UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

FORESIGHT DIAGNOSTICS INC.,
Petitioner,

v.

PERSONALIS, INC.,
Patent Owner.

Case Nos. IPR2023-00224 and IPR2023-00317
U.S. Patent Nos. 11,384,394 and 11,408,033

DECLARATION OF HENRY MORRICE FURNEAUX, PH.D.

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1. I, Henry Morrice Furneaux, Ph.D., make this Declaration in connection with *inter partes* review proceedings at the U.S. Patent & Trademark Office concerning U.S. Patent No. 11,384,394 ("the ′394 patent") and U.S. Patent No. 11,408,033 ("the ′033 patent").

2. 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.

3. As discussed herein, the claims of the ′394 patent are nonobvious over (1) Leary EX1002, (2) Leary EX1002 and Ley EX1012, and (3) Leary EX1002, Chan EX1008, and Liao EX1009. The claims of the ′033 patent are not anticipated by Forshew EX1030. The claims of the ′033 patent are nonobvious over (1) Forshew EX1030 and Wagle EX1033, and (2) Wagle EX1033 and Chan EX1008.

I. INTRODUCTION

4. U.S. Patent Nos. 11,384,394 ("the ′394 Patent") and 11,408,033 ("the ′033 patent") represent novel and nonobvious personalized unbiased approaches to cancer detection and monitoring that were far ahead of their time when the inventions were made in 2012, and they continue to receive acclaim to this day.

5. Briefly, the claims of the ′394 Patent recite a specific process that describes the novel application of "bespoke" preparation and sequencing protocols that enable a much more comprehensive analysis of a whole human genome. This

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comprehensive analysis coupled with a subsequent targeted sequencing assay based on using capture probes in accordance with the claimed method improves diagnosis and treatment of cancer patients. Cancer is essentially caused by alterations in the sequence of DNA. Thus, a more comprehensive analysis that both improves routine analysis of the "charted" regions of the human genome and increases the interrogation of hithertofore "uncharted regions", is now appreciated to be much more likely to identify useful alterations that, serve to diagnose cancer, to monitor cancer trajectory and to predict effective therapies by using the claimed method.

6. Claim 1 of the ′033 Patent recites a specific process that produces capture probes directed to polymorphisms that are associated with a disease or indication. By applying these probes to a nucleic acid sample, one can collect nucleic acid that is likely to encode disease-causing alterations in, for instance, the tumor genome of a cancer patient. DNA sequencing can then be used to identify the specific alterations at issue in the patient. The process of capturing DNA with capture probes and sequencing can be repeated over time, allowing one to monitor disease and response to therapy over time.

7. As the ′394 and ′033 Patents explain, prior art methods using orthodox "one size fits all" sequence protocols were biased in fragment selection and analysis and could "fail to capture many biomedically important variants," not to mention

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perform poorly in certain critical regions of the genome. EX1001 (′394 Patent) at 1:23-34.[1] In addition, it was well acknowledged at that time that the cost of sequencing 16 million DNA fragments (3.2 billion /200 bp fragment size) enough times (estimates varied from 30-500X coverage) to determine a reliable sequence would be extremely cost prohibitive. The cost problem would also be compounded by the accepted belief that the sequence of many of these DNA fragments may be uninformative.

8. Some very preliminary and partial solutions to these problems had been provided by only sequencing genomic segments that were hypothesized to be informative. However, each cancer patient has a unique alteration spectrum arising from the combination of distinct tumor subclones and a unique trajectory in terms of the appearance and disappearance of alterations in particular tumor subclones. Thus, there would be no guarantee that such hypothesis-driven approaches would identify useful alterations in a particular patient. Indeed, the inventors of the ′394 and ′033 patent sought to overcome this problem by developing new personalized approaches.

[1] The ′394 and ′033 patents contain identical disclosures at the same column and line numbers as each other.

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9. Specifically, the inventors of the ′394 Patent recognized that such problems could be overcome by first performing an initial comprehensive sequence analysis of tumor DNA fragments from an individual, using novel and nonobvious methods that significantly decreased fragment bias and enabled increased sequence accuracy. This comprehensive personalized sequence information could then be used to identify informative fragments and to generate capture probes that could isolate such DNA fragments and thus enable an informative yet economic analysis. In particular such capture probes would be of significant utility in the analysis of subsequent patient samples in determining tumor trajectory, response to treatment and to the administration of novel and nonobvious therapies.

10. It was also envisaged by the ′394 patent inventors that the extensive collection of these individual (personal) capture probe sequence databases could help illuminate novel pervasive alteration signatures and enable the prospective (without the first comprehensive DNA sequence analysis) use of such capture probe generated data.

11. The inventors of the ′033 Patent recognized that problems with the prior art could be overcome by using novel and nonobvious methods to generate capture probes that could isolate DNA fragments associated with a plurality of polymorphisms. This would provide an informative yet economic analysis. Such

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capture probes and sequencing assays would be of significant utility in the analysis of subsequent patient samples in determining tumor trajectory, response to treatment, and to the administration of novel and nonobvious therapies.

12. By using the approaches claimed by the ′394 and ′033 patents, Personalis was able to pioneer new frontiers in techniques for detecting and monitoring disease in cancer patients with utmost sensitivity. The Personalis assay that practices the ′394 and ′033 Patents, for instance, can detect tumor DNA in blood plasma at levels of about one part per million (or 0.0001%). Recently, *The Scientist* magazine hailed the Personalis assay as the #1 scientific innovation of 2022:

NeXT Personal™ Personalis

THE SCIENTIST STAFF

Small quantities of cancer cells can linger in the body after tumor removal, a phenomenon known as minimal or molecular residual disease (MRD) that, if left undetected, can lead to recurrence. Personalis's NeXT Personal™ assay, unveiled in December 2021, uses a patient's own tumors to detect, quantify, and monitor circulating tumor DNA in order to spot MRD and track responses to therapy.

Using 1 mm^3 of tumor tissue sampled from a patient, Personalis's lab performs whole genome sequencing to identify up to 1,800 single-nucleotide variants. These patient-specific mutations are used to design a panel of primers targeting those regions for sequencing in future blood biopsies. The assay also includes primers for other known cancer-related genes, says Dan Norton, associate director for product management at Personalis. "We can see if there are other variants emerging that have a precision therapy associated that may be more effective for that patient."

See EX2001 (The Scientist Magazine) at pages 1-2.

13. Now, years after the Personalis inventors conceived of their approach, Petitioner contends that the ′394 and ′033 Patents are invalid in view of certain references.

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14. With respect to the ′394 patent, Petitioner does not identify any anticipatory prior art, but contends that the ′394 Patent is obvious in view of certain references, including Leary, which is Petitioner's primary reference. Yet, as discussed herein, Leary is nowhere close to the ′394 Patent:

- DNA Sequencing is a process to discover the identity and the order of bases present in a duplex DNA structure. Leary only reported the sequence of short segments (25bp) at both ends of a much larger fragment (the mate pairing technique). Thus, Leary is not teaching whole genome ***sequencing*** and perhaps one could argue Leary is not even teaching general ***sequencing***. However, there would be no argument that Leary is teaching a physical ***mapping*** method. Leary uses these disconnected short reads to identify guideposts in the genome that serve to identify gross chromosomal aberrations such as copy number changes and chromosomal rearrangements. It is very important to point out that the extent to which the human genome is mapped in this technique is provided by the term "physical coverage." Physical coverage has been defined as "the number of DNA fragments of which both ends have been sequenced that on average overlie any position in the genome." EX2162 at page 1. Thus, ***physical*** coverage should not be

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confused with ***sequence*** coverage which means the average number of times that a base pair has been sequenced (identified).

- Nowhere does Leary mention whole genome sequencing. Petitioner's contention that Leary teaches "whole genome sequencing" under the correct understanding of this term as used by the ′394 patent is, at best, based on a misunderstanding of Leary and, at worst, a distortion.
- Importantly, as confirmed by Dr. Quackenbush during his deposition, given that there is a large tract of missing sequence between the ends of the DNA fragments, Leary cannot even use the mapping method to report on the sequence of the aberrant chromosomal breakpoints. Such information about what genes are involved and whether the breakpoint has preserved or abolished the reading frame is what a POSA would expect.
- The Petitioner attempts to argue that the alleged "capture probes" (i.e., PCR primers) and the resulting PCR amplification (that in Leary was merely used for confirmation of breakpoint status), are one and the same. The Petitioner attempts to argue that "capture probes", a well-described entity which captures nucleic acids via a non-catalytic reaction, are synonymous with "Polymerase Chain Reaction Primer Capture Probes". "Polymerase Chain Reaction Primer Capture Probes" is a term invented by Dr.

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Quackenbush to illegitimately portray PCR primers as capture probes using improper hindsight. This is quite simply ridiculous. This argument is contrary to not just the intrinsic evidence, but also its own extrinsic evidence.

15. Although Petitioner attempts to plug some of these gaps with a few secondary references, its secondary references simply confirm that Petitioner's obviousness theory is without merit. For instance, while Petitioner contends that Chan can be combined with Leary to render obvious certain claims of the Personalis targeted approach based on the use of capture probes, Chan characterizes such an approach as offering only a "partial glimpse" of a tumor genome in cancer patients. EX1008 (Chan) at page 212. To the extent that Chan even considers such targeted approaches, it is only to vaguely speculate about them as future possibilities, thus confirming how ahead of its time the Personalis approach was. Indeed, an article published in the same issue as, and referring to, Chan teaches away from the use of personalized panels to detect recurrence and instead advocates for the continued use of shotgun sequencing (which is the sequencing of DNA fragments produced in a random unbiased protocol) so as to overcome the problem of tumor heterogeneity. *See* EX2003 (Swanton) at page 7 ("these data support the use of ***unbiased*** shotgun MPS approaches to ctDNA analysis").

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16. Consistent with this, the Ley reference relied upon by Petitioner as allegedly rendering obvious claims 1 and 4-5 expressly teaches against targeted approaches of the type claimed in the ′394 Patent as being "inherently limited" and likely to "miss key mutations:"

> Our 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. Petitioner's reliance on prior art which teaches that capture-based methods are "inherently limited" underscores the weakness of its positions in asserting that a POSA would have been motivated to use such capture-based methods to arrive at the invention claimed by the ′394 patent.

17. Turning to the ′033 patent, Petitioner relies primarily on Forshew as an alleged anticipatory reference. Forshew, however, does not teach the use of capture probes, which is required by every limitation of claim 1 of the ′033 patent. Rather than using capture probes, Forshew relies upon PCR, a method that Petitioner's own prior art repeatedly distinguishes. Importantly, Forshew refers to this method as PCR not as a "PCR primer capture probe" method. Indeed, Forshew itself distinguishes its PCR-based approach from the claimed capture probe methods of the ′033 Patent, criticizing the use of "expensive custom-designed probes" and

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asserting that they "are difficult to implement on a routine basis." EX1030 (Forshew) at page 10.

18. While Petitioner asserts that a POSA would conflate PCR and capture probes, such an assertion is contradicted by the very exhibits cited by Petitioner. The additional obviousness theories that Petitioner presents based on the combination of Forshew and Wagle (or the combination of Wagle and Chan) also are unconvincing. Petitioner relies, for instance, on Wagle as disclosing capture probes. Yet, Wagle simply reports on the use of massively parallel sequencing to analyze samples from FFPE sections. Petitioner acknowledges that Wagle does not expressly disclose repeating steps (b)-(c) on a subsequently obtained sample from said individual. And it would not have been obvious to modify Wagle to repeat steps (b)-(c) on a subsequently obtained sample for several reasons discussed herein.

19. The Chan reference relied upon by Petitioner does not render obvious the personalized approach claimed by the ′033 patent. Chan, in fact, criticizes personalized approaches like the claimed invention because they "might miss important information regarding imminent relapse or disease progression" as follows:

> These data suggest that for an accurate measurement of the total tumor load in a cancer patient, the use of a genomewide shotgun approach

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> might provide a more representative picture, compared with the more traditional approach of targeting specific tumor-associated mutations. For the latter approach, ***if only a subset of the tumor cells possesses the targeted mutations, one might miss important information regarding imminent relapse or disease progression caused by tumor cells*** not possessing the targeted mutations, or one might miss the emergence of a treatment-resistant clone.

EX1008 (Chan) at page 222.

20. For the many reasons discussed in detail in this declaration, the claims of the ′394 and ′033 patents are novel and nonobvious over the references cited by Petitioner.

II. ENGAGEMENT

21. 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 ′394 Patent and ′033 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.

22. 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.

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III. BACKGROUND AND QUALIFICATIONS

23. 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.

24. 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.

25. 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

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the classic textbook "The Nucleic Acids". Thus, this experience provided a rigorous introduction to nucleic acids.

26. 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.

27. 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,

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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.

28. 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.

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29. 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

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antibody-associated paraneoplastic cerebellar degeneration. Proc Nat Acad. Sci, 88:3451-3454, 1991.

30. 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.

31. 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,

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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.

32. 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.

33. 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. <u>Patents Awarded</u>

34. 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);

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- 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. <u>Research And Teaching Experience</u>

35. 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

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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.)

36. 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.

37. 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

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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.

38. 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)

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Technology to Angiogenesis Research, Methods in Endothelial Cell Biology - Springer Lab, (H. Augustin ed., 2004).

39. 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

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biology, hematology and immunology, at the University of Connecticut Medical School (2007-2009).

40. 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. <u>Industry Experience</u>

41. 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

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design novel research reagents based on my understanding of the needs of contemporary researchers.

D. Professional Society Involvement

42. 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."

IV. MATERIALS CONSIDERED

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

- The ′394 and ′033 patents;
- The declarations submitted by Dr. John Quackenbush in these proceedings (Exhibits 1020 in each of IPR2023-00224 and IPR2023-00317) as well as exhibits cited in the Quackenbush declarations.
- The Board's institution decisions in these matters; and
- Materials cited in this declaration.

V. LEGAL STANDARDS

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

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A. Anticipation

45. 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

46. 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

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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.

47. 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.

VI. THE PERSON OF ORDINARY SKILL IN THE ART

48. I understand that the Board has adopted the definition of the person of ordinary skill in the art (POSA) proposed by Petitioner Foresight in these proceedings. The Board has preliminarily determined that a POSA 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

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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 15-19.[2] I have applied this definition of a POSA in forming the opinions described in this declaration.

VII. CLAIM CONSTRUCTION

49. I understand that the Board preliminarily construed two terms: "whole genome sequencing" and "capture probes." I discuss each term below.

A. Construction of "whole genome sequencing"

50. I understand that the Board preliminarily construed "whole genome sequencing" as used in claim limitation 1(b) of the ′394 patent to mean "a non-targeted approach to sequencing all or almost all of a genome."

51. Limitation 1(b) of the ′394 patent recites "conducting a first sequencing assay on said first subset of nucleic acid molecules to yield a first result comprising

[2] The citations in this section of the declaration refer to the Institution Decision from IPR2023-00224. I understand that the Board made equivalent findings on the definition of a POSA in the Institution Decision from IPR2023-00317.

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a first nucleic acid sequence wherein said first sequencing assay comprises ***whole genome sequencing***."

52. I agree with the Board's construction of "whole genome sequencing" as used in claim limitation 1(b). The word "whole" modifies "genome" and thus indicates that the entirety—not a part or subset—of the genome must be sequenced. The specification distinguishes methods that sequence the "whole genome" from methods that sequence only a portion of the genome, such as the "exome." *See* EX1001 (′394 patent) at 1:23-34 ("Often, ***whole genome and/or exome sequencing*** using standard sequencing methods performs poorly in content regions having very high CG content (>70%)"). The specification defines "exome" as only part of the genome (*see id.* at 26:14-19), and consistently refers to "whole genome sequencing" as a separate category of sequencing than "exome sequencing." *See id.* at 33:21-22 ("The sequencing reactions may comprise whole genome sequencing or exome sequencing."); *id.* at 5:38-59 ("Conducting one or more sequencing reactions comprises ***whole genome sequencing or exome sequencing***."). The specification also explains that "genomic regions" can be selected based on various traits, and "may comprise an entire genome or a portion thereof." *Id*. at 24:42-43; *see e.g., id*. at 4:47-52 ("The one or more genomic regions may be selected from the group comprising high GC content, low GC content, low complexity, low mappability,

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known single nucleotide variations (SNVs), known inDels, known alternative sequences, ***entire genome, entire exome, set of genes***, set of regulatory elements, and methylation state."); *id*. at 17:17-35 (same).

B. <u>Construction of "capture probes"</u>

53. I understand that the Board preliminarily construed "capture probes" as encompassing "at a minimum PCR primer capture probes and solution-based hybridization capture probes (e.g., biotinylated oligonucleotide baits)." Institution Decision at pages 23-29. The concurring opinion would have construed "capture probes" as "encompassing nucleic acid structures as small [as] one nucleotide that may but are not required to include linkers and labels." Institution Decision at page 45.

54. I disagree with the Board's preliminary constructions because they do not comply with how a POSA would have understood the plain meaning of "capture probes" for several reasons discussed below.

1. <u>The plain meaning of "capture probes"</u>

55. I understand that claim terms generally are afforded their plain and ordinary meaning to a POSA when read in view of the claims, the patent specification, and the prosecution history. I understand, however, that when a

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specification contains a definition for a term, then that definition governs the meaning of the term.

56. I understand that the Board determined that the specification "provides no express definition of 'capture probes.'" Institution Decision at page 23. I agree that the specification does not provide a definition of "capture probes." The specification describes several features of capture probes, *see e.g.,* EX1001 (the ′394 patent) at 31:4-32:5,[3] but does not describe all known aspects of capture probes.

57. A POSA would have known that "capture probes" has a plain and ordinary meaning. The exhibits cited by Foresight and Dr. Quackenbush consistently demonstrate that a POSA would have understood the plain meaning of "capture probes" to be "***a nucleic acid sequence that hybridizes to target DNA, either in solution or on a solid support, so that one can physically capture and isolate the target DNA***." For example, Mamanova (EX1007) teaches that "capture probes" are the specific type of probes used in "hybrid capture" techniques. *See* EX1007 (Mamanova) at pages 115-16. On pages 115-116, Mamanova exclusively uses the term "capture probes" for describing hybrid capture techniques. On pages

[3] The ′394 and ′033 patents have the same descriptions at the same column and line numbers.

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112-114, Mamanova does not use the term "capture probes" to describe PCR primer amplification techniques. Mertes (EX1006) explains "hybrid capture" techniques as follows:

> 'Hybrid capture': wherein ***nucleic acid strands derived from the input sample are hybridized specifically to preprepared DNA fragments*** complementary to the 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.[4] Mamanova and Mertes provide appropriate examples of the background knowledge of a POSA with respect to capture technologies. The proposed construction in this paragraph appropriately reflects the plain meaning of capture probes described in Mamanova and Mertes as representative examples of the background knowledge of a POSA regarding this term.

2. <u>The preliminary construction does not comply with the plain meaning of "capture probes"</u>

58. The Board's preliminary construction departs from the plain meaning of "capture probe" because it includes "PCR primer capture probe." I note that "PCR

[4] All emphases supplied throughout this declaration unless otherwise noted.

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primer capture probe" is not found in any of the exhibits cited by Foresight or Dr. Quackenbush. However, the phrase "PCR primer" is ubiquitous in the prior art. I am not aware of the phrase "PCR primer capture probe" being used in the prior art literature. The first time I recall ever seeing "PCR primer capture probe" was in Dr. Quackenbush's declaration in this proceeding. It is unclear to me what types of PCR primers are included (or excluded) by the phrase "PCR primer capture probe" because this phrase does not have a commonly understood meaning in the literature. It is unclear what the word "capture" modifies in the term "PCR (polymerase chain reaction)" primer capture probe. Is it the probe, the primer, the polymerase, or the chain reaction?

59. In addition, including the phrase "PCR primer capture probes" in the construction of "capture probes" would expand "capture probes" beyond its plain meaning. The specification treats capture probes and PCR primers as different types of nucleic acids. For example, the specification has separate descriptions of capture probes and primers. EX1001 (the ′394 patent) at 31:3-32:5, 40:29-41:36, 23:23-26, 32:56-60. Several embodiments describe the use of capture probes as a separate step from using PCR primers. EX1001 (the ′394 patent) at 64:29-43, 65:38-50, 66:53-67.

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60. Dr. Quackenbush focuses his understanding of "capture probes" on the portion of the specification that describes a capture probe hybridizing to target DNA. *See* EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶100-101[5]; EX1001 (the ′394 patent) at 31:4-36. While capture probes do hybridize to target DNA, they also must allow one to physically capture the target DNA.

61. I understand that the Board preliminarily determined that it was unclear whether a "pulldown probe" is synonymous with a "capture probe." *See* Institution Decision at pages 27-28. A POSA would have understood "capture probes" to be synonymous with "pulldown probes." For example, Dr. Quackenbush states that "capture probes" refers to "nucleic acid fragments" that "catch" target nucleic acid sequences of interest through hybridization so that target DNA is physically "'pulled down' or 'fished out'" as follows:

> When hybrid capture baits are contacted with the nucleic acid sample (the "pool"), the baits hybridize to the sequences of interest. ***This hybridization results in a "catch" of nucleic acids bearing the targeted sequences, which are "caught" through the hybridization of***

[5] The Quackenbush Declaration (EX1020) from IPR2023-00314 has the same information at paragraphs 98-99. The rest of this section of my declaration cites to only the declaration from IPR2023-00224 for the sake of brevity.

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> ***strand-to-strand base-pairing. The catch is then pulled down or fished out using beads that bind the biotin that was incorporated into the baits, and the catch can then be amplified using PCR before sequencing, for example using NGS workflows***. Ex. 1005 (Gnirke) at 183; Fig. 1 (reproduced below). See also Ex. 1006 (Mertes 2011) at 374 ("By selective recover[y] and subsequent sequencing of genomic loci of interest, costs and efforts can be reduced significantly compared with whole-genome sequencing."); 375 ("Targeted enrichment can be useful in a number of situations where particular portions of a whole genome need to be analyzed.").

EX1020 (Quackenbush Declaration IPR2023-00224) ¶77. He has a similar description at paragraph 238, which is reproduced below:

> To summarize the steps of this process, first, a pool of capture probes (or "baits") consisting of nucleic acid fragments that target a particular nucleic acid sequence (i.e., the complementary nucleic acid sequence that the bait's sequence pairs with by hybridization) is prepared. These capture probes/baits are typically prepared to include a ligand, such as biotin, that allows them to be bound by beads during subsequent steps to facilitate extraction. Second, the prepared baits are contacted with the nucleic acid sample (the "pool"), and the baits bind to the sequences of interest. ***This results in a "catch" of nucleic acids bearing the sequences if interest (i.e., sequences complementary to the baits), which are "caught" through the hybridization of strand-to-strand base-pairing. The catch is then pulled down or fished out using beads***

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> ***that bind the biotin that was incorporated into the baits***, and the catch can then be PCR amplified before analysis with Next-Generation Sequencing.

EX1020 (Quackenbush Declaration IPR2023-00224) ¶238; *see also id.* ¶67 (describing "physically pulling certain pieces of DNA out of a mixture for further analysis").

62. Gnirke (EX1005) treats "pull-down" as synonymous with "capture" in the "hybrid selection method" section of the article as follows:

> The hybridization is driven by the vast excess of RNA baits that cannot self-anneal. ***The 'catch' is pulled down with streptavidin-coated magnetic beads***, PCR amplified with universal primers and analyzed on a next-generation sequencing instrument. The method allows preparation of large amounts of bait from a single oligonucleotide array synthesis that can be tested for quality, stored in aliquots and used repeatedly over the course of a large-scale targeted sequencing project.

EX1005 (Gnirke) at page 183. There is a similar discussion on pages 186-87 of Gnirke as follows:

> ***We perform a simple pull-down with streptavidin-coated magnetic beads***, a generic laboratory technique that does not require customized equipment. It can be performed in almost any tube or multi-well plate format, and there are numerous precedents for processing many

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> samples in parallel. Our method is also largely independent of the sequencing platform.

EX1005 (Gnirke) at pages 186-87.

63. The prosecution history evidences that "pulldown probes" and "capture probes" are synonymous. For example, the Examiner used "capture" and "pulldown" synonymously when conducting prior art searches during prosecution of the ′394 and ′033 patents. *See* EX2024 (′394 File History Excerpts) at pages 3-8. I have reproduced a section of the Examiner's search terms where "captur$5," "pull-down" "pulldown" are searched with the "probe" for search L10:

L10	38	L9 and ((enrich$5 captur$5 pull-down pulldown) with probe with (polymorphism SNP inDel SNV mutation insertion deletion variant variation))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/05/21 20:37
L11	38	L10 and (WGS ("whole genome" near3 sequenc$3) (genom$3 adj sequencing))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/05/21 20:37
L12	38	L11 and ((cancer carcinoma tumor) same (mutation polymorphism SNP indel variant viriation insertion deletion))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/05/21 20:37
L13	15	L9 and ((WGS ("whole genome" near3 sequenc$3) (genom$3 adj sequencing)) same ((enrich$5 captur$5 pull-down pulldown) with (polymorphism SNP inDel SNV mutation insertion deletion variant variation)))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/05/21 20:41

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The Examiner used the same search terms during prosecution of the ′033 patent. *See* EX2025 (′033 File History Excerpts) at pages 3-25. The Examiner did not include "primers" or "PCR" in the search terms.

64. Further, the Examiner agreed during prosecution of related U.S. Application No. 15/996,215 that "pulldown probes" are not PCR primers. *See* EX2005 (File History Excerpts from U.S. Appl. No. 15/996,215) at page 19 (applicant explaining that "The 'primers' of Boers are not pulldown probes."); *id.* at page 24 (amending claim 1 to recite "pulldown probes"); *id.* at page 31 (the examiner stating "it was agreed that the feature of using pulldown probes to selectively enrich, when the selectively enriched first and second subsets of nucleic acids differ by the recited 'one or more features', would distinguish over the Boers reference"). The claims of related U.S. Application No. 14/810,337 were allowed after the applicant explained that "pulldown probes" are not PCR primers. *See* EX2004 (File History Excerpts from U.S. Appl. No. 14/810,337) at page 6 (amending claim 31 to recite "pulldown probes"); *id.* at 25 (applicant explaining that "The 'primers' of Craig are not pulldown probes.").

65. I understand that the Board took issue with Patent Owner's proposed construction by stating:

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> The ′394 patent does not appear to limit the use of capture probes to solution-phase binding. Accordingly, we might imagine potential target nucleic acids affixed to a solid support, which are then exposed to a capture probe. See, e.g., Southern, E.M., "Detection of specific sequences among DNA fragments separated by gel electrophoresis," 98 (3) J. Mol. Biol. 503-508 (1975) (Ex. 3003). Although a subset of the nucleic acids may hybridize to the capture probe, this subset cannot be physically collected or separated from unhybridized nucleic acids because both subsets are affixed to the solid support.

Institution Decision at page 25, footnote 11. It is important to point out that it is "labelled probes" that are used in Southern blot procedures such as identifying DNA fragments in a genomic digest, or identifying DNA clones of interest. In this scenario, the concentration of the probe is much lower than that of the target to enable maximal radioactive specific activity and thus sensitivity. In a capture probe, the molecule is typically in excess over the target. However, these differences notwithstanding, I will comment on the assertion. A labelled probe can be used in a Southern blot protocol to separate hybridized nucleic acids from unhybridized nucleic acids. The location of the hybridized nucleic acids on the nitrocellulose filter is established by comparison with the autoradiogram. The hybridized nucleic acid fragments can be physically separated from other unrelated fragments by cutting out that strip of nitrocellulose and then eluting the nucleic acid. It was well known that

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target DNA or RNA that has been adsorbed to the nitrocellulose leaches off during manipulation. *See* Sambrook, "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, third edition (2001), EX2026, 5.18-5.22 at page 6.57. Thus, immersion of the nitrocellulose strip in typical stripping buffers will suffice to collect the probe and target nucleic acids and they can be further processed as needed. It is also important to point out that such labelled probes are also used in a different Southern blot procedure which directly results in the physical isolation of target DNA. For example, in phage clone screening. *See id.* at pages 2.96-2.100. In this protocol, Phage plaques are transferred to nitrocellulose in a Southern blot method and then the relevant DNA targets identified by hybridization with a labelled probe, the relevant plaque is localized via the autoradiogram and then the DNA is isolated from the plaque.

3. **The specification does not contradict the plain meaning of "capture probe"**

66. The Board notes that the specification describes PCR primers and capture probes as having similar physical characteristics, *see* Institution Decision at pages 26-27, and states that the specification "provides no indication as to whether the categories [of primers and capture probes] are overlapping, *id.* 25. However, a POSA would recognize important physical differences between PCR primers and

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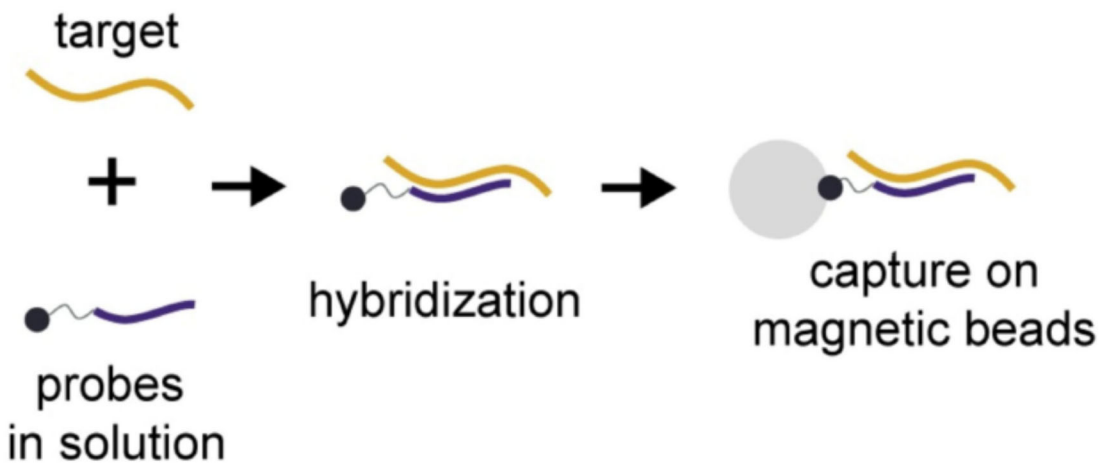
capture probes. If one were to assume that PCR amplification were synonymous with capture (which a POSA would not do), then such capture would require two molecules. In contrast, only ***one*** capture molecule is required to physically pulldown one target DNA molecule. Dr. Quackenbush correctly depicts a single capture probe hybridizing to a single target DNA strand as follows:



EX1020 (Quackenbush Declaration IPR2023-00224) ¶76.

67. Also, a POSA would understand that capture probes and PCR primers have very different ***functions***. The exhibits cited by Foresight and Dr. Quackenbush provide explanations regarding the differences in function between capture probes and PCR primers.

68. For example, Mertes (EX1006) teaches that capture probes and PCR primers are two different enrichment techniques that are "categorized according to the nature of their core reaction principle" as follows:

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> Current techniques for targeted enrichment can be categorized ***according to the nature of their core reaction principle*** (Figure 1):
>
> (i) ***'Hybrid capture'***: wherein nucleic acid strands derived from the input sample are hybridized specifically to preprepared DNA fragments complementary to the targeted regions of interest, ***either in solution or on a solid support, so that one can physically capture and isolate the sequences of interest***;
>
> (ii) 'Selective circularization': also called molecular inversion probes (MIPs), gap-fill padlock probes and selector probes, wherein single-stranded DNA circles that include target region sequences are formed (by gap-filling and ligation chemistries) in a highly specific manner, creating structures with common DNA elements that are then used for selective amplification of the targeted regions of interest;
>
> (iii) ***PCR amplification***: wherein ***polymerase chain reaction (PCR)*** is directed toward the targeted regions of interest by conducting multiple long-range PCRs in parallel, a limited number of standard multiplex PCRs or highly multiplexed PCR methods that amplify very large numbers of short fragments.
>
> ***Given 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. As discussed above, the core reaction principle of capture probes involves hybridizing to "targeted regions of interest, either in solution

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or on a solid support, so that one can physically capture and isolate the sequences of interest." *Id.* In contrast, the core reaction principle of PCR primers is "amplification" of targeted regions by a "polymerase chain reaction (PCR)" that copies target regions. *Id.* Mertes Figure 1 highlights differences between capture probes and PCR primers, and is reproduced below:

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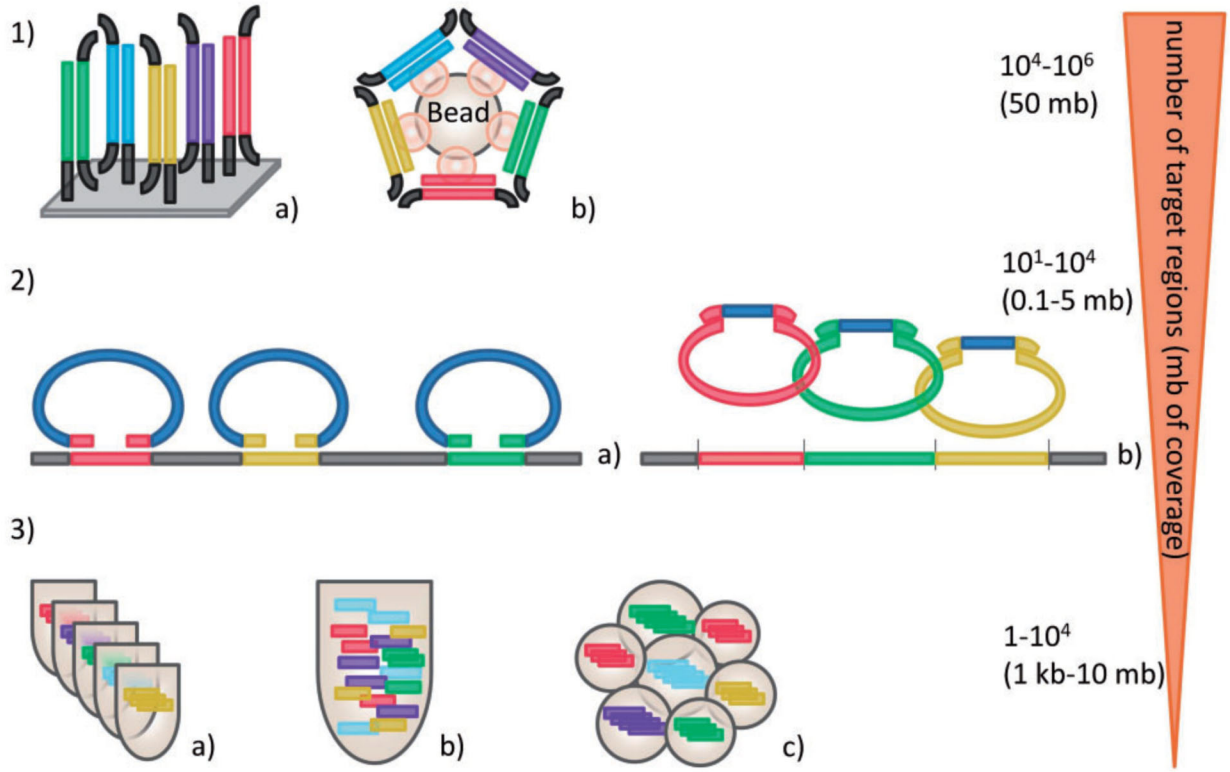

Figure 1: Commonly used targeted enrichment techniques. (1) Hybrid capture targeted enrichment either on solid support-like microarrays (a) or in solution (b). A shot-gun fragment library is prepared and hybridized against a library containing the target sequence. After hybridization (and bead coupling) nontarget sequences are washed away, the enriched sample can be eluted and further processed for sequencing. (2) Enrichment by MIPs which are composed of a universal sequence (blue) flanked by target-specific sequences. MIPs are hybridized to the region of interest, followed by a gap filling reaction and ligation to produce closed circles. The classical MIPs are hybridized to mechanically sheared DNA (a), the Selector Probe technique uses a restriction enzyme cocktail to fragment the DNA and the probes are adapted to the restriction pattern (b). (3) Targeted enrichment by differing PCR approaches. Typical PCR with single-tube per fragment assay (a), multiplex PCR assay with up to 50 fragments (b) and RainDance micro droplet PCR with up to 20 000 unique primer pairs (c) utilized for targeted enrichment.

EX1006 (Mertes) at page 376, Fig. 1. PCR primers function to prime a reaction by allowing two DNA polymerase molecules to bind and start a catalytic energy consuming polymerization reaction to copy both DNA strands and thereby generate what are called "amplicons". Thus, DNA molecules are replicated with PCR primers, which results in exponential amplification of target DNA sequences.

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However, the original DNA is still present (unless the amplicon is purified). Also present are the primers and primer dimers at high concentration, thus has the amplicon been captured? On the other hand, capture probes employ a noncatalytic process, which is simply governed by the concentration of reactants and time for the hybridization event to occur.

69. The Board stated in its Institution Decision that:

> The Specification's physical description aside, it is not clear, on the present record, how a capture probe comprising as few as a one nucleotide could provide the functionality of the capture probe recited in claim step 1(c) and described in the Specification. *See* Concurrence.

Institution Decision at page 26, footnote 12; *see also id.* at pages 43-44 (the Concurrence). However, there are instances where a single nucleotide can function as a capture probe under appropriate conditions. For example, guanine can hybridize in a capture event that allows its physical isolation via the formation of a gel or a precipitant. *See, e.g.,* Gellert et al., "Helix Formation by Guanylic Acid," Proceedings of the National Academy of Sciences, 2013-2018 (1962) at pages 2013-2014. I note that limitation 1(c) further clarifies that the claimed capture probes must

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specifically hybridize to "polymorphisms."[6] A POSA would be able to discern an appropriate length for the specifically claimed capture probes to allow specific hybridization to polymorphisms. *See, e.g.,* EX1006 (Mertes) at page 378 ("Selection libraries for hybrid capture are typically produced by oligonucleotide synthesis upon microarrays, with lengths ranging from ~60 to~180 bases."); EX1007 (Mamanova) at page 115 ("The principal difference between the Agilent and NimbleGen solution capture products is the nature of the capture probe: the NimbleGen product uses 60-90-mer DNA capture probes, whereas the Agilent one uses 150-mer RNA capture probes."); EX1005 (Gnirke) at page 187 ("One advantage of hybrid selection is that long capture probes are more tolerant to polymorphisms than the shorter sequences typically used as primers for PCR or multiplex amplification.").

70. The Board found that claim 13's recitation of "barcode" weighs moderately in favor of Foresight's construction because barcodes are typically used on PCR primers. *See* Institution Decision at page 24. The specification of the ′394

[6] The specification of the ′394 and ′033 patents states that "[t]he one or more polymorphisms may comprise one or more insertions, deletions, structural variant junctions, variable length tandem repeats, single nucleotide mutations, or a combination thereof. EX1001 (′394 patent) at 8:53-56.

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patent states that a "barcode" is a type of "sample identifier." EX1001 ('394 patent) at 55:14-18. It was known that barcodes are also used on capture probes. For example, Francis Barany and colleagues described barcoded capture probes ("unique 24-base oligonucleotides" having "zip-codes") where the barcodes (the "zip codes") direct target DNA having complementary sequences to move to (hybridize) to a unique physical location on an array. *See* Gerry et al., "Universal DNA Microarray Method for Multiplex Detection of Low Abundance Point Mutations," J. Mol. Biol., 292:251-262 (1999) at page 251 ("Our approach uses microarrays of unique 24-base oligonucleotides that are coupled to a three dimensional polymer at known locations. These 24-mers or zip-codes (Table 1) hybridize specifically to molecules containing sequences that are complementary to the zip-codes. … Importantly, because the zip-codes represent unique artificial sequences, zip-code microarrays can be used as a universal platform for molecular recognition simply by changing the gene-specific sequences linked to the zip-code complements").

4. <u>PCR primers and capture probes were two different techniques</u>

71. Foresight and Dr. Quackenbush improperly treat capture probes and PCR primers as being "relatively interchangeable." *See, e.g.,* EX1020 (Quackenbush Declaration IPR2023-00224) ¶69 ("However, these parameters

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would not have been considered as strict limitations, and the techniques can and were used on a relatively interchangeable basis in many experimental protocols, and could also be used together as complements to one another."). Mertes (EX1006), however, has entirely different sections discussing "enrichment by hybrid capture" and "enrichment by PCR" with primers. *See* EX1006 (Mertes) at pages 378-80. 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. Mertes Figure 4 is reproduced below, which shows the very divergent results of available methods in real-world experiments:

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Figure 4: Comparison of different enrichment techniques for regions of interest. The graph shows enrichments for the genes CTNNBI (1), PT53 (2) and ADAMI0 (3) converted to wiggle format on the UCSC genome browser. For every targeted enrichment experiment, the upper graph indicates the coverage obtained after data analysis, the corresponding bar line below specifies the region of interest used for probe design. Enrichment was performed with hybridization-based techniques either in solution (home made, blue; SureSelect whole exome, magenta; FlexGen, green) or on solid support (NimbleGen, yellow), with a PCR-based approach (RainDance, orange) and a circularization method (SelectorProbes, light blue).

72. Mamanova (EX1007) identifies numerous differences between "PCR," "on-array hybrid capture," and "in-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

[a]CTR, capture target region, that is, the regions of the desired target region to which probes could be designed after repeat masking. [b]Mapping 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. [c]Rank-order correlation in capture efficiency distributions between independent samples.

EX1007 (Mamanova) at page 114, Table 1.

73. There are several factors that a POSA 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 POSA 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;

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(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.

74. Similarly, Mertes teaches that a POSA 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 POSA would have understood that capture probes and PCR primers are different techniques, and selecting one or the other is a

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compromise that involves evaluating several parameters (and/or metrics) for each experimental design. 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.

VIII. THE GROUNDS CHALLENGING PATENTABILITY OF THE '394 AND '033 PATENTS

75. I understand that the Board has instituted trial on the following three grounds raised by Foresight in challenging patentability of the claims of the '394 patent:

Ground	'394 patent claims challenged	Type of challenge	Reference(s)
1	1-11, 13-19	Obviousness	Leary (EX1002)
2	1-11, 13-19	Obviousness	Leary (EX1002) & Ley (EX1012)
3	1-19	Obviousness	Leary (EX1002), Chan (EX1008), & Liao (EX1009)

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76. I understand that the Board has instituted trial on the following three grounds raised by Foresight in challenging patentability of the claims of the ′033 patent:

Ground	′033 patent claims challenged	Type of challenge	Reference(s)
1	1-7, 10-23	Anticipation	Forshew (EX1030)
2	1-23	Obviousness	Forshew (EX1002) & Wagle (EX1033)
3	1-9, 11-23	Obviousness	Wagle (EX1033) & Chan (EX1008)

77. The challenges raised by Foresight Diagnostics do not render any of the claims of the ′394 and ′033 patents for reasons described herein.

78. The following table includes a side-by-side comparison of claim 1 from the ′394 and ′033 patents to show some similarities and differences between the claims:

′394 Patent Claim 1	′033 Patent Claim 1
1. A method for analyzing nucleic acid samples isolated from an individual, comprising:	1. A method for analyzing a nucleic acid sample obtained from an individual, comprising:
(a) generating a first subset of nucleic acid molecules from a first nucleic acid sample obtained from said individual;	
(b) conducting a first sequencing assay on said first subset of nucleic acid	

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'394 Patent Claim 1	'033 Patent Claim 1
molecules to yield a first result comprising a first nucleic acid sequence wherein said first sequencing assay comprises whole genome sequencing;	
(c) generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of capture probes, wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof, wherein said first sample or said second sample is obtained from said subject at a first time point;	(a) producing, with the aid of a computer processor, a plurality of capture probes, wherein said plurality of capture probes hybridize to a plurality of polymorphisms, wherein said plurality of polymorphisms are in sequences encoding genes with known biomedically interpretable variants, and wherein said plurality of polymorphisms are based on or extracted from one or more databases of polymorphisms and are observed in a population of one or more samples; (b) contacting said nucleic acid sample with said plurality of capture probes produced in (a);
(d) conducting a second sequencing assay on said second subset of nucleic acid molecules to yield a second result comprising a second nucleic acid sequence;	(c) conducting a sequencing assay on a subset of nucleic acid molecules generated from said nucleic acid sample in step (b) to yield a result comprising a nucleic acid sequence, thereby analyzing said nucleic acid sample; and
(e) using said plurality of capture probes to generate an additional subset of nucleic acid molecules from an additional nucleic acid sample isolated from an additional sample obtained from said subject at an additional time	(d) repeating steps (b)-(c) on a subsequently obtained biological sample from said individual.

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'394 Patent Claim 1	'033 Patent Claim 1
point different from said first time point;	
(f) conducting a third sequencing assay on said additional subset of nucleic acid molecules from said additional nucleic acid sample to yield a third result comprising a third nucleic acid sequence; and	
(g) generating a biomedical report that includes biomedical information of said subject, which biomedical information identifies the presence or absence of said one or more polymorphisms identified by said second result or said third result.	

IX. FORESIGHT HAS NOT PROVEN UNPATENTABILITY OF ANY CLAIM FROM THE '394 PATENT

79. This section of my declaration provides an overview of the '394 patent, followed by an explanation of why each of Grounds 1-3 raised by the Petition do not prove obviousness of the claims of the '394 patent.

A. Overview of the '394 Patent

80. The '394 patent relates to methods for processing and data analysis of nucleic acid samples for producing biomedical reports and the diagnosis, monitoring, treatment, and prevention of diseases. For example, the abstract of the '394 patent states:

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> This disclosure provides systems and methods for sample processing and data analysis. Sample processing may include nucleic acid sample processing and subsequent sequencing. … Methods and systems of the disclosure can aid in the diagnosis, monitoring, treatment, and prevention of one or more diseases and conditions.

EX1001 (the ′394 patent) at Abstract.

81. Claim 1 is the only independent claim, and it recites seven steps (a)-(g) as shown below:

> Claim 1. A method for analyzing nucleic acid samples isolated from an individual, comprising:
> (a) generating a first subset of nucleic acid molecules from a first nucleic acid sample obtained from said individual;
> (b) conducting a first sequencing assay on said first subset of nucleic acid molecules to yield a first result comprising a first nucleic acid sequence wherein said first sequencing assay comprises whole genome sequencing;
> (c) generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of capture probes, wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof, wherein said first sample or said second sample is obtained from said subject at a first time point;

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(d) conducting a second sequencing assay on said second subset of nucleic acid molecules to yield a second result comprising a second nucleic acid sequence;

(e) using said plurality of capture probes to generate an additional subset of nucleic acid molecules from an additional nucleic acid sample isolated from an additional sample obtained from said subject at an additional time point different from said first time point;

(f) conducting a third sequencing assay on said additional subset of nucleic acid molecules from said additional nucleic acid sample to yield a third result comprising a third nucleic acid sequence; and

(g) generating a biomedical report that includes biomedical information of said subject, which biomedical information identifies the presence or absence of said one or more polymorphisms identified by said second result or said third result.

82. The specification of the ′394 Patent explains that prior art methods, including those based on the sequencing of DNA fragments from the "whole genomes" were not only costly, but could "fail to capture many biomedically important variants." EX1001 (the ′394 patent) at 1:23-34. The specification also states that standard methods often "fail[ed] to target biomedically interesting non-exomic and exomic regions," and would perform poorly in certain regions of the genome. EX1001 (the ′394 patent) at 1:23-34. The specification contains similar disclosures elsewhere in the patent. *See, e.g.,* EX1001 (the ′394 patent) at 69:49-

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70:20. In short, the problem was, that if a POSA used the conventional methods, many DNA fragments would either be missing from the analysis or would be hard to sequence.

83. The inventors of the ′394 Patent devised novel bespoke approaches to the processing of DNA fragments together with a subsequent targeted sequencing assay according to the steps recited in the claims. The inventors of the ′394 Patent developed an enrichment pulldown process, customized sample preparation methods and bespoke sequencing protocols to specifically improve performance over the prior art. *See, e.g.,* Claim 1 of EX1001 (the ′394 patent).

B. <u>Ground 1: The claims are nonobvious over Leary (EX1002)</u>

84. Leary (EX1002) does not render obvious independent claim 1 for reasons discussed below. I understand claims 2-19 ultimately depend from claim 1. I understand that dependent claims are nonobvious if the independent claim from which they depend is nonobvious. Therefore, I understand that dependent claims 2-19 are nonobvious over Leary at least because Leary fails to render obvious independent claim 1. Leary further fails to render obvious dependent claims 17, 18 and 19 for additional reasons discussed below.

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1. Claim 1 is nonobvious over Leary (EX1002)

85. Leary (EX1002) fails to render obvious claim 1 because Leary does not teach or suggest at least the following five limitations recited by the claim:

- the "whole genome sequencing" required by limitation 1(b),
- the "second nucleic acid sample" required by limitation 1(c),
- the "capture probes" required by limitation 1(c),
- the "plurality of capture probes" required by limitation 1(e); and
- the "third sequencing assay" required by limitation 1(f).

a. Leary (EX1002) does not teach or suggest limitation 1(b)

86. Limitation 1(b) recites "conducting a first sequencing assay on said first subset of nucleic acid molecules to yield a first result comprising a first nucleic acid sequence ***wherein said first sequencing assay comprises whole genome sequencing***."

87. Leary (EX1002) does not teach or suggest the "whole genome sequencing" (WGS) required by limitation 1(b). I understand that the Board construed WGS to mean "a non-targeted approach to sequencing all or almost all of a genome." *See* Institution Decision at pages 19-23. Leary does not teach or suggest sequencing all or almost all of the genome for reasons discussed below.

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Furthermore, Leary, as conducted, is essentially a physical mapping method, and could be considered to be a targeted approach.

i. <u>Leary does not teach whole genome sequencing</u>

88. Dr. Quackenbush states that "Chan explains that approaches like those of Leary and McBride have a 'targeted nature,' and thus may 'provide only a partial glimpse of the Tumor genome in the plasma of cancer patients.'" EX1020 (Quackenbush Declaration IPR2023-00224) ¶227 (quoting Chan, Exhibit 1008). I agree with Dr. Quackenbush that Leary and McBride have a targeted nature that only provides a partial glimpse of the tumor genome. The "partial glimpse" of a genome provided by Leary does not sequence all or almost all of the genome as required by the Board's construction of the term "whole genome sequencing." For at least this reason, Leary does not teach the whole genome sequencing of limitation 1(b).

89. Rather than teaching whole genome sequencing, Leary teaches at most a physical mapping approach that did not report the actual sequence of the polymorphism. Leary's mapping approach is different from whole genome sequencing. "Mapping" proceeds by evaluating the physical relationship between two or more genomic markers. In contrast, "sequencing" reports the identity and ***order of nucleotides***. If a POSA were to do whole genome sequencing, then it would

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determine the identity and ***order of nucleotides that comprise the whole genome.*** Nowhere does Leary mention whole genome sequencing.

90. Dr. Quackenbush states that "[t]he human genome consists of approximately 3.2 billion nucleotides of DNA, and (most) human cells contain two copies of the genome, one inherited from each biological parent." EX1020 (Quackenbush Declaration IPR2023-00224) ¶47. Dr. Quackenbush refers to "nucleotides," which are different from base pairs. There are two nucleotides in each base pair, thus to sequence the whole human haploid genome, one would need to sequence approximately 6.4 billion nucleotides. To sequence a whole diploid human genome, one would need to sequence approximately 12.8 billion nucleotides. Thus, if one is referring to the sequence coverage of nucleotides, then the denominator must be 12.8 billion. Thus, according to Dr Quackenbush's definition, the actual the nucleotide sequencing coverage of patient Co84 in Leary is a mere 0.22x!

91. Dr. Quackenbush states that "[t]o obtain highly accurate sequencing information of the whole genome, it is necessary to sequence each ***base*** in the genome multiple times" when using next-generation sequencing. EX1020 (Quackenbush Declaration IPR2023-00224) ¶63. I agree that it is necessary to sequence each "base" in each chromosome multiple times to obtain highly accurate sequencing information of the whole genome using next-generation sequencing.

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However, there are approximately 6.4 billion bases (or 3.2 billion base pairs) in the haploid human genome.

92. Leary (EX1002) describes a "PARE" approach, which stands for "personalized analysis of rearranged ends." EX1002 (Leary) at page 1. Leary's PARE approach did not sequence each of the 3.2 billion base pairs in the human genome, and it certainly did not sequence each of the 3.2 billion base pairs multiple times. I will explain Leary's PARE approach in the next several paragraphs.

93. Leary (EX1002) includes the following description of the PARE approach:

> The PARE (personalized analysis of rearranged ends) approach, shown schematically in Fig. 1, requires the identification of patient-specific rearrangements in tumor samples. To determine the feasibility of identifying such alterations using next-generation sequencing approaches, we initially analyzed four tumor samples (two colon and two breast tumors) and their matched normal tissue samples using the Applied Biosystems SOLiD System (Table 1). Genomic DNA from each sample was purified, sheared, and used to generate libraries with mate-paired tags ~1.4 kb apart. Libraries were digitally amplified by emulsion polymerase chain reaction (PCR) on magnetic beads (21), and 25-bp mate-paired tags were sequenced using the sequencing-by-ligation approach (15, 22). An average of 198.1 million 25-bp reads was obtained for each sample, where each read aligned perfectly and

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> was uniquely localized in the reference human genome (hg18), resulting in 4.95 Gb of mappable sequence per sample. An average of 40 million mate-paired reads, where both tags were perfectly mapped to the reference human genome, was obtained for each sample. The total amount of genome base pairs covered by the mate paired analysis (that is, distance between mate-paired tags × number of mate-paired tags) was 53.6 Gb per sample, or an 18-fold physical coverage of the human genome.

EX1002 (Leary) at page 1.

94. Leary's PARE approach that uses mate pair sequencing with the SOLiD sequencing platform may be illustrated by the figure below:

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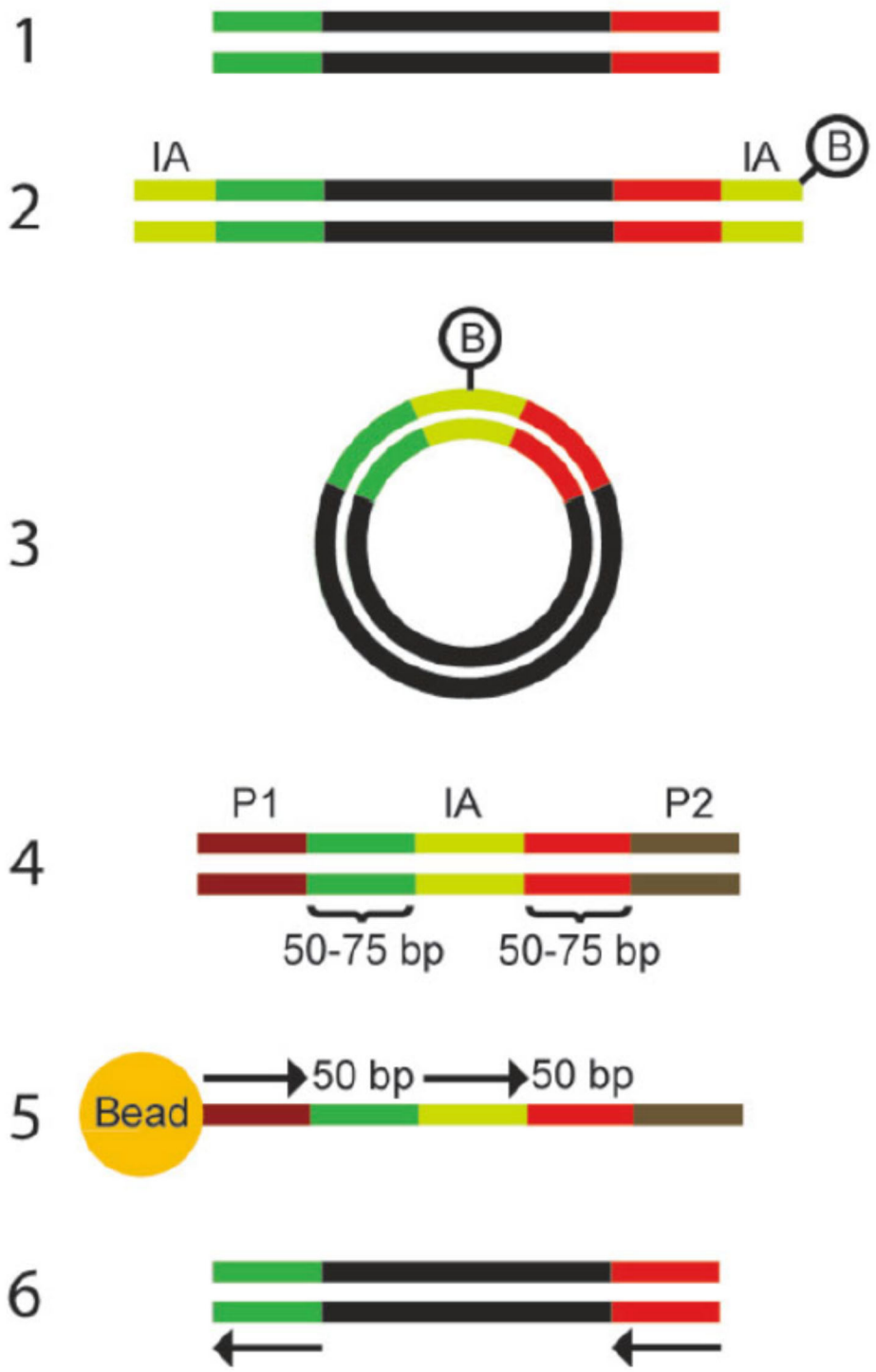

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See Berglund et al., Next-generation sequencing technologies and applications for human genetic history and forensics, Investigative Genetics, 2:23 (2011), EX2017, at page 5, Figure 2(c). As the figure shows, mate pair sequencing using the SOLiD protocol starts with the cleavage of genomic DNA into fragments. These fragments are typically end repaired using a standard kit that utilizes dNTPs and varieties of DNA polymerase. It is well known in the art that not all ends are equal, they have different sequences, some ends are more easily repaired than others and some ends are never repaired. Next, biotin labeled circularization adaptors (IA) are ligated to the ends (step 2) so that the DNA fragments can be circularized and subsequently isolated (step 3). Not all ends can be ligated by DNA ligase!

95. After circularization, the material is fragmented again and only a small fragment containing the ends of the original genomic fragment is retained by adsorption to avidin (steps not shown). Thus, the bulk of the sequence of that fragment is discarded. Library adaptors (PI and P2) are then ligated to the new ends and the resultant material (step 4) is sequenced using two primers (step 5). One primer is complementary to the P1 adaptor yielding tag1 sequence and the other primer is complementary to the 1A adaptor yielding tag2 sequence. Both reads are in the same orientation and each typically are designed to yield 25-75 base pairs (or "bp") of genomic sequence. Not all sequences are equal in terms of their ease of

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sequencing. For example, it was well known that G/C rich sequences are more difficult to sequence.

96. The mate pair sequencing disclosed in Leary is not whole genome sequencing. As Leary explains, it sequences "25-bp mate paired tags." *See, e.g.*, EX1002 (Leary) at page 1 ("Libraries were digitally amplified by emulsion polymerase chain reaction (PCR) on magnetic beads, and ***25-bp mate-paired tags were sequenced*** using the sequencing by ligation approach"). This means that each "mate pair" in Leary's approach only reports 50 bp of genomic DNA sequence (i.e., 2 times 25-bp mate paired tags). As such, to cover the all the base pairs in the whole human genome using the mate pair approach in Leary, one would need to very accurately sequence about 64 million ***non-overlapping*** mate-pairs (*i.e.*, 3.2 billion divided by 50) and one would still need to contend with biases such as those described above (e.g., not all DNA fragment ends can be ligated by DNA ligase, and not all sequences are equal in terms of ease of sequencing, such as G/C rich sequences).

97. None of the samples described in Leary have enough non-overlapping mate-paired tags to cover the whole human genome. Even if we were to make the very unlikely assumption that there were no overlapping mate-pairs, the maximum number of mate-paired tags matching the human genome in Table 2 of Leary is

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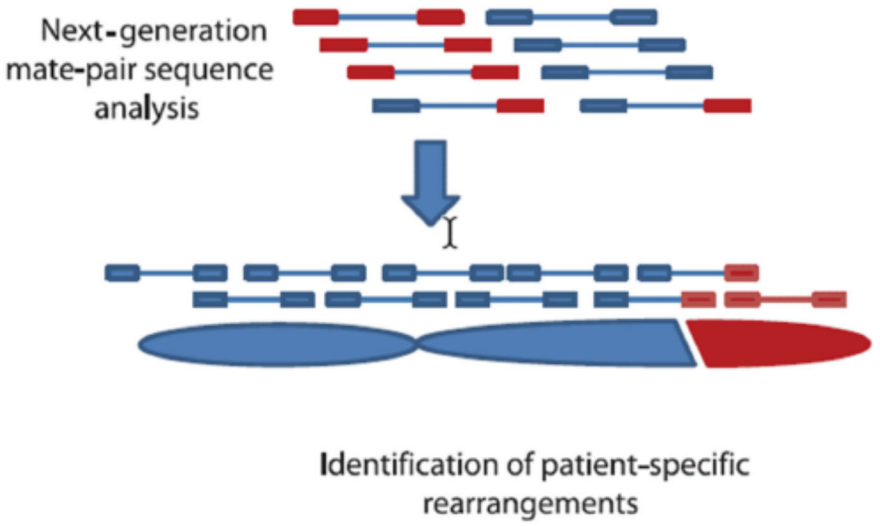
59,019,031, which is for the Co84 normal sample. This accounts for only 2.9 billion bp (59 million times 50-base pairs), which is far less than the 3.2 billion bp acknowledged to be present in the reference genome at this priority date. Thus, in this highest-case scenario, approx. 9% of the human genome is still missing from this analysis. Thus, it is clear that, even in a hypothetical, best of all possible worlds, it is extremely likely that the mate-paired sequencing in Leary will fall substantially short of sequencing all or almost all of the genome.

98. What's more, the mate-paired tags described in Leary are clearly intended to be overlapping. Indeed, there is no indication that the mate-paired tags were selected to be non-overlapping. Just the opposite, Figure 1 of Leary shows the mate tags are overlapping:



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Thus, it is impossible that the mate-paired tags in Table 2 of Leary would suffice to cover all or almost all of the genome. EX1002 (Leary) at Fig. 1.

99. In fact, the data presented in Leary confirms that the mate-paired tags in Leary are very likely overlapping to a very significant degree. Consider again, for example, the Co84 normal sample in Table 1. This sample has the largest number of mate-paired tags, so would ostensibly cover the largest portion of the genome. The data for this sample clearly indicates that some bases aren't sequenced at all and some bases are sequenced more than once. In fact, it appears that the sequenceable bases were actually read (sequenced) on average about 1.7 times, such that to estimate how much of the genome is actually covered in the mate pair analysis, one must divide the total number of sequenced bases by roughly 1.7.

100. The following calculation illustrates this in the case of Co84 normal sample:

- There are 5,457,003,650 total bases sequenced. These sequenced bases come from 25 bp tags, and the tags are placed into 3 kb bins. *See* Leary (EX1002) at Table 1; *id*. at page 3.
- Leary also explains on page 6 that there are 849,434 bins . *Id*. at 6. These 849,434 bins cover only 849,434 bins*(3000 bases of the genome per bin) = 2,548,302,000 bases.

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- Therefore, of the 5,457,003,650 bases sequenced, only about 5,457,003,650*2,548,302,000/3,200,000,000 = 4,345,654,161 are actually placed into a bin.
- Therefore, there are (4,345,654,161 sequenced bases)/(849,434 bins) = 5,115 sequenced bases/bin.
- There are 3000 bases of the genome per bin.
- Therefore, there are (5,115 sequenced bases/bin)/(3000 nucleotides/bin) = 1.70 sequenced bases/nucleotide.

In other words, based on the data in Leary, it appears that each base that is sequenced is, on average, actually sequenced about 1.7 times. Therefore, in reality, the mate-paired analysis for Co84 normal covers, in a best-case scenario, only about 1.71 Gb = 2.9/1.70 of the genome, not 2.9 Gb.

101. I understand Petitioner quotes a portion of Leary that refers to "18-fold physical coverage of the human genome" in connection with the mate-pair sequencing to support its contention that Leary is performing "whole genome sequencing." *See* Petition at pages 32-33. This reference to "18-fold physical coverage of the human genome," however, does not mean that on average every base pair in the entire genome was sequenced 18 times. It means instead "the number of DNA fragments of which both ends have been sequenced that on average overlie

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any position in the genome." EX2162 at page 1. In other words, there are on average 18 potentially informative guideposts that surround that particular locus. However, as discussed below, because not all ends are equal, some locations have many more or less guideposts than others (and some will have none)!

102. In fact, this sentence in Leary is referring to the circularized molecules that are generated before sequencing. This distinction between sequence coverage and physical mapping is clear from the text of Leary, which Petitioner only quotes in part. The relevant sentence of Leary (with the excluded portion indicated in bold and italics) reads in full as follows:

> The total amount of genome base pairs covered by the mate paired analysis (***that is, distance between mate-paired tags × number of mate-paired tags***) was 53.6 Gb per sample, or an 18-fold physical coverage of the human genome.

EX1002 (Leary) at page 1. Thus, as Leary makes clear, the "18-fold physical coverage" merely indicates the physical "distance between mate-paired tags." Leary never says what the sequence coverage is for the PARE assay. "Physical coverage" does not mean sequence coverage. In mate-pair sequencing, only the ends of the original fragment (the tags) are sequenced, not the very much larger segment between the ends. The vast majority of the original DNA genome between the ends has been removed by the second fragmentation step. Only the ends—i.e., the "mate

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pairs"—of these molecules were actually sequenced. Petitioner has simply misunderstood what Leary is teaching when it refers to "18-fold physical coverage of the human genome." Specifically, Petitioner has confused "physical coverage" and "sequence coverage."

103. Finally, it is important to rebut Dr. Quackenbush's assertion that the missing sequence between the ends of the Fragments could be provided by increasing sequence coverage. Firstly, Dr. Quackenbush merely suggests and does not provide a calculation as to the sequence coverage level that may be needed. Sequence coverage in the provided prior art varies from less than 1x to 500x. However, Dr. Quackenbush has made the fatal flaw of assuming that all ends of the fragments are equal. No matter how many reads are attempted, it is most likely that there will be many fragments that evade sequencing.

104. I understand that the Board found it pertinent that Forshew described "Leary's PARE approach as whole genome sequencing." Institution Decision at page 22. The phrase "whole genome sequencing" has been used in the literature in different ways and in different contexts. As discussed above, Leary's PARE approach does not sequence all or almost all of the human genome, and therefore fails to meet "whole genome sequencing" as the Board has construed that term in this proceeding.

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105. I note that there are several examples in the literature that specifically ***exclude*** Leary's PARE approach when describing whole genome sequencing. For example, Feldman (EX2008) states:

> ***Whole-genome sequencing currently demands resources beyond the reach of most cancer investigators***. Transcriptome sequencing has been used to identify novel fusion genes derived from chromosomal translocations in solid tumors. In lymphomas, however, translocations often do not produce a fusion gene and, in some cases, disrupt a putative tumor suppressor gene. Transcriptome sequencing is unlikely to detect such translocations. ***Therefore, we used a mate-pair genomic DNA sequencing approach for lymphoma translocation discovery***.

EX2008 (Feldman) at page 918.

106. As another example, Meyerson (EX1031) has a table that summarizes "whole-genome sequencing studies of cancer," which is reproduced below:

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Table 1 | **Whole-genome sequencing studies of cancer**

Study	Method	Cancer type	Number of samples sequenced	Aberration type	Refs
Ley *et al.*, 2008	Deep single-end whole-genome sequencing	AML	1	Point mutations, insertions, deletions	10
Campbell *et al.*, 2008	Shallow paired-end whole-genome sequencing	Lung	2	Deletions, amplifications, tandem duplications, interchromosomal rearrangements	22
Stephens *et al.*, 2009	Shallow paired-end whole-genome sequencing	Breast	24	Deletions, amplifications, tandem duplications, interchromosomal rearrangements, inversions	39
Pleasance *et al.*, 2010	Deep paired-end whole-genome sequencing	Melanoma	1	Point mutations, insertions, deletions, amplifications, interchromosomal rearrangements	12
Pleasance *et al.*, 2010	Deep paired-end whole-genome sequencing	Small-cell lung	1	Point mutations, insertions, deletions, amplifications, interchromosomal rearrangements	13
Mardis *et al.*, 2009	Deep paired-end whole-genome sequencing	AML	1	Point mutations, insertions, deletions, amplifications, interchromosomal rearrangements	11
Shah *et al.*, 2009	Deep paired-end whole-genome sequencing	Breast	1	Point mutations, insertions, deletions, amplifications, interchromosomal rearrangements	15
Ding *et al.*, 2010	Deep paired-end whole-genome sequencing	Breast	1	Point mutations, insertions, deletions, amplifications, interchromosomal rearrangements, inversions	35
Lee *et al.*, 2010	Deep paired-end whole-genome sequencing	Lung	1	Point mutations, insertions, deletions, amplifications, interchromosomal rearrangements, inversions	14

AML, acute myelogenous leukaemia.

EX1031 (Meyerson) at page 687, Table 1. Leary is cited as reference 99 in Meyerson, but it is not included in the list of whole genome sequencing studies in Table 1.

107. Newman (EX2152) is an article that was co-authored by Ash Alizadeh and Maximilian Diehn, who are two founders of Foresight.[7] Newman was cited by Foresight as Exhibit 1044 in the two co-pending matters of IPR2023-00545 and

[7] Foresight was founded by Ash Alizadeh, Maximilian Diehn, David Kurtz, and Jake Chabon. *See* EX2151 (Foresight website) available at https://foresight-dx.com/about.

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IPR2023-00546 that challenge two more of Personalis' patents. Newman referenced several whole genome sequencing references as follows:

> Following therapy, ctDNA concentrations typically drop, thus requiring even lower detection thresholds. Previously published methods employing amplicon[8,10,11], whole exome[12], 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).

EX2152 (Newman) at page 7. Leary is not included in the list of exemplary whole genome sequencing references by Newman. However, Leary is cited elsewhere in Newman as reference 5. *See* Ex2152 (Newman) at page 11.

108. McBride (EX1015) explains that Leary and McBride use "similar paired-end rearrangement screen" that "require only low coverage sequencing of the cancer genome" as follows:

> ***While this manuscript was in preparation, another group published similar paired-end rearrangement screens in six patients at diagnosis of colorectal cancer, with studies of plasma DNA in two patients (Leary et al., 2010)***. As we do, this article shows that tumor-specific rearrangements can be sensitively and specifically detected in plasma, with serial studies in one patient showing that levels correlated with decreasing disease burden during treatment. …

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> To date, we have completed screens for somatically acquired genomic rearrangements in >100solid tumor samples, including breast, pancreatic, ovarian, bone, and lung cancers. In all but one of these samples, we have successfully identified rearrangement breakpoints, and ~85% of samples have >10 rearrangements (Campbell et al., 2008; Mardis et al., 2009; Stephens et al., 2009; Stratton et al., 2009; Pleasance et al., 2010). This underscores the widespread applicability of the approach described here for patients with solid tumors. Protocols have been developed that allow massively parallel sequencing to be performed on formalin-fixed, paraffin-embedded pathology blocks (Schweiger et al., 2009), which further broadens accessibility since most patients undergo biopsy or resection of their cancer. ***The primary paired-end screen for rearrangements requires only low coverage sequencing of the cancer cell genome***, with subsequent validation of likely somatic rearrangements by PCR in cancer and normal DNAs.

EX1015 (McBride) at page 9. McBride and Leary used similar "paired-end screening" that provide "only low coverage sequencing of the cancer cell genome," rather than covering all or almost all of the genome as required for whole genome sequencing. As discussed above, Leary describes a breakpoint ***mapping*** technique that only uses the sequences of the mate-pair ends to identify the putative ***location*** of the breakpoint rather than determining the order of the nucleotides in all (or almost all) of the genome.

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ii. Leary does not suggest WGS

109. A POSA would not have been motivated to modify Leary's targeted PARE approach to use whole genome sequencing for at least two reasons: (1) whole genome sequencing imposed a large data burden in the 2012-2013 timeframe, and (2) whole genome sequencing was expensive in the 2012-2013 timeframe.

(a) The data burden of WGS

110. The exhibits filed by Foresight in this proceeding consistently describe whole genome sequencing as being undesirable because it imposes a large data burden that is orders of magnitude higher than targeted approaches (such as Leary's PARE approach). For example, Meyerson (EX1031) teaches that whole genome sequencing requires the approximately 3 billion bases of the human genome to be covered at least 30-fold on average, requiring the generation of 90 billion bases of sequence data per sample:

> Two important issues to consider when planning whole-genome sequencing experiments are depth of coverage and physical coverage. Sequence depth is measured by the amount of over-sampling: typically, to detect nucleotide alterations with high sensitivity, ***the 3 billion bases of the human genome are covered at least 30-fold on average, requiring the generation of 90 billion bases of sequence data per sample***. For cancer samples, this number needs to be increased to

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> account for the decreased purity and often increased ploidy of each sample.

EX1031 (Meyerson) at page 688. The data burden is even higher for cancer samples "to account for the decreased purity and often increased ploidy of each sample." *Id.* Meyerson further explains that whole genome sequencing at 30-fold coverage requires 90 Gb of sequence, which is an order (and nearly two orders) of magnitude higher than the 3 Gb of sequence required for targeted approaches:

> Because the exome represents only approximately 1% of the genome, or about 30 Mb, vastly higher sequence coverage can be readily achieved using second-generation sequencing platforms with considerably less raw sequence and cost than whole-genome sequencing. For example, whereas 90 Gb of sequence is required to obtain 30-fold average coverage of the genome, 75-fold average coverage is achieved for the exome with only 3 Gb of sequence using the current state-of-the-art platforms for targeting.

EX1031 (Meyerson) at page 690. Meyerson states that there are "computational issues" caused by "the huge quantity of experimental data" as follows:

> ***Computational issues***
>
> Tools for computational analysis need to develop rapidly to keep up with ***the huge quantity of experimental data*** produced by second-generation sequencing. Many of the computational tools for second-

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> generation sequencing were developed for analysing non-cancer samples for human population genetic studies.

EX1031 (Meyerson) at page 692. Meyerson states that it was a "major challenge" "to make biological sense of the mountains of genomic data" as follows:

> It is likely that second-generation sequencing methods will continue to transform cancer genomics, leading to the comprehensive discovery of all the major alterations in the cancer genome, followed by the application of comprehensive sequencing approaches to cancer diagnostics. Computational analysis will become a central part of these discovery and diagnostic efforts. ***The major challenge will be to make biological sense of the mountains of genomic data***.

EX1031 (Meyerson) at page 694.

111. Mertes (EX1006) teaches that targeted exome sequencing, as opposed to whole genome sequencing, provides "simpler data interpretation" that is a "crucial consideration" as follows:

> The alternative of fully sequencing many complete genomes to high average coverage (~30x or higher) to enable things like genetic variation analysis, was ***simply not affordable***. Another reason for assaying, e.g. exome rather than whole-genome sequencing is the ***simpler data interpretation*** of the former. ***This is a crucial consideration as it is generally much more challenging to find the functional impact of variants in noncoding regions***.

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EX1006 (Mertes) at page 382.

112. Mamanova (EX1013) teaches that a POSA would pursue targeted methods because the demands "on laboratory time and funding" required by whole genome sequencing "would place a substantial burden on a research center's informatics infrastructure" as follows:

> Nevertheless, it is not yet feasible to sequence large numbers of complex genomes in their entirety because ***the cost and time taken are still too great***. To obtain 30-fold coverage of a human genome (90 Gb in total), would currently require several sequencing runs and would cost tens of thousands of dollars. ***In addition to the demands such a project would place on laboratory time and funding, the primary analysis during which the captured image files are processed, as well as storage of the sequences, would place a substantial burden on a research center's informatics infrastructure***.

EX1007 (Mamanova) at page 111.

113. Shendure (EX1013) explains that large quantities of sequencing data raises "new challenges for experimental design, data analysis and interpretation" as follows:

> Over the past several years, next-generation DNA sequencing technologies have catapulted to prominence, with increasingly widespread adoption of several platforms that individually implement different flavors of massively parallel cyclic-array sequencing.

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> ***Common characteristics extend beyond the technologies themselves, to the quantity and quality of data that are generated, such that all raise a similar set of new challenges for experimental design, data analysis and interpretation***.

EX1013 (Shendure) at page 1143.

114. Ross (EX1014) teaches that large amounts of sequencing data presents "significant data management and interpretation challenges" as follows:

> NGS sequencing can produce upwards of 1 billion sequences per instrument in a 4-day run, and the cost per base sequenced is far cheaper than for the traditional dye-terminator methods. ***NGS is highly dependent on sophisticated bioinformatic analysis programs and faces significant data management and interpretation challenges*** for confirming whether a specific locus in a single cancer-related gene is wild type, an SNP, or a somatic mutation.

EX1014 (Ross) at page 537.

115. Forshew (EX1030) disparages whole genome sequence by explaining that it "generates a large amount of data on genomic regions that do not, at present, inform clinical decisions" as follows:

> Advances in massively parallel sequencing make new approaches possible. These have largely focused on large-scale analyses, including whole-genome or whole-exome sequencing. ***This generates a large***

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> ***amount of data on genomic regions that do not, at present, inform clinical decisions***.

EX1030 (Forshew) at page 9.

116. Anderson (EX1004) recommends "sequencing targeted subsets of the genome" over whole genome sequencing as follows:

> To meet the expectations associated with these emerging technologies, diagnostic laboratories may be anticipated to offer more comprehensive sequence analysis than ever before, encompassing the entire genome instead of single genes. ***However, the most successful initial clinical applications of next-generation sequencing may be through sequencing targeted subsets of the genome***, either to identify sequence variants associated with pharmacogenetics, or with inherited and somatic genetic diseases (cancers), by the parallel sequencing of multiple genes or by investigating such changes in candidate regions.

EX1004 (Anderson) at page 59.

117. I note that Dr. Quackenbush wrote in 2019 (several years after the '394 patent was filed) that "[s]equencing technologies have improved to the point where the greatest barrier to obtaining scientific insights is more related to data storage, analysis, and interpretation than its generation." *See* Hersch and Quackenbush et al., "High-Throughput Sequencing in Respiratory, Critical Care, and Sleep Medicine Research," Ann Am Thorac Soc, 16:1-16 (2019), EX2019, at page 7. I note that in

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2012 (closer to the time the ′394 patent was filed), Dr. Quackenbush wrote "[t]he pace of acquisition of genomic data for cancer patients far outstrips the utility of that information for choosing specific therapeutic avenues for individualized patient care… [t]here are both conceptual and technical challenges to assessing the wealth of information for individual tumors obtained with next-generation sequencing (NGS) technologies." Milbury and Quackenbush et al., "COLD-PCR Enrichment of Rare Cancer Mutations prior to Targeted Amplicon Resequencing," Clinical Chemistry, 58:580-589 (2012), EX2020, at page 580. He also wrote in 2011 that "[t]he cost and analytical complexity of sequencing still limit the number of whole genomes that can be sequenced in any single project…. Thus, whole-exome sequencing presents an effective alternative to whole-genome sequencing and provides an unbiased, cost-effective and time-efficient tool for the study of the genetic basis for disease." Sathirapongsasuti and Quackenbush et al., "Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV," Bioinformatics 27:2648-2654 (2011), EX2158, at page 2648.

118. Two of Foresight's founders (Ash Alizadeh and Maximilian Diehn) characterized Leary was being undesirable because it was more "technically challenging and expensive" than targeted approaches as follows:

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> Current strategies for ctDNA analysis may be divided into three categories: ***(1) patient-specific approaches that utilize personalized assays (1–4);*** (2) tumor type–specific targeted sequencing that does not require patient-specific optimization (5–7); and (3) tumor type–independent genome-wide analyses (8–11). ***The first approach is highly sensitive but is technically challenging and expensive***.

Przybyl, Alizadeh, and Diehn et al., "Combination Approach for Detecting Different Types of Alterations in Circulating Tumor DNA in Leiomyosarcoma," Clin Cancer Res, 24:2688-99 (2018), EX2021, at page 2688. Leary is cited reference number 2 in the Przybyl article.

119. In 2010, Blighe wrote that "the majority of current NGS [next generation sequencing] platforms follow similar protocols and are prone to error, including: the incorporation of incorrect bases during the PCR step; shorter read lengths (compared to Sanger di-deoxy sequencing); and sequence detection errors." Blighe, "Molecular analysis of circulating cell-free DNA in breast cancer patients," University of Leicester, 2012, EX2155, at page 22. He further explained "[f]or cancer genomes, greater coverage and specific algorithms may be required to compensate for aneuploidy and heterogeneity. Hence NGS is currently considered a research tool – rather than an aid – for diagnosis and treatment." *Id.* at page 22-23. He went on to state that "[a]nalysis of NGS data is a challenging issue that must be

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considered." *Id.* at page 23. He explained that "[t]he management and processing of raw sequence data necessitates the availability of both high-end hardware and dedicated manpower (Meldrum et al., 2011) – storage issues are also important (Haimovich, 2011)." *Id.* He further explained that "[t]he methods for this purpose are still under research and development (Haimovich, 2011 and Meldrum et al., 2011). The application of NGS to DNA extracted from FFPE tissue is another technical challenge (Meldrum et al., 2011)." *Id.* He concluded that "sequencing technologies are still in their infancy" and recommended targeted sequencing over whole genome sequencing as follows:

> However, sequencing technologies are still in their infancy and are not yet ready for use in diagnostics (Meldrum et al., 2011). Such methods also generate tremendous amounts of data - much more than microarrays - that equally require intensive interpretation. For example, solely from pilot data, the 1,000 Genomes Project (The 1,000 Genomes Project Consortium, http://www.1000genomes.org/) has already discovered around 15 million SNPs and 1 million insertions and deletions, but to what end? ***Whole genome sequencing may merely provide another more expensive way of determining potential targets that then have to be examined, as usual, in the laboratory.*** However, targeted sequencing could be a way forward if it can be developed to cover key somatic mutations and disease-causing SNPs/CNVs.

Id. at page 230.

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120. A POSA would not have been motivated to modify Leary's targeted PARE assay to use whole genome sequencing due to the substantial burden of processing the very large amounts of data associated with whole genome sequencing. The above exhibits repeatedly emphasize that a POSA would have avoided making such a modification to Leary due to the substantial burden of processing the very large amounts of data associated with whole genome sequencing.

(b) At this time WGS was at least an order of magnitude more expensive than targeted approaches

121. The exhibits filed by Foresight in this proceeding consistently explain that whole genome sequencing was undesirable because it is significantly more expensive than targeted or mapping approaches, such as Leary's PARE approach. The difference in expense is not trivial. For example, Sims (EX2022) is an exhibit cited by Foresight as Exhibit 1040 in the two co-pending matters of IPR2023-00545 and IPR2023-00546 that challenge two more of Personalis' patents. Sims explains that the difference in expense between targeted and whole genome sequencing approaches "can be an order of magnitude" as follows:

> Although costs vary depending on the sequence capture solution, ***WES [whole exome sequencing] can be an order of magnitude less***

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> ***expensive than WGS to achieve an approximately equivalent breadth of coverage of protein-coding exons***. These reduced costs offer the potential to greatly increase sample numbers, which is a key factor for many studies.

EX2022 (Sims) at page 123. Sims describes the expenses as being "substantial" and "often set limits to the amount of sequences that can be generated" such that investigators sought experimental designs "for the lowest sequencing cost" as follows:

> Sequencing technologies have placed a wide range of genomic analyses within the capabilities of many laboratories. However, ***sequencing costs often set limits to the amount of sequences that can be generated and***, consequently, the biological outcomes that can be achieved from an experimental design. …
>
> Despite sharp falls in recent years, ***sequencing costs remain substantial*** and vary for different types of experiment. Consequently, in all of these fields ***investigators are seeking experimental designs that generate robust scientific findings for the lowest sequencing cost***.

EX2022 (Sims) at page 121.

122. Mertes (EX1006) teaches that targeted approaches allow "costs and efforts [to] be reduced significantly compared with whole-genome sequencing" as follows:

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> As part of this, methods have been developed that aim to achieve 'targeted enrichment' of genome subregions, also sometimes referred to as 'genome partitioning'. Strategies for direct selection of genomic regions were already developed in anticipation of the introduction of NGS. ***By selective recover[y] and subsequent sequencing of genomic loci of interest, costs and efforts can be reduced significantly compared with whole-genome sequencing***.

EX1006 (Mertes) at page 374.

123. Gnirke (EX1005) teaches that there is a "substantial economy in targeting" that "substantially lower[s] the sequencing costs of a project" as follows:

> Sequencing entire human genomes will be an important application of next-generation sequencing. However, many research and diagnostic goals may be achieved by sequencing a specific subset of the genome in large numbers of individual samples. ***For example, there may be substantial economy in targeting the protein-coding fraction, the 'exome', which represents only ~ 1 % of the human genome***. The economy is even greater for many key resequencing targets, such as genomic regions implicated by whole genome association scans and the exons of sets of protein-coding genes implicated in specific diseases. ***Efficient and cost-effective targeting of a specific fraction of the genome could substantially lower the sequencing costs of a project, independent of the sequencing technology used.***

EX1005 (Gnirke) at page 182.

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124. Liao (EX1009) teaches that it was logical for a POSA to avoid whole genome sequencing as follows:

> Massively parallel sequencing has recently been used in noninvasive prenatal diagnosis. The current costs of this technology are still relatively expensive, however, and sample throughput is still relatively low when it is used as a molecular diagnostic tool. ***Rather than nonselectively sequencing the genome, target enrichment provides a logical approach for more efficient and cost-effective massively parallel sequencing because it increases the proportion of informative data from the targeted region(s)***.

EX1009 (Liao) at page 92.

125. In 2021, two of Foresight's founders (Ash Alizadeh and Maximilian Diehn) wrote that "the amount of sequencing required for the much larger targeted genomic space makes the per sample costs of WGS significantly higher" than other approaches as follows:

> In theory, application of whole-genome sequencing (WGS) to posttreatment plasma could allow detection of an even greater number of mutations than the aforementioned NGS approaches and not require custom panel design. However, due to assay background error rates, there are diminishing returns of increasing the number of tracked mutations past a certain point (see below). Therefore, recent work suggests that when combined with customized bioinformatics, direct

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> WGS of plasma can potentially achieve similar but not superior limits of MRD detection as the other NGS approaches. ***Additionally, the amount of sequencing required for the much larger targeted genomic space makes the per sample costs of WGS significantly higher***.

Moding, Alizadeh, and Diehn et al., "Detecting Liquid Remnants of Solid Tumors: Circulating Tumor DNA Minimal Residual Disease," Cancer Discovery, 11:2968-86 (2021), EX2023, at page 2969.

126. Further, Leary's PARE approach was considered to be undesirably limited due to high costs. For example, Newman (EX2152) is an article that was co-authored by Ash Alizadeh and Maximilian Diehn, who are two founders of Foresight. Newman was cited by Foresight as Exhibit 1044 in the two co-pending matters of IPR2023-00545 and IPR2023-00546 that challenge two more of Personalis' patents. Newman criticized Leary as being "limited" due to its high cost among other things as follows:

> NSCLC, PCR-based assays have been used to detect recurrent point mutations in genes such as KRAS (kirsten rat sarcoma viral oncogene homolog) or EGFR (epidermal growth factor receptor) in plasma DNA[1–4], but the majority of patients lack mutations in these genes. ***Recently, approaches employing massively parallel sequencing have been used to detect ctDNA[5–12]. However, the methods reported to date have been limited by*** modest sensitivity[13], applicability to only a

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> minority of patients, the need for patient-specific optimization, ***and/or cost***. To overcome these limitations, we developed a novel strategy for analysis of ctDNA.

EX2152 (Newman) at page 2. Leary is cited as reference 5 in Newman. *See* EX2152 (Newman) at page 11.

127. The exhibits filed by Foresight in this proceeding describe the real-world cost constraints imposed by whole genome sequencing as compared to targeted approaches. The cost of whole genome sequencing was often at least an order of magnitude higher than targeted approaches. A POSA reading Leary would not be motivated to perform whole genome sequencing because it would involve sequencing what was believed at that time to be useless parts of the genome. *See* EX2155 (Blighe) at 230 (explaining that WGS "discovered around 15 million SNPs and 1 million insertions and deletions, ***but to what end?*** Whole genome sequencing may merely provide another more expensive way of determining potential targets that then have to be examined, as usual, in the laboratory."). In fact, such parts were not only "useless" but expensive to sequence! A POSA in 2012 would have focused on targeted sequencing of exomic regions, which were the regions believed to be the clinically relevant. *See id.* ("targeted sequencing could be a way forward if it can be developed to cover key somatic mutations and disease-causing SNPs/CNVs"). A

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POSA would not have been motivated to modify Leary's already cost-limited approach to employ a whole genome sequencing approach because it would increase sequencing costs by up to at least an order of magnitude to sequence what was believed to be clinically irrelevant portions of the genome. A POSA would have avoided such a modification.

iii. Simply increasing Leary's coverage depth would not convert Leary's PARE to WGS

128. I note that the Board's Institution Decision cited Dr. Quackenbush's "discussion of increasing coverage depth when required by an experiment" as potentially motivating a POSA to modify Leary to sequence all or almost all of a genome. *See* Institution Decision at pages 22-23. A POSA would not have been so motivated for several reasons.

129. First, I note that Dr. Quackenbush elsewhere wrote that "[d]espite widespread perceptions to the contrary, increasing the number of reads generally does not improve the signal-to-noise ratio or lower the detection limit for either COLD-PCR or conventional PCR." *See* Milbury and Quackenbush et al., "COLD-PCR Enrichment of Rare Cancer Mutations prior to Targeted Amplicon Resequencing," Clinical Chemistry, 58:580-589 (2012), EX2020, at page 587. Simply increasing the number of reads for Leary's PARE method, which uses

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conventional PCR for amplification, would not improve the signal-to-noise ratio or lower the detection limit.

130. Second, increasing coverage depth would not, by itself, be expected to sequence all or almost all of a genome due to real world saturation limits. For example, Sims (EX2022) is an exhibit cited by Foresight as Exhibit 1040 in the two co-pending matters of IPR2023-00545 and IPR2023-00546 that challenge two more of Personalis' patents. Sims explains that "[s]aturation" is the "point at which the addition of extra reads to an analysis yields no improvement in the number of significant effects identified." EX2022 (Sims) at page 126. Similarly, two of Foresight's founders explain "due to assay background error rates, there are diminishing returns of increasing the number of tracked mutations past a certain point." Moding, Alizadeh, and Diehn et al., "Detecting Liquid Remnants of Solid Tumors: Circulating Tumor DNA Minimal Residual Disease," Cancer Discovery, 11:2968-86 (2021), EX2023, at page 2969.

131. Third, if a POSA were to be challenged to complete a whole genome sequence and thereby identify a comprehensive catalogue of DNA alterations by using Leary's mapping method (with an increased number of reads), he or she would not be optimistic of success. A POSA would know that Leary's method was useful for identifying chromosomal breakpoint translocations, but it was not

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designed to identify SNPs, inDels, methylation, or other types of polymorphisms. A POSA would know that Leary's fragment preparation steps undoubtedly have biases that prevent portions of the genome from being sequenced. Further, a POSA would need to consider the number of aligned 50bp mate-pair tags that would be needed to tile the entire 3.2 billion bases in the human genome. Even if all of these obstacles were overcome and the protocol worked efficiently (which is very unlikely), it would significantly increase the time and expense of Leary's assay. Thus, Leary's approach was very limited in what it could identify, and a POSA with the desire and means to compile a comprehensive catalogue of DNA variations would have sought ways to sequence the entire genome that had more versatile and bespoke applications than starting with Leary's mapping approach.

132. Fourth, Foresight's challenge to the patentability of the ′394 patent is based on a theory that Leary teaches whole genome sequencing, which is incorrect for reasons discussed above. A POSA would not have modified Leary to increase coverage depth to the point of sequencing all or almost all of the genome in view of the many fragments that are missing from sequence analysis or are sequenced very inefficiently, and the data burden and real-world expense constraints associated with whole genome sequencing for reasons discussed above.

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b. <u>Leary does not teach or suggest the "second nucleic acid sample" of limitation 1(c)(i)</u>

133. Limitation 1(c) recites "[i][8] generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of capture probes, [ii] wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof, [iii] wherein said first sample or said second sample is obtained from said subject at a first time point."

134. Limitation 1(c)(i) recites "generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of capture probes."

135. Foresight and Dr. Quackenbush have not established that Leary teaches the "second nucleic acid sample of said individual." In particular, Foresight and Dr. Quackenbush rely on the ***same*** tumor sample from Leary to supposedly meet the

[8] I have used the same bracketed numbers (e.g., "[i]" and "[ii]") as Dr. Quackenbush solely for the purpose of rreferencing the language in limitation 1(c). *See* EX1020 (Quackenbush Declaration IPR2023-00224) ¶68.

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"first nucleic acid sample" required by limitation 1(a) and the "second nucleic acid sample" required by limitation 1(c)(i). Specifically, Foresight and Dr. Quackenbush rely on Leary "obtaining a nucleic acid sample from a patient's tumor sample" for limitation 1(a). *See* EX1020 (Quackenbush Declaration IPR2023-00224) ¶123, *see also* ¶¶121-124. Foresight and Dr. Quackenbush rely on the ***same*** tumor sample for limitation 1(c)(i). *See id.* ¶131 (asserting Leary "generates a second subset of DNA from ***the*** tumor sample"), *see also* ¶¶128-131. By relying on the same tumor sample for Leary for both limitations 1(a) and 1(c)(i), Foresight and Dr. Quackenbush have not identified a first and second nucleic acid sample in Leary, as required by the claims.

136. A POSA would not have been motivated to obtain a second tumor sample to use in Leary's method. A POSA would have been dissuaded from obtaining a second tumor sample from Leary's patient because it would have involved an invasive procedure. For example, Newman (EX2152) is an article that was co-authored by two founders of Foresight (Ash Alizadeh and Maximilian Diehn). Newman was cited by Foresight as Exhibit 1044 in the two co-pending matters of IPR2023-00545 and IPR2023-00546 that challenge two more of Personalis' patents. Newman states that tumors "cannot be repeatedly sampled without invasive procedures" as follows:

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> Noninvasive access to malignant DNA is particularly attractive for solid tumors, which cannot be repeatedly sampled without invasive procedures.

EX2152 (Newman) at page 2.

c. <u>Leary does not teach or suggest the "capture probes" of limitation 1(c)(i)-(ii)</u>

137. Limitation 1(c)(i)-(ii) recites "[i] generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of capture probes, [ii] wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof."

i. <u>Leary does not teach or suggest "capture probes"</u>

138. Foresight and Dr. Quackenbush's theory that Leary renders obvious the claims of the ′394 patent depends upon a POSA equating Leary's PCR primers with the claimed "capture probes." *See* EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶128-134. Under the appropriate construction of "capture probes," a POSA would not have considered Leary's PCR primers to be "capture probes" because Leary's PCR primers do not hybridize to target DNA so that one can physically capture the target DNA.

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139. As discussed above, the exhibits filed by Foresight and Dr. Quackenbush repeatedly distinguish capture probes and PCR primers as two different enrichment techniques that differ according to their very different core reaction principles. *Supra* Section VII.B. A POSA would not view Leary's PCR primers as teaching the claimed capture probes and, accordingly, Leary does not teach the "capture probes" required by limitation 1(b).

140. A POSA would not have been motivated to modify Leary's PCR primer-based PARE assay to use the hybridization capture technique of capture probes. As discussed above, *supra* Section VII.B.4, a POSA 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 Leary's already working PCR primer PARE assay and replace it with capture probes. For example, Mamanova teaches seven parameters that need evaluation:

> (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;

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(v) cost;

(vi) ease of use; and

(vii) amount of DNA required per experiment, or per megabase of target.

EX1007 (Mamanova) at page 111. The vast majority of these parameters would have counseled a POSA to avoid attempting to redesign Leary'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." *Id.* Further, Mamanova teaches that PCR has higher ***reproducibility*** of "up to 100%" than capture probes. *Id.* at page 114, Table 1. Leary 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 Leary's already-working assay could very well cause the assay to lose sensitivity or even worse, not work at all.

141. It is unclear to me how Foresight and Dr. Quackenbush propose to change the workflow of Leary's PARE assay and what such a changed workflow would look like if a POSA were to somehow have been motivated to use capture

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probes instead of PCR primers. Leary's PARE approach requires DNA from each patient sample to be "purified, sheared, and used to generate libraries with mate-paired tags ~1.4 kb apart" followed by the libraries being "digitally amplified by emulsion polymerase chain reaction (PCR) on magnetic beads," and then sequencing 25-bp mate-paired tags "using the sequencing-by-ligation approach." EX1002 (Leary) at page 1. One PCR step is then "used to identify somatic rearrangements from these data (fig. S1)," and another PCR step is used to assay the presence of the

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rearrangement in patient samples.

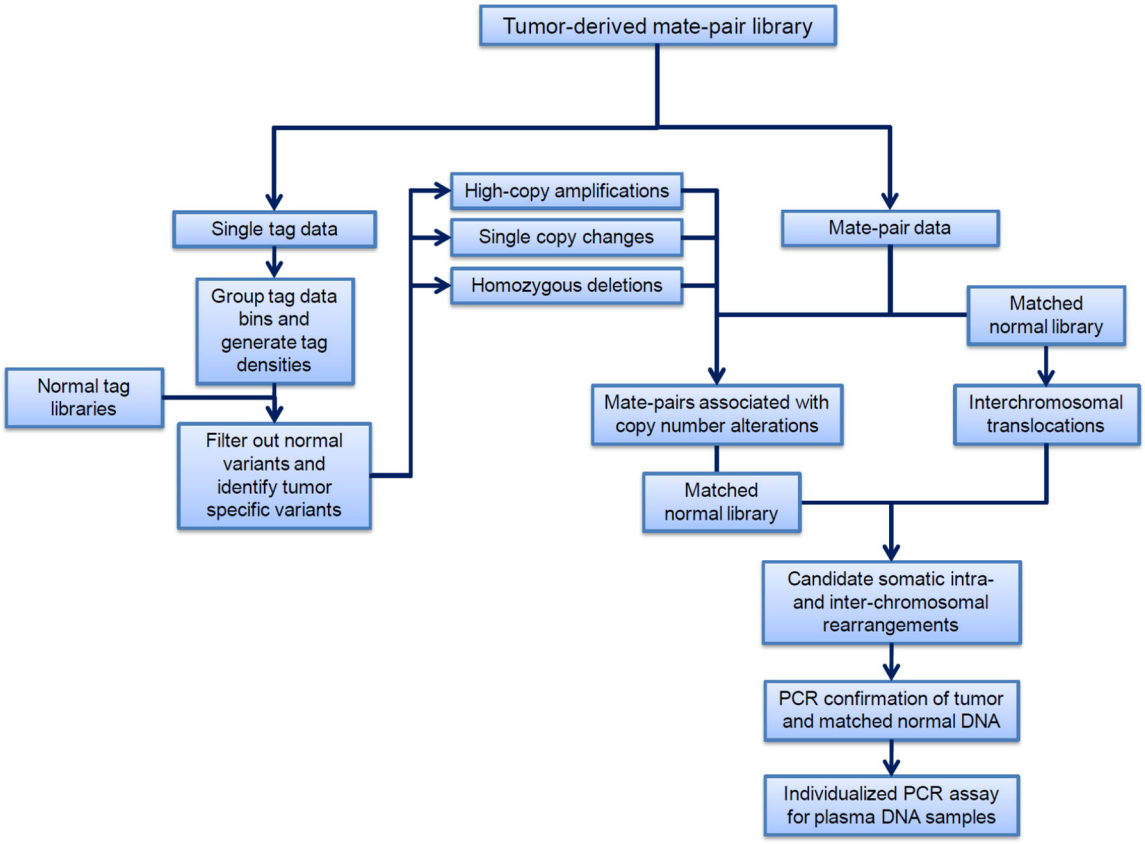

Fig. S1. Flow chart of approach used to identify rearranged sequences

EX1002 (Leary) at page 1; EX1026 (Leary Supplemental Material) at page 4, Fig. S1. Dr. Quackenbush does not articulate which PCR step of the PARE approach a POSA would have been motivated to change to capture probes, and which steps in the PARE workflow would need to be kept, discarded, or rearranged to accommodate such a change with a reasonable expectation of success. Further, Leary's assay uses PCR ***as its detection method*** for identifying chromosomal

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rearrangements. EX1002 (Leary) at page 4 ("To determine the utility of these rearranged sequences to serve as potential biomarkers, we designed PCR assays to detect them in the presence of increasing amounts of normal DNA."); *id.* at page 4 ("Digital PCR analyses of plasma samples from patient Hx402 indicated that rearranged tumor DNA was detectable at all six time points analyzed throughout the course of treatment (Fig. 4B)."). Dr. Quackenbush does not explain how capture probes could replace PCR to serve as the detection method for Leary's assay.

d. <u>Leary does not teach or suggest the "plurality of capture probes of limitation 1(e)</u>

142. Limitation 1(e) recites "***using said plurality of capture probes*** to generate an additional subset of nucleic acid molecules from an additional nucleic acid sample isolated from an additional sample obtained from said subject at an additional time point different from said first time point." Thus, limitation 1(e) requires a "***plurality*** of capture probes."

143. As discussed above, Leary's PCR primers are not "capture probes," and Leary fails to teach limitation 1(e) for at least this reason. However, even if Leary's PCR primers were to be considered "capture probes"—which they should not and is ridiculous as discussed above—Leary fails to disclose a ***plurality*** of probes that would meet limitation 1(e).

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144. Dr. Quackenbush relies on Leary Figure 4 for limitation 1(e). EX1020 (Quackenbush Declaration) ¶146. However, only a ***single*** chromosomal rearrangement was analyzed for each patient in Leary Figure 4. That is, patient Hx402 was only analyzed with a single primer pair that produces a single amplicon for the fragment spanning the Chr 4:8 rearrangement. Patient Hx403 was only analyzed with a single primer pair that produces a single amplicon for the fragment spanning the Chr 16 rearrangement. *See* EX1002 (Leary) at page 4 ("A chromosome 4:8 translocation associated with an amplification was used in tumor Hx402, and an intrachromosomal rearrangement associated with a homozygous deletion of chromosome 16 was used in tumor Hx403."); *see also id.* at page 6 "we used serial dilutions of tumor-normal DNA mixtures as templates for PCR using primers for the chromosome 4:8 translocation in Hx402.").

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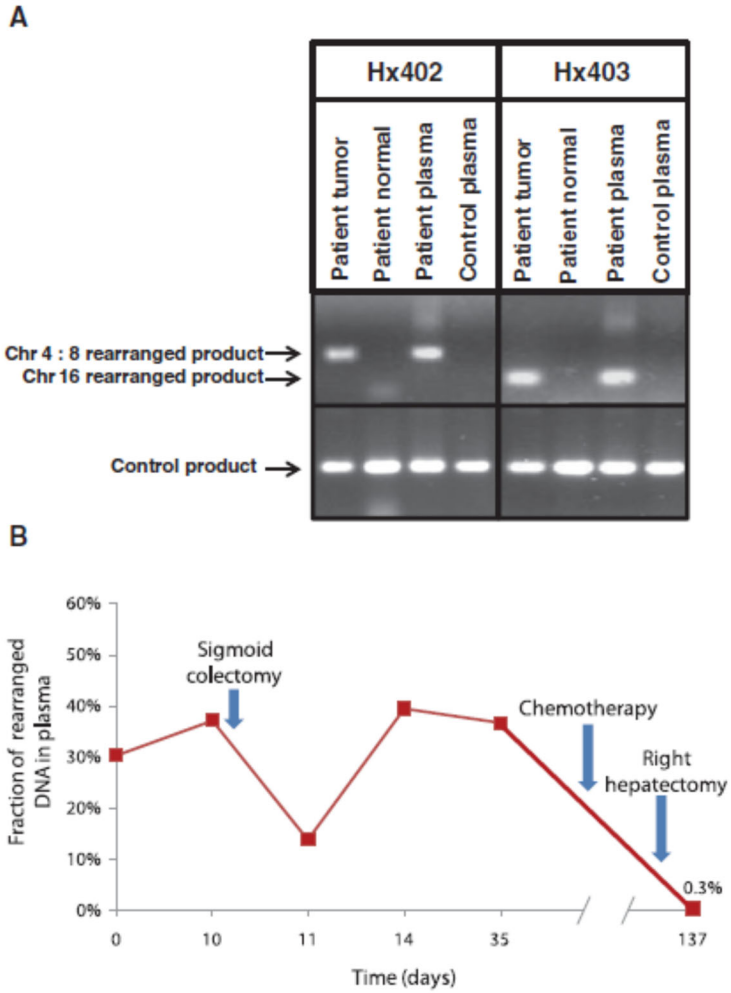

Fig. 4. Detection of tumor-specific rearrangements in plasma of cancer patients. (**A**) The identified chromosome 4:8 and 16 rearrangements were used to design PCR primers spanning breakpoints and to amplify rearranged DNA from tumor tissue and plasma from patients Hx402 and Hx403, respectively. A plasma sample from an unrelated healthy individual was used as a control for both rearrangements. (**B**) Plasma samples from patient Hx402 were analyzed at different time points using digital PCR to determine the fraction of genomic equivalents of plasma DNA containing the chromosome 4:8 rearrangement. The fraction of rearranged DNA at day 137 was 0.3%, consistent with residual metastatic lesions present in the remaining lobe of the liver.

In addition, Leary's Figure 3 is reproduced below:

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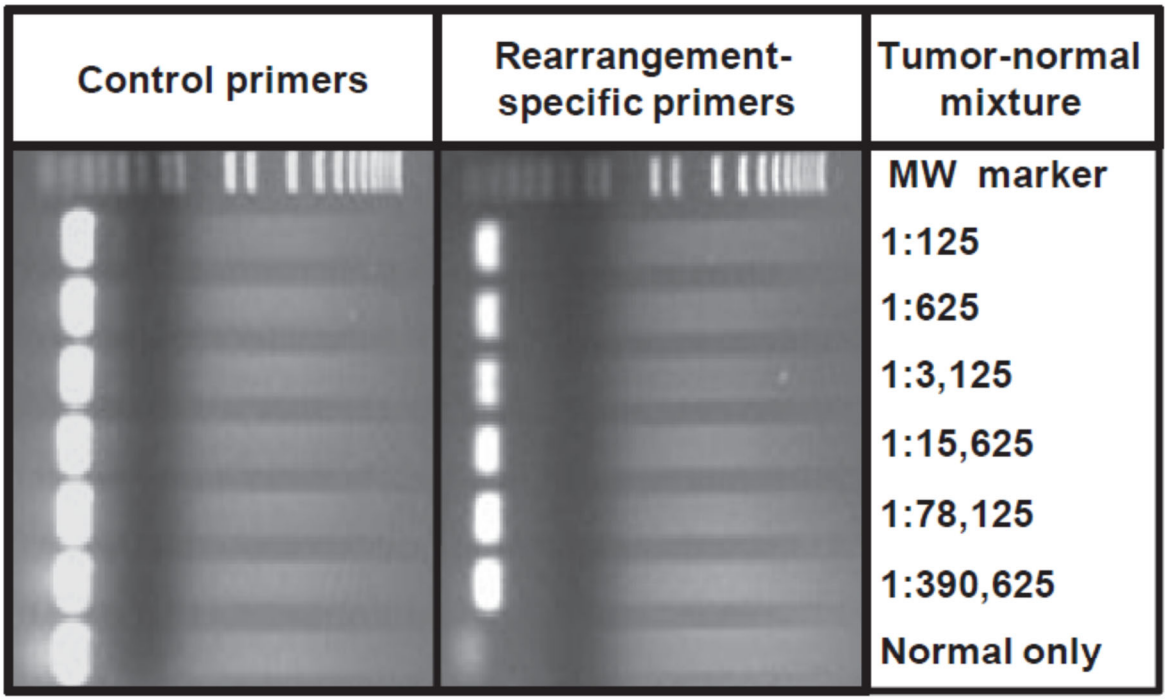

Fig. 3. Detection of tumor-specific rearrangements in mixtures of tumor and normal DNA. Decreasing amounts of tumor DNA were mixed with increasing amounts of normal tissue DNA (300 ng total) and were used as template molecules for PCR using chromosome 4:8 translocation-specific primers or chromosome 3 control primers (see Materials and Methods for additional information).

145. The single pair of primers generates a single amplicon because amplification "is achieved only when each member of a primer pair anneals in a stable fashion to its target sequence." Sambrook, "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, third edition (2001), EX2160, at page 8.13. Dr. Quackenbush does not explain how Leary's production of a single amplicon via a single primer pair functioning as a single unit constitutes the "plurality" of capture probes required by limitation 1(e).

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146. Dr. Quackenbush also relies on the "monitoring of plasma samples or other bodily fluids" depicted in Leary Figure 1 for limitation 1(e). EX1020 (Quackenbush Declaration) ¶¶143-44. Leary Figure 1, which is reproduced below with green and red annotations added, shows that multiple mate pair tags are prepared only for the tumor sample to identify "patient-specific rearrangements," which Foresight relies on for the first sample required by claim limitation 1(a). Figure 1 shows that 2 mate pair tags surround the breakpoint. Primer pairs were designed from the sequence of one set of mate pair tags that surrounded the break point Leary Figure 1 shows that only a single amplicon ("personalized biomarker for tumor monitoring") for the "plasma or other bodily fluids" was measured. Leary's Figure 1 depiction of only monitoring a single biomarker (amplicon) is consistent with the actual experiment conducted by Leary that reported just one chromosomal rearrangement in each of the Hx402 and Hx403 patients.

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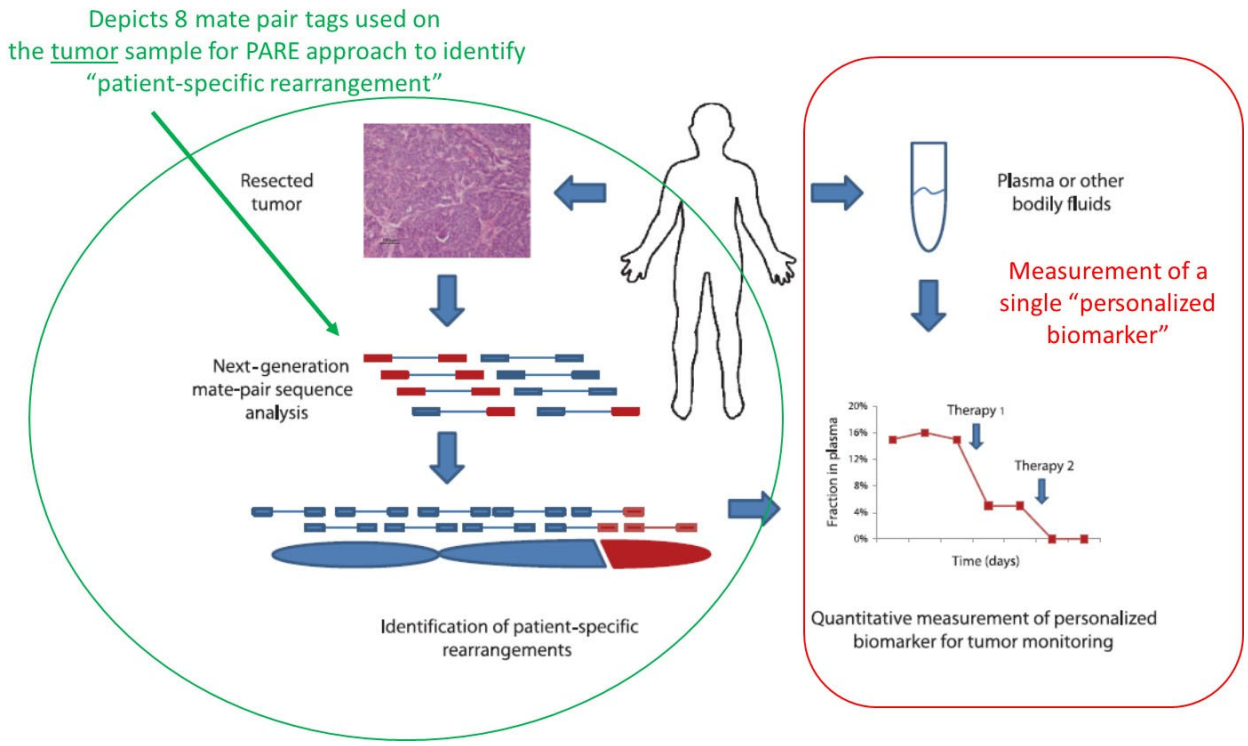

EX1002, Fig. 1 (green and red annotations added). Leary used a single amplicon produced from a single primer pair to measure the personalized biomarker. As a result, Foresight fails to establish that Leary teaches a "plurality" of capture probes that meets limitation 1(e), and Foresight fails to provide any reason that a POSA would have been motivated to modify Leary with a reasonable expectation of success to arrive at limitation 1(e).

e. <u>Leary does not teach or suggest the "third sequencing assay" of limitation 1(f)</u>

147. Foresight and Dr. Quackenbush argue that Leary "discloses, and at a minimum renders obvious, conducting a sequencing assay on PCR products

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generated from each plasma sample" for the purpose of "verify[ing] that the selected PCR primer capture probes selectively bind the tumor sequences they were intended to target." EX1020 (Quackenbush Declaration IPR2023-00224) ¶147. Foresight and Dr. Quackenbush, however, relied on Leary's verification sequencing to supposedly meet limitation 1(d). EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶138-140. Leary does not disclose conducting an additional verification (i.e., a re-verification) sequencing step. Foresight and Dr. Quackenbush do not articulate a reason why a POSA would have been motivated to modify Leary to conduct such an additional verification step, which would appear to be unnecessary because Leary's PARE approach was already verified to be working. The Forshew reference (EX1030) states that Leary's existing workflow was considered to be "difficult to implement on a routine basis" as follows:

> Previously proposed methods for personalized monitoring of tumor dynamics relied on expensive custom-designed probes (3) or identification of rearrangements using whole-genome sequencing (18, 19). These have better analytical sensitivity than currently achieved by TAm-Seq, ***but are difficult to implement on a routine basis***.

EX1030 (Forshew) at page 10 (reference 18 is Leary). Forshew also states that Leary was not "widely adopted" as follows:

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> Various methods have been suggested to monitor changes in mutation load in plasma. These can have enhanced sensitivity compared to TAm-Seq for tracking individual mutations, but require design of personalized assays (3, 18, 19). ***None of these methods have been widely adopted***.

EX1030 (Forshew) at page 8 (reference 18 is Leary).

148. A POSA would not have been motivated to add more sequencing steps to Leary's workflow. Leary's workflow only sequences the tumor and matched normal sample once (although there were seven cases where the amplicons were sequenced for verification, the sequence was not reported). Leary used a PCR generated amplicon for detection, not sequencing. Leary's workflow was already "difficult to implement on a routine basis" and more sequencing steps would be expected to make the workflow even more difficult to implement and lead to even less adoption. Adding sequencing steps to Leary's workflow would add more data analysis, cost, and time. Foresight and Dr. Quackenbush do not adequately explain why a POSA would want to take on these additional burdens to conduct an undisclosed additional verification sequencing step.

f. <u>Lack of motivation to modify Leary</u>

149. As discussed above, a POSA would not have been motivated to redesign Leary's PCR primer-based targeted PARE assay to (1) increase sequencing

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by an order of magnitude to cover all or almost all of the genome, (2) conduct a second sequencing assay on a second tumor sample, (3) change the core reaction principle from PCR primers to capture probes, and (4) include a third sequencing assay. Foresight and Dr. Quackenbush do not provide a convincing motivation for any one of these modifications, much less a motivation to do all of the modifications together.

2. Claims 17 and 18 are nonobvious over Leary

150. Dependent claims 17 and 18 require that the "whole genome sequencing produces at least [750,000,0000 or 1,000,000,000] sequencing reads."

151. Foresight and Dr. Quackenbush argue that a POSA would have been motivated to increase Leary's 18x physical coverage to "30x" sequence coverage ! EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶201-206. It is thus clear, that they are confusing physical coverage with sequence coverage. Leary's sequence coverage was only 0.9x, and could not be simply increased to 30x for reasons discussed above.

152. Also, Foresight and Dr. Quackenbush do not provide an explanation why a POSA would select 30x as opposed to 5x or 100x (or some other multiplier). EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶205.

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3. Claim 19 is nonobvious over Leary

153. Dependent claim 19 requires "repeating said third sequencing assay one or more times" on "a fourth nucleic acid sequence."

154. As discussed above, Foresight and Dr. Quackenbush relied on Leary's verification sequencing (i.e., of the same tumor sample) to supposedly meet limitation 1(d), EX1020 (Quackenbush Declaration IPR2023-00224) ¶147, and also asserted that a POSA would conduct additional ***re***-verification sequencing (i.e., of the plasma) to meet limitation 1(f). EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶138-140; *supra* Section IX.B.1.e. Foresight and Dr. Quackenbush allege that a POSA would conduct another re-verification sequencing (i.e., a ***re-re***-verification sequencing of subsequent plasma samples) to meet dependent claim 19. EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶207-209. A POSA would not have been motivated to complicate Leary's already "difficult to implement" method with even more data analysis, cost, and time to conduct an undisclosed and unnecessary ***re-re***-verification sequencing step, let alone multiple times. *See* EX1030 (Forshew) at page 10 (describing Leary as "difficult to implement on a routine basis").

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C. Ground 2: The claims are nonobvious over Leary and Ley

155. Foresight and Dr. Quackenbush rely on Ley (EX1012) as supposedly rendering obvious limitation 1(c)(ii). EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶210-217.

156. Limitation 1(c)(ii) recites "[ii] wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof."

157. Ley (EX1012) does not address the deficiencies with Leary discussed above regarding the lack of modification necessary to modify Leary's PCR primer-based targeted PARE assay to (1) increase sequencing by an order of magnitude to cover all or almost all of the genome (WGS), (2) conduct a second sequencing assay on a second tumor sample, (3) change the core reaction principle from PCR primers to capture probes, and (4) include a third sequencing assay.

158. A POSA would not be motivated to increase the coverage of Leary since Ley actually confirms that, going forward, WGS (even using radically increased sequence coverage) is not desirable. Ley (EX1012) actually confirms that a POSA would not have been motivated to modify Leary's assay to perform whole genome sequencing because Ley teaches sequencing less than the whole genome is

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preferable because it would be "faster, cheaper, and more efficient" for finding polymorphisms as follows:

> Although we performed whole-genome sequencing on this cancer sample, we restricted our initial validation studies to the 1-2% of the genome that encodes genes. ***This raises the issue of whether sequencing the complementary DNA transcriptome of this tumor would have been a faster, cheaper and more efficient way of finding the mutations***.

EX1012 (Ley) at page 71.

159. Ley (EX1012) states that its method in not "feasible" without performing whole genome sequencing two times (once on the patient's normal genome, and again on the tumor genome) as follows:

> We cannot overemphasize the importance of parallel sequencing of the patient's normal genome to determine which variants were inherited; the identification of the true somatic mutations in this tumour genome would not have been feasible without this approach.

EX1012 (Ley) at page. 71. A POSA would have been dissuaded from selecting a method that performs whole genome sequencing twice for reasons discussed above regarding data burden and expense constraints.

160. Also, both Leary (EX1002) and Ley (EX1012) use PCR primers. For example, Ley states that it performed PCR as follows:

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> For all putative variants, we attempted validation using custom PCR and capillary sequencing on the ABI 3730 platform. All validated somatic mutations were further analysed by Roche/454 sequencing of PCR-generated amplicons made from primary genomic DNA to compare readcounts of wild-type and mutant alleles in the primary tumour, skin and relapse tumour samples.

EX1012 (Ley) at page 71. Ley's PCR primers do not teach or suggest the recited "capture probes" for reasons discussed above. *Supra* Section VII.B, IX.B.1.c.

161. In addition, the facilities available to Ley at The Genome Center at Washington University were not available to the POSA in the 2012-2013 timeframe. The Genome Center at Washington University was one of the most sophisticated sequencing laboratories in the world at that time. The Ley article was published in Nature, one of the premiere journals in the world. Foresight and Dr. Quackenbush do not explain how a person of ***ordinary*** skill in the art would have been able to modify Leary based on Ley in view of the differences in resources that a POSA would have had versus those available to Ley at The Genome Center at Washington University.

162. Further, Dr. Quackenbush does not describe with specificity what his proposed combination of a Leary/Ley assay would look like to result in an assay that meets all of claim limitations 1(b), 1(c), 1(e), and 1(f). Leary (EX1002) discloses a

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PARE approach. EX1002 (Leary) at page 1. Ley (EX1012) discloses a different approach that involves identifying "putative variants" with "validation using custom PCR and capillary sequencing" as well as sequencing of "validated somatic mutations" "by Roche/454 sequencing of PCR-generated amplicons made form primary genomic DNA." EX1012 (Ley) at page 71. These approaches have significantly different workflows, and Dr. Quackenbush has not explained with particularity which steps of Leary's PARE assay would be retained, replaced, or discarded by something from Ley's assay to arrive at a reconfigured assay that meets all limitations of the claim. Further, a POSA would have expected replacing Leary's PCR-based approach with capture probes would cause Leary's assay to undesirably lose specificity and uniformity in view of Mamanova's teaching 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. Using PCR primers and capture probes have significantly different workflows, and switching from PCR to capture probes would entail significantly reworking Leary's assay. Some of the differences in workflows are highlighted in the following table:

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Exemplary PCR workflow	**Exemplary Capture probe workflow**
Design primers, synthesize primers, conduct PCR reaction on, thermocycle, run amplicon on gel	Design capture probe, synthesize biotinylated capture probe, prepare target DNA fragment library, (including discrete steps of fragmentation, fragment end repair, linker ligation reaction), hybridize primer probe to target DNA library, isolate captured fragments via beads, elute from beads and sequence

D. Ground 3: The claims are nonobvious over Leary, Chan, and Liao

163. Ground 3 does not prove obviousness of the claims for several reasons, including that Chan is not prior art to the ′394 patent.

1. Chan is not prior art

164. First, I have been informed that Chan is not prior art to the ′394 patent. I have been informed that the inventors conceived of their invention no later than May 8, 2012 and diligently pursued reducing it to practice by the filing of their provisional application on January 17, 2013. Chan published in October of 2012, which is after the inventors' conception of the claimed invention.

2. Leary, Chan, and Liao do not render the claims obvious

165. The combination of Leary (EX1002), Chan (EX1008), and Liao (EX1009) fail to disclose at least the following limitations of claim 1:

- the "whole genome sequencing" required by limitation 1(b),

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- the "second nucleic acid sample" required by limitation 1(c),
- the "capture probes" required by limitation 1(c),
- the "third sequencing assay" required by limitation 1(f).

As a result, Ground 3 fails to render obvious claim 1.

a. <u>The combination of references do not teach or suggest the whole genome sequencing of limitation 1(b)</u>

166. Leary (EX1002) fails to teach or suggest whole genome sequencing for reasons discussed above. *Supra* Section IX.B.1.a. Foresight and Dr. Quackenbush assert that Chan's "shotgun sequencing of a plasma sample" constitutes the whole genome sequencing required by limitation 1(b). *See* EX1020 (Quackenbush Declaration IPR2023-00224) ¶231. I disagree for reasons discussed below.

167. Forshew (EX1030) explains that the ctDNA circulating in plasma has "an average length of 140 to 170 base pairs (bp) and is present in only a few thousand amplifiable copies per milliliter of blood, of which only a fraction may be diagnostically relevant." EX1030 (Forshew) at page 1. Cell free DNA arises from the digestion of genomic DNA into small fragments by a variety of endo and exonucleases. The DNA fragments that are bound to nucleosomes survive digestion, whereas unbound DNA is almost completely degraded and lost. As a result, the portions of the genome that are not bound to nucleosomes are largely missing from

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the DNA circulating in plasma. Importantly, such digestion into fragments is not random, since nucleosomes occupy specific segments of the genome.

168. Chan's "shotgun sequencing of plasma DNA," EX1008 (Chan) at page 223, does not constitute sequencing all or almost all of the patient's genome because the fragmented DNA circulating in the plasma does not include the entire genome. Chan merely describes its method as performing "cancer genome ***scanning***," EX1008 (Chan) at page 223, but does not characterize its technique as "whole genome sequencing."

169. Liao (EX1009) instructs a POSA that a target enrichment approach is a logical choice to use instead of WGS based on efficiency and expense as follows:

> Rather than nonselectively sequencing the genome, target enrichment provides a logical approach for more efficient and cost-effective massively parallel sequencing because it increases the proportion of informative data from the targeted region(s).

EX1009 (Liao) at page 92. Liao therefore teaches a POSA that whole genome sequencing is undesirable.

170. Based on at least the foregoing, none of Leary, Chan, or Liao teach or suggest the whole genome sequencing required by limitation 1(b).

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b. <u>The combination of references do not teach or suggest limitation 1(c)</u>

171. Foresight and Dr. Quackenbush rely on a statement in Chan (EX1008) that discusses combining "targeted and shotgun approaches" in view of "solution-phase hybridization-based capture approaches hav[ing] already been successfully" used in the prior art to assert that a POSA would have modified Leary's PCR primer approach to arrive at the "capture probes" recited by limitation 1(c). EX1020 (Quackenbush Declaration IPR2023-00224) ¶¶232-241.

172. As discussed above, a POSA was aware that PCR primers and capture probes have a different "core reaction principles," EX1006 (Mertes) at page 375, and "[g]iven the operational characteristics of these different targeted enrichment methods, they naturally vary in their suitability for different fields of application," *id.*, and provide "very divergent results" with "very different average coverage, evenness and specificity," *id.* 383; *supra* Section VII.B. The statements in Chan do not provide a reason why a POSA would have modified Leary's specific PCR-based assay to use capture probes and additional sequencing steps. For example, Dr. Quackenbush did not explain how a POSA would have evaluated any of the seven parameters (and/or metrics) associated with the disadvantages of capture probes before discarding the core reaction principle of Leary's PCR primers and switching

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it for capture probes. Foresight also fails to explain how capture probes could conceivably replace PCR ***to serve as the detection method*** for Leary's assay.

173. The claimed invention recites a specific method that first conducts whole genome sequencing (which neither Leary, Chan, or Liao teach), and then uses capture probes ***after*** whole genome sequencing on a second sample. Chan does not teach using capture probes ***after*** whole genome sequencing, and further does not teach a second sample. Dr. Quackenbush asserts that Chan's supposed use of solution-phase hybridization capture is conducted ***before*** the sequencing—not after like claimed. EX1020 (Quackenbush Declaration IPR2023-00224) ¶238 ("The catch is then pulled down or fished out using beads that bind the biotin that was incorporated into the baits, and the catch can then be PCR amplified ***before*** analysis with Next-Generation Sequencing.").

174. Foresight and Dr. Quackenbush also assert that a POSA would use solution-phase hybridization probes "in addition to the PCR primer capture probes" taught by Leary. EX1020 (Quackenbush Declaration IPR2023-00224) ¶232. However, Mamanova (EX1007) teaches that PCR amplification has a "negative influence" on hybrid capture, that combining them should be "avoided," and if used at all, only "after hybridization" as follows:

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> ***We noted a negative influence of PCR amplification on the uniformity of enrichment in both on-array and in-solution methods***: performing 18 cycles of PCR amplification of libraries both before and after hybridization can introduce severe bias toward neutral G+C content in the resulting sequences (Fig. 5b). ***Avoiding the PCR step altogether before hybridization greatly improved the situation (Fig. 5c), so it is desirable to keep PCR amplification to a minimum and only perform it after hybridization***.

EX1007 (Mamanova) at page 116.

c. <u>The combination of references do not teach or suggest limitation 1(f)</u>

175. Foresight and Dr. Quackenbush rely on Leary (EX1002) for allegedly performing the "third sequencing assay" for limitation 1(f), and further asserts that a POSA would replace Leary's PCR primers with capture probes (and additional sequencing steps) based on Chan (EX1008). EX1020 (Quackenbush Declaration IPR2023-00224) ¶244. As discussed above, a POSA would not have been motivated to modify Leary to include unnecessary re-verification sequencing, *supra* Section IX.B.1, and a POSA would not have been motivated to replace Leary's PCR primers with capture probes based on Chan, *supra* Section IX.D.2.

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176. Based on at least the foregoing, Foresight and Dr. Quackenbush have not proved obviousness of independent claim 1 based on the combination of Leary, Chan, and Liao.

E. Objective Indicia of Nonobviousness

177. As explained above, Foresight and Dr. Quackenbush failed to show that a POSA 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

178. I understand that the evidence of objective indica 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 '394 Patent.

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1. A method for analyzing nucleic acid samples isolated from an individual, comprising:	The Next Personal test performs a method for analyzing samples isolated from an individual. **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 1mm^3 of FFPE tumor tissue. EX2012 (Personalis NeXT Personal Brochure) at page 15.

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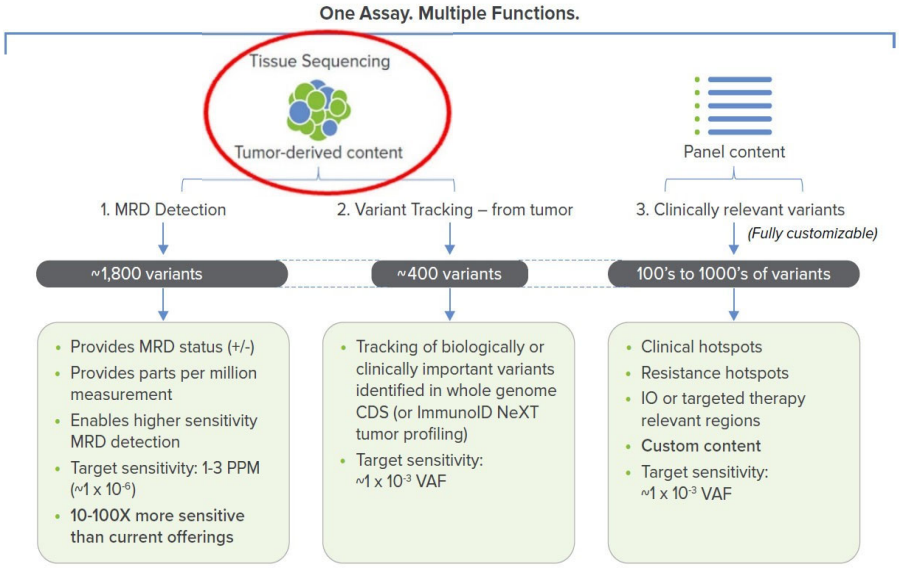
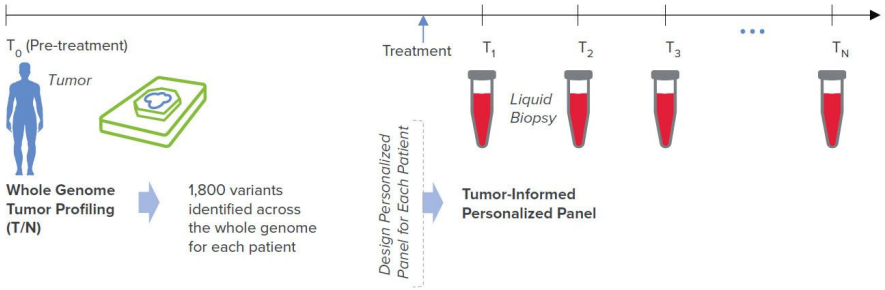
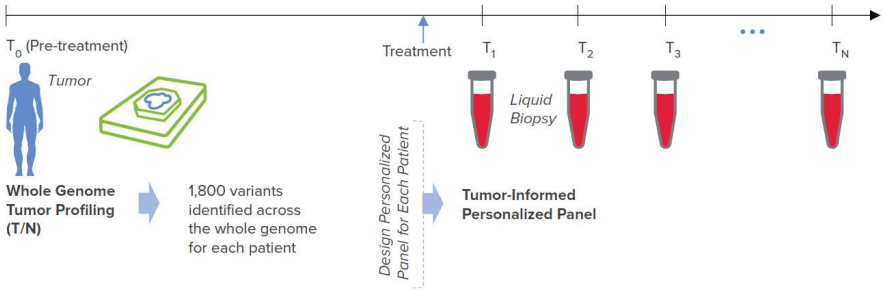
(a) generating a first subset of nucleic acid molecules from a first nucleic acid sample obtained from said individual;	The NeXT Personal test generates a first subset of nucleic acid molecules from a first nucleic acid sample obtained from a subject. The first subset of nucleic acid molecules is obtained from a tumor biopsy obtained from an individual. **Figure 2:** NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD & Variant Tracking  EX2012 (Personalis NeXT Personal Brochure) at page 16, Fig. 2. **Figure 3:** NeXT Personal: Advanced, Higher Sensitivity, Personalized, Tumor-Informed Liquid Biopsy for MRD & Variant Tracking  EX2012 (Personalis NeXT Personal Brochure) at page 17, Fig. 3.

'394 Patent Claim Language	Personalis' NeXT Personal® assay
(b) conducting a first sequencing assay on said first subset of nucleic acid molecules to yield a first result comprising a first nucleic acid sequence wherein said first sequencing assay comprises whole genome sequencing;	The NeXT Personal test conducts a whole genome sequencing assay on the first subset of 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  EX2012 (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 EX2012 (Personalis NeXT Personal Brochure) at page 17.

(c) generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by Contacting said second nucleic acid sample with a plurality of capture probes, wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof, wherein said first sample or said second sample is obtained from said subject at a first time point;	The method of conducting the NeXT Personal test generates a second subset of nucleic acid molecules from a second nucleic acid sample from an individual by contacting nucleic acids isolated from a blood, plasma, cfDNA, or tumor sample from an individual with a personalized capture probe set that is designed to hybridize to one or more polymorphisms, observed in either the blood, plasma, plasma, cfDNA or tumor biopsy sample obtained from the patient at a first time point. 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. EX2012 at page 29, ¶6.

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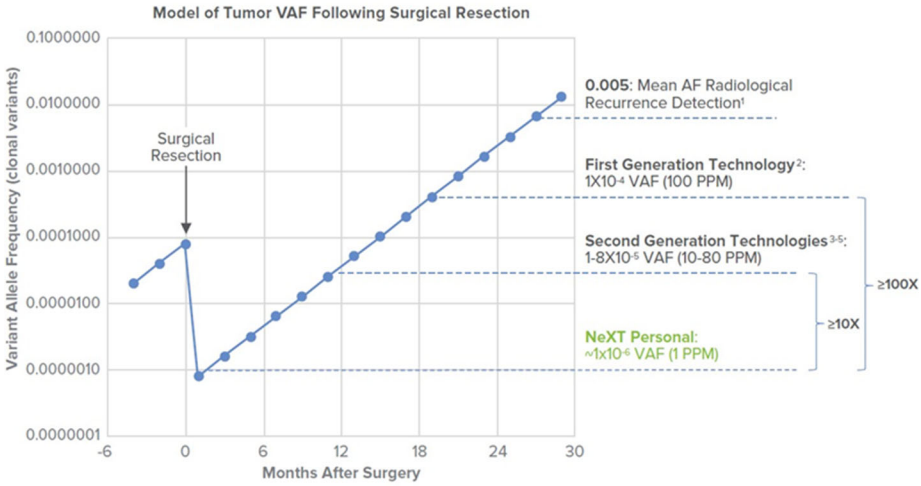
(d) conducting a second sequencing assay on said second subset of nucleic acid molecules to yield a second result comprising a second nucleic acid sequence;	The NeXT Personal test conducts a second sequencing assay on the second subset of nucleic acids generated from the plasma DNA sample. **Delivering 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 1mm^3 of FFPE tumor tissue. EX2012 (Personalis NeXT Personal Brochure) at page 15. **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 study[6]; Residual tumor from surgery: 1%; Volume doubling time (actual VAF time): 2 months EX2012 (Personalis NeXT Personal Brochure) at page 15, Fig. 1

'394 Patent Claim Language	Personalis' NeXT Personal® assay
(e) using said plurality of capture probes to generate an additional subset of nucleic acid molecules from an additional nucleic acid sample isolated from an additional sample obtained from said subject at an additional time point different from said first time point;	The personalized probe set used in the NeXT Personal test is used to generate an additional subset of nucleic acid molecules from an additional sample taken from an additional time point different from the first time point. First, the NeXT Personal test involves the use of capture probes.

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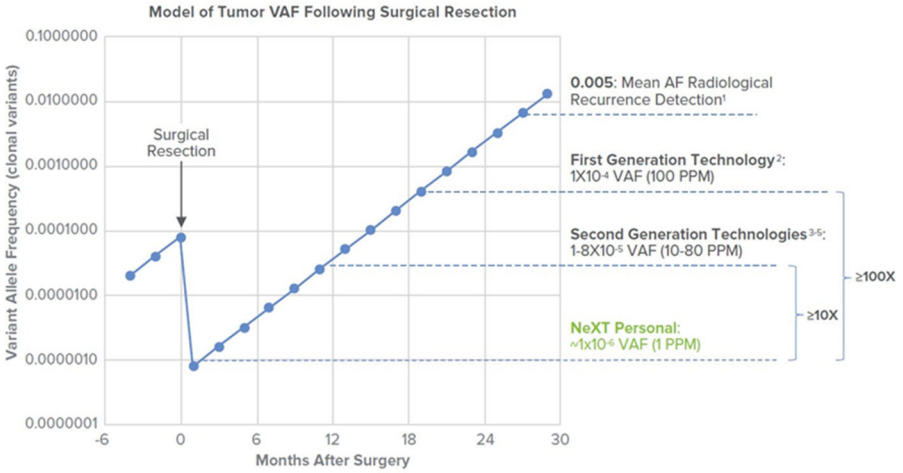
6. As background, both Foresight and Personalis offer testing products that can be used to monitor for cancer recurrence by detecting and analyzing tiny amounts of tumor DNA that are present in the bloodstream of a cancer patient. It is important to understand that both Foresight and Personalis offer 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.

EX2012 at page 29, ¶6

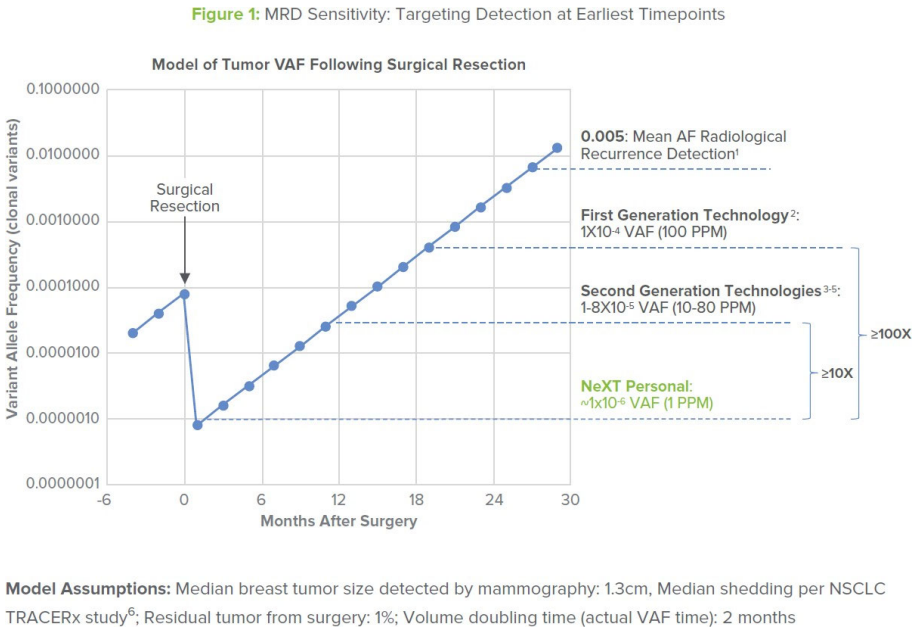
Additionally, Fig. 1 shows that tumor variant allele frequencies can be tracked at multiple time points before and after surgery.

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 study[6]; Residual tumor from surgery: 1%; Volume doubling time (actual VAF time): 2 months

EX2012 (Personalis NeXT Personal Brochure) at page 15, Fig. 1

'394 Patent Claim Language	Personalis' NeXT Personal® assay
(f) conducting a third sequencing assay on said additional subset of nucleic acid molecules from said additional nucleic acid sample to yield a third result comprising a third nucleic acid sequence; and	The NeXT Personal test conducts a third sequencing assay on the additional nucleic acid molecules from the additional sample to yield an additional result. For example, Fig, 1 teaches that the additional samples are sequenced and the nucleic acids identified.  EX2012 (Personalis NeXT Personal Brochure) at page 15, Fig. 1

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(g) generating a biomedical report that includes biomedical information of said subject, which biomedical information identifies the presence or absence of said one or more polymorphisms identified by said second result or said third result.	The NeXT Personal test generates a biomedical report that includes biomedical information, including whether the polymorphisms identified in the prior sequencing of the plasma samples is or is not present. 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. EX2012 at page 15, Fig. 1.

2. Industry Praise

179. 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 '394 Patent earned the #1 ranking. *See* EX2001 at 1-2.

180. The differences between the method used in Leary (EX1002) and the method claimed in the '394 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%). EX2012 at page 15. By comparison, the best sensitivity from plasma

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DNA in the Leary reference was about 0.3%, which is several hundred times less sensitive than the claimed method. EX1002 (Leary) at page 4 ("However, the fraction of mutant tumor DNA did not reach zero (remaining at 0.3% at day 137), consistent with the fact that this patient had residual metastatic lesions in the remaining left lobe of the liver"). This is shown below by the right most end of the graph in Fig. 4B of Leary:

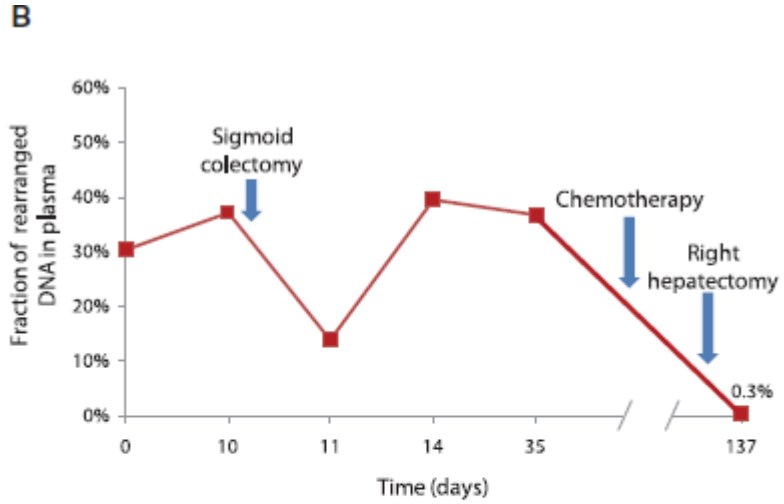

Fig. 4. Detection of tumor-specific rearrangements in plasma of cancer patients. (**A**) The identified chromosome 4:8 and 16 rearrangements were used to design PCR primers spanning breakpoints and to amplify rearranged DNA from tumor tissue and plasma from patients Hx402 and Hx403, respectively. A plasma sample from an unrelated healthy individual was used as a control for both rearrangements. (**B**) Plasma samples from patient Hx402 were analyzed at different time points using digital PCR to determine the fraction of genomic equivalents of plasma DNA containing the chromosome 4:8 rearrangement. The fraction of rearranged DNA at day 137 was 0.3%, consistent with residual metastatic lesions present in the remaining lobe of the liver.

EX1002 (Leary) at Fig. 4.

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3. Skepticism of Experts

181. 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 capture probes. 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 capture probes Forshew (EX1030) described as "difficult to implement on a routine basis." EX1030 (Forshew) at page 10.

182. Other references outline industry-wide problems with next-generation sequencing (NGS) at the time, which were unexpectedly solved by the methods in the ′394 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.

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183. This objective real-world evidence supports a finding that the claims of the ′394 patent are nonobvious.

X. FORESIGHT HAS NOT PROVEN UNPATENTABILITY OF ANY CLAIM FROM THE ′033 PATENT

184. This section of my declaration provides an overview of the ′033 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 ′033 patent.

A. Overview of the ′033 Patent

185. The ′033 patent relates to systems and methods for sample processing and analysis to aid in the diagnosis, monitoring, treatment, and prevention of diseases. For example, the abstract of the ′033 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. … Methods and systems of the disclosure can aid in the diagnosis, monitoring, treatment, and prevention of one or more diseases and conditions.

EX1001 (the ′033 patent) at Abstract.

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

> 1. A method for analyzing a nucleic acid sample obtained from an individual, comprising:

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(a) producing, with the aid of a computer processor, a plurality of capture probes, wherein said plurality of capture probes hybridize to a plurality of polymorphisms, wherein said plurality of polymorphisms are in sequences encoding genes with known biomedically interpretable variants, and wherein said plurality of polymorphisms are based on or extracted from one or more databases of polymorphisms and are observed in a population of one or more samples;

(b) contacting said nucleic acid sample with said plurality of capture probes produced in (a);

(c) conducting a sequencing assay on a subset of nucleic acid molecules generated from said nucleic acid sample in step (b) to yield a result comprising a nucleic acid sequence, thereby analyzing said nucleic acid sample; and

(d) repeating steps (b)-(c) on a subsequently obtained biological sample from said individual.

187. The specification of the ′033 Patent explains that prior art methods such as whole genome sequencing were not only costly, but could "fail to capture many biomedically important variants." EX1001 (the ′033 patent) at 1:23-34. The specification also states that standard methods often "fail[ed] to target biomedically interesting non-exomic and exomic regions," and would perform poorly in certain regions of the genome. EX1001 (the ′033 patent) at 1:23-34. The specification contains similar disclosures elsewhere in the patent. *See, e.g.,* EX1001 (the ′033

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patent) at 69:49-70:20. In short, the problem was, that if a POSA used the conventional methods, many DNA fragments would either be missing from the analysis or would be hard to sequence.

188. The inventors of the ′033 Patent devised novel bespoke approaches to the processing of DNA fragments together with a subsequent targeted sequencing assay according to the steps recited in the claims. The inventors of the ′033 Patent developed an enrichment pulldown process and sequencing protocols to specifically improve performance over the prior art. *See, e.g.,* Claim 1 of EX1001 (the ′033 patent).

B. Ground 1: Forshew (EX1030) does not anticipate the claims

189. Forshew (EX1030) does not anticipate independent claim 1 at least because (1) Forshew is not prior art to the ′033 patent, and (2) Forshew fails to teach the "capture probes" recited by limitations 1(a), 1(b), 1(c), and 1(d).

1. Forshew is not prior art

190. I have been informed that Forshew (EX1030) is not prior art to the ′033 patent. I have been informed that the inventors conceived of their invention no later than May 8, 2012 and diligently pursued reducing it to practice by the filing of their provisional application on January 17, 2013. Forshew published on May 30, 2012, which is after the inventors' conception of the claimed invention.

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2. Forshew does not teach the "capture probes" recited by claim 1

191. Forshew (EX1030) fails to anticipate claim 1 because it does not teach the "plurality of capture probes" recited by limitations 1(a), 1(b), 1(c), and 1(d). I understand that because Forshew does not anticipate independent claim 1, it also does not anticipate any of the dependent claims.

a. Forshew does not teach the "capture probes" of limitation 1(a)

192. Limitation 1(a) recites "producing, with the aid of a computer processor, ***a plurality of capture probes***, wherein said plurality of capture probes hybridize to a plurality of polymorphisms, wherein said plurality of polymorphisms are in sequences encoding genes with known biomedically interpretable variants, and wherein said plurality of polymorphisms are based on or extracted from one or more databases of polymorphisms and are observed in a population of one or more samples."

193. Foresight and Dr. Quackenbush's theory that Forshew (EX1030) anticipates the claims of the ′033 patent depends upon a POSA equating Forshew's PCR primers with the claimed "capture probes." *See* EX1020 (Quackenbush Declaration IPR2023-00317) ¶¶119-123. For example, Dr. Quackenbush states that "Forshew discloses the design and use of 'capture probes' in the form of PCR

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primers that hybridize to and amplify (and thus 'capture') a plurality of polymorphisms in sequences encoding genes with known biomedically interpretable variants." *Id.* ¶121.

194. Under the appropriate construction of "capture probes" discussed above, however, a POSA would not have considered Forshew's PCR primers to be "capture probes" because Forshew's PCR primers do not hybridize to target DNA so that one can physically capture the target DNA. The exhibits filed by Foresight and Dr. Quackenbush repeatedly distinguish capture probes and PCR primers as being two different techniques that differ according to their very different core reaction principles. *Supra* Section VII.B. A POSA would not view Forshew's PCR primers as teaching the claimed capture probes and, accordingly, Forshew does not teach the "capture probes" required by limitation 1(a).

b. <u>Forshew does not teach the "plurality of capture probes" of limitation 1(b)</u>

195. Limitation 1(b) recites "contacting said nucleic acid sample ***with said plurality of capture probes*** produced in (a)."

196. As discussed above, Forshew (EX1030) discloses PCR primers, and a POSA would not have confused Forshew's PCR primers with the claimed capture

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probes. Forshew fails to anticipate limitation 1(b) for at least this reason. *Supra* Section X.B.2.a.

c. <u>Forshew does not teach limitation 1(c)</u>

197. Limitation 1(c) recites "conducting a sequencing assay on a subset of nucleic acid molecules ***generated from said nucleic acid sample in step (b)*** to yield a result comprising a nucleic acid sequence, thereby analyzing said nucleic acid sample."

198. Forshew (EX1030) does not anticipate limitation 1(c) because its sequencing assay is conducted on molecules that result from PCR amplification, rather than on molecules captured by contacting capture probes with the nucleic acid sample. *Supra* Section X.B.2.a. Forshew fails to anticipate limitation 1(c) for at least this reason.

d. <u>Forshew does not teach limitation 1(d)</u>

199. Limitation 1(d) recites "repeating steps (b)-(c) on a subsequently obtained biological sample from said individual."

200. Forshew (EX1030) does not teach this limitation because Forshew does not teach the "capture probes" required by limitations 1(b) and 1(c), *see supra* Section X.B.2.a-c, much less repeat them. Forshew fails to anticipate limitation 1(d) for at least this reason.

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C. Ground 2: The claims are nonobvious over Forshew and Wagle

201. The combination of Forshew (EX1030) and Wagle (EX1033) does not render obvious independent claim 1 for several reasons.

202. First, Forshew (EX1030) is not prior art to the ′033 patent. Second, a POSA would not have been motivated to use Wagle's capture probes in place of Foreshew's PCR-based amplification assay at least because Forshew's assay is designed to operate on minuscule amounts of DNA, and Foresight's exhibits repeatedly explain that hybridization capture is not desirable for assays that use such small amounts of DNA. Third, Foresight provides no credible explanation why a POSA would have been motivated to discard the core reaction principle of Forshew's already working PCR-based assay and replace it with Wagle's capture probes in view of numerous parameters and metrics associated with the known risks and disadvantages of capture probes that are repeatedly described throughout Foresight's exhibits. Fourth, a POSA would not have been motivated to add Wagle's capture probes to Forshew's PCR-based assay in view of Foresight's own exhibits that counsel against combining the different core reaction principles of PCR primers and capture probes. I discuss each of these four reasons in more detail below.

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1. Forshew is not prior art

203. As discussed above, I have been informed that Forshew (EX1030) is not prior art to the ′033 patent because the inventors conceived of their invention no later than May 8, 2012 and diligently pursued reducing it to practice by the filing of their provisional application on January 17, 2013. Forshew published on May 30, 2012, which is after the inventors' conception of the claimed invention. I understand that Forshew therefore does not qualify as prior art to the ′033 patent.

2. No motivation due to Forshew's miniscule amounts of DNA

204. Foresight and Dr. Quackenbush assert that a POSA would have been motivated and "reasonably expected to succeed in using Wagle's RNA bait-based probes in place of Forshew's PCR primer capture probes in the capture step." *See* EX1020 (Quackenbush Declaration IPR2023-00317) ¶183. This assertion, however, only serves to highlight why a POSA would not conflate PCR primers and capture probes, and also why a POSA would not have been motivated to replace Forshew's PCR primers with Wagle's capture probes for reasons discussed below.

205. Forshew's TAm-Seq PCR-based assay is designed to operate on "minute" amounts of DNA. EX1030 (Forshew) at page 8 ("Thus, we screened 62 samples across sizeable genomic stretches, ***using minute amounts of plasma DNA (median, 4 ng)***, and obtained 97.5% sensitivity with PPV of 100% for identifying

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mutations at AF >2% in plasma by TAm-Seq."); *id.* at page 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***."). The minute amounts of DNA range from 0.9-19.7 nanograms of DNA per amplification reaction, as shown, for example, in Table 2 of Forshew reproduced below:

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Table 2. Mutations identified by TAm-Seq in a set of 62 plasma samples from ovarian cancer patients. Forty mutations were identified by TAm-Seq using stringent parameters for mutation calling. Plasma samples described in this table are distinct from those in Table 1, but patients included overlap. Additional data on patients and mutations are provided in table S6.

Sample number	Plasma volume per amplification reaction (μl)	DNA amount per amplification reaction (ng)	Gene	Protein change	Mean depth (sequencing reads)	Mean AF using TAm-Seq	Mean AF using digital PCR
1	70	0.9	*TP53*	p.R273C	640	0.260	0.167
2	160	4.2	*TP53*	p.R248Q	340	0.244	0.150
3	160	5.7	*TP53*	p.R248Q	640	0.507	0.410
4	120	9.9	*TP53*	p.R213X	810	0.059	0.035
5	120	1.4	*TP53*	p.C141Y	680	0.021	0.013
6	120	2.1	*TP53*	p.C141Y	720	0.044	0.038
7	190	17.9	*TP53*	p.I195N	800	0.091	0.081
8	160	14.8	*TP53*	p.R175H	510	0.608	0.627
9	160	10.7	*TP53*	p.R175H	550	0.526	0.604
10	160	6.1	*TP53*	p.R175H	530	0.651	0.682
11	160	4.9	*TP53*	p.R175H	490	0.526	0.581
13	160	2.8	*TP53*	p.C135R	480	0.039	0.045
14	160	2.5	*TP53*	p.C135R	610	0.046	0.120
15	160	3.0	*TP53*	p.C135R	470	0.091	0.068
16†	130	3.7	*TP53*	p.R196P	1070	0.088	0.135
			EGFR	p.R832H	614	0.048	0.050
17	160	4.2	*TP53*	p.C176S	580	0.113	0.432
18	160	4.4	*TP53*	p.C176S	620	0.029	0.108
20	140	5.2	*TP53*	p.R175H	650	0.201	0.226
21	140	3.6	*TP53*	p.R175H	650	0.085	0.074
22	140	4.1	*TP53*	p.R175H	630	0.081	0.125
23	140	3.7	*TP53*	p.R175H	710	0.074	0.106
24	140	7.1	*TP53*	p.R175H	760	0.269	0.286
25	130	3.9	*TP53*	p.R273H	750	0.094	0.099
26	160	5.7	*TP53*	p.R282W	640	0.048	0.061
27	150	3.6	*TP53*	p.C141Y	480	0.321	0.364
29	150	9.5	*TP53*	p.E258K	190	0.548	0.253
31	160	3.6	*TP53*	p.C135Y	620	0.040	0.034
32	140	2.4	*TP53*	p.E56X	1480	0.137	0.122
33	160	13.2	*TP53*	p.K132N	740	0.216	0.206
34	60	5.3	*TP53*	p.K132N	570	0.151	0.201
36	160	5.8	*TP53*	p.K132N	620	0.191	0.275
37	160	9.4	*TP53*	p.K132N	530	0.287	0.362
38	160	10.1	*TP53*	p.K132N	590	0.275	0.331
39	160	16.4	*TP53*	p.K132N	700	0.315	0.323
40	160	19.7	*TP53*	p.K132N	830	0.435	0.482
41	160	15.0	*TP53*	p.K132N	730	0.452	0.445
42	160	8.5	*TP53*	p.K132N	560	0.185	0.245
43	150	3.6	*TP53*	Splicing	680	0.143	0.121
44‡	170	5.2	*TP53*	p.C238R	1543	0.071	0.073

†Both a *TP53* and an *EGFR* mutation were identified in this sample, collected from patient 27 (Table 1), 25 months after initial surgery (Fig. 4A). ‡The amplicon containing the mutation failed amplification in this sample in the initial experiment and was identified successfully in repeat analysis.

EX1030 (Forshew) at page 6, Table 2 (red box added).

206. Forshew explains that its assay is designed to operate on the amount of DNA present in just microliters of blood plasma as follows:

- "Using the equivalent amount of DNA present ***in 30 to 120 µl of plasma***, we performed duplicate preamplification reactions for each sample." EX1030 (Forshew) at page 4;
- "DNA extracted from 62 additional plasma samples collected at different time points from 37 patients with advanced HGSOC was amplified in duplicate (table S6), ***using DNA present in ~0.15 ml of plasma per reaction*** (range, 0.06 to 0.2 ml)." EX1030 (Forshew) at page 4. I note that 0.15 milliliters of plasma is 150 µl of plasma.

Table 1 of Forshew uses between 30 to 120 µl of plasma per amplification reaction, which is reproduced below:

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Table 1. Mutations identified by TAm-Seq in plasma samples from seven ovarian cancer patients. TAm-Seq was used to sequence DNA extracted from plasma of subjects with HGSOC (stage III/IV at diagnosis). Plasma was collected when patients presented with relapse disease, before initiation of chemotherapy. For patient 46, DNA from a formalin-fixed, paraffin-embedded (FFPE) sample was not included in the TAm-Seq set and the mutation was validated in FFPE by Sanger sequencing. CA125 was measured at time of plasma collection. Mean depth of coverage at the mutation locus in the TAm-Seq data was averaged over the repeats (RMS deviation = 850). AF, allele frequency; N, no; Y, yes.

Patient ID	Age at diagnosis	Time elapsed since surgery (months); number of previous lines of chemotherapy	CA125 (U/ml)	Plasma per amplification reaction (µl)	Gene	Mutation and base change (genome build hg19)		Protein change	Detected in FFPE	Mean depth (sequencing reads)	Mean AF using TAm-Seq	Mean AF using digital PCR
8	60	13; 1	2122	50	*TP53*	17:7577120	C>T	p.R273H	Y	5000	0.09	0.10
12	62	27; 3	365	50	*TP53*	17:7577579	G>T	p.Y234*	Y	5000	0.10	0.08
14	58	50; 3	260	120	*TP53*	17:7578212	G>A	p.R213*	Y	5800	0.15	0.12
25	61	9; 1	944	110	*TP53*	17:7578404	A>T	p.C176S	Y	4800	0.04	0.08
27†	68	15; 1	1051	90	*TP53*	17:7578262	C>G	p.R196P	Y	7700	0.06	0.14
					EGFR	7:55259437	G>A	p.R832H	N	5700	0.06	0.05
31	64	12; 1	313	30	*TP53*	17:7578406	C>T	p.R175H	Y	4500	0.44	0.56
46	56	30; 2	1509	30	*TP53*	17:7578406	C>T	p.R175H	Y	4200	0.23	0.30

*Indicates stop codon. †Both a *TP53* and an *EGFR* mutation were identified in this sample (Fig. 4A).

EX1030 (Forshew) at page 5, Table 1 (red box added). Table 2, which is reproduced above, uses between 70 to 170 µl of plasma volume per amplification reaction. *See* EX1030 (Forshew) at page 6, Table 2.

207. Forshew explains that the DNA circulating in plasma exists in a fragmented state with "an average length of 140 to 170 base pairs (bp) and is present in only a few thousand amplifiable copies per milliliter of blood, of which only a fraction may be diagnostically relevant." EX1030 (Forshew) at page 1. Forshew teaches that PCR is required to "amplify" the small amount of circulating DNA to generate enough DNA to use in its TAm-Seq assay. EX1030 (Forshew) at page 2; *id.* at page 9 ("Our TAm-Seq method uses a combination of short amplicons, two-step amplification, sample barcodes, and high-throughput PCR. Because the

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amplicons are short, this method effectively amplifies even small amounts of fragmented DNA such as are present in circulating DNA."). The amount of DNA used in Forshew's TAm-Seq assay is so small that Forshew uses ***three rounds*** of PCR amplification to generate sufficient amounts of DNA to use in its assay. The first round consists of 15 cycles of "preamplification" PCR, which is followed by a second round of 35 cycles of "single-plex PCR," and a third round of 15 cycles of "barcoding PCR." EX1030 (Forshew) at pages 2-3 and Fig. 1(B); *see also* EX1032 (Forshew Supplemental Material) at pages 2-3.

208. A POSA would not have been motivated to replace Forshew's PCR-based amplification with capture probes with a reasonable expectation of success because capture probes do not amplify DNA. Capture probes only capture the amount of DNA that is already present in a sample. Because capture probes do not amplify DNA, the maximum amount that they can capture is simply the amount of DNA that is already present in the microliters of plasma sample. Foresight's Mamanova exhibit teaches that approximately 3 micrograms of capture probe DNA are required for efficient operation . EX1007 (Mamanova) at page 114, Table 1. Similarly, Wagle teaches that its capture probe assay requires "3,600 nanograms" of DNA (i.e., 3.6 micrograms) as follows:

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> DNA libraries were pooled by mixing 300 ng of each library in a single 1.5-mL polypropylene sample tube, lyophilizing by the use of a speedvac evaporator, and resuspending in 4 µL of nuclease-free water. ***This entire amount (3,600 ng DNA in 4 µL) was used for hybrid selection***. ... The final volume of the hybridization reaction was 19 µL, consisting of the following components: ***4 µL of pooled DNA libraries***, 2.5 µL of 1.0 mg/mL human Cot-1 DNA, 2.5 µL of 10.0 mg/mL salmon sperm DNA, 1.5 µL of 200 µM blocking oligo 1.0, 1.5 µL of total of the twelve 200 µM blocking oligonucleotides, 5.0 µL of 12.5 ng/µL biotinylated oligonucleotide baits, 1.0 µL of 20 U/µL Superase-In RNAse inhibitor, and 1 µL of nuclease-free water.

EX1033 (Wagle) at page 91. The 3.6 ***micro***gram amounts of DNA required for Wagle's capture probes is at least 100 times more—and in many cases over 3,000 times more—than the 0.9-19.7 ***nano***gram amounts of DNA present in Forshew's plasma samples.

209. Forshew itself criticizes Wagle's capture probes for having "not been applied to analysis of circulating tumor nucleic acids" precisely because capture probes require more DNA than is present in circulating plasma as follows:

> 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%

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> AF, ***but has not been applied for analysis of circulating tumor nucleic acids***.

EX1030 (Forshew) at page 9. Reference 35 in Forshew is Wagle. *Id.* at page 11.

210. Foresight and Dr. Quackenbush do not provide any reason why a POSA would have been motivated to use Wagle's capture probes in Forshew's assay in view of the vast discrepancy between the microgram amounts of DNA required to use Wagle's capture probes and the nanogram amounts of DNA in Forshew's plasma samples. Also, Foresight and Dr. Quackenbush do not provide any reason why a POSA would have reasonably expected to succeed in using Wagle's capture probes in Forshew's assay in view of the vastly different amounts of DNA required by the two assays.

3. No motivation to change the core reaction principle of Forshew

211. Foresight and Dr. Quackenbush do not provide a convincing reason why a POSA would have been motivated to discard Forshew's PCR primer-based assay to instead use Wagle's capture probes. As discussed above, 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 POSA would have needed to

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evaluate seven parameters associated with the disadvantages of capture probes before replacing the core reaction principle of Forshew's PCR primer-based assay with capture probes.

212. 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 POSA would have needed to evaluate seven metrics associated with the disadvantages of capture probes before discarding the already-working core reaction principle of Forshew's PCR primers for Wagle's capture probes. Dr. Quackenbush did not explain how a POSA would have evaluated any of the seven parameters (and/or metrics) associated with the disadvantages of capture probes before discarding the core reaction principle of Forshew's PCR primers and switching it for capture probes. A POSA would not have been motivated to make such a drastic change to Forshew's the core reaction principle.

213. For example, Mamanova teaches seven parameters that need evaluation:

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> (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.

EX1007 (Mamanova) at page 111. The vast majority of these parameters would have counseled a POSA to avoid attempting to redesign Forshew'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." *Id.* 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

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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.

214. In addition, the facilities available to Forshew at The Cancer Research UK Cambridge Research Institute were not available to the POSA in the 2012-2013 timeframe. The Cancer Research UK Cambridge Research Institute was one of the most sophisticated sequencing laboratories in the world at that time. Foresight and Dr. Quackenbush do not explain how a person of ***ordinary*** skill in the art would have been able to modify Forshew in view of the differences in resources that a POSA would have had versus those available to Forshew at The Cancer Research UK Cambridge Research Institute.

4. <u>No motivation to add capture probes to Forshew</u>

215. Foresight and Dr. Quackenbush assert that a POSA would have been motivated and "reasonably expected to succeed in using Wagle's hybridization-based probes ***in addition to*** Forshew's PCR-based capture step." *See* EX1020 (Quackenbush Declaration IPR2023-00317) ¶184. This assertion does not demonstrate obviousness for several reasons discussed below.

216. First, Dr. Quackenbush does not articulate what this proposed combination of Forshew and Wagle would look like. Instead, his declaration

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includes the following nondescript statement that "a POSA could have been motivated to use hybridization followed by PCR, or vice-versa, in certain applications, for example, in some scenarios in order to further enrich a target sequence to further improve and refine results as compared to performing either step alone." *See* EX1020 (Quackenbush Declaration IPR2023-00317) ¶184. It is unclear in what manner Dr. Quackenbush is proposing that Forshew's workflow would be modified to include both PCR-based amplification and hybridization-based capture with capture probes. I note that Forshew's TAm-Seq assay requires "[a]n initial preamplification step" for 15 cycles "using a pool of the target-specific primer pairs to preserve representation of all alleles in the template material," followed by a "single-plex PCR" where "[r]egions of interest in the preamplified material were then selectively amplified in individual (single-plex) PCR, thus excluding nonspecific products," followed by "sequencing adaptors and sample-specific barcodes [being] attached to the harvested amplicons in a further PCR," *see* EX1030 (Forshew) at page 2, Fig. 1(B), followed by performing "100-base single-end sequencing (GAIIx sequencer, Illumina), with an additional 10 cycles using the barcode sequencing primer," EX1030 (Forshew) at page 3. Dr. Quackenbush does not explain which step within Forshew's workflow he believes would benefit from the addition of Wagle's capture probes. His declaration also does not explain which

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steps in Forshew's workflow would need to be removed, rearranged, or further supplemented to accommodate such a combination of two different core reaction principles with a reasonable expectation of success.

217. Second, Forshew criticizes capture probes as being "expensive" and "difficult to implement on a routine basis" as follows:

> ***Previously proposed methods for personalized monitoring of tumor dynamics relied on expensive custom-designed probes*** (3) or identification of rearrangements using whole-genome sequencing (18, 19). These have better analytical sensitivity than currently achieved by TAm-Seq, ***but are difficult to implement on a routine basis***.

EX1030 (Forshew) at page 10. A POSA would not have been motivated to further complicate Foreshew's already-working and already-complicated process with unnecessary, "expensive," and "difficult to implement" steps.

218. Third, Mamanova (EX1007) teaches that PCR amplification has a "negative influence" on hybrid capture, that combining them should be "avoided," and if used at all, only "after hybridization" as follows:

> ***We noted a negative influence of PCR amplification on the uniformity of enrichment in both on-array and in-solution methods***: performing 18 cycles of PCR amplification of libraries both before and after hybridization can introduce severe bias toward neutral G+C content in the resulting sequences (Fig. 5b). ***Avoiding the PCR step***

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> ***altogether before hybridization greatly improved the situation (Fig. 5c), so it is desirable to keep PCR amplification to a minimum and only perform it after hybridization***.

EX1007 (Mamanova) at page 116. Dr. Quackenbush did not explain how a POSA would overcome the incompatibilities of PCR primers and capture probes described by Mamanova when implementing his proposed combination of Forshew and Wagle.

D. Ground 3: The claims are nonobvious over Wagle and Chan

219. The combination of Wagle (EX1033) and Chan (EX1008) does not render obvious independent claim 1 for at least two reasons. First, Chan is not prior art to the ′033 patent. Second, Wagle in view of Chan does not teach or suggest limitation 1(d).

1. Chan is not prior art

220. I have been informed that Chan (EX1008) is not prior art to the ′033 patent. I have been informed that the inventors conceived of their invention no later than May 8, 2012 and diligently pursued reducing it to practice by the filing of their provisional application on January 17, 2013. Chan published in October of 2012, which is after the inventors' conception of the claimed invention.

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2. Wagle and Chan do not teach or suggest limitation 1(d)

221. Limitation 1(d) recites "repeating steps (b)-(c) on a subsequently obtained biological sample from said individual."

222. Wagle (EX1033) in view of Chan (EX1008) does not teach or suggest limitation 1(d). Wagle discloses a "massively parallel sequencing approach to detect tumor genomic alteration in formalin-fixed, paraffin-embedded (FFPE) tumor samples." EX1033 (Wagle) at page 83. Wagle's approach uses "a solution-based exon capture/massively parallel sequencing approach in which a pool of long oligonucleotides complimentary to these exons of interest were used to reduce the complexity of tumor genomic DNA for clinically oriented sequencing." EX1033 (Wagle) at page 84. Dr. Quackenbush states that "Wagle did not repeat steps (b)-(c) because of the sample type and also because it was an interrogation of tumor tissue to personalize cancer treatment (as opposed to tumor monitoring over time) and did not require repeating." EX1020 (Quackenbush Declaration IPR2023-00317) ¶218. I agree that Wagle does not disclose repeating steps (b)-(c) of the claimed invention.

223. Foresight and Dr. Quackenbush assert that a POSA would have been motivated to repeat Wagle's solution-based exon capture and massively parallel sequencing approach on (i) "subsequently obtained tumor samples," or (ii) "plasma samples that included cell-free tumor DNA." EX1020 (Quackenbush Declaration

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IPR2023-00317) ¶219. Foresight and Dr. Quackenbush have not established that a POSA would have repeated Wagle's approach on either of these two sample types.

224. First, a POSA would not have been motivated to repeat Wagle's assay on a subsequently obtained tumor sample from the same patient because obtaining a second tumor sample from Wagle's patient would require an undesirable invasive procedure to be performed on the patient. For example, Forshew explains that "[b]iopsies are invasive and costly." EX1030 (Forshew) at page 1. As another example, Newman (EX2152) is an article that was co-authored by two founders of Foresight (Ash Alizadeh and Maximilian Diehn). Newman was cited by Foresight as Exhibit 1044 in the two co-pending matters of IPR2023-00545 and IPR2023-00546 that challenge two more of Personalis' patents. Newman states that tumors "cannot be repeatedly sampled without invasive procedures" as follows:

> Noninvasive access to malignant DNA is particularly attractive for solid tumors, which cannot be repeatedly sampled without invasive procedures.

EX2152 (Newman) at page 2. I note that Chan does not teach or suggest obtaining a second tumor sample from a patient; rather, Chan discusses plasma samples that include cell-free tumor DNA. *See* EX1008 (Chan) at 211 ("In this study, we

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explored the use of shotgun massively parallel sequencing (MPS) of plasma DNA from cancer patients to scan a cancer genome noninvasively.”).

225. Wagle also explains that cost remained “a limiting barrier” of his current approach and that his approach had a long turnaround time of 2 weeks as follows:

> ***Toward this end, the potential turnaround time for the current approach is ~2 weeks***. Emerging sequencing instruments promise vast reductions in turnaround time. Cost, a significant consideration in clinical sequencing, can also be reduced dramatically by sample pooling. Indeed, it is likely that a combination of multiplexing together with falling sequencing costs ***may ultimately eliminate cost as a limiting barrier*** to sequencing data generation.

EX1033 (Wagle) at page 90. In view of the invasiveness, expense, and length of Wagle’s original assay, a POSA would not have been motivated to repeat the assay on a subsequently obtained tumor.

226. Second, a POSA would not have been motivated to repeat Wagle’s assay on a plasma sample that included cell-free tumor DNA. As discussed above, cell-free tumor DNA is not present in sufficient quantities to have motivated a POSA to use Wagle’s capture probes on a plasma sample containing nanogram amounts of DNA with a reasonable expectation of success. *Supra* Section X.C.2. Forshew

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states that capture probes had "not been applied to analysis of circulating tumor nucleic acids" precisely because capture probes require more DNA than is present in circulating plasma as follows:

> 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. Foresight and Dr. Quackenbush do not provide an explanation of how a POSA would have been motivated to modify Wagle's workflow to analyze the miniscule amounts of DNA in Chan's cell-free tumor DNA plasma samples with a reasonable expectation of success.

E. <u>Objective Indicia of Nonobviousness</u>

227. As explained above, Foresight and Dr. Quackenbush failed to show that a POSA 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.

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1. Nexus

228. I understand that the evidence of objective indica 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 '033 Patent.

'033 Patent Claim Language	Personalis' NeXT Personal® assay
1. A method for analyzing a nucleic acid sample obtained from an individual, comprising:	The Next Personal test performs a method for analyzing samples isolated from an individual. **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 1mm^3 of FFPE tumor tissue. EX2008 (Personalis NeXT Personal Brochure) at page 10.

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'033 Patent Claim Language	Personalis' NeXT Personal® assay
(a) producing, with the aid of a computer processor, a plurality of capture probes, wherein said plurality of capture probes hybridize to a plurality of polymorphisms, wherein said plurality of polymorphisms are in sequences encoding genes with known biomedically interpretable variants, and wherein said plurality of polymorphisms are based on or extracted from one or more databases of polymorphisms, and are observed in a population of one or more samples,	The NeXT Personal test utilizes computer processors to produce capture probes hybridized to a plurality of polymorphisms. 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. EX2008 at page 24, ¶6.

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'033 Patent Claim Language	Personalis' NeXT Personal® assay
(b) contacting said nucleic acid sample with said plurality of capture probes produced in (a)	The method of conducting the NeXT Personal test involves contacting nucleic acid samples with capture probes. 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. EX2008 at page 24, ¶6.

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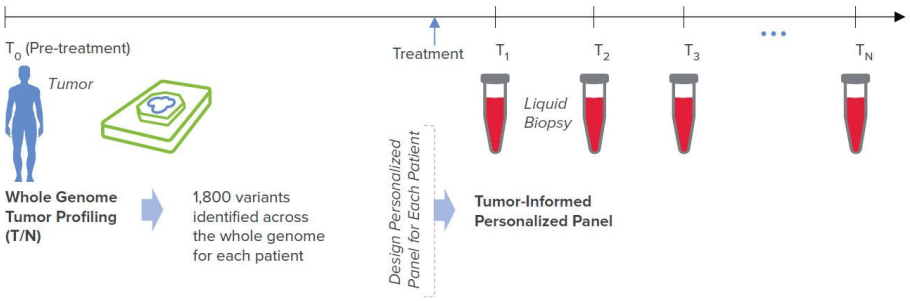
'033 Patent Claim Language	Personalis' NeXT Personal® assay
(c) conducting a sequencing assay on a subset of nucleic acid molecules to yield a result comprising a nucleic acid sequence, thereby analyzing said nucleic acid sample; and	The Foresight Diagnostics Solid Tumor Recurrence test conducts a second sequencing assay on the second subset of nucleic acids generated from the plasma DNA sample. **Figure 3:** NeXT Personal: Advanced, Higher Sensitivity, Personalized, Tumor-Informed Liquid Biopsy for MRD & Variant Tracking  EX2008 (Personalis NeXT Personal Brochure) at page 12, 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 EX2008 (Personalis NeXT Personal Brochure) at page 12.

'033 Patent Claim Language	Personalis' NeXT Personal® assay
(d) repeating steps (b)-(c) on a subsequently obtained biological sample from said individual.	The NeXT Personal test repeats the sequencing assay performed on the plasma sample multiple times over time. For example, Figure 1 shows the tracking and identification of variant allele frequencies at multiple timepoints over the course of 30 months after surgery. **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 1mm^3 of FFPE tumor tissue. EX2008 (Personalis NeXT Personal Brochure) at page 10.

2. <u>Industry Praise</u>

229. 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 '033 Patent earned the #1 ranking. *See* EX2001 at 1-2.

230. The differences between the method used in Leary (EX1002) and the method claimed in the '033 patent yield very different results. Personalis' assay can detect tumor in DNA in blood plasma at levels of about one part per million (or

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0.0001%). EX2008 at 10. By comparison, the best sensitivity from plasma DNA in the Leary reference was about 0.3%, which is several hundred times less sensitive than the claimed method. EX1002 (Leary) at page 4 ("However, the fraction of mutant tumor DNA did not reach zero (remaining at 0.3% at day 137), consistent with the fact that this patient had residual metastatic lesions in the remaining left lobe of the liver"). This is shown below by the right most end of the graph in Fig. 4B of Leary:

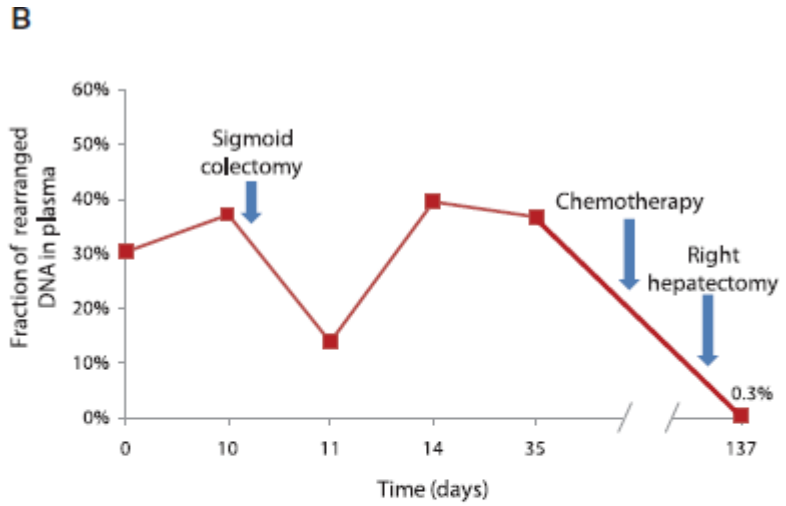

Fig. 4. Detection of tumor-specific rearrangements in plasma of cancer patients. (**A**) The identified chromosome 4:8 and 16 rearrangements were used to design PCR primers spanning breakpoints and to amplify rearranged DNA from tumor tissue and plasma from patients Hx402 and Hx403, respectively. A plasma sample from an unrelated healthy individual was used as a control for both rearrangements. (**B**) Plasma samples from patient Hx402 were analyzed at different time points using digital PCR to determine the fraction of genomic equivalents of plasma DNA containing the chromosome 4:8 rearrangement. The fraction of rearranged DNA at day 137 was 0.3%, consistent with residual metastatic lesions present in the remaining lobe of the liver.

EX1002 (Leary) at Fig. 4.

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3. Skepticism of Experts

231. 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 capture probes. 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 capture probes Forshew (EX1030) described as "difficult to implement on a routine basis." EX1030 (Forshew) at page 10.

232. Other references outline industry-wide problems with next-generation sequencing (NGS) at the time, which were unexpectedly solved by the methods in the '033 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.

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233. This objective real-world evidence supports a finding that the claims of the '033 patent are nonobvious.

XI. CERTIFICATION

234. 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: October 6, 2023 By: _____

Henry Morrice Furneaux, Ph.D.

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