Ley to meet the “additional biological sample from said subject” required for limitation 1(b).

99. Dr. Quackenbush does not articulate a motivation to modify Ley’s assay to include more samples from Ley’s patient. Adding more tumor sampling steps to Ley’s assay does not appear to be necessary for the purposes described by Ley of discovering cancer-initiating mutations. Also, a person of ordinary skill in

the art would not have been motivated to obtain more samples from Ley's patient at least because Ley's patient was deceased at the time Ley's assay was performed. See EX1042 (Ley Supplementary Information) at pages 2-3.

c. Limitation 1(c)

100. Limitation 1(c) requires “subjecting said sequencing library to sequencing ***to identify*** a presence or absence of at least a subset of said set of genetic variants.”

101. I am not aware of any discussion in Dr. Quackenbush's declaration regarding how he believes a person of ordinary skill in the art would have been motivated to modify Ley's assay to involve “subjecting said sequencing library to sequencing ***to identify*** a presence or absence of at least a subset of said set of genetic variants” as required by limitation 1(c). For example, the obviousness section of Dr. Quackenbush's declaration includes some discussion of limitation 1(a) and limitation 1(d), but it does not discuss limitation 1(c). See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶179-193. It appears that Dr. Quackenbush's obviousness position simply relies on a person of ordinary skill in the art treating Ley's validation step as being equivalent to the claimed “to identify” recited by limitation 1(c). See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶122-

124. A person of ordinary skill in the art would not confuse these distinct steps for reasons discussed above. *Supra* Section VII.B.1.c.

102. A person of ordinary skill in the art would understand that Ley teaches an identification step that is distinct and separate from Ley’s validation step. This understanding is supported by Dr. Quackenbush’s explanation that “[o]nce the variants of interest, i.e., the ‘putative variants,’ **were identified, Ley then describes ‘validation** using custom PCR and capillary sequencing’ as well as sequencing of ‘validated somatic mutations’ ‘by Roche/454 sequencing of PCR-generated amplicons made from primary genomic DNA.” EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. I note that the “validation” step referenced by Dr. Quackenbush actually has two separate steps. For the “custom PCR and capillary sequencing” step, Ley confirms the exon in which the mutation is present. For the “Roche/454 sequencing” step, Ley determines the prevalence of the genetic variants by quantifying readcount data. Thus, Ley initially identifies variants of interest, and then separately performs the “validation” referenced by Dr. Quackenbush. The result of Ley’s “validation” step is the quantification of variant frequencies by tallying readcount sequencing data rather than establishing the identity of a presence or absence of variants as claimed by limitation 1(c). A person of ordinary skill in

the art would not have been motivated to *re*-establish the identity of mutations that had already been established.

103. The innovation of the claimed invention to require subjecting said sequencing library to sequencing to establish the identify of a presence or absence of at least a subset of said set of genetic variants is apparent when considered in view of the state of the art at the time of the invention. Firstly, it is important to make some overarching comments about the state of the field of endeavor and the nature of the problems that a person of ordinary skill in the art would have encountered at that time. A person of ordinary skill in the art would know that there were at least three principal problems to be overcome with studies of tumors tissues that used next-generation sequencing: (1) The typical “sequence machines” available at that time could only perform short reads. A person of ordinary skill would understand that short reads posed very significant problems in the correct mapping of sequence reads. This is a problem of particular significance in sequencing tumor tissue that one expects to contain genetic variants. (2) Tumor tissue not only contains normal cells, but also contains many subclones of tumor cells that differ in the composition and nature of their mutant alleles. This is called the tumor heterogeneity problem. *See, e.g.,* Burell (EX1071). Thus, any proposed methodology should be able to detect mutant alleles that are present at very low frequency. In this time period, it

was agreed that such methods should at least be able to detect mutant alleles present at 2%. See EX1030 Editor's Summary. (3) A person of ordinary skill would be well aware that cost of sequencing is a significant factor in any typical lab environment and thus consider that the design of assays should be cost effective.

104. With this understanding of the state of the art, a person of ordinary skill considering Ley's assay would not have been motivated to adapt the assay to include "subjecting said sequencing library to sequencing **to identify** a presence or absence of at least a subset of said set of genetic variants" as required by limitation 1(c). Ley did not successfully address any of problems listed above. Ley merely provided a short read study that sidestepped the heterogeneity issue by using an atypical tumor sample composed of almost 100% tumor cells. See EX1042 (Ley Supplementary Materials) at 1-2 (requiring a "high sample purity"). In a typical tumor sample, there is a mixture of tumor and normal cells. The normal cells are problematic because they cause a high background level of normal cell signal that makes it difficult to establish the identity of a presence or absence of genetic variants. This high background level was understood to make it difficult to establish the identity of genetic variants that might exist in a sample with an allele frequency below 5%. Further, a person of ordinary skill in the art would not have been impressed by the meager collection of just 8 validated somatic SNVs reported by Ley's workflow that

started with parallel whole genome sequencing of the tumor and normal genome. A person of ordinary skill would have been surprised (and indeed skeptical) that Ley’s meager haul did not include the “typical suspects” of SNVs reported in previous studies. Ley’s workflow would have been cost prohibitive to a person of ordinary skill working in a typical lab, and indeed could perhaps only have been conducted in one of the centers historically associated with and originally funded by the human genome project. Ley’s authors are from The Genome Center at Washington University—one of the most sophisticated sequencing laboratories in the world. A person of ordinary skill in the art would not have access to such resources.

d. Limitation 1(d)

105. There are at least two reasons why Ley (EX1012) does not suggest “generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said biological sample based on said identified at least a subset of (c) and (ii) genetic variants ***which inform a therapy choice or a change in therapy for the subject***” as required by limitation 1(d).

106. First, the mutations identified by Ley’s assay have unknown clinical utility. For example, Ley (EX1012) explains “until hundreds (or perhaps thousands) of normal genomes and other AML tumours are sequenced, ***the contextual relevance of the mutations found in this genome will be unknown.***” EX1012 (Ley)

at page 71. For example, one mutation might indicate a particular clinical course, yet another different mutation in another gene might contraindicate that course. Because the contextual relevance of Ley's mutations were unknown, a person of ordinary skill in the art would not have been motivated to use them to make a therapy choice or a change in therapy for a subject. Further a person of ordinary skill in the art may have been reluctant to report Ley's variants of unknown significance to a physician.

107. Second, Ley's patient was deceased at the time Ley's assay was performed. EX1042 (Ley Supplementary Information) at pages 2-3. I am unaware of therapy choices or changes in therapy to make for a deceased patient.

e. No motivation to modify Ley

108. In summary, a person of ordinary skill in the art would not have been motivated to modify Ley's assay to (1) add PCR primer pairs having a "variable portion" and "fixed portion" to meet limitation 1(a), (2) add an additional biological sample from the subject to meet limitation 1(b), (3) add a protocol to *re*-establish the identity of mutations that Ley had already been established to meet limitation 1(c), and (4) make a therapy choice or change in therapy based on mutations of unknown clinical utility to a deceased patient to meet limitation 1(d).

2. Claims 3, 8, 15, and 16

109. Claim 3 depends from claim 1 and recites:

Claim 3. The method of claim 2, wherein said plurality of biological samples comprises a tissue sample ***and a plasma sample.***

110. Claim 8 depends from claim 1 and recites:

Claim 8. The method of claim 1, wherein said additional biological sample of (c) ***is a blood sample.***

111. Claim 15 depends from claim 1 and recites:

Claim 15. The method of claim 1, wherein said additional biological sample ***is a blood sample*** comprising: (i) cfDNA, (ii) cfRNA, (iii) DNA or RNA from circulating tumor cells, or (iv) RNA or DNA from a tumor metastasis.

112. Claim 16 depends from claim 1 and recites:

Claim 16. The method of claim 1, wherein said additional biological sample and one or more subsequent biological samples ***are each blood samples*** comprising: (i) cfDNA, (ii) cfRNA, (iii) DNA or RNA from circulating tumor cells, or (iv) RNA or DNA from a tumor metastasis.

113. Claims 3, 8, 15, and 16 each require either a “plasma sample” or a “blood sample.” Dr. Quackenbush asserts that a person of ordinary skill in the art would have been motivated to use Ley’s assay on “cell-free tumor DNA circulating

in the blood” to allegedly meet claims 3, 8, 15, and 16. *See* EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶197-199, 203-205, 212-219.

114. A person of ordinary skill in the art would not have been motivated to use Ley’s assay on cell-free tumor DNA circulating in the blood with a reasonable expectation of success. Ley teaches that its assay requires “high sample purity” “to eliminate the need to further purify malignant cells” and that “contaminating normal cells would complicate our analysis.” EX1042 (Ley Supplementary Information) at page 1. In contrast to the highly pure cancer cell sample required for Ley’s assay, it was known that cell-free tumor DNA is typically only 1% of the total DNA in blood. Moreover, cell free tumor DNA in blood is also impure because it is contaminated with many types of normal cells such as white blood cells and red blood cells and normal cellular debris. Further, each of claims 8, 15, and 16 require the “additional biological sample” to be a “blood sample.” Dr. Quackenbush does not provide any explanation as to how he believes a person of ordinary skill in the art would go about obtaining an additional blood sample from the deceased patient.

3. Claim 22

115. Claim 22 depends from claim 1 and requires the “personalized probe set comprises hybrid capture probes.”

116. Dr. Quackenbush asserts that a person of ordinary skill in the art would have found it obvious to modify Ley's assay by replacing its undisclosed PCR primers with hybrid capture probes because they were "relatively interchangeable" and could be obtained "out of the box." See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶223-226. Dr. Quackenbush's assertion is made without specificity, so it is unclear if he is suggesting that a person of ordinary skill would replace PCR primers with capture probes for only the 8 "validated as somatic SNVs," or the "31,632 new SNV's," or for something else in Ley's workflow from, e.g., Figure 2:

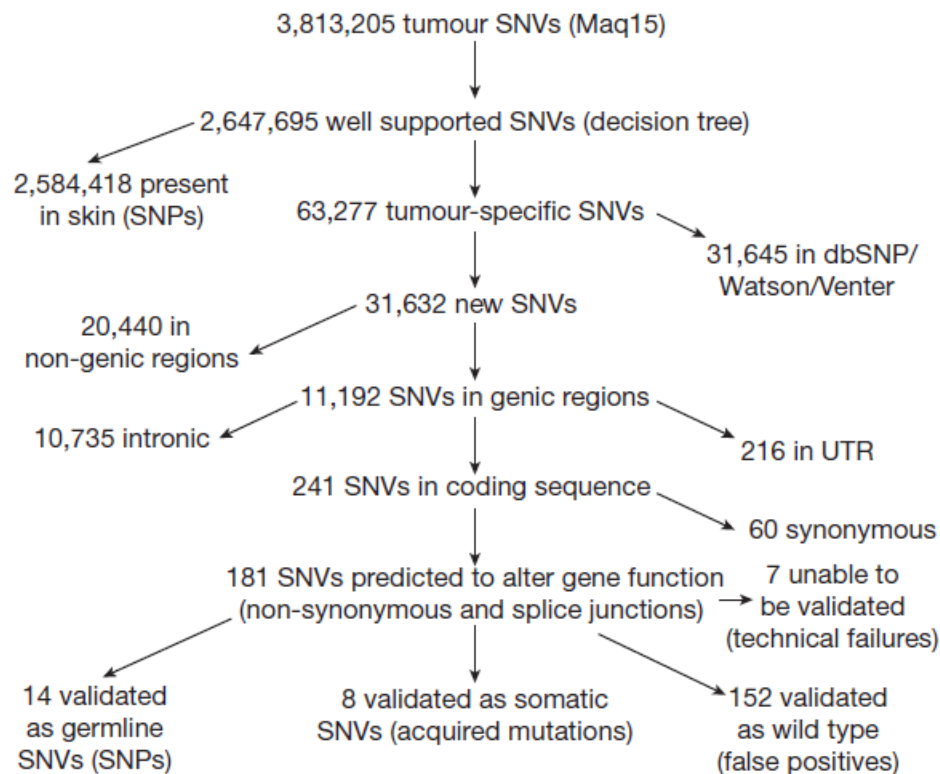


Figure 2 | Filters used to identify somatic point mutations in the tumour genome. See text for details. UTR, untranslated regions.

EX1012 (Ley) at page 68, Figure 2. A person of ordinary skill in the art would not treat capture probes and PCR primers as being interchangeable in Ley’s assay. For example Mertes (EX1006) teaches that capture probes and PCR are two different enrichment techniques that are “categorized according to the nature of their **core reaction principle**.” EX1006 (Mertes) at page 375. The core reaction principle of capture probes involves hybridizing to “targeted regions of interest, either in solution or on a solid support, so that one can physically capture and isolate the sequences of interest.” EX1006 (Mertes) at page 375. In contrast, the core reaction principle of

PCR primers is “amplification” of targeted regions by a “polymerase chain reaction (PCR)” that copies target regions. EX1006 (Mertes) at page 375. Dr. Quackenbush’s colleagues at the Dana-Farber Cancer Institute wrote that PCR and capture probe arrays are “orthogonal technologies.” EX2177 (Sholl) at pages 23-24.

117. Foresight’s founders stated that “hybrid capture-based workflows” are limited because they “typically have longer turnaround times, since the target enrichment process often takes days (instead of hours with PCR), and are more expensive, since they involve sequencing a larger genomic space,” which “may provide diminishing additional value.” Dudley and Diehn, “Detection and Diagnostic Utilization of Cellular and Cell-Free Tumor DNA,” *Annu. Rev. Pathol. Mech. Dis.* 16:199-222 (2021), EX2166, at page 203. They further wrote that hybrid capture assays are limited because they “can only detect alterations in genomic regions that are covered” by the capture probes. See Chaudhuri, Alizadeh, and Diehn et al., “Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA,” *Semin. Radiat. Oncol.* 25:305-312 (2015), EX2165, at page 308.

118. The exhibits cited by Dr. Quackenbush describe different benefits and drawbacks of capture probes and PCR primers. Mertes (EX1006) has different

sections discussing “enrichment by hybrid capture” and “enrichment by PCR” with primers. See EX1006 (Mertes) at pages 378-379 (section entitled “Enrichment by hybrid capture”) and pages 379-380 (section entitled “Enrichment by PCR”). Mertes explains that “[g]iven the operational characteristics of these different targeted enrichment methods, they naturally vary in their suitability for different fields of application.” EX1006 (Mertes) at page 375. Mertes demonstrates “how very divergent the results of” hybrid capture and PCR primer amplification can be, as they “produce very different average coverage, evenness and specificity” as follows:

The comparison of different genome partitioning methods in Figure 4 gives a real-world indication of how very divergent the results of the available methods can be. Even for the same genetic locus, processed by the same people in the same laboratory, the different enrichment methods produce very different average coverage, evenness and specificity.

EX1006 (Mertes) at 383. Similarly, Mamanova (EX1007) identifies numerous differences between “PCR,” “[o]n-array hybrid capture,” and “[i]n-solution hybrid capture” in Table 1, which is reproduced below:

Table 1 | Performance of target-enrichment methods

	PCR	MIP	On-array hybrid capture	In-solution hybrid capture
Cost	High	<10 samples, high; >100 samples, low	Medium	<10 samples, medium; >10 samples, low
Ease of use	Low	High	Medium	High
Mass DNA	~8 µg for 1 Mb of 2× tiled, 5 kb amplicons	As little as 200 ng	10-15 µg per array for up to 30 Mb target	3 µg for up to 30 Mb target
Sensitivity	>99.5%	>98%, with stringent design constraints	98.6% of CTR ^a	>99.5% of CTR ^a
Specificity	93% for HapMap DNA samples, 72% for whole genome– amplified samples	>98%	Up to 70% mapping to CTR ^a for exons; higher for contiguous regions	Up to 80% mapping to CTR ^a for exons; higher for contiguous regions
Uniformity	80% of bases within twofold range of median	58% of CTR within tenfold coverage range; 88% within 100-fold coverage range	60% of CTR ^a within 0.5–1.5-fold of mean coverage (mapping quality ^b 30)	61% of CTR ^a within 0.5–1.5-fold of mean coverage (mapping quality ^b 30)
Reproducibility	Up to 100%	0.92 rank-order correlation ^c	For 10 ⁷ paired-end sequences, >95% reproducibility at tenfold between two samples	For 10 ⁷ paired end-sequences, >96% reproducibility at tenfold between two samples

^aCTR, capture target region, that is, the regions of the desired target region to which probes could be designed after repeat masking. ^bMapping qualities were calculated by the mapping software, MAQ, and indicate the probability that the mapping location is correct. A score of 30 or greater indicates that the quality of a read was good, and that it mapped unambiguously to that location with few mismatches. ^cRank-order correlation in capture efficiency distributions between independent samples.

EX1007 (Mamanova) at page 114, Table 1.

119. Based on the differences in core reaction principles of the techniques, Mamanova (EX1007) explains that there are many factors that a person of ordinary skill in the art must weigh when selecting either capture probes or PCR primers for a given experiment. Mamanova teaches “[t]here are several enrichment approaches, each with unique advantages and disadvantages” such that a user must “find the best compromise between sensitivity, specificity and uniformity for their particular project.” EX1007 (Mamanova) at page 111. Mamanova further teaches that there are seven “parameters” by which a person of ordinary skill in the art should evaluate capture probes or PCR primers before selecting one over the other:

- (i) sensitivity, or the percentage of the target bases that are represented by one or more sequence reads;

- (ii) specificity, or the percentage of sequences that map to the intended targets;
- (iii) uniformity, or the variability in sequence coverage across target regions;
- (iv) reproducibility, or how closely results obtained from replicate experiments correlate;
- (v) cost;
- (vi) ease of use; and
- (vii) amount of DNA required per experiment, or per megabase of target.

Id.

120. Similarly, Mertes (EX1006) teaches that a person of ordinary skill in the art must “rationally decide which technologies are most suitable when designing a research project” by weighing the following **seven** “series of metrics” to evaluate each “method’s innate enrichment capability” for each experiment:

- (i) Region of interest (size): ROI;
- (ii) Average read depth (in ROI): D;
- (iii) Fraction of ROI sufficiently covered (at a specified D): F;
- (iv) Specificity (fraction of reads in ROI): S;
- (v) Enrichment Factor (D for ROI versus D for rest of genome): EF;
- (vi) Evenness (lack of bias): E and
- (vii) Weight (input DNA requirement): W.

EX1006 (Mertes) at page 380. A person of ordinary skill in the art would have understood that capture probes and PCR primers are different techniques, and selecting one or the other is a compromise that involves evaluating several parameters (and/or metrics) for each experimental design. Forshew teaches that custom designed capture probes are “expensive” and have not been applied to the “analysis of circulating tumor nucleic acids” as follows:

Previously proposed methods for personalized monitoring of tumor dynamics relied on expensive custom-designed probes.

EX1030 (Forshew at page 10).

The intermediate scale of sequencing is most likely to have immediate impact on clinical genomics. Targeted sequencing has been applied for tumor DNA (34, 35) and cyst fluid (33) to detect mutations down to 5% AF, *but has not been applied for analysis of circulating tumor nucleic acids.*

EX1030 (Forshew) at page 9 (Reference 35 in Forshew is Wagle, which uses capture probes). **Perhaps, most importantly, capture probes are uniquely different from PCR in that capture probes physically isolate native DNA. In other words, the DNA that is captured retains its original features. In particular, DNA is naturally modified by the methylation of Cytosine, forming 5-Methylcytosine. Capture probe technology would enable the reporting of 5-Methylcytosine,**

whereas, PCR cannot. The amplified DNA can only contain cytosine. Thus, PCR cannot truly “capture” DNA, it can only copy it.

121. As discussed above, a person of ordinary skill in the art would have needed to evaluate seven parameters (and/or seven metrics) associated with the disadvantages of capture probes before deciding to change the core reaction principle of Ley’s PCR assay and replacing it with capture probes. The vast majority of these parameters would have counseled a person of ordinary skill in the art to avoid attempting to redesign Ley’s experiment. For example, Mamanova teaches that “[t]he *specificity* of PCR will almost certainly always exceed that of hybrid capture, and its *uniformity* may never be matched by either hybrid capture of MIPs.” EX1007 (Mamanova) at page 111. Further, Mamanova teaches that PCR has higher *reproducibility* of “up to 100%” than capture probes. *Id.* at page 114, Table 1. Ley already had a working assay, so switching the core reaction principle to capture probes would entail more *costs* for redesigning the experiment, and risk making the assay *harder to use*. The *sensitivity* and *amount of DNA required* are parameters that would, at best, be considered neutral; but the risks associated with modifying Ley’s already-working assay could very well cause the assay to lose sensitivity or even worse, not work at all.

122. It is unclear to me how Dr. Quackenbush proposes to change the workflow of Ley's PCR assay or what such a changed workflow would look like if a person of ordinary skill in the art were to somehow have been motivated to use capture probes instead of PCR. Dr. Quackenbush does not articulate which portion of Ley's PCR-based workflow a person of ordinary skill in the art would have been motivated to change to capture probes, and which steps in Ley's workflow would need to be kept, jettisoned, or rearranged to accommodate such a change in the core reaction principle of Ley's assay with a reasonable expectation of success.

123. Also, if a POSA were to obtain hybrid capture probes "out of the box," as suggested by Dr. Quackenbush, EX1020 (Quackenbush Declaration IPR2023-00545) ¶224, then the genomic content of such probes would not meet the claimed "variable portion" which must change from one subject to another and result from sequencing data for a biological sample from the subject.

D. GROUND 3: NONOBVIOUSNESS OVER FORSHEW AND DAWSON

124. The combination of ForsheW (EX1030) and Dawson (EX1070) does not prove obviousness of claim 1. These references further do not prove obviousness of dependent claim 22 for additional reasons discussed below.

1. Claim 1

125. The combination of Forshew (EX1030) and Dawson (EX1070) do not render claim 1 obvious at least because it does not teach or suggest the following two limitations of the claim:

- “(i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data” as required by limitation 1(a), and
- “generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject” as required by limitation 1(d).

a. Limitation 1(a)

126. Limitation 1(a) recites “(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of

genetic variants are identified in sequence data for said at least one biological sample, ***further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data.***”

127. The combination of Forshew (EX1030) and Dawson (EX1070) does not teach or suggest a personalized probe set comprising nucleic acid probe molecules having oligonucleotide-directed genomic content comprising “(i) ***at least one variable portion*** from a result of said sequence data ***and*** (ii) ***at least one fixed portion*** independent of said result of said sequencing data” as required by limitation 1(a).

i. **Forshew does not teach the claimed “variable portion”**

128. Dr. Quackenbush asserts that Forshew “expressly discloses the use of a variable portion” based on a set of 10 PCR primers for Forshew’s breast cancer patient that he alleges meets the “variable portion” of limitation 1(a). See EX1020 (Quackenbush Declaration IPR2023-00545) ¶255. There is no such express disclosure in Forshew (EX1030). Forshew does not disclose the sequences of the PCR primers used for its breast cancer patient. Forshew merely discloses that

“standard amplification primers tailored to the mutation loci” were used for the breast cancer patient as follows:

Using standard amplification primers tailored to the mutation loci, we also used TAm-Seq to monitor the dynamics of 10 mutations in plasma DNA of a single patient with metastatic breast cancer, using minute amounts of input DNA.

EX1030 (Forshaw) at 10.

129. Forshaw does not provide details regarding the PCR primers that were used for the breast cancer patient in the article (EX1030) or the supplemental materials (EX1032 from IPR2023-00317). It is possible that Forshaw’s “standard amplification primers” refers to a standard probe set that was purchased from a commercial supplier. If Forshaw used a standard probe set that was purchased from a commercial supplier, then such a probe set would not constitute the “variable portion” of limitation 1(a). The appropriate construction of “variable portion” requires that the portion “changes from one subject to another and results from said sequence data.” *Supra* Section VII.B.1.a.i. First, a standard probe set purchased from a commercial supplier would not change from one subject to another. Second, a standard probe set would not “result from said sequence data” from “at least one biological sample” as required by limitation 1(a) because the standard probe set

would be the result of whatever criteria was applied by the commercial supplier in creating the standard probe set.

ii. **No suggestion for the claimed “variable portion”**

130. Forshew (EX1030) does not suggest using PCR primers that would meet the claimed “variable portion.” Forshew teaches that its TAm-Seq assay should be used “without requiring any specially designed probes” because “custom-designed probes” are “expensive” and are “difficult to implement on a routine basis” as follows:

Previously proposed methods for personalized monitoring of tumor dynamics relied on *expensive custom-designed probes* or identification of rearrangements using whole-genome sequencing. These have better analytical sensitivity than currently achieved by TAm-Seq, *but are difficult to implement on a routine basis*. TAm-Seq strikes a balance between sensitivity and ease of use and could facilitate study and application of circulating DNA. Using TAm-Seq, we identified cancer mutations in the plasma of most advanced ovarian cancer patients and tracked dynamics of TP53 mutations *without requiring any specially designed probes*.

EX1030 (Forshew) at page 10. Based on Forshew’s teaching to avoid custom-designed probes, a person of ordinary skill in the art would have been dissuaded from using the claimed personalized probe set recited by limitation 1(a) comprising

nucleic acid probe molecules having oligonucleotide-directed genomic content comprising at least one variable portion that changes from one subject to another and results from said sequenced data. A person of ordinary skill in the art would have considered such a personalized probe set to be expensive and difficult to implement in TAm-Seq based on Forshew's express statements to that effect.

131. Dawson (EX1070) does not suggest using PCR primers that would meet the claimed "variable portion." Dawson contrasts the TAm-Seq assay with digital PCR. EX1070 (Dawson) at page 1207 ("Here we have demonstrated the use of two strategies to quantify circulating tumor DNA: digital PCR assay and targeted deep sequencing [TAm-Seq]."). Dawson teaches that TAm-Seq is desirable over digital PCR because digital PCR "requires the design of personalized assays, an expensive and rate-limiting step." *Id.* Dawson teaches that targeted deep sequencing is "a cost-effective alternative" to digital PCR as follows:

Here we have demonstrated the use of two strategies to quantify circulating tumor DNA: digital PCR assay and targeted deep sequencing. ***Digital PCR assay provides high accuracy and sensitivity but requires the design of personalized assays, an expensive and rate-limiting step. Targeted deep sequencing of plasma DNA provides a cost-effective alternative*** for high-throughput analysis and may overcome limitations of initial tumor-tissue assessment by virtue of allowing for the direct identification of mutations in plasma.

Id. In view of Dawson’s teaching to avoid personalized assays because they are expensive and rate-limiting, a person of ordinary skill in the art would have been dissuaded from using the claimed personalized probe set required by limitation 1(a) in a TAm-Seq assay.

iii. **No motivation to combine “variable portion” and “fixed portion”**

132. If I were to assume for argument’s sake that Forshew’s undisclosed PCR primers for the breast cancer patient constitute a “variable portion” as recited by limitation 1(a)—which they do not for reasons discussed above—Forshew still does not teach or suggest combining a “variable portion” with a “fixed portion” in a personalized probe set as required by limitation 1(a).

133. Dr. Quackenbush admits that Forshew (EX1030) did not teach combining a “variable portion” with a “fixed portion” in a personalized probe set as required by limitation 1(a) as follows:

In the example studying the breast cancer patient, ***Forshew does not expressly disclose including PCR primers (probes) for sequences or markers that constitute a fixed portion that is selected independent of the result of said sequencing data.***

See EX1020 (Quackenbush Declaration IPR2023-00545) ¶256. Dr. Quackenbush asserts that a person of ordinary skill in the art would have been motivated to

complicate Forshew's TAm-Seq assay (which already included a set of 10 PCR primer pairs for a breast cancer patient) with even more PCR primer pairs for variants in tumor suppressor gene *TP53* mutations for ovarian cancer as follows:

Thus, Forshew disclosed including probes for variants in *TP53* independent of any prior sequencing data in its other assays. Including such additional probes in addition to sequence-based probes in a single panel would require nothing more than the predictable use of techniques used in other examples of the very same reference.

EX1020 (Quackenbush Declaration IPR2023-00545) ¶257.

134. As an initial matter, Dr. Quackenbush did not establish that the undisclosed PCR primer pairs that Forshew used for its breast cancer patient constitute the claimed "variable portion" for reasons discussed above. *Supra* Section VII.B.1.a. Thus, even if a person of ordinary skill in the art had been motivated to combine Forshew's undisclosed PCR primers for its breast cancer patient with the PCR primers Forshew used to detect *TP53* mutations for ovarian cancer, Dr. Quackenbush would still not have established that such a combination would result in a "variable portion" and a "fixed portion" in a personalized probe set as required by limitation 1(a).

135. Also, a person of ordinary skill in the art would not have been motivated to redesign Forshew's TAm-Seq assay in the manner proposed by Dr. Quackenbush.

Forsheiw's TAm-Seq assay already included a set of 10 PCR primer pairs for the breast cancer patient. EX1030 (Forsheiw) at 10 ("Using standard amplification primers tailored to the mutation loci, we also used TAm-Seq to monitor the dynamics of 10 mutations in plasma DNA of a single patient with metastatic breast cancer, using minute amounts of input DNA."). Forsheiw separately used a set of 16 other PCR primer pairs to amplify TP53 mutations from a small portion of the TP53 gene, which are disclosed in Table S1 (the relevant portion of which is excerpted below):

SUPPLEMENTARY TABLES

Table S1: Target-specific primers. Each target-specific primer consists of a universal 5' end and a target-specific 3' end as follows:

5'-ACACTGACGACATGGTTCTACA-[Target Specific-Forward]-3'.

5'-TACGGTAGCAGAGACTTGGTCT-[Target Specific-Reverse]-3'

Gene name and Amplicon ID	Target Specific Primer – Forward	Target Specific Primer- Reverse	Chr	Amplicon Start	Amplicon End

TP53_E00001757276_1	GACCCAAAACCCAAAATGGC	TCCCTGCTTCTGTCTCCTAC	17	7572850	7573030
TP53_E00001728015_1	GGAATCCTATGGCTTTCCAACC	CCCCCTCCTCTGTTGCTG	17	7573859	7574054
TP53_00001404886_13	TCTGTATCAGGCAAAGTCATAGAA	GCCTCAAAGACAATGGCTCC	17	7576584	7576734
TP53_E00001789298_1	AGAAAACGGCATTGAGTGT	AAGGGTGCAGTTATGCCTCA	17	7576786	7576983
TP53_E00001789298_2	CTGGTGTGTTGGGCAGT	ATCTCCGCAAGAAAGGGGAG	17	7576908	7577075
TP53_E00001789298_3	TGTCCTGCTTGCTTACCTCG	GCCTCTTGCTTCTCTTTTCCT	17	7577003	7577187
TP53_E00001665758_1	GGGGTCAGAGGCAAGCAG	CTTGGGCCTGTGTTATCTCC	17	7577432	7577631
TP53_E00001255919_1	GAGAAAAGCCCCCTACTGC	AGCATCTTATCCGAGTGGAAAGG	17	7578091	7578274
TP53_E00001255919_3	TCCAAATACTCCACACGCAAA	GCTGCCCCCACCATGAG	17	7578229	7578406
TP53_E00001255919_5	AGCTGCTCACCATCGCTA	CCAACTGGCCAAGACCT	17	7578361	7578525
TP53_E00001255919_6	TGTGCTGTGACTGCTTGTAG	TGCCCTGACTTTCAACTCTGT	17	7578425	7578594
TP53_E00001612188_1	ATACGGCCAGGCATTGAAGT	CCTCTGGCCCCCTGTC	17	7579260	7579421
TP53_E00001612188_2	GGAAACCGTAGCTGCCCTG	AAGACCCAGGTCCAGATGAA	17	7579359	7579520
TP53_E00002359670	CAGCCTCTGGCATTCTGG	CCTGGTCTCTGACTGCTCT	17	7579479	7579626
TP53_E00002419584	TCAAATCATCCATTGCTTGG	CCATGGGACTGACTTTCTGC	17	7579557	7579754
TP53_E00001596491_1	TTTCGCTTCCACAGGTCTC	CAGCCAGACTGCCTTCCG	17	7579758	7579940

EX1032 (Forsheiw Supplementary Materials) at page 10 (excerpted). These 16 PCR primer pairs generates amplicons that only span exons 5 and 6 (approximately 295 bases) from the TP53 gene. At the same ratio of PCR primers to bases, it would take over 1,000 PCR primer pairs to cover the entire length of the TP53 gene (which

spans approximately 19,000 bases). A person of ordinary skill in the art would not have been motivated to complicate Forshew's TAm-Seq assay that already included a set of ten PCR primer pairs for breast cancer with over 1,000 additional PCR primer pairs for TP53 for several reasons.

136. First, Forshew teaches that the undisclosed PCR primer pairs for the breast cancer patient were "not included in the other sets of samples analyzed." EX1030 (Forshew) at page 7, Fig. 4(D) (legend). Thus, Forshew did not actually combine the breast cancer patient PCR primers with PCR primer pairs for TP53.

137. Second, Dr. Quackenbush testified that a person of ordinary skill in the art would have been motivated by four factors when designing assays related to tumors:

- (1) a desire to increase accuracy for an assay, *see* EX2163 (Quackenbush Transcript) at 17:19-20:1, 21:12-23:10;
- (2) a desire to reduce the cost of an assay, *see* EX2163 (Quackenbush Transcript) at 27:15-30:22;
- (3) a desire to simplify an assay, *see* EX2163 (Quackenbush Transcript) at 36:7-21; and
- (4) a desire to simplify the computational analysis for data generated by an assay, *see* EX2163 (Quackenbush Transcript) at 41:18-44:19.

Dr. Quackenbush's obviousness position that relies on a person of ordinary skill in the art redesigning Forshe's TAM-Seq assay that already included a set of ten PCR primer pairs for breast cancer to include over 1,000 additional PCR primer pairs for TP53 is counterproductive to each of these four factors.

138. For example, Dr. Quackenbush's proposal to add more PCR primer pairs to Forshe's set of ten PCR primer pairs for breast cancer would have been avoided by a person of ordinary skill in the art because it was expected to decrease accuracy and complicate the assay with amplification issues. Foresight's founders explained that Forshe's assay already suffered from "technical limitations related to multiplexing of PCR assays [that] have to date limited the number of genomic positions that can be interrogated," and explained that Forshe's methods can only use a "small combination of amplicons" as follows:

Although early NGS [next generation sequencing]-based ctDNA detection platforms had insufficient sensitivity for most clinical applications (>1%) [13,14], several groups, including ours, have recently developed NGS-based methods that permit ultrasensitive ctDNA detection [1,5,11]. Two of these utilized deep sequencing of a limited number of amplicons targeting commonly mutated cancer genes [1,2,11,15,16]. Although low detection thresholds are achievable with such methods, ***technical limitations related to multiplexing of PCR assays have to date limited the number of genomic positions that can***

be interrogated. This complicates potential clinical applications because a given **small combination of amplicons** will not identify a mutation in the majority of patients with most cancers.

Bratman, Alizadeh, and Diehn et al., “Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq,” *Expert Rev. Mol. Diagn.* 15:715-719 (2015), EX2164, at page 716 (Reference 15 is Forshew).

139. Foresight’s founders explained that Forshew’s existing assays were limited to the “interrogation of relatively few genomic loci owing to difficulties related to multiplexing of PCR assays” as follows:

The focused NGS-based approaches have mainly used PCR amplicon-based strategies. Specifically, **PCR is used to amplify a handful of regions of the genome** and the resulting amplified products are subject to NGS. For example, **Forshew et al developed Tagged-Amplicon Sequencing (TAm-Seq)** and used it to detect mutations in plasma samples from patients with advanced ovarian cancer, showing that this technique can achieve a detection limit of ~2%. ... **Limitations of these approaches include interrogation of relatively few genomic loci owing to difficulties related to multiplexing of PCR assays**, the inability to detect somatic copy number alterations, and the inability to detect breakpoints or rearrangements without patient-specific optimization of amplicons.

Chaudhuri, Alizadeh, and Diehn et al., “Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA,” *Semin. Radiat. Oncol.* 25:305-312 (2015), EX2165, at pages 307-308. In 2021, Foresight founder Diehn wrote that “PCR-based assays have limited multiplexability.” Dudley and Diehn, “Detection and Diagnostic Utilization of Cellular and Cell-Free Tumor DNA,” *Annu. Rev. Pathol. Mech. Dis.* 16:199-222 (2021), EX2166, at page 202.

140. Mamanova (EX1007) states that “PCR is difficult to multiplex to any useful degree: the simultaneous use of many primer pairs can generate a high level of nonspecific amplification, caused by interaction between the primers, and moreover amplicons can fail to amplify.” EX1007 (Mamanova) at page 112. Mertes (EX1006) teaches that PCR “does not scale easily, in any format” and “any significant extent of parallelized” multiplex PCR “would need to be supported by the use of automated robotics” and multiplex products “need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible)” as follows:

The main downside of the method is that it does not scale easily, in any format, to enable the targeting of very large genome subregions or many DNA samples. To use this method effectively, any significant extent of parallelized singleplex or multiplex PCR would need to be supported by the use of automated robotics, individual PCR amplicons

(or multiplex products) need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible), and the amount of DNA material a study requires can be substantial as this requirement grows linearly with the number of utilized PCR reactions.

EX1006 (Mertes) at page 379.

141. I note that Forshew used the Illumina GAIIx sequencer. *See* EX1030 (Forshew) at page 3; EX1032 (Forshew Supplementary Materials from IPR2023-00317) at page 3. Forshew also used an ABI 3730 Sanger sequencer. *See* EX1032 (Forshew Supplementary Materials) at page 1. Ross (EX1014) teaches that the Illumina sequencer had “low multiplex capability.” EX1014 (Ross) at pages 530-31, Table 3. Ross teaches that Sanger sequencing is limited “to single-gene or hot-spot sequence analysis,” *id.* at page 535, and was believed to be “incapable of scaling sufficiently to support the breadth of information required to manage an ever-growing arsenal of targeted anticancer drugs,” *id.* at page 528.

142. The Editor’s Summary of Forshew criticizes the TAm-Seq assay as “need[ing] to achieve a more sensitive detection limit (<2% allele frequency) to identify mutations in the plasma of patients with less advanced cancers.” EX1030 (Forshew) at Editor’s Summary. Ross (EX1014) teaches that “[c]linical applications, particularly in oncology, demand high levels of accuracy, sensitivity,

and specificity for sequence-based tests focused on calling out specific genetic alterations.” Ross (EX1014) at page 535. Foresight’s founders wrote that “only highly sensitive detection techniques are likely to be of clinical use.” See Chaudhuri, Alizadeh, and Diehn et al., “Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA,” *Semin. Radiat. Oncol.* 25:305-312 (2015), EX2165, at page 306. Adding a set of over 1,000 PCR primer pairs for TP53 to the undisclosed set of 10 PCR primers that Forshew used for breast cancer would have been undesirable because it would have been expected to reduce accuracy, sensitivity, and specificity due to amplification complications discussed above. *Supra* Section VII.C.1.a.ii. Such a reduction in accuracy, sensitivity, and specificity is precisely what The Editor’s Summary of Forshew and Ross teach a person of ordinary skill in the art would want to avoid. Dr. Quackenbush does not explain how the undisclosed set of 10 PCR primer pairs that Forshew used for the breast cancer patient would have been expected to be compatible with a set of over 1,000 PCR primer pairs necessary to cover the TP53 gene in the multiplexed assay that he proposes in view of the many concerns raised by Foresight’s exhibits and founders with multiplexing PCR primers.

143. Dawson uses the same TAm-Seq assay as Forshew. See EX1070 (Dawson) at page 1200 (describing the use of “tagged-amplicon deep sequencing²²”

where reference 22 is Forshew). Newman (EX1044) is an article that was co-authored by two founders of Foresight (Alizadeh and Diehn). Newman criticizes Forshew and Dawson's TAM-Seq assay because it lacked sensitivity as follows:

Previously published methods employing amplicon^{8,10,11}, whole exome¹², or whole genome^{9,32,33,24} sequencing would not be sensitive enough to detect ctDNA in most patients with NSCLC, even at 10-fold or greater sequencing costs (Fig. 1d and Supplementary Fig. 8).

EX1044 (Newman) at page 7 (Refence 8 is Forshew and Reference 11 is Dawson). Newman repeats its criticisms of Forshew and Dawson as being limited due to modest sensitivity and cost, among other things, as follows:

Recently, approaches employing massively parallel sequencing have been used to detect ctDNA⁵⁻¹². However, the methods reported to date have been limited by modest sensitivity¹³, applicability to only a minority of patients, the need for patient-specific optimization, and/or cost.

EX1044 (Newman) at page 2 (Refence 8 is Forshew and Reference 11 is Dawson). As discussed above, adding a set of over 1,000 more PCR primers for TP53 to Forshew's existing assay that already uses a set of 10 undisclosed PCR primers for breast cancer would have been expected to decrease the sensitivity of the assay and increase its cost.

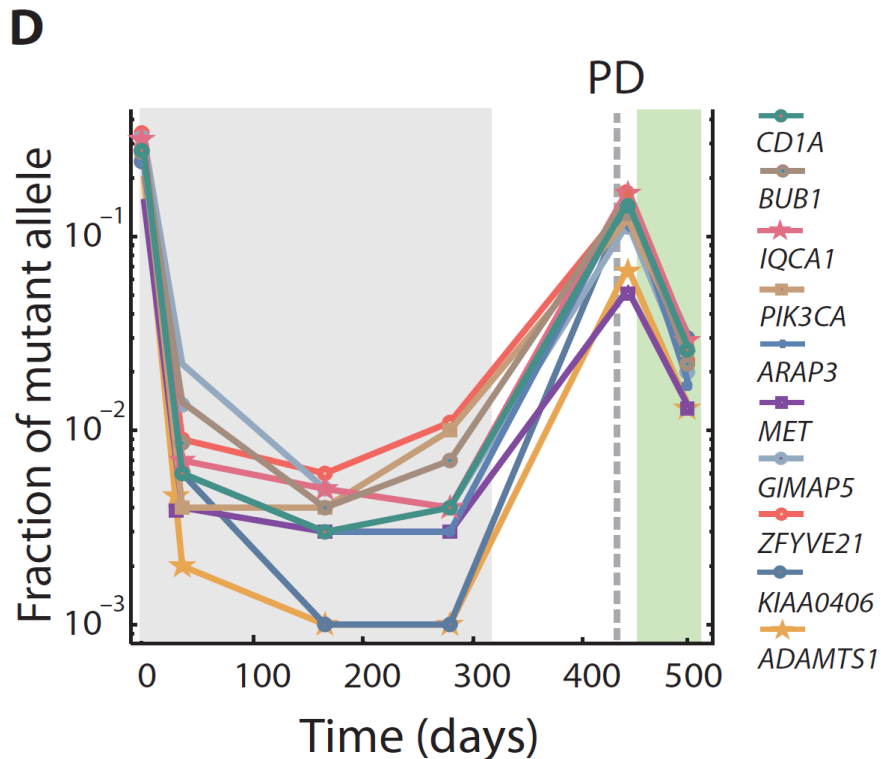
b. Limitation 1(d)

144. Limitation 1(d) requires “generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said biological sample based on said identified at least a subset of (c) and (ii) genetic variants ***which inform a therapy choice or a change in therapy for the subject.***”

145. Dr. Quackenbush asserts that Figure 4(D) of Forshew “shows a report depicting the fraction of the various mutant alleles over time, including in response to therapy (grey box).” EX1020 (Quackenbush Declaration IPR2023-00545) ¶264. Forshew Figure 4(D), however, does not constitute a report that informs a therapy choice or a change in therapy for the subject based on Forshew’s TAM-Seq assay for at least several reasons.

146. First, Forshew explains that the patient had been diagnosed with metastatic cancer. Thus, the patient has or had had a primary breast tumor. The treatment of the primary tumor had not been described, and so it is not clear whether any ctDNA is arising from the tumor or the metastasis. It is common knowledge that the development of metastatic lesions is expected. Given that treatment commenced at time 0 in Forshew’s Figure 4(D), it is certain that a clinical or radiological diagnosis of metastatic lesions had already been determined. Forshew indicated the time at which plasma samples were collected, but does not indicate

if/when the TAm-Seq determination of the mutant allele frequency was available to the physician. It is noticeable that during treatment with epirubicin some mutant allele frequencies decreased to a baseline level during treatment with epirubicin, but some (GIMAP5, BUB1, PIK3CA) had risen. Thus, it is unclear how such a report with inconsistent results would inform a physician in making a therapy choice or a change in therapy for the subject. In addition, the patient was treated with two phases of chemotherapy. EX1030 (Forsheew) at page 9. In the first chemotherapy phase, “[t]he patient was treated with a single-agent epirubicin (gray-shaded region).” *Id.* The patient was treated with epirubicin from time zero to a few days after day 300, as shown by the gray-shaded region in Figure 4(D):



Id. at page 7, Fig. 4(D). ForsheW does not teach that the patient was treated with epirubicin for this period of time due to any aspect of the TAm-Seq assay. Rather, the patient was treated with epirubicin based on her having metastatic breast cancer (which was known **before** the fraction of her mutant allele was tracked using the TAm-Seq assay of Figure 4(D)). In the second chemotherapy phase, “[a]fter 4 months off treatment, **a CT scan** showed **progressive disease** and the patient commenced further treatment with paclitaxel therapy.” EX1030 (ForsheW) at page 9. Further evidence of this includes “PD” in Figure 4(D), which refers to “Progressive Disease,” which was based on the CT scan. Thus, the patient received

paclitaxel therapy based on **a CT scan (the dotted line in Fig 4D)**, not based on any result from the TAm-Seq assay. The change in therapy for Forshew's patient that Dr. Quackenbush relies upon was not based on the results of TAm-Seq assay from Figure 4(D). Instead, it was based on knowledge that the patient had metastatic breast cancer (which was known before running Forshew's TAm-Seq assay for the epirubicin treatment). The decision was based on the observation of recurrence or non-response (there is no information that the metastatic tumors had in fact been down regulated) as visualized by a CT scan. Thus, it was the scan that indicated paclitaxel treatment, not the TAm-Seq assay results.

147. Second, the Editor's Summary of Forshew cautions that the TAm-Seq assay must be further "optimized" before it can "inform clinical decision-making on an individual basis." EX1030 (Forshew) at Editor's Summary ("Nevertheless, once optimized, this 'liquid biopsy' approach will be amenable to personalized genomics, where the level and type of mutations in ctDNA would inform clinical decision-making on an individual basis"). Forshew further cautions that its TAm-Seq assay will require faster and more accurate sequencing technologies or amplification strategies before it would potentially be considered for use in with patients at the point of care. EX1030 (Forshew) at page 2 ("Combined with faster, more accurate sequencing technologies or rare allele amplification strategies, this approach could

potentially be used for personalized medicine at point of care.”). Based on these express cautions, a person of ordinary skill in the art would not have believed that the TAm-Seq assay was ready to be used to inform a therapy choice or a change in therapy for a patient.

148. Third, Dr. Quackenbush does not discuss the expected delay in obtaining data from Forshew’s TAm-Seq assay due to the length of time it would take to perform the assay and process the resulting data. Dr. Quackenbush does not explain how a person of ordinary skill in the art would obtain data from Forshew’s TAm-Seq assay within a clinically relevant timeframe for making a therapy choice or change in therapy for the Forshew’s patient. When a therapy choice was made for Forshew’s patient in Figure 4D, it was made based on, for example, a CT scan that provides results much faster than can be obtained with the TAm-Seq assay. Moreover, Dr. Quackenbush does not explain why the assay was conducted at the indicated time points. It would seem very likely that if there was an intent to utilize the TAm-Seq assay results reported in Figure 4(D) to make a therapy choice for the patient, then there would have been a consensus to determine allele fraction prior to the CT scan. In fact, Dr. Quackenbush’s assertions appear to be a retrospective analysis designed to give credence to the notion that this allele frequency report could have influenced clinical decisions. Further, there is no rigorous discussion in

Forsheew or by Dr. Quackenbush as to how the tumor biology in Foresheew's patient that was produced by the alteration of the indicated genetic functions could have in any way influenced the clinical decisions made for the patient.

2. Claim 22

149. Claim 22 depends from claim 1 and requires the “personalized probe set comprises hybrid capture probes.”

150. Dr. Quackenbush asserts that a person of ordinary skill in the art would have found it obvious to modify Forsheew and Dawson's TAm-Seq assay by replacing the PCR primers used for the breast cancer patient with hybrid capture probes because they were “relatively interchangeable” and could be obtained “out of the box.” See EX1020 (Quackenbush Declaration IPR2023-00545) ¶¶316-319. Dr. Quackenbush repeats the same arguments he previously made regarding claim 22 in Ground 2.

151. For reasons discussed above, a person of ordinary skill in the art would not treat capture probes and PCR primers as being interchangeable in the TAm-Seq assay. *Supra* Section VII.C.3. For example, Mertes (EX1006) teaches that capture probes and PCR are two different enrichment techniques that are “categorized according to the nature of their **core reaction principle**.” EX1006 (Mertes) at page 375. Mamanova (EX1007) teaches that “[t]here are several enrichment approaches,

each with unique advantages and disadvantages” such that a user must “find the best compromise between sensitivity, specificity and uniformity for their particular project.” EX1007 (Mamanova) at page 111. Mamanova teaches that a person of ordinary skill in the art would have needed to evaluate seven parameters associated with the disadvantages of capture probes before discarding the core reaction principle of the PCR-based TAm-Seq assay and replacing it with capture probes.

152. The vast majority of these parameters would have counseled a person of ordinary skill in the art to avoid attempting to redesign the TAm-Seq assay. For example, Mamanova teaches that “[t]he **specificity** of PCR will almost certainly always exceed that of hybrid capture, and its **uniformity** may never be matched by either hybrid capture of MIPs.” EX1007 (Mamanova) at page 111. Further, Mamanova teaches that PCR has higher **reproducibility** of “up to 100%” than capture probes. *Id.* at page 114, Table 1. Forshew already had a working assay, so switching the core reaction principle to capture probes would entail more **costs** for redesigning the experiment (and adding a different detection mechanism), and risk making the assay **harder to use**. The **sensitivity** and **amount of DNA required** are parameters that would, at best, be considered neutral; but the risks associated with modifying Forshew’s already-working assay could very well cause the assay to lose sensitivity or even worse, not work at all.

153. Similarly, Mertes (EX1006) explains that PCR primers and capture probes have different “operational characteristics” that “naturally vary in their suitability for different fields of application.” EX1006 (Mertes) at page 375. Mertes demonstrates “how very divergent the results of” hybrid capture and PCR primer amplification can be, as they “produce very different average coverage, evenness and specificity.” EX1006 (Mertes) at page 383. Mertes teaches that a person of ordinary skill in the art would have needed to evaluate seven metrics associated with the disadvantages of capture probes before forsaking the already-working core reaction principle of the PCR-based TAM-Seq assay for capture probes. A person of ordinary skill in the art would not have been motivated to make such a change to the assay’s core reaction principle with a reasonable expectation of success.

154. Also, if a POSA were to obtain hybrid capture probes “out of the box,” as suggested by Dr. Quackenbush, EX1020 (Quackenbush Declaration IPR2023-00545) ¶317, then the genomic content of such probes would not meet the claimed “variable portion” which must change from one subject to another and result from sequencing data for a biological sample from the subject.

E. OBJECTIVE INDICIA OF NONOBVIOUSNESS

155. As explained above, Foresight and Dr. Quackenbush failed to show that a person of ordinary skill in the art would have been motivated to modify the asserted

references to arrive at the claimed invention with a reasonable expectation of success. Even if they had made such a showing, however, I understand that objective indicia of nonobviousness must be carefully evaluated to avoid hindsight bias.

1. Nexus

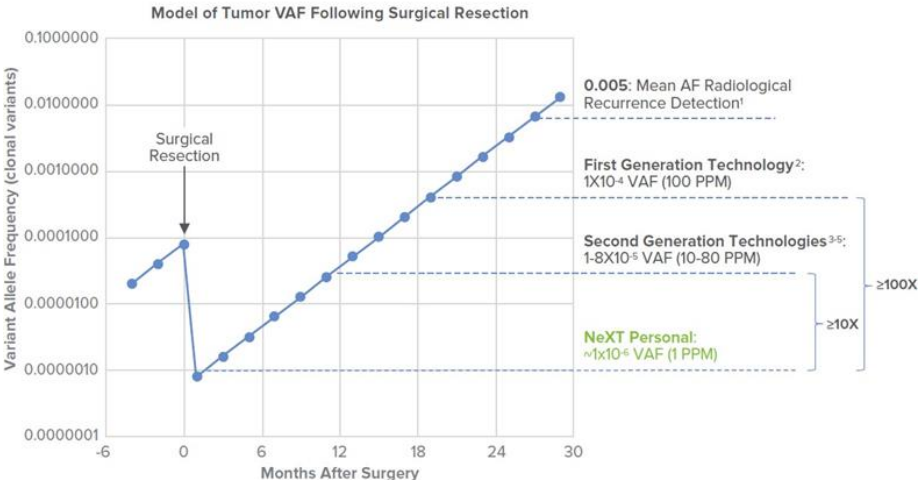
156. I understand that the evidence of objective indicia of nonobviousness must have a ‘nexus’ to the claims. I understand that a nexus is presumed when the patentee shows that the objective evidence is tied to a specific product and that product embodies the claimed features, and is coextensive with them. Here, Personalis is entitled to a presumption of nexus because, as detailed in the following chart, Personalis’ NeXT Personal[®] assay embodies claim 1 of the ’783 patent.

'783 Patent Claim Language	Personalis' NeXT Personal [®] assay
1. A method for personalized genetic testing, comprising:	The Next Personal Test performs a method for personalized genetic testing. Delivering Industry-Leading Sensitivity to Detect Residual Disease at the Earliest Timepoints NeXT Personal, an advanced, personalized, and tumor-informed liquid biopsy assay, is designed to detect molecular residual disease (MRD) and cancer recurrence at the earliest timepoints — prior to, during or after treatment — in patients previously diagnosed with cancer. While circulating tumor-derived DNA (ctDNA) is an emerging biomarker for many cancers, the limited sensitivity of current detection methods reduces its utility for diagnosing MRD across a variety of clinical applications. Standard-of-care (SOC) radiological-based technologies, including CT, PET and MRI scans, also remain limited in their ability to detect residual disease during or after surgical or systemic therapy due to the minimum tumor volume required.¹ Therefore reliable, sensitive detection and quantification of MRD remains a key challenge, particularly in early-stage cancers, where timely detection of small micrometastatic lesions may enable treatment that prevents progression to advanced metastatic, incurable disease. To address these challenges, NeXT Personal was developed to deliver industry-leading MRD sensitivity in the range of 1-3 parts per million (PPM) representing a 10-100X increase over other available methods (Figure 1), while requiring only a single tube of blood (4mL plasma/15ng cfDNA), and 1mm³ of FFPE tumor tissue. EX2003 (Personalis NeXT Personal Brochure) at page 13.

(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data	The method of conducting the NeXT Personal test involves contacting nucleic acid samples with capture probes, which are configured to selectively amplify sequences with particular genetic variants in a biological sample. 6. As background, both Foresight and Personalis offer testing products that can be used to monitor for cancer recurrence by detecting and analyzing tiny amounts of tumor DNA that are present in the bloodstream of a cancer patient. It is important to understand that both Foresight and Personalis offer testing products that operate according to the same basic principles, specifically, by using whole genome sequencing of a tumor sample to identify mutations that can subsequently be detected in circulating tumor DNA by capturing the mutation carrying DNA with capture probes and then sequencing the captured DNA. This unique approach distinguishes the Foresight and Personalis tests from other testing products, and allows the products from Foresight and Personalis to achieve greater sensitivity relative to other testing products currently on the market. EX2003 at page 27, ¶6. The NeXT Personal Test involves using personalized probes as well as known cancer-related clinical variants. Here we report a performance update of the NeXT Personal® platform. NeXT Personal utilizes hybridization-capture-based technology to quantify ctDNA in liquid biopsy samples. Its unified probe panel design and workflow simultaneously detects and monitors MRD, surveys known cancer-related clinical variants, and quantifies and tracks investigational variants of interest, by leveraging both tumor-informed and tumor-agnostic genomic information (Fig. 1). The tumor-informed MRD content is dedicated to personalized MRD detection and longitudinal monitoring, which utilizes cumulative tumor-derived signals to achieve ultra-high sensitivity down to 1 ~ 3 parts per million (PPM) limit of detection (LOD). The tumor-agnostic clinically relevant content surveys known and validated actionable genomic variants in 90 clinically relevant genes, and serves to inform therapy selection and detect existing or potentially emerging events such as new subclones or metastases. The investigational content provides capacity to track important cancer-related variants for research or investigational purposes. EX2003 at page 38. The NeXT Personal test also detects SNVs at >30x WGS coverage of a patient's tumor. This translates into sequencing over 90 gigabases.
---	--

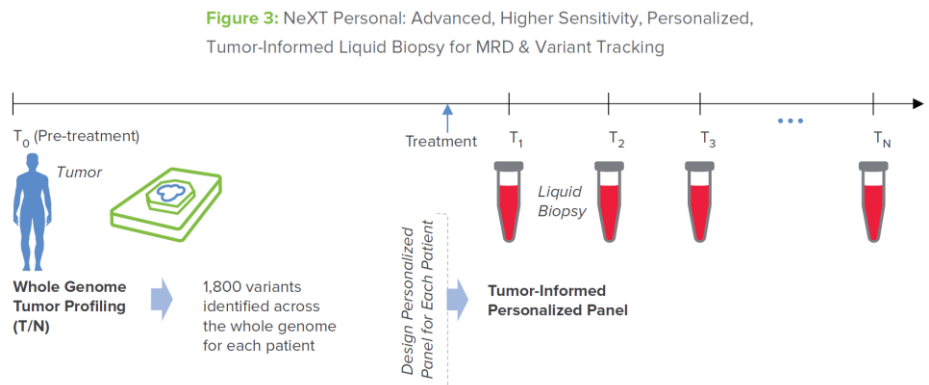
'783 Patent Claim Language	Personalis' NeXT Personal® assay
for said at least one biological sample, further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data;	Leveraging Tissue-Based Whole Genome Sequencing (WGS) Yields Best-In-Class MRD Performance in Plasma: 1-3 PPM Sensitivity Central to the MRD performance of NeXT Personal is the number of tumor-derived mutations interrogated in patient plasma samples. Analytical and clinical MRD sensitivity can be increased by expanding coverage of loci containing tumor-specific mutations.⁷ To this end, NeXT Personal leverages the broad array of SNVs detected with >30x WGS coverage of a patient's tumor (and accompanying normal) to inform the selection of 1,800 SNVs for inclusion in a personalized MRD panel (Figure 3). The majority of these patient-specific SNVs are located in non-coding regions of the genome. Finally, Personalis' extensive investment into the Illumina® NovaSeq® series enables ultra-deep sequencing (~100,000 raw depth), while keeping costs in line with other MRD tests that interrogate many fewer loci. EX2003 (Personalis NeXT Personal Brochure) at page 15.

(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;	The capture probes selectively amplify sequences comprising the set of genetic variants over other sequences in additional biological samples taken from the subject, perhaps post-surgery, to generate a sequencing library. 6. As background, both Foresight and Personalis offer testing products that can be used to monitor for cancer recurrence by detecting and analyzing tiny amounts of tumor DNA that are present in the bloodstream of a cancer patient. It is important to understand that both Foresight and Personalis offer testing products that operate according to the same basic principles, specifically, by using whole genome sequencing of a tumor sample to identify mutations that can subsequently be detected in circulating tumor DNA by capturing the mutation carrying DNA with capture probes and then sequencing the captured DNA. This unique approach distinguishes the Foresight and Personalis tests from other testing products, and allows the products from Foresight and Personalis to achieve greater sensitivity relative to other testing products currently on the market. EX2003 at page 27, ¶6. Fig. 1 shows the tracking of tumor variant allele frequencies in the subsequently analyzed samples from additional time points.
---	---

'783 Patent Claim Language	Personalis' NeXT Personal® assay
	Figure 1: MRD Sensitivity: Targeting Detection at Earliest Timepoints  Model Assumptions: Median breast tumor size detected by mammography: 1.3cm, Median shedding per NSCLC TRACERx study⁶; Residual tumor from surgery: 1%; Volume doubling time (actual VAF time): 2 months EX2003 (Personalis NeXT Personal Brochure) at page 13, Fig. 1.

(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and

The NeXT Personal test involves sequencing the sequencing library to identify a subset of genetic variants in the additional biological sample.



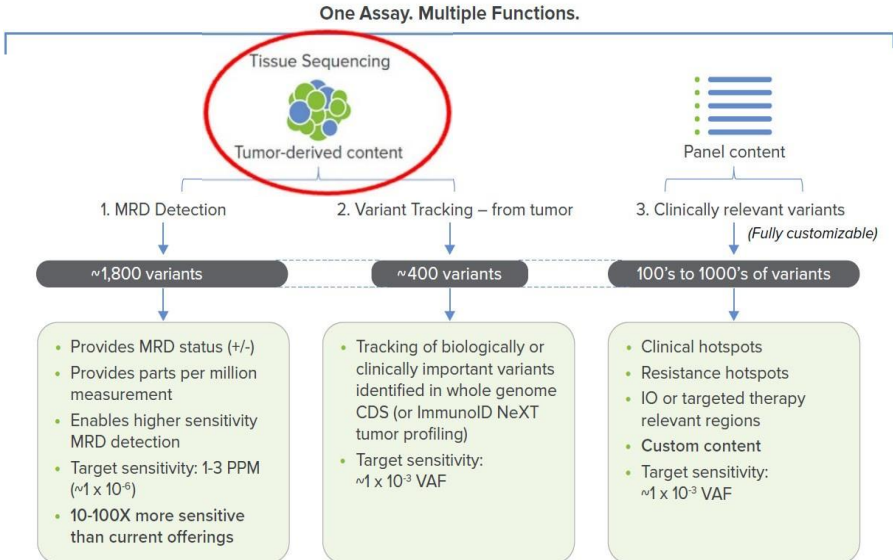
EX2003 (Personalis NeXT Personal Brochure) at page 15, Fig. 3.

Leveraging Tissue-Based Whole Genome Sequencing (WGS) Yields Best-In-Class MRD Performance in Plasma: 1-3 PPM Sensitivity

Central to the MRD performance of NeXT Personal is the number of tumor-derived mutations interrogated in patient plasma samples. Analytical and clinical MRD sensitivity can be increased by expanding coverage of loci containing tumor-specific mutations.⁷ To this end, NeXT Personal leverages the broad array of SNVs detected with >30x WGS coverage of a patient's tumor (and accompanying normal) to inform the selection of 1,800 SNVs for inclusion in a personalized MRD panel (Figure 3). The majority of these patient-specific SNVs are located in non-coding regions of the genome. Finally, Personalis' extensive investment into the Illumina® NovaSeq® series enables ultra-deep sequencing (~100,000 raw depth), while keeping costs in line with other MRD tests that interrogate many fewer loci.

EX2003 (Personalis NeXT Personal Brochure) at page 15.

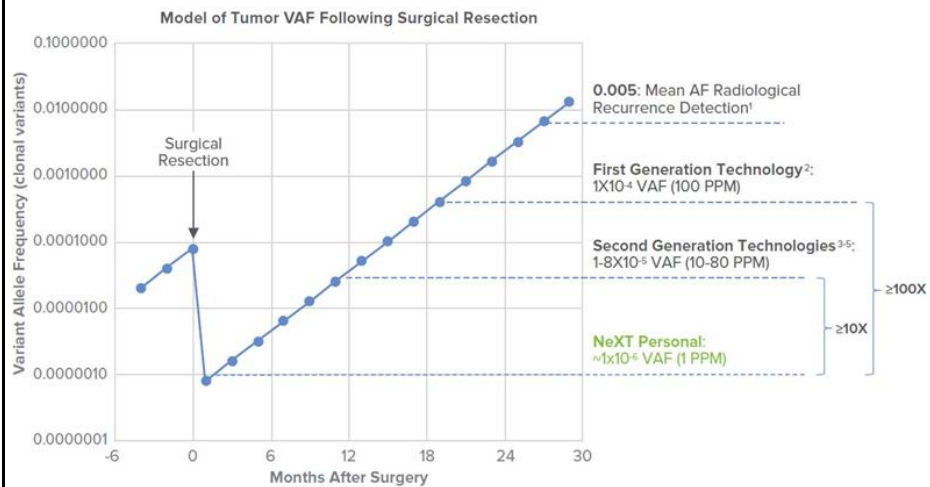
The NeXT Personal test also involves a variant tracking panel that identifies a subset of ~400 sequences, with a sequencing coverage footprint that is less than the sequencing footprint of the sequence data in step (a)

'783 Patent Claim Language	Personalis' NeXT Personal [®] assay
	Figure 2: NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD & Variant Tracking One Assay. Multiple Functions.  <pre> graph TD TS[Tissue Sequencing] --> TDC[Tumor-derived content] TDC --> MRD[1. MRD Detection] TDC --> VT[2. Variant Tracking - from tumor] TDC --> CRV[3. Clinically relevant variants (Fully customizable)] MRD --> V1[~1,800 variants] VT --> V2[~400 variants] CRV --> V3[100's to 1000's of variants] V1 --> F1[Provides MRD status +/- Provides parts per million measurement Enables higher sensitivity MRD detection Target sensitivity: 1-3 PPM (~1 x 10^-6) 10-100X more sensitive than current offerings] V2 --> F2[Tracking of biologically or clinically important variants identified in whole genome CDS (or ImmunolD NeXT tumor profiling) Target sensitivity: ~1 x 10^-3 VAF] V3 --> F3[Clinical hotspots Resistance hotspots IO or targeted therapy relevant regions Custom content Target sensitivity: ~1 x 10^-3 VAF] </pre> EX2003 (Personalis NeXT Personal Brochure) at page 14, Fig. 2.

(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.

The NeXT Personal test generates a biomedical report that includes biomedical information, including the presence or absence of polymorphisms identified in the prior sequencing of the plasma samples. For example, NeXT Personal test shows in Fig. 1 shows the tracking of tumor variant allele frequencies in the subsequently analyzed samples from additional time points.

Figure 1: MRD Sensitivity: Targeting Detection at Earliest Timepoints



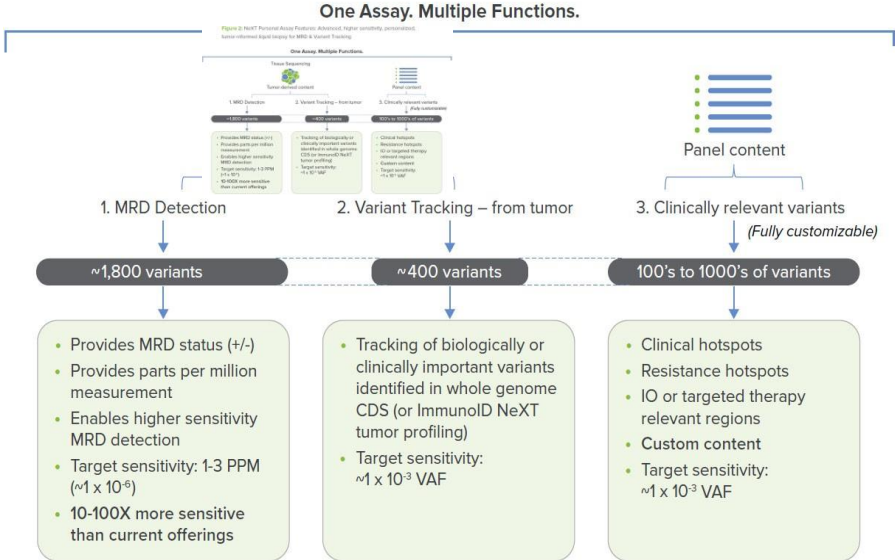
Model Assumptions: Median breast tumor size detected by mammography: 1.3cm, Median shedding per NSCLC TRACERx study⁶; Residual tumor from surgery: 1%; Volume doubling time (actual VAF time): 2 months

EX2003 (Personalis NeXT Personal Brochure) at page 13, Fig. 1.

The identified genetic variants are used to inform the understanding of the tumor biology and its dynamic response to therapy

Unique Assay Design Provides Unprecedented Insights Into Longitudinal Disease Monitoring

While the MRD component of NeXT Personal provides a highly sensitive and aggregated measurement of tumor burden in plasma, the ability to simultaneously track individual variants longitudinally can also be utilized to further our understanding of tumor biology and its dynamic response to therapy. Therefore, in addition to MRD, NeXT Personal provides the ability to track and annotate individual variants over the whole evolution of a cancer patient's trajectory in a single panel design. Variants tracked by NeXT Personal in the blood are derived from those detected in the tissue, as well as those specified for panel inclusion by the user (Figure 2).

'783 Patent Claim Language	Personalis' NeXT Personal [®] assay
	Figure 2: NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD & Variant Tracking  One Assay. Multiple Functions. 1. MRD Detection ~1,800 variants - Provides MRD status (+/-) - Provides parts per million measurement - Enables higher sensitivity MRD detection - Target sensitivity: 1-3 PPM ($\sim 1 \times 10^{-6}$) 10-100X more sensitive than current offerings 2. Variant Tracking – from tumor ~400 variants - Tracking of biologically or clinically important variants identified in whole genome CDS (or ImmunID NeXT tumor profiling) - Target sensitivity: $\sim 1 \times 10^{-3}$ VAF 3. Clinically relevant variants (Fully customizable) 100's to 1000's of variants - Clinical hotspots - Resistance hotspots - IO or targeted therapy relevant regions - Custom content - Target sensitivity: $\sim 1 \times 10^{-3}$ VAF EX2003 (Personalis NeXT Personal Brochure) at page 14, Fig. 2.

2. Industry Praise

157. Personalis' assay has received industry attention and praise due in part to its sensitivity relative to other methods. For example, *The Scientist* magazine published its list of the Top 10 Innovations of 2022. The patented approach disclosed in the '783 patent earned the #1 ranking. See EX2001 at 1-2.

158. The differences between the method used in Leary (EX1002) and the method claimed in the '783 patent yield very different results. Personalis' assay can detect tumor in DNA in blood plasma at levels of about one part per million (or

0.0001%). EX2003 at page 13. Personalis' assay therefore can detect allele frequencies of 0.0001%. By comparison, the Editor's Summary of Forshew explains that the field sought allele frequency detections of less than 2%. EX1030 (Forshew) Editorial Summary ("TAm-Seq will need to achieve a more sensitive detection limit (<2% allele frequency) to identify mutations in the plasma of patients with less advanced cancers."). Ley's assay was only able to detect allele frequencies at approximately 3% for the "skin" sample. EX1012 (Ley) at 69, Figure 3. Thus, Forshew and Ley were several hundred times less sensitive than the claimed method.

3. Skepticism of Experts

159. The references cited by Foresight and Dr. Quackenbush provide evidence that the industry's experts held skepticism toward someone conceiving of a method that would solve the challenges of conducting highly sensitive genome sequencing using personalized probe sets. Forshew (EX1030) acknowledges that using capture probes for cancer monitoring was "expensive" and that achieving sensitive monitoring for mutations across entire genes has been "more challenging." EX1030 (Forshew) at page 10 and 1; *see also* EX1031 (Meyerson) at 686 (discussing "the key challenges in cancer genome sequencing"). Experts in the field were clearly skeptical of the personalized probes Forshew (EX1030) described as "difficult to implement on a routine basis." EX1030 (Forshew) at page 10.

160. Other references outline industry-wide problems with next-generation sequencing (NGS) at the time, which were unexpectedly solved by the methods in the '783 Patent. For example, Anderson (EX1004) emphasizes that “difficult engineering challenges must be overcome before these technologies could become commercially viable.” EX1004 (Anderson) at page 48. Further, Lo (EX1017) identifies difficulties facing experts regarding “how to handle the massive amount of sequencing data” when utilizing NGS for profiling cancer genomes.” EX1017 at page 3.

161. This objective real-world evidence supports a finding that the claims of the '783 patent are nonobvious.

VIII. FORESIGHT HAS NOT PROVEN UNPATENTABILITY OF ANY CLAIM FROM THE '611 PATENT

162. This section of my declaration provides an overview of the '611 patent, followed by an explanation of why each of Grounds 1-4 raised by the Petition do not prove anticipation or obviousness of the claims of the '611 patent.

A. OVERVIEW OF THE '611 PATENT

163. The '611 patent relates to methods and systems for personalized genetic testing of a subject for use in the diagnosis, monitoring, treatment, and prevention of diseases. For example, the abstract of the '611 patent states:

This disclosure provides systems and methods for sample processing and data analysis. Sample processing may include nucleic acid sample processing and subsequent sequencing. Some or all of a nucleic acid sample may be sequenced to provide sequence information, which may be stored or otherwise maintained in an electronic storage location. The sequence information may be analyzed with the aid of a computer processor, and the analyzed sequence information may be stored in an electronic storage location that may include a pool or collection of sequence information and analyzed sequence information generated from the nucleic acid sample. Methods and systems of the present disclosure can be used, for example, for the analysis of a nucleic acid sample, for producing one or more libraries, and for producing biomedical reports. Methods and systems of the disclosure can aid in the diagnosis, monitoring, treatment, and prevention of one or more diseases and conditions.

EX1001 (the '611 patent) at Abstract.

164. Claim 1 is the only independent claim and it recites five steps (a)-(e) as shown below:

Claim 1. A method for personalized genetic testing, comprising:

(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;

(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set of genetic variants,

wherein said set of genetic variants are identified with respect to a reference;

(c) obtaining a probe set comprising a plurality of nucleic acid probe molecules having said plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in said at least one biological sample;

(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and

(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

EX1001 (the '611 patent) at Claim 1.

165. The specification of the '611 patent explains that high costs previously limited “clinical adoption of exome-scale sequencing,” and that “[t]his problem is even worse in cancer, where the depth of deoxynucleic acid (DNA) sequencing required can be much higher (e.g., >500×) than that for inherited diseases (e.g., 30-

100×).” EX1001 (the ‘611 patent) at 4:23-24; 4:26-29. And “[w]hile array-based DNA synthesis is now widely used to capture whole exomes, transcriptomes, or application-specific subsets of exomes (e.g., the genes involved with a specific Mendelian disease), a limitation of the field, as recognized herein, is the potential to leverage array synthesis of DNA in a personalized manner.” *Id.* at 4:30-35. Instead, “[t]he field has largely used array-based synthesis to develop standard products which are broadly applicable across a whole set of human patients and/or research subjects.” *Id.* at 4:35-38; *see also id.* at 4:38-40 (“Even where custom array synthesis is proposed, it is to sequence regions of the genome defined independent of a specific sample.”).

166. The methods claimed by the ‘611 patent are directed to a method for “personalized genetic testing.” First, the claimed method requires sequencing nucleic acid molecules from at least one biological sample, which can be a tissue sample, blood sample, or tumor tissue (*see* EX1001 (the ‘611 patent) at 8:63-65, 10:7-8), to generate sequencing data of 12 gigabases (GB) or more of coverage. *See, e.g., id.* at 62:31-38 (“In the standard cancer sequencing process, a deep tumor sample can be exome sequenced (e.g., 30-75 billion bases) and a germline DNA sample can be exome sequenced (e.g., 12 billion bases). The data can be used to determine which variants are somatic. Also, a tumor RNA (e.g., 22 billion bases,

e.g., 50-70 million paired-end reads) can be deep transcriptome sequenced.”); *see also id.* at 69:18-21, which is limitation 1(a).

167. Second, the sequencing data undergoes computer processing to identify genetic variants and their nucleic acid sequences. *See, e.g.*, EX1001 (the ‘611 patent) at 11:46-55. Genetic variant “generally refers to an alteration, variant or polymorphism in a nucleic acid sample or genome of a subject.” *Id.* at 25:4-6. The variants are identified with respect to a reference, including genomes or sequences from the same subject or another individual. *Id.* at 25:6-9; *see also id.* at 69:22-25, which is limitation 1(b).

168. Third, the claimed method involves obtaining a probe set. A probe “generally refer[s] to a nucleic acid molecule comprising a single-stranded portion capable of hybridizing to a complementary nucleic acid sequence,” and “can be used for detection or enrichment of [said] nucleic acid molecules.” EX1001 (the ‘611 patent) at 26:1-6. “A probe may be included in a set, or plurality, of probes.” *Id.* at 26:11-12. The claimed probe set includes probes that are complementary to and thereby configured to enrich or amplify the identified genetic variants. *Id.* at 26:1-19; *see also generally id.* at Example 7 (describing exemplary methods to synthesize probes); *see also id.* at 69:26-32, which is limitation 1(c).

169. Fourth, the probe set is then applied to an “additional biological sample” to selectively enrich or amplify sequences comprising the genetic variants. *See, e.g.* EX1001 (the ‘611 patent) at 26:1-19. The additional biological sample may be a sample of the same tissue from same subject taken at a later time point, a sample of different tissue from the same subject, a sample from a biological relative of the subject, etc.... *See, e.g., id.* at Example 1; *id.* 5:4-9. The enriched and amplified sequences of the genetic variants generate a sequencing library. *See, e.g., id.* at 4:55-63; *see also id.* at 69:33-37, which is limitation 1(d).

170. Finally, the sequencing library is sequenced to identify a presence or absence of the genetic variants in the additional biological sample. The sequencing coverage of this step is 175 kilobases (kb) or less. *See* EX1001 (the ‘611 patent) at 34:65-35:3 (“As a non limiting example, 500 variants may require the synthesis of at least about 500 sequences. The genomic region captured by each probe can be at least about 350 bases. Therefore, for at least about 500 sequences, the footprint of this assay may be about 175,000 bases.”). This is far less than the 12 GB sequenced during the first step, and, when “[c]ompared to an exome, where the footprint of the assay is typically at least 35 million bases, this may result in 200× less sequencing.” *Id.* at 35:3-5. Use of targeted probes allows for a “dramatic reduction in the amount of sequencing required,” making it “much more affordable to sequence additional

pedigree members” or other additional biological samples. *Id.* at 35:5-9. With the reduction in sequencing footprint, the sequencing depth can be greater than the depth of the first sequencing step without being cost prohibitive, which the specification explains as follows:

To increase the confidence of detecting a potentially mosaic variant in the parents, they will need to be sequenced at greater depth than the child. If the assay for additional members of a family pedigree is exome sequencing, it may be cost prohibitive to sequence at that depth. Using method provided herein, the footprint of the assay for incremental family members is much smaller (e.g., 175,000 bases vs 35 million bases, as discussed above). This smaller footprint makes it affordable to sequence the incremental samples at much greater depth, thus improving the sensitivity for detection of mosaic variants in the parents.

EX1001 (the '611 patent) at 61:37-49; *see also id.* at 69:38-45, which is limitation 1(e). I note that the specification estates that “[t]he term ‘mosaicism,’ as used herein, generally refers to genetic changes which occur after an embryo has started to develop. These changes will only be found in a fraction of the cells of a human body.” *Id.* at 27:19-22. In other words, mosaicism refers to genetic changes which occur after the sperm fuses with the egg.

B. GROUND 1: NOVELTY OVER LEY

171. Ley does not anticipate independent claim 1, and therefore fails to anticipate any of the challenged dependent claims. Ley further fails to anticipate dependent claims 5, 10, and 28 for additional reasons discussed herein.

1. Claim 1

172. Ley (EX1012) fails to anticipate claim 1 because it does not teach at least three distinct requirements recited by limitation 1(e). In particular, Ley fails to teach:

- “a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a),”
- “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less,” and
- “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less **to identify** a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative.”

a. Ley does not teach the required sequencing depth

173. Limitation 1(e) requires “a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).”

174. Dr. Quackenbush asserts that Ley meets this requirement of limitation 1(e) based on adding together variant and reference read counts found in Ley’s Table 3. EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶123-125. Dr. Quackenbush assumes that by “[s]umming the variant and reference read counts for each gene in each sample” shown in Ley’s Table 3 “one can see that the variant loci in the additional relapse sample (‘additional biological sample from said subject’) have been sequenced at depths ranging from 588-fold to 12,365-fold.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶125.

175. Table 3 from Ley is reproduced below:

Table 3 | 454 Readcount data for somatic mutations and known SNPs

Gene	Consequence	Primary AML (100% blasts)			Skin			Relapse (78% blasts)		
		Variant	Ref	Variant (%)	Variant	Ref	Variant (%)	Variant	Ref	Variant (%)
CDH24	Y590X	5672	4890	53.70	564	10358	5.16	3108	4599	40.33
SLC15A1	W77X	3817	4962	43.48	875	10773	7.51	4714	7173	39.66
KNDCC1	L799F	4640	4848	48.90	770	8972	7.90	3883	6342	37.98
PTPRT	P1235L	998	1058	48.54	126	1489	7.80	350	493	41.52
GRINL1B	R176H	2211	2674	45.26	318	4461	6.65	1447	2070	41.14
GPR123	T38I	4618	4569	50.27	850	9751	8.02	3660	6057	37.67
EBI2	A338V	12750	15453	45.21	458	10088	4.34	2646	3627	42.18
PCLKC	P1004L	992	855	53.71	341	3153	9.76	705	773	47.70
FLT3	ITD	4220	7810	35.08	3475	23159	13.05	3870	8495	31.30
NPM1	CATG ins	1550	1974	43.98	143	2390	5.65	2303	3910	37.07
BRCA2	N372H	778	752	50.85	763	876	46.55	285	303	48.47
TP53	P72R	8989	1	99.99	8161	0	100.00	7914	6	99.92

The differences between variant frequencies in primary or relapse tumour samples and skin were highly significant for all somatic mutations ($P < 0.000001$, Fisher’s exact test, one tailed). The BRCA2 variant is a known heterozygous SNP in this genome, and the TP53 variant is a known homozygous SNP.

EX1012 (Ley) at page 70, Table 3. This table provides “454 Readcount data for somatic mutations and known SNPs.” *Id.* The read counts found in Ley’s Table 3 do not provide sufficient information to calculate the sequencing depth. I note that Dr. Quackenbush explained in the background “overview” section of his declaration

that a person of ordinary skill in the art would have known that sequencing depth is calculated using the Lander/Waterman equation:

$$C=L*(N/G)$$

Where C stands for “coverage” or “sequencing depth,” G is the length of the genes being studied, L is the read length, and N is the number of reads. EX1061 (Quackenbush Declaration IPR2023-00546) ¶66.

176. Ley’s Table 3 only provides N, the number of reads (i.e., Ley’s “readcount”). Knowing the number of reads (N) alone does not allow a person of ordinary skill in the art to calculate the sequencing depth (C). To calculate sequencing depth (C), the person of ordinary skill in the art must also know the relevant read length (L) and the relevant length of genes being studied (G). Dr. Quackenbush’s declaration does not provide the relevant read length (L) or length of genes being studied (G) that a person of ordinary skill in the art would have used to calculate Ley’s sequencing depth (C).

177. Ley does not provide the primer sequences or the exon that was analyzed to generate the data in Table 3. The primer sequences impact the read length of the resulting amplicon based on which part of the exon the primers hybridize to. If the primers hybridize to a part of the exon that is closer to its starting or ending points, then the resulting amplicon will be longer than if the primers

hybridize to a part of the exon that is farther from the starting or ending points. Because Ley does not provide the primer sequences or the exon that was analyzed to generate the data in Table 3, the read length (L) is unknown.

178. Ley does not provide the length of genes studied (G) because Ley does not provide the exact exon that was analyzed. Some exons are longer than others.

179. Thus, the read length (L) and length of genes studied (G) that Ley used to generate the data in Table 3 are unknown. Therefore, Ley does not provide sufficient information to calculate the sequencing depth (C) for the experiments from Ley that Dr. Quackenbush relied upon to allegedly meet the portion of limitation 1(e) that recites “a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).”

180. Because Ley fails to disclose sufficient information to calculate Ley’s sequencing depth, Dr. Quackenbush has not demonstrated that Ley discloses the portion of limitation (e) that recites “a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).”

b. Ley does not teach 175 kilobases or less

181. Limitation 1(e) requires “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less.”

182. Dr. Quackenbush asserts that Ley sequenced at most 45.254 kilobases. EX1061 (Quackenbush Declaration IPR2023-00546) ¶121. In making this assertion, Dr. Quackenbush makes at least three assumptions. First, he assumes that the genetic variant will simply be revealed by primers that in some way bracket the position of the asserted variant. However, the vast majority of the putative variants appeared to be “classified” as normal sequence. This opens the possibility that the putative variants are indeed variants, but variants that map to a different locus. Second, he assumes an “upperbound” on the sequence coverage of Ley based on a PCR primer pair that would generate an amplicon for the entire length of each of the genes that contained a genetic variant identified by Ley as follows:

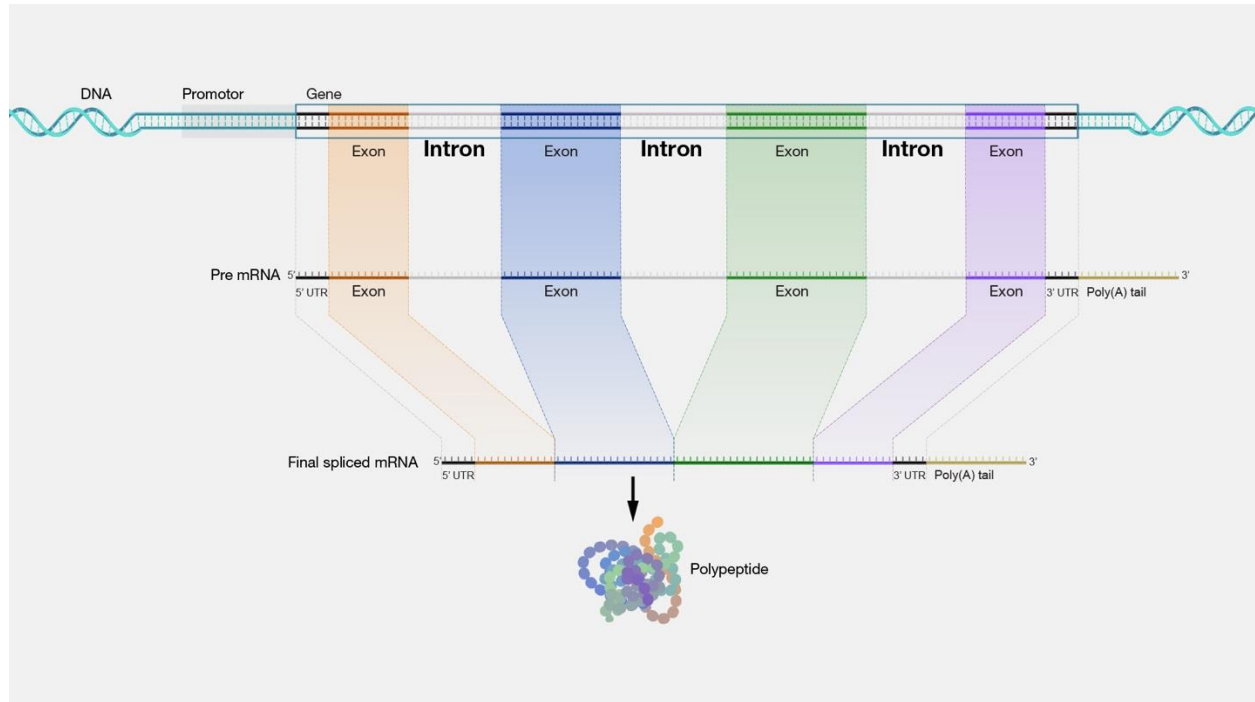
While Ley does not provide the primer sequences or the exact exons that were analyzed, *one can easily set an upper bound on the sequence coverage Ley teaches by using the entire length of the genes* that contained the genetic variant rather than the much smaller sequence of the variant-containing exon.

EX1061 (Quackenbush Declaration IPR2023-00546) ¶121. Third, he assumes that “the messenger RNA corresponding to the coding region of these ten genes” allows a person of ordinary skill in the art to “easily obtain the length of the entire coding sequence for each of these genes.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶121.

183. Dr. Quackenbush's third assumption is incorrect. Messenger RNA does not correspond to the length of the ten genes used by Ley. The length of the messenger RNA only corresponds to the length of the **exons** within Ley's genes. It was well known that the entire length of a gene includes both exons **and introns**. For example, the Lewin textbook explains this as follows:

The simple view of the gene was upset in 1977 by the discovery of interrupted genes. The primary evidence for the existence of these interruptions was a comparison between the structure of DNA and the corresponding messenger RNA. The messenger RNA always included a nucleotide sequence that corresponds exactly with the protein product according to the rules of the genetic code. *But the gene includes additional sequences that lie within the coding region, interrupting the sequence that represents the protein.*

Lewin, Genes V, Oxford University Press, 1994, EX2172, at page 149 (emphasis in original). "Exons are the regions that are represented in the messenger RNA. Introns are regions that are missing from the messenger RNA." *Id.* A graphic conveying this information from the National Human Genome Research Institute (<https://www.genome.gov/genetics-glossary/Intron>) is provided below:



In many genes, the introns are much longer than the exons. In fact, “the exome represents only approximately 1% of the genome.” EX1031 (Meyerson) at page 690. Thus, while the exons for a given gene could span, for example, 1,000 bases, those exons could be distributed over tens of thousands of bases when including the introns for the gene. When Dr. Quackenbush sets an “upper bound on the sequence coverage Ley teaches by using the entire length of the genes,” EX1061 (Quackenbush Declaration IPR2023-00546) ¶121, the number of kilobases would greatly exceed 45,254 bp. It would amount to far more than 175 kilobases because the entire length of the genes includes both introns and exons rather than just the exons found in the much shorter messenger RNA. It is important to point out that

although Dr. Quackenbush indicates that only coding segments should be considered, he includes the non-coding segments of mRNA in his analysis. The mRNA included by Dr. Quackenbush has 5' and 3' UTR (untranslated region) segments, which are non-coding segments. This further supports that all noncoding segments should be considered.

c. Ley does not teach sequencing “to identify”

184. Limitation 1(e) requires “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less *to identify* a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative.”

i. Claim construction of “to identify”

185. I understand that claim terms generally are afforded their plain and ordinary meaning to a person of ordinary skill in the art.

186. I also understand that the Board preliminarily determined that “step 1(e) is silent as to whether a genetic variant is identified de novo in a previously untested ‘additional’ sample, or identified in a previously tested sample for some validating or confirmatory purpose.” Institution Decision at page 27. I understand that the Board also preliminarily determined that “[i]n showing that variant alleles were present in both primary and relapse tumor samples, Ley’s Figure 3 satisfies claim

step 1(e) by ‘identifying a presence of at least a subset of said set of genetic variants’ in said additional biological sample.” *Id.*

187. I disagree with the Board’s preliminary determinations because they do not comply with how a person of ordinary skill in the art would have understood the plain meaning of “to identify” as recited by limitation 1(e).

188. The specification does not define “to identify.” I understand that this term should therefore be accorded its plain and ordinary meaning to a person of ordinary skill in the art.

189. The dictionary definition of “identify” is “to establish the identity of.” See EX2170 (American Heritage Dictionary, 4th edition, 2000) at page 871. Thus, a person of ordinary skill in the art would have understood the plain meaning of “to identify” as recited by limitation 1(c) to mean “*to establish the identity of.*”

ii. **Ley does teach limitation 1(e) under the plain meaning of “to identify”**

190. Dr. Quackenbush believes that Ley (EX1012) performs separate steps of identification and validation. Specifically, he states “[o]nce the variants of interest, i.e., the ‘putative variants,’ *were identified, Ley then describes ‘validation* using custom PCR and capillary sequencing’ as well as sequencing of ‘validated somatic mutations’ ‘by Roche/454 sequencing of PCR-generated amplicons made

from primary genomic DNA.” EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. I note that the “validation” step referenced by Dr. Quackenbush actually has two separate steps. For the “custom PCR and capillary sequencing” step, Ley confirms the exon in which the mutation is present. For the “Roche/454 sequencing” step, Ley determines the prevalence of the genetic variants by quantifying readcount data. Thus, Ley initially identifies variants of interest, and then separately performs the “validation” referenced by Dr. Quackenbush.

191. Ley’s identification step uses sequencing data to establish the identity of somatic mutations in the typical AML genome. See EX1012 (Ley) at page 68-69 (section of Ley entitled “Identification of somatic mutations in coding sequences”). For this identification, Ley sequenced PCR-generated amplicons to “determine[] that 152 of these variants were false positive (that is, wild type) calls, 14 were inherited SNPs, and eight were somatic mutations in both the original tumor and the relapse sample (Table 2).” EX1012 (Ley) at page 69.

192. The “validation” protocol from Ley that Dr. Quackenbush relies upon to allegedly meet limitation 1(e) is a separate protocol from Ley’s identification step. For example, Dr. Quackenbush relies on Ley Figure 3. See EX1020 (Quackenbush Declaration IPR2023-00545) ¶122. Ley’s Figure 3 depicts the quantities of genetic variants present in Ley’s subject based on readcount data:

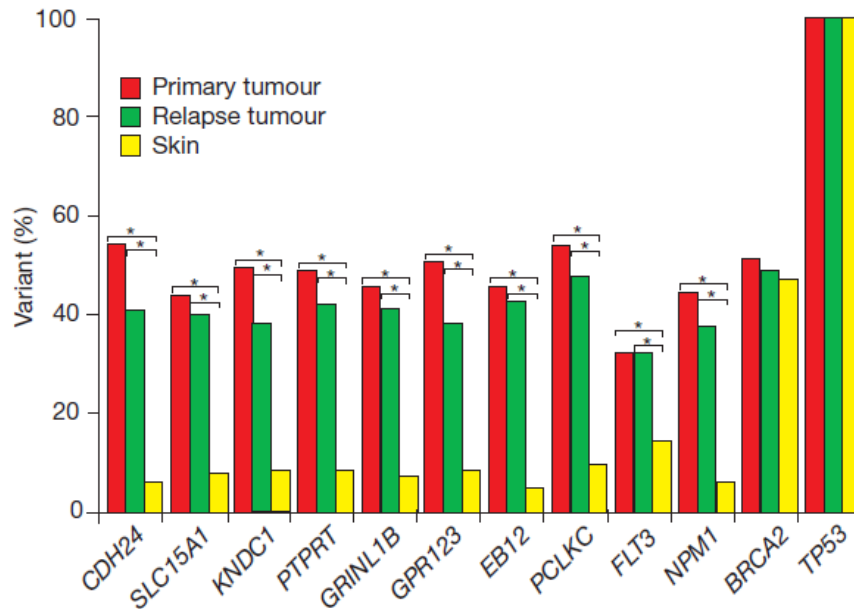


Figure 3 | Summary of Roche/454 FLX readcount data obtained for ten somatic mutations and two validated SNPs in the primary tumour, relapse tumour and skin specimens. The readcount data for the variant alleles in the primary tumour sample and relapse tumour sample are statistically different from that of the skin sample for all mutations ($P < 0.000001$ for all mutations, Fisher's exact test, denoted by a single asterisk in all cases). Note that the normal skin sample was contaminated with leukaemic cells containing the somatic mutations. The patient's white blood cell count was 105,000 (85% blasts) when the skin punch biopsy was obtained.

EX1012 (Ley) at page 69, Figure 3. The mutation frequencies are quantified during the validation protocol by tallying readcounts. The identity of the mutations was established in the proceeding (and separate) protocol described in the section entitled “Identification of somatic mutations in coding sequences” on pages 68-69 of Ley. In fact, to pursue this analysis, it must be the case that the variant is no longer regarded as putative but is already regarded as validated.

193. A person of ordinary skill in the art would not have confused Ley's "validation" protocol with the claimed "to identify" step of limitation 1(e). Rather, a person of ordinary skill in the art would understand that Ley teaches an identification step that is distinct and separate from Ley's "validation" step. This understanding is supported by Dr. Quackenbush's explanation that "[o]nce the variants of interest, i.e., the 'putative variants,' **were identified, Ley then describes 'validation** using custom PCR and capillary sequencing' as well as sequencing of 'validated somatic mutations' 'by Roche/454 sequencing of PCR-generated amplicons made from primary genomic DNA.'" EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. The result of Ley's "validation" step is the quantification of variant frequencies by tallying readcount sequencing data rather than establishing the identity of a presence or absence of variants as claimed by limitation 1(e).

2. Claim 5

194. Claim 5 depends from claim 1 and requires "each of said plurality of nucleic acid probe molecules includes oligonucleotide-directed genomic content comprising (i) at least one variable portion from a result of said sequencing of (a) and (ii) at least one fixed portion independent of said result of said sequencing of (a)."

195. Ley does not teach a probe set comprising a plurality of nucleic acid probe molecules having oligonucleotide-directed genomic content comprising “(i) **at least one variable portion** from a result of said sequencing of (a) and (ii) **at least one fixed portion** independent of said result of said sequencing of (a)” as required by claim 5.

a. **Claim construction of “variable portion” and “fixed portion”**

196. I understand that claim terms generally are afforded their plain and ordinary meaning to a person of ordinary skill in the art. I understand, however, that when a specification contains a definition for a term, then that definition governs the meaning of the term.

197. The recited “variable portion” and “fixed portion” do not have a plain meaning to a person of ordinary skill in the art. However, the specification of the '783 patent provides an express definition of “variable portion” and “fixed portion” as follows:

In some applications, it may be desirable for the oligo-directed genomic content of the personalized assay to contain both ***a variable portion (defined based on the genetic characteristics originally determined for the individual subject) and at least one fixed portion (which does not change from one subject to another).***

EX1001 (the '611 patent) at 38:42-47. The variable portion changes from subject to subject because it is “defined based on the genetic characteristics originally determined for the individual subject,” whereas the fixed portion “does not change from one subject to another.” *Id.*; *see also id.* at 63:33-36 (“Thus, as discussed above, the genomic content of a personalized genetic assay may include **a portion which is variable subject to subject, and another portion which is fixed.**”). The title of the section describing variable and fixed content is “Combinations of **Fixed and Variable (personal)** Genomic Content in the Array-Synthesized Nucleic Acids.” EX1001 (the '611 patent) at 38:37-38. The variable portion is “personal” to the subject, whereas the fixed portion does not change from subject to subject.

198. Claim 5 provides further clarification regarding the “variable portion” and “fixed portion” by stating that the variable portion is “from a result of said sequence data” and the fixed portion must be “independent of said result of said sequencing data.” EX1001 (the '611 patent) at 69:63-67.

199. Based on the definition in the specification and the claim language that provides further clarification on the meaning of “variable portion” and “fixed portion” within the context of the claimed invention, *the “variable portion” recited by claim 5 should be construed to refer to a portion “which changes from one subject to another and results from said sequence data,” and the “fixed portion”*

should be construed to refer to a portion “which does not change from one subject to another and is independent of said result of said sequencing data.”

b. Ley does not teach a variable portion or fixed portion

200. Dr. Quackenbush states in his declaration that “Ley does not provide the primer sequences or the exact exons that were analyzed.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶121. Ley does not disclose PCR primer sequences in either the body of the paper (EX1012) or the Supplementary Information (EX1042). In fact, the word “primer” does not appear in Ley (EX1012) or Ley’s Supplementary Information (EX1042).

201. Dr. Quackenbush asserts that Ley (EX1012) discloses PCR primers “for known mutations of the BRCA2 and TP53 genes” that supposedly meet the “fixed portion” of claim 5. EX1061 (Quackenbush Declaration IPR2023-00546) ¶136. Ley reports that the amino acid at position 372 in the BRCA2 protein, derived from one allele, had changed from asparagine to histidine, namely Ley reports N372H. Ley reports that the amino acid at position 72 in the TP53 protein, derived from both alleles, had changed from proline to arginine, namely P72R. See Ley (EX1012) at page 70, Table 3.

202. The asparagine (N) at position 372 of the amino acid sequence is coded by an AAT codon in the reference DNA sequence of the human BRCA2 gene. This

is easily determined using simple software that converts the nucleotide sequence provided in the Genbank Record for BRCA2 in Exhibit 1058. The amino acid histidine (H) is coded by either a CAT codon or a CAC codon. See *e.g.*, EX2172 (Lewin) at page 172 (providing DNA codons for amino acids). Thus, the genetic variant reported in Ley's subject could have had either of these two codons in the affected allele. Ley does ***not disclose*** which of these two possible codons were present in the subject's BRCA2 gene. Dr. Quackenbush's assertion that Ley's subject had "known mutations of the BRCA2" gene does not answer whether the mutation in the DNA sequence of the subject's BRCA2 gene changes from one subject to another. Because there are two different potential codons that Ley's subject may have had, the genomic content created by Ley's PCR primers will change from one subject to another. If the genomic content of Ley's PCR primers changes from one subject to another, then they would not meet the "fixed portion" recited by claim 5 because the "fixed portion" must "not change from one subject to another" under the appropriate construction of that term.

203. The proline (P) at position 72 of the amino acid sequence is coded by a CCC codon in the reference DNA sequence of the TP53 gene. This is easily determined using simple software that converts the nucleotide sequence provided in the Genbank Record for TP53 in Exhibit 1059. The amino acid arginine (R) is coded

by CGC, CGT, CGA, CGG, AGA, or AGG codons. *See e.g.*, EX2172 (Lewin) at pages 172 (providing DNA codons for amino acids). Thus, Ley's subject could have had any of these six codons in each TP53 allele. Ley does not disclose which of these six possible codons were present in the subject's alleles. Dr. Quackenbush's assertion that Ley's subject had "known mutations" of the TP53 gene does not answer whether the mutation in the DNA sequence of the subject's TP53 gene changes from one subject to another. Because there are at least six different potential codons that Ley's subject may have had, the genomic content created by Ley's PCR primers could very well change from one subject to another. If the genomic content of Ley's PCR primers changes from one subject to another, then they would not meet the "fixed portion" recited by claim 5 because the "fixed portion" must "not change from one subject to another" under the appropriate construction of that term.

204. If Dr. Quackenbush had asserted that the PCR primers used by Ley to generate amplicons of the subject's BRCA2 and TP53 mutations do not change from one subject to another, then he should have also explained how the PCR primers used by Ley to generate amplicons of the other eight mutations from Ley that he alleges meet the claimed "variable portion," *see* EX1061 ¶135, do not actually constitute "fixed portions" that do not change from one subject to another. I note that the other eight mutations were located in a patient with "a **typical** acute myeloid

leukaemia genome,” rather than an atypical genome. EX1012 (Ley) at page 66. Ley explains that specific patient being studied, UPN 933124, was selected because the patient had “[t]ypical features of M1 AML” that was “**representative of most patients** with M1 AML.” EX1042 (Ley Supplementary Information) at page 1. Ley “required a gene expression profile **typical** of M1 AML with **normal cytogenetics**, to assure that the sample was **not an outlier** for this subtype.” EX1042 (Ley Supplementary Information) at page 1. Because Ley’s patient was selected to be typical and representative of most patients with M1 AML, then the PCR primers used by Ley to generate amplicons of the other eight mutations that Dr. Quackenbush relies upon to supposedly meet the “variable portion” could very well **not** change from one subject to another. Ley states that “[a]ll of the PCR-amplified exons from the eight genes containing validated somatic mutations were sequenced in 187 further cases of AML using samples from our discovery and validation sets; no further somatic mutations were detected in these genes (data not shown).” EX1012 (Ley) at page 69. The data from these patients is not provided. There is no information contained within Ley (EX1012) or Ley’s Supplementary Information (EX1042) to know with certainty what the genomic content of Ley’s undisclosed PCR primers was to make a determination if Ley discloses something that meets the claimed “variable portion” and/or “fixed portion.”

3. Claims 10 and 28

205. Claim 10 depends from claim 1 and recites:

Claim 10. The method of claim 1, further comprising ***providing a therapeutic intervention*** at least based on said presence or absence of said at least said subset of said genetic variants identified in (e).

206. Claim 28 depends from claim 1 and recites:

Claim 28. The method of claim 1, further comprising using said presence or absence of said at least said subset of said set of genetic variants identified in (e) ***to treat said subject or said at least one biological relative for a disorder.***

207. Dr. Quackenbush asserts that Ley (EX1012) discloses a therapeutic intervention and treating the subject for a disorder based on the patient's implication of the SLC15A1, positive mutation in the FLT3 and NMP1 genes, and Ley's Figure 3. See EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶139-142 (claim 10), ¶¶151-155 (claim 28).

208. Ley (EX1012) does not disclose a contemporaneous therapeutic intervention or using the presence/absence of genetic variants to contemporaneously treat the subject because the subject of Ley's assay ***was deceased*** at the time when Ley's assay was performed. The subject of Ley's assay (UPN 933124) had "***expired*** 24 months after her initial diagnosis" and "[i]nformed consent for whole genome

sequencing ***was subsequently obtained*** from her next of kin and family members.” EX1042 (Ley Supplementary Information) at page 2. “***Subsequent to the death of patient 933124***, the WU HSC [Washington University Human Studies Committee] approved an amendment to the protocol that incorporated language specifically permitting the use of banked DNA for whole genome sequencing, to which her husband consented.” EX1042 (Ley Supplementary Information) at page 3. I am not aware of any therapeutic intervention or treatment that is available for the deceased subject of Ley’s assay.

C. GROUND 2: NONOBVIOUSNESS OVER LEY

209. Ley does not render obvious independent claim 1. Ley also does not render obvious dependent claims 5, 10, and 28 for additional reasons discussed in this section.

1. Claim 1

210. Ley does not suggest at least three distinct requirements recited by limitation 1(e). Specifically, Ley does not suggest:

- “a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a),”
- “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less,” and

- “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less **to identify** a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative.”

a. Ley does not suggest the required sequencing depth

211. As discussed above, Ley does not disclose sufficient information to determine the sequencing depth for the experiment used to generate the data in Ley’s Table 3. *Supra* Section VIII.B.1.a. Accordingly, Dr. Quackenbush has not demonstrated that Ley discloses the sequencing depth required by limitation 1(e).

212. Dr. Quackenbush asserts that there was a general motivation to “achiev[e] a higher sequence depth” for the purpose of “increasing accuracy of detecting and quantifying mutations in tumors.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶167. Dr. Quackenbush does not explain why he believes a person of ordinary skill in the art would have thought that the accuracy and/or quantification aspects of Ley’s assay that generated the data in Table 3 required improvement. Moreover, as discussed above, Ley (EX1012) did not disclose sufficient information to determine the sequencing depth for the data in Table 3. Without knowing Ley’s sequencing depth, a person of ordinary skill in the art would have no baseline

sequencing depth from which to measure whether it was desirable to achieve a higher (or lower) sequencing depth for the assay that generated Ley's Table 3.

213. Dr. Quackenbush's assertion that a person of ordinary skill in the art would want to achieve a higher sequence depth to increase accuracy is counterproductive to other factors that Dr. Quackenbush explained a person of ordinary skill in the art would try to achieve when designing assays for tumors. Dr. Quackenbush testified that a person of ordinary skill in the art would have been motivated by several factors when designing assays related to tumors:

- a desire to reduce the cost of an assay, *see* EX2163 (Quackenbush Transcript) at 27:15-30:22;
- a desire to simplify an assay, *see* EX2163 (Quackenbush Transcript) at 36:7-21; and
- a desire to simplify the computational analysis for data generated by an assay, *see* EX2163 (Quackenbush Transcript) at 41:18-44:19.

214. Increasing sequencing depth of Ley's assay would complicate the assay with more sequencing. It would also increase the computational analysis by generating more data to analyze. And it would increase the cost of the assay by increasing the amount of sequencing and data analysis. Here, the prior art does not give an indication or direction as to whether these factors would have been

considered to be less important to a person of ordinary skill in the art than attempting to increase the accuracy of Ley's assay.

b. Ley does not suggest 175 kilobases or less

215. Limitation 1(e) requires "subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less."

216. As discussed above, Dr. Quackenbush is incorrect that Ley discloses the portion of limitation 1(e) that requires "sequencing at a coverage of 175 kilobases (kb) or less." *Supra* Section VIII.B.1.b.

217. Dr. Quackenbush asserts that the specification of the '611 patent "does not attach any significance" to the claimed coverage. EX1061 (Quackenbush Declaration IPR2023-00546) ¶165. The specification, however, explains that the 175 kilobase limitation dramatically reduces the amount of sequencing required and makes the claimed methods much more affordable than prior art methods as follows:

To increase the confidence of detecting a potentially mosaic variant in the parents, they will need to be sequenced at greater depth than the child. If the assay for additional members of a family pedigree is exome sequencing, it may be cost prohibitive to sequence at that depth. Using method provided herein, the footprint of the assay for incremental family members is much smaller (e.g., 175,000 bases vs 35 million bases, as discussed above). This smaller footprint makes it affordable

to sequence the incremental samples at much greater depth, thus improving the sensitivity for detection of mosaic variants in the parents.

EX1001 (the '611 patent) at 61:37-49; *see also generally id.* at 59:29-62:3 (Example 1). The specification further explains:

As a non-limiting example, 500 variants may require the synthesis of at least about 500 sequences. The genomic region captured by each probe can be at least about 350 bases. Therefore, for at least about 500 sequences, the footprint of this assay may be about 175,000 bases. Compared to an exome, where the footprint of the assay is typically at least 35 million bases, this may result in 200× less sequencing. This dramatic reduction in the amount of sequencing required, per additional family pedigree member, can make it much more affordable to sequence additional pedigree members (e.g., the parents and other children of the same parents).

EX1001 (the '611 patent) at 34:66-35:9; *see also generally id.* at 34:42-35:56 (explaining that the 175 kilobase limitation results in 200x less sequencing by providing at least a 10x reduction in the sequencing footprint and discussing Figures 3-4); *id.* at Figures 3-4.

218. Dr. Quackenbush asserts that there was a general motivation to “target smaller genomic regions” and “reduce sequencing costs” at the time of the invention. EX1061 (Quackenbush Declaration IPR2023-00546) ¶167. Dr. Quackenbush,

however, does not explain why he believes a person of ordinary skill in the art would have thought that Ley's specific assay that generated the data in Table 3 would benefit from targeting a smaller genomic region or a reduction in sequencing costs. Ley teaches "[o]ur results strongly support the notion that hypothesis-driven (for example, candidate gene-based) examination of tumour genomes by PCR-directed" methods "is inherently limited, and will miss key mutations." EX1012 70. Thus, targeted examination of smaller genomic regions is the type of "hypothesis-driven" examination of genomic regions that Ley strongly counsels are inherently limited and will miss key mutations. Thus, Ley itself would dissuade a POSA from targeting smaller genomic regions.

c. Ley does not suggest sequencing "to identify"

219. Limitation 1(e) requires "subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less ***to identify*** a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative."

220. I am not aware of any discussion in Dr. Quackenbush's declaration regarding how he believes a person of ordinary skill in the art would have been motivated to modify Ley's assay to involve "subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less ***to identify*** a presence or

absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative” as required by limitation 1(e). For example, the obviousness section of Dr. Quackenbush’s declaration includes some discussion of “sequencing at a coverage of 175 kilobases (kb) or less,” but it does not discuss the “to identify” requirement of limitation 1(e). See EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶163-168. It appears that Dr. Quackenbush’s obviousness position simply relies on a person of ordinary skill in the art treating Ley’s validation step as being equivalent to the claimed “to identify” recited by limitation 1(e). See EX1061 (Quackenbush Declaration IPR2023-00546) ¶122. A person of ordinary skill in the art would not confuse these distinct steps for reasons discussed above. *Supra* Section VIII.B.1.c.

221. A person of ordinary skill in the art would understand that Ley teaches an identification step that is distinct and separate from Ley’s validation step. This understanding is supported by Dr. Quackenbush’s explanation that “[o]nce the variants of interest, i.e., the ‘putative variants,’ **were identified, Ley then describes ‘validation** using custom PCR and capillary sequencing’ as well as sequencing of ‘validated somatic mutations’ ‘by Roche/454 sequencing of PCR-generated amplicons made from primary genomic DNA.’ EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. Thus, Ley initially identifies variants of interest, and then

separately validates the variants of interest. The result of Ley's validation step is the quantification of variant frequencies by tallying readcount sequencing data rather than establishing the identity of a presence or absence of variants as claimed by limitation 1(e). A person of ordinary skill in the art would not have been motivated to *re*-establish the identity of mutations that had already been established.

222. The innovation of the claimed invention to require subjecting said sequencing library to sequencing to establish the identify of a presence or absence of at least a subset of said set of genetic variants is apparent when considered in view of the state of the art at the time of the invention. Firstly, it is important to make some overarching comments about the state of the field of endeavor and the nature of the problems that a person of ordinary skill in the art would have encountered at that time. A person of ordinary skill in the art would know that there were at least three principal problems to be overcome with studies of tumors tissues that used next-generation sequencing: (1) The typical "sequence machines" available at that time could only perform short reads. A person of ordinary skill would understand that short reads posed very significant problems in the correct mapping of sequence reads. This is a problem of particular significance in sequencing tumor tissue that one expects to contain genetic variants. (2) Tumor tissue not only contains normal cells, but also contains many subclones of tumor cells that differ in the composition

and nature of their mutant alleles. This is called the tumor heterogeneity problem. *See, e.g.*, Burell (EX1071). Thus, any proposed methodology should be able to detect mutant alleles that are present at very low frequency. In this time period, it was agreed that such methods should at least be able to detect mutant alleles present at 2%. *See* EX1030 Editor's Summary. (3) A person of ordinary skill would be well aware that cost of sequencing is a significant factor in any typical lab environment and thus consider that the design of assays should be cost effective.

223. With this understanding of the state of the art, a person of ordinary skill considering Ley's assay would not have been motivated to adapt the assay to include "subjecting said sequencing library to sequencing ***to identify*** a presence or absence of at least a subset of said set of genetic variants" as required by limitation 1(e). Ley did not successfully address any of problems listed above. Ley merely provided a short read study that sidestepped the heterogeneity issue by using an atypical tumor sample composed of almost 100% tumor cells. *See* EX1042 (Ley Supplementary Materials) at 1-2 (requiring a "high sample purity"). In a typical tumor sample, there is a mixture of tumor and normal cells. The normal cells are problematic because they cause a high background level of normal cell signal that makes it difficult to establish the identity of a presence or absence of genetic variants. This high background level was understood to make it difficult to establish the identity of

genetic variants that might exist in a sample with an allele frequency below 5%. Further, a person of ordinary skill in the art would not have been impressed by the meager collection just 8 validated somatic SNVs reported by Ley's workflow that started with parallel whole genome sequencing of the tumor and normal genome. A person of ordinary skill would have been surprised (and indeed skeptical) that Ley's meager haul did not include the "typical suspects" of SNVs reported in previous studies. Ley's workflow would have been cost prohibitive to a person of ordinary skill working in a typical lab, and indeed could perhaps only have been conducted in one of the centers historically associated with and originally funded by the human genome project. Ley's authors are from The Genome Center at Washington University—one of the most sophisticated sequencing laboratories in the world. A person of ordinary skill in the art would not have access to such resources.

d. No motivation to modify Ley

224. In summary, a person of ordinary skill in the art would not have been motivated to modify Ley's assay to (1) increase the undisclosed and unknown sequencing depth of Ley's assay to meet "a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a)" recited by limitation 1(e), (2) change Ley's sequencing to be performed "at a coverage to 175 kilobases (kb) or less" as required by limitation 1(e), and (3) add a protocol to *re*-establish the

identity of mutations that Ley had already established to meet the “to identify” requirement of limitation 1(e).

225. During deposition, Dr. Quackenbush invoked the “Profile” study at the Dana-Farber Cancer Institute as being a relevant prior art study. EX2163 (Quackenbush Transcript) at 27:15-29:5, 51:16-52:1. He explains that “Profile was an attempt to sequence cancer-associated genes in every patient who walked through the doors of the Dana-Farber Cancer Institute.” *Id.* A news article discussing Profile explains that “[a]n important consideration in shifting Profile toward a clinical service s reimbursement.” EX2176. “As a research test, we get no reimbursement. The cost is borne 100 percent by the institution, and we use philanthropic funds to cover what is about a \$6 million per year ongoing run rate.” *Id.* “Once it becomes clinical or even quasi-clinical we’ll start getting some reimbursement, but you may know that the ability to get reimbursement doesn’t mean you actually get it, so we expect that the majority of costs will still be philanthropy-supported.” *Id.* “Most insurance companies are still not covering a test like this and when they do, reimbursement doesn’t usually cover the cost of running the assay.” *Id.* “But we think it’s important to do this for our patients, and thankfully, the institution has an endowment to cover the gap.” *Id.*

226. The news article highlights two very different scenarios. In the first scenario, you have a rare institution like Dana-Farber that was not cost constrained to cover \$6 million in fees to run the Profile study based on philanthropic donations. Such a rare laboratory is not cost constrained. If such a rare laboratory had been motivated to perform the large-scale sequencing of “12 gigabases (GB) or more” required by limitation 1(a) of the claims, the laboratory would not have been simultaneously motivated to reduce costs to arrive “a sequencing depth of said sequencing of (e) [that] is greater than a sequencing depth of said sequencing of (a)” as required by limitation 1(e). In the second scenario, you have a typical laboratory that faces real-world cost constraints. Assuming for argument’s sake that such a typical laboratory had been motivated to reduce costs to arrive at “a sequencing depth of said sequencing of (e) [that] is greater than a sequencing depth of said sequencing of (a),” the laboratory would not have been simultaneously motivated to arrive at the expensive large-scale sequencing of “12 gigabases (GB) or more” required by limitation 1(a).

2. Claim 5

227. Dr. Quackenbush’s alleges that Ley’s assay included twelve PCR primer pairs: ten PCR primer pairs for “non-synonymous” mutations and two PCR primer pairs for “control” mutations as follows:

Those skilled in the art would know that the creation of “PCR-generated amplicons made from genomic DNA” for sequencing by the Roche/454 platform necessarily involves the use of a probe set, specifically PCR primers directed to the validated somatic mutations. ***In Ley, the probe set would thus necessarily include PCR primers that hybridize to the 10 non-synonymous (variable) mutations identified in the patient’s tumor (CDH24, SLC15A1, KNDC1, PTPRT, GRINL1B, GPR123, EB12, PCLKC, FLT3, NPM1) and the two fixed (control) mutations (BRCA2, TP53).***

See EX1020 (Quackenbush Declaration IPR2023-00545) ¶109. As discussed above, Ley does not disclose the sequence of any of the PCR primers that Dr. Quackenbush alleges were necessarily included in Ley’s assay. *Supra* Section VII.B.1.a.

228. Dr. Quackenbush asserts that a person of ordinary skill in the art would have been motivated to modify Ley’s assay (which he asserts already includes twelve undisclosed PCR primer pairs) to include even more PCR primer “probes targeted to [1] genetic variants involved in the pharmacogenomics of cancer drugs, i.e., genetic variants that were known to have companion diagnostics, as well as to [2] tumor-driver mutations that were known in the art” to allegedly meet the “fixed portion” of claim 5. See EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶172-177.

229. There are at least three reasons why a person of ordinary skill in the art would not have been motivated to further complicate Ley's assay with even more PCR primer pairs directed to genetic variants involved in the pharmacogenomics of cancer drugs and tumor-driver mutations that were known in the art. Each of the three reasons are discussed below.

a. **Ley counsels against the modification proposed by Dr. Quackenbush**

230. First, Ley teaches "[o]ur results strongly support the notion that hypothesis-driven (for example, candidate gene-based) examination of tumor genomes by PCR-directed or capture-based methods is inherently limited, and will miss key mutations." EX1012 (Ley) at page 70. Dr. Quackenbush's obviousness position requires a person of ordinary skill in the art to add more PCR primer pairs to Ley's assay, where the additional PCR primer pairs would be directed to (1) genetic variants involved in pharmacogenomics of cancer drugs and (2) tumor-driver mutations known in the prior art. See EX1061 (Quackenbush Declaration IPR2023-00546) ¶172. Adding PCR primer pairs directed to genetic variants involved in pharmacogenomics of cancer drugs and tumor-driver mutations known in the prior art are the type of "hypothesis-driven" examination of tumor genomes by PCR-directed methods that Ley describes as inherently limited and that will miss key

mutations. Thus, Ley's own teaching counsels a person of ordinary skill in the art to avoid Dr. Quackenbush's proposed modification of Ley's assay.

231. Rather than increase the number of PCR primers used in Ley's assay, a person of ordinary skill in the art would have instead sought to **decrease** the number of PCR primers for several reasons. I have reviewed the transcript of Dr. Quackenbush's October 20 deposition for this case. Dr. Quackenbush testified that a person of ordinary skill in the art would have been motivated by four factors when designing assays related to tumors:

- (1) a desire to increase accuracy for an assay, *see* EX2163 (Quackenbush Transcript) at 17:19-20:1, 21:12-23:10;
- (2) a desire to reduce the cost of an assay, *see* EX2163 (Quackenbush Transcript) at 27:15-30:22;
- (3) a desire to simplify an assay, *see* EX2163 (Quackenbush Transcript) at 36:7-21; and
- (4) the desire to simplify the computational analysis for data generated by an assay, *see* EX2163 (Quackenbush Transcript) at 41:18-44:19.

Dr. Quackenbush's proposal to increase the number of PCR primers used by Ley's assay is counterproductive to each of these four factors.

232. Increasing the number of PCR primers would decrease the accuracy of the assay due to amplification issues with PCR primers that are discussed in the very next subsection of this Declaration entitled “Adding more PCR primers would complicate the assay.”

233. Increasing the number of PCR primers would have been expected to increase the cost of the assay by requiring the design and synthesis of more PCR primers (as well as increasing costs due to issues with potential complications associated with adding more PCR primers to an assay that are discussed in the next subsection of this Declaration). Cost is an important driver of assay design because if an assay is too expensive, then a person of ordinary skill in the art would not have the resources to run the assay. Labor costs in performing an assay is most often greater than the reagent costs required to perform an assay. I note that Dr. Quackenbush testified that cost was “really driving some of the science” because “[i]f the assay was simply too expensive, then one could not effectively run this assay” as follows:

Q. Do you feel that reducing sequencing costs was a real consideration for the person of skill in the art? Or is it really just merely a dollar sign? You can always write another grant application to get more dollars and it is not really that much of a consideration.

MR. KRAUSE: Objection, calls for speculation.

THE WITNESS: So, you know, based on your questions, you have never written a grant application, so you don't know how challenging it is to receive one. ***The budgets are fixed and often require balancing many different things.***

In our assays, you know, with a fixed budget, as a laboratory scientist, we have always striven to be as cost efficient as possible, because that typically allows us to collect data on larger numbers of subjects, which in some applications is, is really driving some of the science that we do.

So, a person of ordinary skill in the art would consider that. ***If the assay was simply too expensive, then one could not effectively run this assay.***

EX2163 (Quackenbush Transcript) at 29:6-30:10.

234. Adding PCR primers to Ley's assay would complicate the assay rather than simplify it. There were known complication issues with adding more PCR primers to an assay that are discussed in the very next subsection of this Declaration entitled "Adding more PCR primers would complicate the assay."

235. Also, adding PCR primers to Ley's assay would generate more data, which would increase the amount of computational analysis for the assay. Computation analysis for Ley's assay would include processing the sequencing data

as described, for example, in the “Methods Summary” section of Ley. *See, e.g.*, Ley (EX1012) at page 71 (section entitled “Methods Summary”).

b. Adding more PCR primers would complicate the assay

236. Second, a person of ordinary skill in the art would not have been motivated to add even more PCR primer pairs to Ley’s assay (which Dr. Quackenbush asserts already had twelve PCR primer pairs) because it would complicate the assay. For example, Mamanova (EX1007) states that “PCR is difficult to multiplex to any useful degree: the simultaneous use of many primer pairs can generate a high level of nonspecific amplification, caused by interaction between the primers, and more-over amplicons can fail to amplify.” EX1007 (Mamanova) at page 112. Mertes (EX1006) teaches that PCR “does not scale easily, in any format” and “any significant extent of parallelized singleplex or multiplex PCR would need to be supported by the use of automated robotics” and the products “need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible)” as follows:

The main downside of the method is that it does not scale easily, in any format, to enable the targeting of very large genome subregions or many DNA samples. To use this method effectively, any significant extent of parallelized singleplex or multiplex PCR would need to be

supported by the use of automated robotics, individual PCR amplicons (or multiplex products) need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible), and the amount of DNA material a study requires can be substantial as this requirement grows linearly with the number of utilized PCR reactions.

EX1006 (Mertes) at page 379.

237. As another example, two of Foresight’s founders (Ash Alizadeh and Maximilian Diehn) wrote that “technical limitations related to multiplexing of PCR assays have to date limited the number of genomic positions that can be interrogated” and that PCR assays can only use a “small combination of amplicons.” See Bratman, Alizadeh, and Diehn et al., “Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq,” *Expert Rev. Mol. Diagn.* 15:715-719 (2015), EX2164, at page 716. They also wrote that the “[l]imitations of [PCR-based] approaches include interrogation of relatively few genomic loci owing to difficulties related to multiplexing of PCR assays.” See Chaudhuri, Alizadeh, and Diehn et al., “Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA,” *Semin. Radiat. Oncol.* 25:305-312 (2015), EX2165, at pages 307-308. In 2021, Foresight founder Diehn wrote that “PCR-based assays have limited multiplexability.” See Dudley and Diehn, “Detection and Diagnostic

Utilization of Cellular and Cell-Free Tumor DNA,” Annu. Rev. Pathol. Mech. Dis. 16:199-222 (2021), EX2166, at page 202.

c. Ley’s mutations had unknown clinical utility

238. Third, Dr. Quackenbush cites Ross (EX1014) and Burell (EX1071) to assert that a person of ordinary skill in the art would have been motivated to add to Ley’s assay more PCR primer pairs for genetic variants involved in the pharmacogenomics of cancer drugs and tumor-driver mutations known in the prior art. See EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶173-177. Ley (EX1012), however, explains that the mutations identified by Ley’s assay had unknown clinical utility. For example, Ley explains “until hundreds (or perhaps thousands) of normal genomes and other AML tumours are sequenced, ***the contextual relevance of the mutations found in this genome will be unknown.***” EX1012 (Ley) at page 71. Ley further states that the function of the mutations found by its assay “***is not yet known.***” EX1012 (Ley) at page 66.

239. A person of ordinary skill in the art would not have been motivated to combine PCR primers for Ley’s mutations of unknown clinical significance with PCR primers for genetic variants and tumor-driver mutations known in the prior art because Ross (EX1014) teaches to avoid assays that include “abnormalities of unknown clinical importance” as follows:

Given the potential generation of ‘actionable’ gene sequence abnormalities and abnormalities of unknown clinical importance, the successful NGS-based clinical service will have a result report that is focused on the abnormalities that can lead directly to the best available therapy, direct a patient to an ongoing clinical trial, or result in some other actionable response from the treating oncologist.

EX1014 (Ross) at page 537. Ross further teaches it is not practical or feasible to test for all mutations of potentially important clinical significance in a given tumor “given the amount of tumor sample, time, and money it would take to perform so many individual tests.” EX1014 (Ross) at page 537.

240. Ross (EX1014) also teaches that “[c]linical applications, particularly in oncology, demand high levels of accuracy, sensitivity, and specificity for sequence-based tests focused on calling out specific genetic alterations.” EX1014 (Ross) at page 535. Foresight’s founders wrote that “only highly sensitive detection techniques are likely to be of clinical use.” See Chaudhuri, Alizadeh, and Diehn et al., “Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA,” *Semin. Radiat. Oncol.* 25:305-312 (2015), EX2165, at page 306. Adding even more PCR primers to the set of twelve PCR primers that Dr. Quackenbush asserts are already used with Ley’s assay would have been expected to raise concerns about reducing the accuracy, sensitivity, and

specificity of Ley's assay due to amplification issues associated with PCR primers discussed above, *see supra* Section VIII.C.2.b. A reduction in accuracy, sensitivity, and specificity is something that Ross teaches a person of ordinary skill in the art to avoid.

241. I note that Ley's assay used the Illumina sequencer, ABI 3730 Sanger sequencer, and the Roche/454 sequencer. *See* EX1012 (Ley) at page 71. Ross (EX1014) teaches that the Illumina sequencer had "low multiplex capability." EX1014 (Ross) at 530-31, Table 3. Ross teaches that Sanger sequencing is limited "to single-gene or hot-spot sequence analysis," *id.* at page 535, and was believed to be "incapable of scaling sufficiently to support the breadth of information required to manage an ever-growing arsenal of targeted anticancer drugs," *id.* at page 528. Ross also teaches the Roche/454 sequencer had "low parallelism." EX1014 (Ross) at pages 530-31, Table 3. Based on at least the foregoing, a person of ordinary skill in the art would not have been motivated to modify Ley's sequencing workflow to add more PCR primers directed to genetic variants involved in the pharmacogenomics of cancer drugs and tumor-driver mutations known in the prior art.

242. As mentioned in the first sentence of the first paragraph of this subsection of this Declaration, Dr. Quackenbush cites Burell (EX1071) to assert that

a person of ordinary skill in the art would have been motivated to add to Ley's assay more PCR primer pairs for genetic variants involved in the pharmacogenomics of cancer drugs and tumor-driver mutations known in the prior art. See EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶176-177. Burell (EX1071) is a review paper that discusses genetic heterogeneity in cancer evolution. Burell does not discuss PCR primers or how to use next generation sequencing instruments.

3. Claim 10

243. Claim 10 depends from claim 1 and recites:

Claim 10. The method of claim 1, further comprising ***providing a therapeutic intervention*** at least based on said presence or absence of said at least said subset of said genetic variants identified in (e).

244. I note that Dr. Quackenbush does not provide any explanation as to why he believes that the claimed “providing a therapeutic intervention at least based on said presence or absence of said at least said subset of said genetic variants identified in (e)” of claim 10 is obvious over Ley. See EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶161-179.

4. Claim 28

245. Claim 28 depends from claim 1 and recites:

Claim 28. The method of claim 1, further comprising using said presence or absence of said at least said subset of said set of genetic variants identified in (e) ***to treat said subject or said at least one biological relative for a disorder.***

246. There are at least two reasons why Ley (EX1012) does not suggest “using said presence or absence of said at least said subset of said set of genetic variants identified in (e) ***to treat said subject or said at least one biological relative for a disorder***” as required claim 28.

247. First, the mutations identified by Ley’s assay have unknown clinical utility. For example, Ley (EX1012) explains “until hundreds (or perhaps thousands) of normal genomes and other AML tumours are sequenced, ***the contextual relevance of the mutations found in this genome will be unknown.***” EX1012 (Ley) at page 71. For example, one mutation might indicate a particular clinical course, yet another different mutation in another gene might contraindicate that course. Because the contextual relevance of Ley’s mutations were, a person of ordinary skill in the art would not have been motivated to use them to treat a subject for a disorder. Further a person of ordinary skill in the art may have been reluctant to report Ley’s variants of unknown significance to a physician.

248. Second, Ley's patient was deceased at the time Ley's assay was performed. EX1042 (Ley Supplementary Information) at pages 2-3. I am unaware of treatments to make for a deceased patient.

D. GROUND 3: NOVELTY OVER NEWMAN

249. Newman (EX1043) does not anticipate independent claim 1. Newman further does not anticipate dependent claims 5, 10, 11, and 28 for additional reasons discussed below.

1. Claim 1

250. Newman (EX1043) fails to anticipate claim 1 because it does not teach at least four requirements of the claim:

- “wherein said sequencing comprises sequencing 12 gigabases (GB) or more” as required by limitation 1(a),
- “an additional biological sample from said subject or at least one biological relative of said subject” as required by limitation 1(d), and
- “said additional biological sample from said subject or said at least one biological relative” as required by limitation 1(e), and
- “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less” as required by limitation 1(e).

a. Limitation 1(a)

251. Limitation 1(a) recites “sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, ***wherein said sequencing comprises sequencing 12 gigabases (GB) or more.***”

252. Dr. Quackenbush relies on Newman’s disclosure of sequencing its GBM Relapse tumor sample and GBM PBL (peripheral blood leukocyte) sample to allegedly meet this limitation. EX1061 (Quackenbush Declaration IPR2023-00546) ¶189. Newman (EX1043) discloses that sequencing these samples was performed using either “2 x 100 or 2 x 150 paired-end reads.” EX1043 (Newman) at Online Methods section at first page. Newman does not disclose whether “2 x 100” or “2 x 150” was used for the two GBM samples. If 2 x 100 was used, then sequencing the 82,247,848 reads for the GBM Relapse sample would only constitute 8.2 GB (100 bp read length * 82,247,848) and sequencing the 86,781,192 reads for the GBM PBL sample would only constitute 8.7 GB (100 bp read length * 86,781,192). Such sequencing does not meet the “12 gigabases (GB) or more” requirement of limitation 1(a).

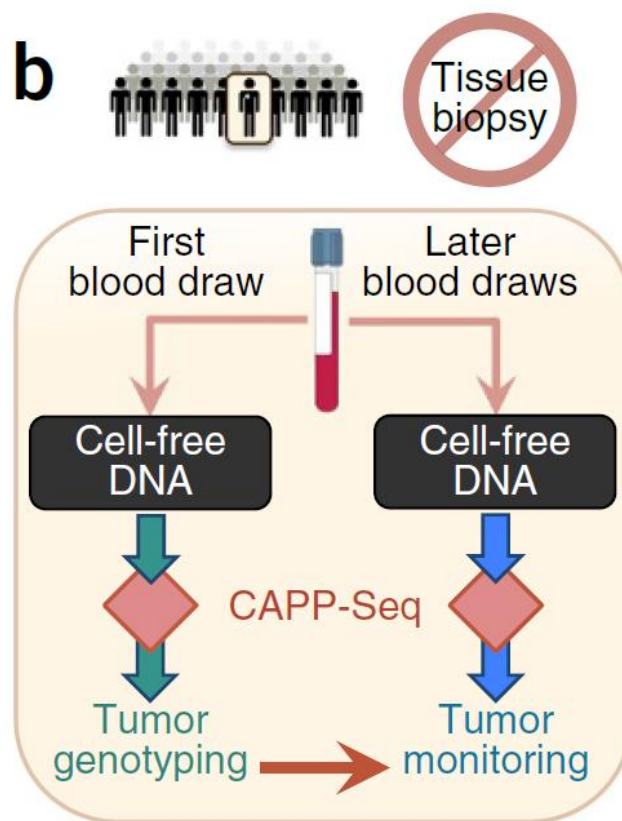
b. Limitation 1(d)

253. Limitation 1(d) requires “***an additional biological sample*** from said subject or at least one biological relative of said subject.”

254. As discussed above for limitation 1(a), Dr. Quackenbush relies on two tissue samples from Newman (EX1043) to allegedly meet the “at least one biological sample of a subject” required by limitation 1(a): a GBM (glioblastoma multiforme) Relapse sample and a GBM PBL (peripheral blood leukocyte) sample. EX1061 (Quackenbush Declaration IPR2023-00546) ¶189. The glioblastoma samples were used by Newman to “establish the detection limit” of its CAPP-Seq assay. EX1043 (Newman) at page 551.

255. Turning to limitation 1(d), Dr. Quackenbush relies on a different CAPP-Seq assay from Newman (EX1043) to allegedly meet the “additional biological sample” required by limitation 1(d). Dr. Quackenbush specifically relies on Newman’s Figure 1b to allegedly meet the additional biological sample of limitation 1(d). EX1061 (Quackenbush Declaration IPR2023-00546) ¶195. The caption for Newman’s Figure 1b states that it is a “[s]chematic illustrating the potential application of CAPP-Seq to *noninvasive (biopsy-free)* genotyping and monitoring of ctDNA.” EX1043 (Newman) at page 548, Fig. 1b. Newman Figure 1b and its caption are reproduced below:

Figure 1 Framework for noninvasive profiling of ctDNA. (a) Theoretical ctDNA detection limits are shown for single-mutation noninvasive genotyping and multi-mutation monitoring based on a typical cfDNA yield from 10 ml blood (assuming ~50% molecule recovery⁸; top) (Online Methods). Background noise of CAPP-Seq is shown to increase as a function of lower ctDNA allele fractions. (b) Schematic illustrating the potential application of CAPP-Seq to noninvasive (biopsy-free) genotyping and monitoring of ctDNA.



EX1043 (Newman) at page 548, Fig. 1b. Newman's Figure 1b crosses out "Tissue biopsy" to emphasize that no tissue is taken in this noninvasive application of CAPP-Seq. Thus, Newman teaches that the CAPP-Seq protocol in Figure 1b is not to be used with invasively obtained tissue samples. Thus, the protocol in Fig 1b is not

-162-

relevant to the GBM Relapse and GBM PBL tissue samples because obtaining such tissue samples would involve an invasive procedure.

256. Dr. Quackenbush additionally relies on Newman Figure 5d to supposedly meet the “additional biological sample” required by limitation 1(d). EX1061 (Quackenbush Declaration IPR2023-00546) ¶195. The sample used to generate Figure 5d is not from the same subject or relative of the subject as the GBM patient. Glioblastoma multiforme is a type of brain cancer. Newman Figure 5d concerns data from a different subject that had lung cancer. For example, Newman describes figure 5d as involving NSCLC, which is non-small cell lung cancer, as follows:

We studied all *NSCLC patients* in our cohort with a known tumor genotype from a tissue biopsy, a matching peripheral blood leukocyte sample, and at least three longitudinal plasma time points for correlation assessments (n = 8). Regardless of whether mutations were identified directly from pretreatment plasma with iDES-enhanced CAPP-Seq or determined from tumor biopsies, ctDNA measurements were highly correlated (**Fig. 5d, top**; Supplementary Fig. 14). Moreover, mutations detected in plasma were confirmed in a matching primary tumor in seven of eight evaluable patients for ctDNA fractions as low as 0.04%. When comparing the various error-suppression strategies for monitoring ctDNA, iDES continued to outperform the other methods tested (**Fig. 5d, bottom**).

EX1043 (Newman) at page 553. Thus, Figure 5d is not an additional biological sample from *the same* subject or a relative of the subject as required by limitation 1(d).

c. Limitation 1(e): said additional biological sample

257. Limitation 1(e) requires “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants *in said additional biological sample from said subject or said at least one biological relative*, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).”

258. Dr. Quackenbush relies on the same GBM Relapse and GBM PBL samples from Newman (EX1043) to supposedly meet the “at least one biological sample of a subject” required by limitation 1(a) and the “additional biological sample” required by limitation 1(e). That is, Dr. Quackenbush relies on Newman’s GBM Relapse and GBM PBL samples to supposedly meet the “at least one biological sample of a subject” required by limitation 1(a). EX1061 (Quackenbush Declaration IPR2023-00546) ¶189 (citing rows 286-87 of Table 2). Dr. Quackenbush relies on these *same* samples for limitation 1(e). EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶197-98 (again citing rows 286-87 of

Table 2). By relying on the same tumor samples from Newman for both limitations 1(a) and 1(e), Dr. Quackenbush did not identify an “additional biological sample” required by limitation 1(e).

d. Limitation 1(e): coverage of 175 kilobases or less

259. Limitation 1(e) requires “*subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less* to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative.”

260. Dr. Quackenbush asserts that the sequencing of the DNA fragments captured by Newman’s “157 kb selector meets the “coverage of 175 kilobases or less” required by limitation 1(e). EX1061 (Quackenbush Declaration IPR2023-00546) ¶198. Firstly, there are no details on the composition of this selector, all that Newman provides is the undocumented assurance that the sum of the individual probes (of undocumented size and composition) comes to 157kb. Further, although Newman’s selector for the glioblastoma tumor, comprised small capture probes that summed to 157kb, this does not axiomatically mean that the total unique sequences pulled down by the capture probes will total to (and thereby cover) only 157 kb of sequence. Cell free DNA (samples 262-263 from table 2) was contacted with Newman’s 157 kb selector panel of capture probes to pull down GBM tumor DNA

having sequences complementary to the capture probes on the panel. It is important to point out that not all the bases in the capture probe need to be paired to capture the target. Thus, there's significant potential "bridging" annealing resulting in increased coverage. In fact the sum of the unique sequences captured (in other words the genome as described in the Lander/Waterman equation) can be calculated from the data in table 2 using $G = L * (N/C)$

So for sample 262, and using the "on target" mapped reads, the selector will have covered a genome of $17,345,145 \times 150 / 4780 = 544.3$ kb. This is much greater than 157kb and 175kb. For this calculation, the length of the read (L) is 150, the number of reads (N) is the "No. on-target reads (deduped)" from table 2 (EX1045), and sequencing depth (C) is the "median deduplicated depth" from table 2 (EX1045).

261. As a result, Dr. Quackenbush has not demonstrated that Newman's 157 kb selector meets the requirement of limitation 1(e) of "subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less."

e. Limitation 1(e): sequencing depth

262. Limitation 1(e) requires "wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a)."

263. Newman states that it applied the 157kb "selector to perform deep resequencing of the GBM tumor (1/2 of a MiSeq lane), and confirmed 97% of

expected SNVs, 99.7% of which had duplex support (n=1,502). EX1043 (Newman) Online Methods at last page. Dr. Quackenbush calculates the read depths for Newman's 157kb selector based on an assumption that Newman's "MiSeq lane produced 4.5-5.1 Gb of sequencing data for 2 x 150 paired end reads." EX1061 (Quackenbush Declaration IPR2023-00546) ¶198. Newman does not provide a detailed analysis of the sequencing data that was produced in this experiment with the 157kb selector. Newman does not report the quantity or quality of DNA that was used on the MiSeq lane. Low quantity and/or quality of DNA would reduce the amount of data produced by the MiSeq lane. It is simply unknown how much sequencing data was produced by Newman's use of one-half of a MiSeq lane. Dr. Quackenbush has not demonstrated that Newman's use of one-half of a MiSeq lane produced 4.5-5.1 GB of sequencing data.

2. Claim 5

264. Claim 5 depends from claim 1 and requires "each of said plurality of nucleic acid probe molecules includes oligonucleotide-directed genomic content comprising (i) at least one variable portion from a result of said sequencing of (a) and (ii) at least one fixed portion independent of said result of said sequencing of (a)." The plurality of nucleic acid probe molecules must include both "at least one variable portion" **and** "at least one fixed portion."

265. Dr. Quackenbush relies on the 157kb personalized selector panel of capture probes that Newman used for the GBM (glioblastoma multiforme) sample to allegedly meet the claimed “variable portion.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶207. He relies on a *separate* 175-kb NSCLC (lung cancer) selector panel of capture probes to allegedly meet the claimed “fixed portion.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶208.

266. Newman explains that these two selectors are distinct from each other—they are not combined into a single selector. For example, Newman describes the 157-kb GBM selector as follows:

We designed a 157-kb “personalized” selector to cover these mutations, employing the same biotinylated oligonucleotide synthesis process as described for NSCLC selector design, above. We then applied this selector to perform deep resequencing of the GBM tumor (1/2 of a MiSeq lane), and confirmed 97% of expected SNVs, 99.7% of which had duplex support (n = 1,502).

EX1043 (Newman) Online Methods at last page. Newman describes the separate and distinct 175-kb NSCLC panel as follows:

Two distinct, but overlapping NSCLC selectors were applied in this work. *The first was developed to maximize analytical sensitivity in NSCLC cfDNA samples (‘cfDNA’ selector) and consisted of a 175-kb panel that was designed to target a median of at least eight mutations*

per NSCLC patient. To improve coverage for nonsmokers (whose tumors generally have lower mutation burdens) and to improve clinical applicability, additional clinically relevant regions were included in the cfDNA selector in order to target actionable fusions in ALK, ROS1, and RET using intronic probes, and to ensure coverage of selected regions containing other actionable variants. The final panel spanned 203 kb, consisting of regions from 207 exons, 4 introns, and 134 genes. We also designed a larger ~300 kb NSCLC ‘tumor genotyping’ selector, which contained the entire cfDNA selector, but also targeted additional regions including complete exon coverage of selected genes and the addition of other regions of clinical or biological interest. ***The specific selector used for each sample is provided in Supplementary Table 2. Details of the selector source data and coordinate selection algorithm are provided in Supplementary Note (“Additional details related to NSCLC selector design”).***

EX1043 (Newman) Online Methods at third page. Newman does not instruct a person of ordinary skill in the art to combine the 157kb GBM selector with the separate 175kb NSCLC selector. Therefore, Newman does not disclose a plurality of nucleic acid probe molecules that includes both “at least one variable portion” ***and*** “at least one fixed portion” as required by claim 5.

3. Claims 10 and 28

267. Claim 10 depends from claim 1 and recites:

Claim 10. The method of claim 1, further comprising ***providing a therapeutic intervention*** at least based on said presence or absence of said at least said subset of said genetic variants identified in (e).

268. Claim 28 depends from claim 1 and recites:

Claim 28. The method of claim 1, further comprising using said presence or absence of said at least said subset of said set of genetic variants identified in (e) ***to treat said subject or said at least one biological relative for a disorder.***

269. The mutations identified by Newman (EX1043) were of unknown clinical utility. For example, Newman states that they are merely “likely to play” a role in personalized cancer therapy. EX1043 (Newman) at page 554. Newman does not teach that its assay was actually used to play a role in a therapeutic intervention or to treat a subject for a disorder. Even if Newman’s assay was “likely to play” a role, it does not establish that the assay was actually used to provide a therapeutic intervention or to treat a subject for a disorder. Newman therefore does not disclose the requirement of claim 10 to provide a therapeutic intervention, and Newman also does not disclose the requirement of claim 28 to treat the subject for a disorder.

4. Claim 11

270. Claim 11 depends from claim 1 and recites:

Claim 11. The method of claim 1, wherein said at least one biological sample includes a tumor sample and said nucleic acids molecules are from cells in said tumor sample.

271. Claim 11 refers to “said at least one biological sample,” which refers back to the “at least one biological sample recited by limitation 1(a).

272. As discussed above for limitation 1(a), Dr. Quackenbush relies on two GBM (glioblastoma multiforme) samples from Newman to supposedly meet the “at least one biological sample of a subject” required for limitation 1(a). EX1061 (Quackenbush Declaration IPR2023-00546) ¶189. Glioblastoma Multiforme is a type of brain cancer.

273. For claim 11, Dr. Quackenbush turns to a different subject who has lung cancer by relying on “lung tumor samples” disclosed by Newman to allegedly meet the “said at least one biological sample” of claim 11. EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶214-215. Because Dr. Quackenbush relies on a sample from a different subject, he has not demonstrated that Newman discloses the “said at least one biological sample” recited by claim 11.

E. GROUND 4: NONOBVIOUSNESS OVER NEWMAN

274. Newman does not render obvious independent claim 1. Newman also does not render obvious dependent claims 5, 10, 11, and 28 for additional reasons discussed in this section.

1. Claim 1

275. Newman does not suggest at least four requirements of claim 1:

- “an additional biological sample from said subject or at least one biological relative of said subject” as required by limitation 1(d),
- “said additional biological sample from said subject or said at least one biological relative” as required by limitation 1(e),
- “wherein said sequencing comprises sequencing 12 gigabases (GB) or more” as required by limitation 1(a), and
- “subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less” as required by limitation 1(e).

a. Limitation 1(d)

276. As discussed above, to allegedly meet the “at least one biological sample of a subject” required by limitation 1(a), Dr. Quackenbush relies on two tissue samples from Newman: the GBM Relapse and GBM PBL samples. *Supra* Section VIII.D.1.b; EX1061 (Quackenbush Declaration IPR2023-00546) ¶189.

277. Also as discussed above, to allegedly meet the “additional biological sample from said subject” required by limitation 1(**d**), Dr. Quackenbush relies on Newman’s Figure 1b to allegedly meet the additional biological sample of limitation 1(d). *Supra* Section VIII.D.1.b; EX1061 (Quackenbush Declaration IPR2023-00546) ¶195.

278. Newman (EX1043) instructs the person of ordinary skill in the art **not** to combine the GBM tissue samples with the noninvasive biopsy free application of CAPP-Seq described in Newman’s Figure 1b. The caption for Figure 1b states that it is a “[s]chematic illustrating the potential application of CAPP-Seq **to noninvasive (biopsy-free)** genotyping and monitoring of ctDNA.” EX1043 (Newman) at page 548, Fig. 1b. Newman Figure 1b even includes a “prohibition sign” (also known as a “no symbol,” a “do not” symbol, a “universal no” symbol, and a “don’t do it” symbol), which consists of a red circle with a slash mark through “Tissue biopsy” as reproduced below:



EX1043, Fig. 1b (excerpted); *see also* https://en.wikipedia.org/wiki/No_symbol (describing the “prohibition sign”), EX2175. Newman’s instruction ***not*** to use an invasive tissue biopsy sample would have led the person of ordinary skill in the art in a divergent direction from the combination proposed by Dr. Quackenbush. The procedure to obtain a GBM tumor sample is most likely to require the type of invasive procedure that Newman Figure 1b instructs a person of ordinary skill in the art to avoid.

b. Limitation 1(e)

279. As discussed above, Newman does not teach the “additional biological sample” required by limitation 1(e). *Supra* Section VIII.D.1.c. To allegedly meet limitation 1(e), Dr. Quackenbush relies on the same GBM Relapse and GBM PBL samples from Newman that it also relies upon to supposedly meet the “at least one biological sample of a subject” required by limitation 1(a). EX1061 (Quackenbush

Declaration IPR2023-00546) ¶189 (limitation 1(a)); *id.* ¶¶197-98 (limitation 1(d)). By relying on the same tumor samples from Newman for both limitations 1(a) and 1(e), Dr. Quackenbush did not identify an “additional biological sample” as required by limitation 1(e).

280. Dr. Quackenbush does not explain why he believes a person of ordinary skill in the art would have been motivated to obtain an additional biological sample from the GBM patient to use in Newman’s method. For example, Forsheew teaches that “[b]iopsies are invasive and costly.” EX1030 (Forsheew) at page 1. Foresight’s founders explained that tumors “cannot be repeatedly sampled without invasive procedures.” EX1044 (Newman 2014) at page 2. Here, obtaining an additional biological sample from Newman’s glioblastoma patient would have been undesirable because it likely would have involved an especially invasive procedure—brain surgery.

c. Limitation 1(a)

281. As discussed above, Newman does not teach “sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, ***wherein said sequencing comprises sequencing 12 gigabases (GB) or more***” as required by limitation 1(a). *Supra* Section VIII.D.1.a.

282. Dr. Quackenbush relies on Newman's disclosure of whole genome sequencing of the GBM Relapse and GBM PBL samples to allegedly meet this limitation. EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶231-232. Newman discloses that sequencing all of the at least 287 samples throughout Newman was performed using either "2 x 100 or 2 x 150 paired-end reads." EX1043 (Newman) Online Methods at first page. Newman does not disclose whether "2 x 100" or "2 x 150" was used for the two GBM samples. If 2 x 100 was used, then sequencing the 82,247,848 reads for the GBM Relapse sample would only constitute 8.2 GB (100 bp read length * 82,247,848) and sequencing the 86,781,192 reads for the GBM PBL sample would only constitute 8.7 GB (100 bp read length * 86,781,192). Such sequencing does not meet the 12 GB limitation.

283. Using the "2 x 100" paired-end read length instead of the "2 x 150" read length would provide 50% less data. Selecting the lower read length conforms with Dr. Quackenbush's testimony that a person of ordinary skill in the art would have found it desirable to reduce the cost of an assay, EX2163 (Quackenbush Transcript) at 27:15-30:22, to simplify an assay, *id.* at 36:7-21, and to simplify computational analysis for data generated by an assay, *id.* at 41:18-44:19.

d. Limitation 1(e)

284. As discussed above, Newman does not disclose “sequencing at a coverage of 175 kilobases (kb) or less” as required by limitation 1(e). *Supra* Section VIII.D.1.d. Dr. Quackenbush does not explain why he believes a person of ordinary skill in the art would have been motivated to modify Newman’s CAPP-Seq assay on the GBM samples to arrive at the claimed “sequencing at a coverage of 175 kilobases (kb) or less.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶230-234. Therefore, he has not demonstrated that a person of ordinary skill in the art would have arrived at the claimed invention.

e. No motivation to modify Ley

285. In summary, a person of ordinary skill in the art would not have been motivated to modify Newman’s assay to (1) combine Newman’s GBM tissue samples with the “non-invasive (biopsy-free)” CAPP-Seq application described in Newman Figure 1b to arrive at the “additional biological sample” required by limitation 1(d), (2) perform an invasive brain surgery procedure to obtain an additional biological sample from Newman’s glioblastoma patient to arrive at the “additional biological sample” required by limitation 1(e), (3) generate 50% more data by using a “2 x 150” read length that would increase the time, expense, and complexity of Newman’s assay to arrive at the “sequencing 12 gigabases (GB) or

more” required by limitation 1(a), and (4) change Newman’s assay to be performed “at a coverage to 175 kilobases (kb) or less” as required by limitation 1(e).

2. Claim 5

286. Dr. Quackenbush asserts that Newman’s lung cancer “NSCLC selector” meets the claimed “fixed portion” and that “a person of ordinary skill in the art knew that a panel of 175 kb or less **could** be designed and produced with fixed and variable sequences.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶236-237. I understand that obviousness is not demonstrated by showing what a person of ordinary skill in the art “could” have done; instead it requires demonstrating what a person of ordinary skill in the art “would” have been motivated to do.

287. Also, Dr. Quackenbush’s anticipation and obviousness positions for claim 1 rely on Newman’s GBM (glioblastoma multiforme) samples—not Newman’s lung cancer patient. Newman uses a 157-kb personalized selector panel on the GBM samples for the purpose of determining the “ctDNA detection limits for iDES” enhanced CAPP-Seq. EX1043 (Newman) Online Methods at last page. Dr. Quackenbush does not explain why he believes Newman’s detection limit determination using GBM samples and a personalized selector panel designed for

the GBM samples **would** benefit from being combined with the separate and distinct 175-kb panel designed for lung cancer samples.

288. Dr. Quackenbush asserts that a person of ordinary skill in the art would have “found it obvious to include probes targeted to genetic variants involved in the pharmacogenomics of cancer drugs, i.e., genetic variants that were known to have companion diagnostics, as well as to tumor-driver mutations that were known in the art.” EX1061 (Quackenbush Declaration IPR2023-00546) ¶238. First, Dr. Quackenbush provides no explanation why he believes Newman’s process for determining the ctDNA detection limits with GBM samples would benefit from adding more probes targeted to genetic variants involved in the pharmacogenomics of cancer drugs and tumor-drive mutations that were known in the art. Second, adding probes would increase the size of Newman’s 157kb selector, which would pulldown even more kilobases that were already significantly higher than the “175 kilobases or less” required by limitation 1(e). *Supra* Section VIII.D.1.d.

289. Third, Dr. Quackenbush confusingly refers to “methods expressly disclosed by Ley” and “the types of assays described in Ley” to assert obviousness of claim 5 in its Newman-only ground 4. EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶240. Ley’s assay was PCR-based, which has a different core reaction principle from the capture probes used by Newman’s selector panels. A

person of ordinary skill in the art would not have been motivated to combine Ley's PCR-based assay with Newman's capture probes within Newman's overall workflow to arrive at the invention recited by claim 5 with a reasonable expectation of success. *Supra* Section VII.C.3 (discussion of claim 22 from the '783 patent).

290. Rather than increasing the number of capture probes in Newman's selector panel, a person of ordinary skill in the art would have instead sought to **decrease** the number of capture probes for several reasons. Dr. Quackenbush testified that a person of ordinary skill in the art would have been motivated by four factors when designing assays related to tumors:

- (1) a desire to increase accuracy for an assay, *see* EX2163 (Quackenbush Transcript) at 17:19-20:1, 21:12-23:10;
- (2) a desire to reduce the cost of an assay, *see* EX2163 (Quackenbush Transcript) at 27:15-30:22;
- (3) a desire to simplify an assay, *see* EX2163 (Quackenbush Transcript) at 36:7-21; and
- (4) the desire to simplify the computational analysis for data generated by an assay, *see* EX2163 (Quackenbush Transcript) at 41:18-44:19.

Dr. Quackenbush's proposal to increase the number of capture probes used by Newman's selector panel is counterproductive to these motivations. For example,

increasing the number of capture probes would increase the cost of the assay by requiring the design and synthesis of more capture probes. Adding more capture probes would complicate the assay as well as the computation analysis required for the assay, rather than simplify them.

3. Claims 10, 11, and 28

291. Dr. Quackenbush does not explain why he believes the specific requirements of dependent claims 10, 11 and 28 are obvious over Newman. EX1061 (Quackenbush Declaration IPR2023-00546) ¶¶230-234. Newman does not teach or suggest claims 10, 11, and 28 for reasons discussed above. *Supra* Section VIII.D.3-4.

F. OBJECTIVE INDICIA OF NONOBVIOUSNESS

292. As explained above, Foresight and Dr. Quackenbush failed to show that a person of ordinary skill in the art would have been motivated to modify the asserted references to arrive at the claimed invention with a reasonable expectation of success. Even if they had made such a showing, however, I understand that objective indicia of nonobviousness must be carefully evaluated to avoid hindsight bias.

1. Nexus

293. I understand that the evidence of objective indicia of nonobviousness must have a ‘nexus’ to the claims. I understand that a nexus is presumed when the

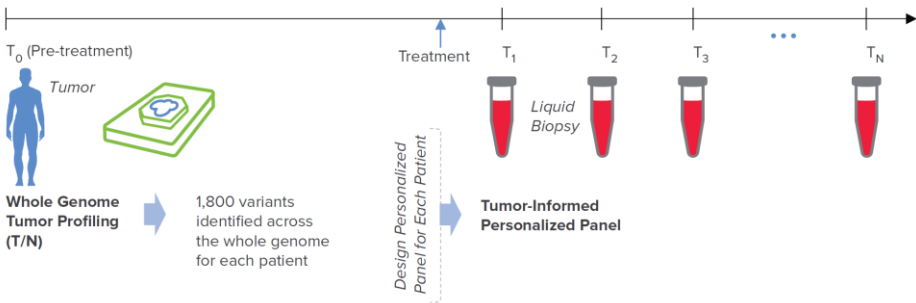
patentee shows that the objective evidence is tied to a specific product and that product embodies the claimed features, and is coextensive with them. Here, Personalis is entitled to a presumption of nexus because, as detailed in the following chart, Personalis' NeXT Personal[®] assay embodies claim 1 of the '611 patent.

'611 Patent Claim Language	Personalis' NeXT Personal [®] assay
1. A method for personalized genetic testing, comprising:	The Next Personal Test performs a method for personalized genetic testing. Delivering Industry-Leading Sensitivity to Detect Residual Disease at the Earliest Timepoints NeXT Personal, an advanced, personalized, and tumor-informed liquid biopsy assay, is designed to detect molecular residual disease (MRD) and cancer recurrence at the earliest timepoints — prior to, during or after treatment — in patients previously diagnosed with cancer. While circulating tumor-derived DNA (ctDNA) is an emerging biomarker for many cancers, the limited sensitivity of current detection methods reduces its utility for diagnosing MRD across a variety of clinical applications. Standard-of-care (SOC) radiological-based technologies, including CT, PET and MRI scans, also remain limited in their ability to detect residual disease during or after surgical or systemic therapy due to the minimum tumor volume required.¹ Therefore reliable, sensitive detection and quantification of MRD remains a key challenge, particularly in early-stage cancers, where timely detection of small micrometastatic lesions may enable treatment that prevents progression to advanced metastatic, incurable disease. To address these challenges, NeXT Personal was developed to deliver industry-leading MRD sensitivity in the range of 1-3 parts per million (PPM) representing a 10-100X increase over other available methods (Figure 1), while requiring only a single tube of blood (4mL plasma/15ng cfDNA), and 1mm³ of FFPE tumor tissue. EX2004 (Personalis NeXT Personal Brochure) at page 15.

(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;

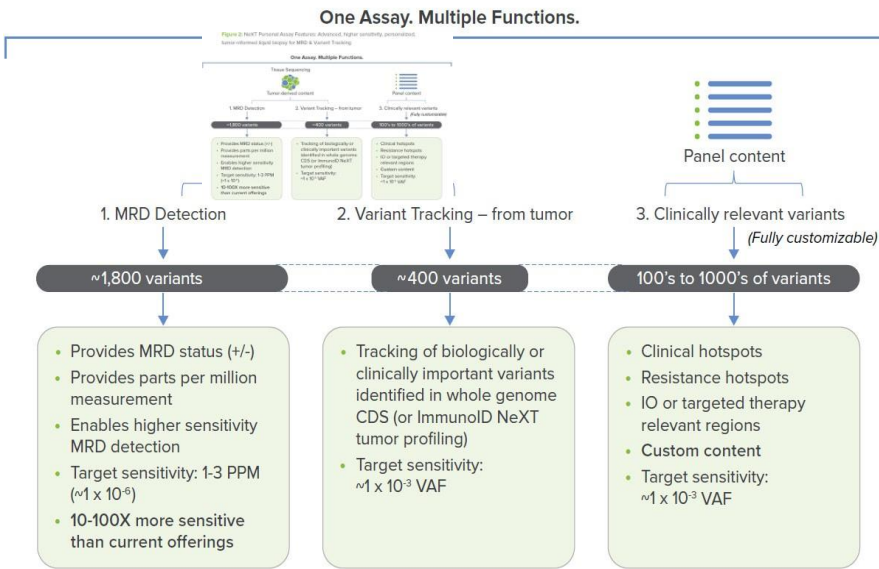
The NeXT Personal test conducts a whole genome sequencing assay on nucleic acid molecules obtained from the tumor biopsy sample from the individual.

Figure 3: NeXT Personal: Advanced, Higher Sensitivity, Personalized, Tumor-Informed Liquid Biopsy for MRD & Variant Tracking



EX2004 (Personalis NeXT Personal Brochure) at page 17, Fig. 3.

Figure 2: NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD & Variant Tracking



EX2004 (Personalis NeXT Personal Brochure) at page 16, Fig. 2.

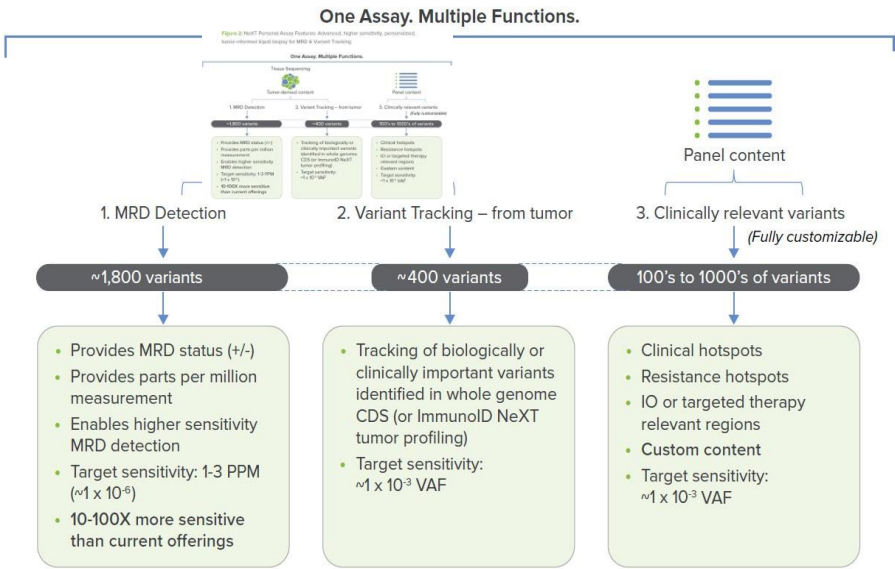
The NeXT Personal test detects SNVs at >30x WGS

'611 Patent Claim Language	Personalis' NeXT Personal [®] assay
	coverage of a patient's tumor. This translates into sequencing over 90 gigabases. Leveraging Tissue-Based Whole Genome Sequencing (WGS) Yields Best-In-Class MRD Performance in Plasma: 1-3 PPM Sensitivity Central to the MRD performance of NeXT Personal is the number of tumor-derived mutations interrogated in patient plasma samples. Analytical and clinical MRD sensitivity can be increased by expanding coverage of loci containing tumor-specific mutations.⁷ To this end, NeXT Personal leverages the broad array of SNVs detected with >30x WGS coverage of a patient's tumor (and accompanying normal) to inform the selection of 1,800 SNVs for inclusion in a personalized MRD panel (Figure 3). The majority of these patient-specific SNVs are located in non-coding regions of the genome. Finally, Personalis' extensive investment into the Illumina[®] NovaSeq[®] series enables ultra-deep sequencing (~100,000 raw depth), while keeping costs in line with other MRD tests that interrogate many fewer loci. EX2004 (Personalis NeXT Personal Brochure) at page 17.

(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set of genetic variants, wherein said set of genetic variants are identified with respect to a reference;

The NeXT Personal test analyzes the sequencing data to identify sequences having genetic variants, and automatically filters the variants, prioritizing variants in the gene with known relevance to cancer.

Figure 2: NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD & Variant Tracking



EX2004 (Personalis NeXT Personal Brochure) at page 16, Fig. 2.

For every panel, NeXT Personal boasts the capacity to include up to 400 single nucleotide variants (SNVs) detected in the tumor to track in plasma. NeXT Personal automatically filters for variants that lie in coding regions of clinically relevant genes, prioritizing those in genes with known relevance to cancer. This ensures that the most biologically and clinically important variants are tracked longitudinally to deliver insights on evolving tumor biology (Figure 2.2).

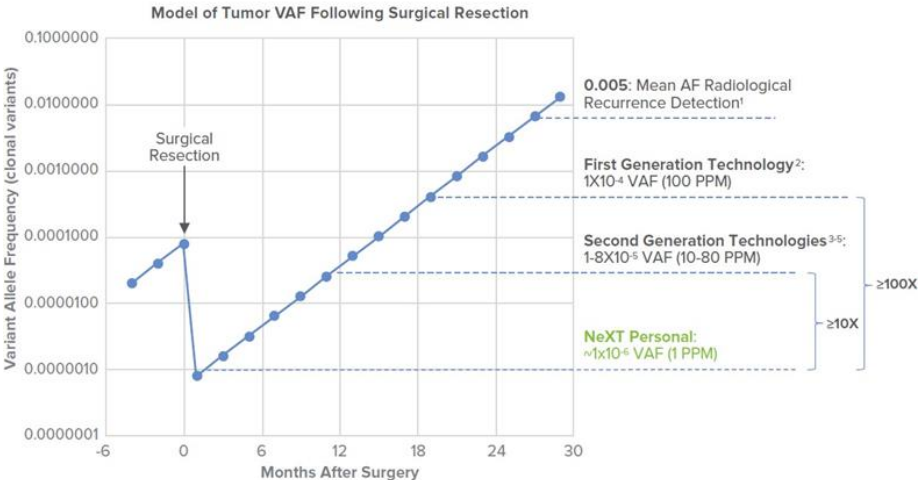
EX2004 (Personalis NeXT Personal Brochure) at page 17.

Additionally, the NeXT Personal test uses the Illumina NovaSeq series to enable deep sequencing

'611 Patent Claim Language	Personalis' NeXT Personal [®] assay
	Leveraging Tissue-Based Whole Genome Sequencing (WGS) Yields Best-In-Class MRD Performance in Plasma: 1-3 PPM Sensitivity Central to the MRD performance of NeXT Personal is the number of tumor-derived mutations interrogated in patient plasma samples. Analytical and clinical MRD sensitivity can be increased by expanding coverage of loci containing tumor-specific mutations.⁷ To this end, NeXT Personal leverages the broad array of SNVs detected with >30x WGS coverage of a patient's tumor (and accompanying normal) to inform the selection of 1,800 SNVs for inclusion in a personalized MRD panel (Figure 3). The majority of these patient-specific SNVs are located in non-coding regions of the genome. Finally, Personalis' extensive investment into the Illumina[®] NovaSeq[®] series enables ultra-deep sequencing (~100,000 raw depth), while keeping costs in line with other MRD tests that interrogate many fewer loci. EX2004 (Personalis NeXT Personal Brochure) at page 17.

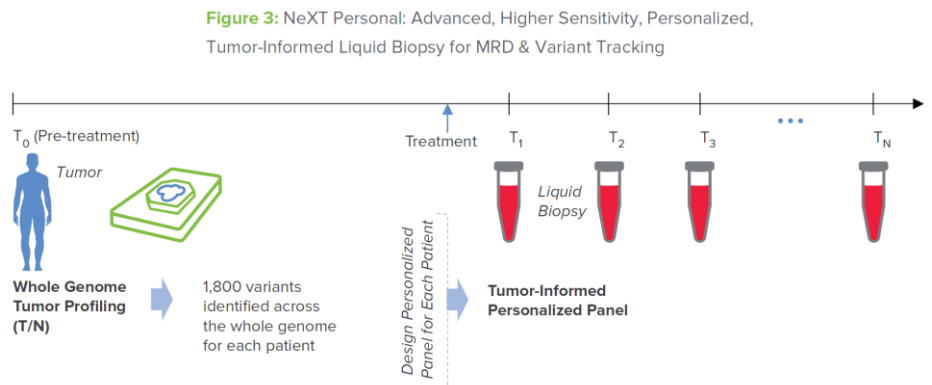
'611 Patent Claim Language	Personalis' NeXT Personal® assay
(c) obtaining a probe set comprising a plurality of nucleic acid probe molecules having said plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in said at least one biological sample;	The NeXT Personal test involves using capture probes to capture mutation carrying DNA. 6. As background, both Foresight and Personalis offer testing products that can be used to monitor for cancer recurrence by detecting and analyzing tiny amounts of tumor DNA that are present in the bloodstream of a cancer patient. It is important to understand that both Foresight and Personalis offer testing products that operate according to the same basic principles, specifically, by using whole genome sequencing of a tumor sample to identify mutations that can subsequently be detected in circulating tumor DNA by capturing the mutation carrying DNA with capture probes and then sequencing the captured DNA. This unique approach distinguishes the Foresight and Personalis tests from other testing products, and allows the products from Foresight and Personalis to achieve greater sensitivity relative to other testing products currently on the market. EX2004 at page 29, ¶6.

(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and	The capture probes selectively amplify sequences comprising the set of genetic variants over other sequences in additional biological samples taken from the subject perhaps post-surgery, to generate a sequencing library. 6. As background, both Foresight and Personalis offer testing products that can be used to monitor for cancer recurrence by detecting and analyzing tiny amounts of tumor DNA that are present in the bloodstream of a cancer patient. It is important to understand that both Foresight and Personalis offer testing products that operate according to the same basic principles, specifically, by using whole genome sequencing of a tumor sample to identify mutations that can subsequently be detected in circulating tumor DNA by capturing the mutation carrying DNA with capture probes and then sequencing the captured DNA. This unique approach distinguishes the Foresight and Personalis tests from other testing products, and allows the products from Foresight and Personalis to achieve greater sensitivity relative to other testing products currently on the market. EX2004 at page 29, ¶6. Fig. 1 shows that tumor variant allele frequencies are tracked at multiple time points with additional biological samples being taken from the subject after surgery
---	---

'611 Patent Claim Language	Personalis' NeXT Personal [®] assay
	Figure 1: MRD Sensitivity: Targeting Detection at Earliest Timepoints  Model Assumptions: Median breast tumor size detected by mammography: 1.3cm, Median shedding per NSCLC TRACERx study⁶; Residual tumor from surgery: 1%; Volume doubling time (actual VAF time): 2 months EX2004 (Personalis NeXT Personal Brochure) at page 15, Fig. 1.

(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

The NeXT Personal test involves sequencing the sequencing library to identify a subset of genetic variants in the additional biological sample.



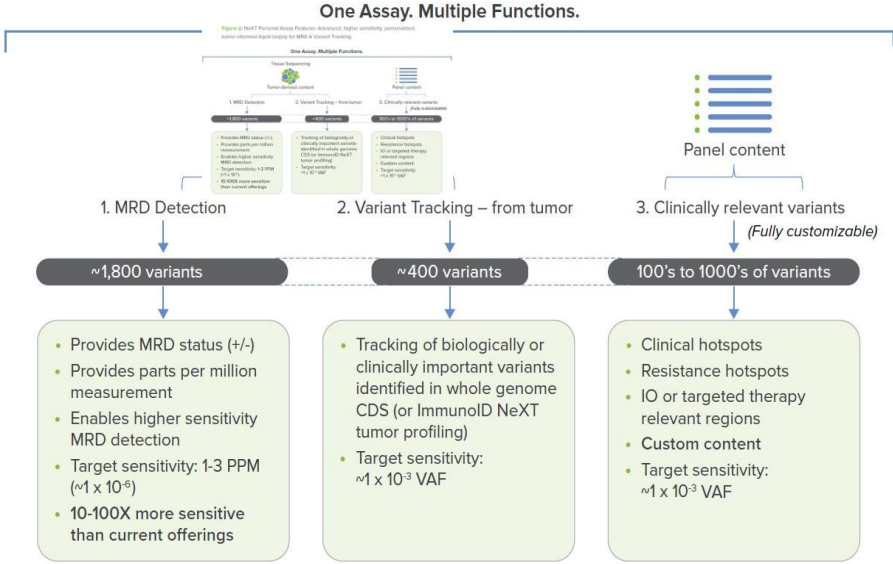
EX2004 (Personalis NeXT Personal Brochure) at page 17, Fig. 3.

Leveraging Tissue-Based Whole Genome Sequencing (WGS) Yields Best-In-Class MRD Performance in Plasma: 1-3 PPM Sensitivity

Central to the MRD performance of NeXT Personal is the number of tumor-derived mutations interrogated in patient plasma samples. Analytical and clinical MRD sensitivity can be increased by expanding coverage of loci containing tumor-specific mutations.⁷ To this end, NeXT Personal leverages the broad array of SNVs detected with >30x WGS coverage of a patient's tumor (and accompanying normal) to inform the selection of 1,800 SNVs for inclusion in a personalized MRD panel (Figure 3). The majority of these patient-specific SNVs are located in non-coding regions of the genome. Finally, Personalis' extensive investment into the Illumina® NovaSeq® series enables ultra-deep sequencing (~100,000 raw depth), while keeping costs in line with other MRD tests that interrogate many fewer loci.

EX2003 (Personalis NeXT Personal Brochure) at page 15.

The NeXT Personal test also involves a variant tracking panel that identifies a subset of ~400 sequences, at a coverage of less than 175 kilobases.

'611 Patent Claim Language	Personalis' NeXT Personal [®] assay
	Figure 2: NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD & Variant Tracking  One Assay. Multiple Functions. 1. MRD Detection - Provides MRD status (+/-) - Provides parts per million measurement - Enables higher sensitivity MRD detection - Target sensitivity: 1-3 PPM ($\sim 1 \times 10^{-6}$) 10-100X more sensitive than current offerings 2. Variant Tracking – from tumor - Tracking of biologically or clinically important variants identified in whole genome CDS (or ImmunID NeXT tumor profiling) - Target sensitivity: $\sim 1 \times 10^{-3}$ VAF 3. Clinically relevant variants (Fully customizable) - Clinical hotspots - Resistance hotspots - IO or targeted therapy relevant regions - Custom content - Target sensitivity: $\sim 1 \times 10^{-3}$ VAF EX2003 (Personalis NeXT Personal Brochure) at page 14, Fig. 2.

2. Industry Praise

294. Personalis' assay has received industry attention and praise due in part to its sensitivity relative to other methods. For example, *The Scientist* magazine published its list of the Top 10 Innovations of 2022. The patented approach disclosed in the '611 patent earned the #1 ranking. See EX2001 at 1-2.

295. The differences between the method used in Leary (EX1002) and the method claimed in the '611 patent yield very different results. Personalis' assay can

detect tumor in DNA in blood plasma at levels of about one part per million (or 0.0001%). EX2003 at page 13. Personalis' assay therefore can detect allele frequencies of 0.0001%. By comparison, Ley's assay was only able to detect allele frequencies at approximately 3% for the "skin" sample. EX1012 (Ley) at 69, Figure 3. Thus, Ley was several hundred times less sensitive than the claimed method.

3. Skepticism of Experts

296. The references cited by Foresight and Dr. Quackenbush provide evidence that the industry's experts held skepticism toward someone conceiving of a method that would solve the challenges of conducting highly sensitive genome sequencing using personalized probe sets. Forshew (EX1030) acknowledges that using capture probes for cancer monitoring was "expensive" and that achieving sensitive monitoring for mutations across entire genes has been "more challenging." EX1030 (Forshew) at page 10 and 1; *see also* EX1031 (Meyerson) at 686 (discussing "the key challenges in cancer genome sequencing"). Experts in the field were clearly skeptical of the personalized probes Forshew (EX1030) described as "difficult to implement on a routine basis." EX1030 (Forshew) at page 10.

297. Other references outline industry-wide problems with next-generation sequencing (NGS) at the time, which were unexpectedly solved by the methods in the '611 Patent. For example, Anderson (EX1004) emphasizes that "difficult

engineering challenges must be overcome before these technologies could become commercially viable.” EX1004 (Anderson) at page 48. Further, Lo (EX1017) identifies difficulties facing experts regarding “how to handle the massive amount of sequencing data” when utilizing NGS for profiling cancer genomes.” EX1017 at page 3.

298. This objective real-world evidence supports a finding that the claims of the '611 patent are nonobvious.

IX. CERTIFICATION

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

Dated: November 3, 2023

By:  _____

Henry Morrice Furneaux, Ph.D.