

**IN THE UNITED STATES DISTRICT COURT
FOR THE MIDDLE DISTRICT OF NORTH CAROLINA**

NATERA, INC.,	)	
	)	
Plaintiff,	)	
	)	
v.	)	
	)	C.A. No. 1:23-cv-629
NEOGENOMICS LABORATORIES,	)	
INC.,	)	
	)	
Defendant.	)	

**DECLARATION OF DR. MICHAEL L. METZKER IN SUPPORT OF
PLAINTIFF NATERA, INC.'S MOTION FOR A PRELIMINARY INJUNCTION**

FILED UNDER SEAL

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1. I have been retained by counsel for Plaintiff Natera Inc. (“Natera”) in connection with the above-captioned matter. I understand that Natera has filed suit against Defendant NeoGenomics Laboratories, Inc. (“NeoGenomics”), alleging infringement of U.S. Patent Nos. 11,530,454 (the “’454 Patent”)¹ and 11,519,035 (the “’035 Patent”)² (collectively, the “Asserted Natera Patents”). I have been asked to offer opinions regarding infringement of the Asserted Natera Patents by Defendant’s RaDaR™ assay (the “Accused Assay”), how it compares with Natera’s Signatera™ assay, and whether Signatera™ practices the claims of the Asserted Natera Patents.

2. This Declaration is based on information currently available to me. I may continue my investigation and study as new information comes to light and circumstances evolve. Accordingly, I may supplement my opinions and/or provide rebuttal opinions and reserve the right to do so, including through testimony given at deposition or trial. I would be able to testify regarding the matters stated in this Declaration, if asked by the Court or by the parties’ attorneys.

I. SUMMARY OF OPINIONS

3. It is my opinion that the Accused Assay infringes Claims 1-6, 8, and 10-13 of the ’454 Patent and Claims 1, 12 and 13 of the ’035 Patent (collectively, the “Asserted Claims”). It is also my opinion that Signatera™ practices the inventions claimed in the

¹ See Babiarz *et al.*, *Detecting mutations and ploidy in chromosomal segments*, U.S. Patent No. 11,530,454 (2022) (the “’454 Patent”).

² See Rabinowitz *et al.*, *Methods for simultaneous amplification of target loci*, U.S. Patent No. 11,519,035 (2022) (the “’035 Patent”).

Asserted Patents. It is further my opinion that there is no evidence of any clinical advantage of RaDaR over Signatera™.

II. BACKGROUND AND QUALIFICATIONS

4. I am currently Founder, President, and Chief Executive Officer of RedVault Biosciences, LP (“RedVault”), which I founded in April 2013. RedVault is a biotechnology company committed to creating innovative technologies to advance genomic medicine, which I founded in April 2013. I am also a Co-founder and Chief Technology Officer at 454 Bio, a next-generation sequencing company developing low-cost devices and reagents. Before taking my positions at RedVault and 454 Bio, I was a tenured and adjunct Associate Professor in both the Department of Molecular & Human Genetics and at the Human Genome Sequencing Center (“HGSC”) at Baylor College of Medicine. I have also been a Senior Manager at the HGSC at Baylor College of Medicine. I have also been an adjunct Associate Professor in the Cell & Molecular Biology Program at the Baylor College of Medicine. Prior to my tenured positions, I held the position of tenure-track Assistant Professor in Molecular and Human Genetics and Senior Manager at the HGSC and the position of adjunct Associate and Assistant Professor in Chemistry at Rice University.

5. In March 2002, I founded LaserGen, Inc. (“LaserGen”), which provides technology and reagents used in, among other things, nucleic acid sequencing methods. From March 2002 to April 2012, I was President and Chief Executive Officer of LaserGen, and from April 2012 to October 2012, I held the position of Chief Technology Officer. I

was also a member of the Board of Directors from March 2002 to October 2012. I currently have no working relationship with LaserGen. In March 2016, Agilent Technologies invested \$80M in LaserGen to convert its reversible terminator technology into a clinical diagnostic platform. In March 2018, Agilent Technologies purchased the remaining shares of LaserGen for \$105M.

6. Since 1988, I have performed scientific research in the fields of molecular biology and chemical synthesis of nucleoside and nucleotide inhibitors. In particular, I have extensive experience with technology development in DNA sequencing methods including genomic DNA isolation, polymerase chain reaction (“PCR”), fragmenting genomic DNA, genomic DNA and complementary DNA (“cDNA”) library construction, bacterial cloning, nucleic acids chemistry, DNA modifying enzymes, polymerase engineering, fluorescent dyes, fluorescence imaging, and data analysis of multi-color images. I also have extensive experience in the use and development of methods utilized to detect sequence variation, including single nucleotide variants (“SNVs”) and single nucleotide polymorphisms (“SNPs”), in all organisms, with a particular focus in humans and human immunodeficiency virus (“HIV”).

7. My research has been devoted in large part to developing next-generation sequencing (“NGS”) technologies, developing novel methods to study HIV transmission between individuals, and identifying molecular causes of novel forms of diabetes and their treatment. My colleagues and I have been deeply involved in PCR, DNA fragmentation, library construction, cloning, and the development of sequencing technologies. My

industrial experience entails performing analytical chemical testing of inorganic materials (Aerojet-General), organic synthesis and characterization of porous polystyrene beads for applications in high performance liquid chromatography (Bio-Rad), development and applications of a robotic platform in Sanger sequencing (Applied Biosystems), and development and applications of high-throughput DNA sequencing to identify novel gene targets for small molecule screening (Merck Research Laboratories).

8. I received my Bachelor of Science degree in Biochemistry & Biophysics from the University of California, Davis in 1984. Between 1985 and 1988, I completed the required coursework with an emphasis in Organic Chemistry as part of the Master of Science program in the department of Chemistry at San Francisco State University. Instead of completing this degree, I pursued a Doctor of Philosophy in 1991 in the Department of Molecular and Human Genetics from Baylor College of Medicine in Houston, Texas. I received my Ph.D. in 1996 in Molecular and Human Genetics.

9. From 2000 to 2018, I gave lectures to graduate and medical students in the Molecular Methods course at Baylor College of Medicine. The lectures were titled, *cDNA and Genomic Libraries*, *First-generation Sequencing*, and *Genotyping*, and *Next-generation Sequencing*. From 2001 to 2003, I also gave a lecture titled *Mammalian Genome Analysis* in the Mammalian Genomics course.

10. I have also presented lectures at a variety of academic and industry conferences. In May 2010, I was an invited speaker at the *Next-Generation Sequencing* Workshop at Lübeck University in Germany. In October 2010, I was an invited speaker at

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

11. I have reviewed numerous manuscripts for peer-reviewed publications that present data for many of the techniques described above. I have also authored several extensive reviews on sequence technologies and their applications used for the detection of sequence variation, including SNPs and SNVs. I have also received research grants and fellowships both from government and private agencies to fund my investigations of technology development in Sanger sequencing and NGS methods, chemical synthesis of nucleoside and nucleotide inhibitors, development of NGS approaches for HIV forensics, the detection of genomic variation in diabetic cohorts using novel methods, and technology development of a novel diagnostic method involving microRNAs. I have received or have been associated with numerous grants largely focusing on gene and genome sequencing totaling over \$170 million since 1999. The source of these grants has been the National

Institutes of Health (“NIH”), the National Institute of Justice (“NIJ”), the United States Department of Agriculture (“USDA”), and private foundations.

12. I have authored 58 peer-reviewed papers, seven book chapters, and am an inventor on 58 issued patents and patent applications. Several of my review papers discuss the emerging technologies and advances in DNA sequencing. The patents relate to various aspects of molecular biology, DNA sequencing, and chemical synthesis. For example, I co-invented the pulsed- multiline excitation (“PME”) method in collaboration with Nobel Laureate Robert F. Curl, Ph.D., which has resulted in U.S. Patent Nos. 6,995,841; 7,511,811; and 8,089,628. By way of collaboration with my scientific team at LaserGen, I have also co-invented novel nucleotide terminators that have resulted in numerous patents including U.S. Patent Nos. 7,893,227; 7,897,737; 7,964,352; 8,148,503; 8,198,029; 8,361,727; 8,497,360; 8,877,905; 8,889,860; 8,969,535; 9,200,319; 9,399,798; 9,689,035; and 10,041,115.

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

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

15. I have also served as an expert witness in several criminal trials in the United States involving the transmission of human immunodeficiency virus (HIV) between individuals. The criminal case involving Richard Schmidt was portrayed in an episode titled *Shot of Vengeance* on Forensic Files in 2003. The criminal case involving Philippe Padieu was reported on ABC News 20/20 by Elizabeth Vargas and on Oprah, both in 2009, and helped inspired an episode titled *Quickie* on Law & Order: Special Victims Unit in 2010.

16. I belong to the American Association for the Advancement of Science, the American Chemical Society, and the Texas Genetics Society. My professional experience, education, and publications are presented in greater detail in my curriculum vitae, including publications and patents authored in the previous 10 years, as well as cases that I have provided expert testimony at deposition or trial.³

³ See Ex. 1.

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

18. In formulating my opinions, I have relied upon my 30+ years of education, training, knowledge, and experience in the relevant art. But at all times, I have attempted to understand and interpret the claims of the '454 Patent and '035 Patent from the point of view of one of ordinary skill in the art, as discussed in Section IV.

19. I am being compensated for services provided in this case at my usual and customary consulting rate of \$750/hour. My compensation does not depend in any way on the opinions I express or the outcome of this case.

III. UNDERSTANDING OF THE LAW

20. I am not an attorney, nor have I independently researched the law. I have been informed by counsel for Natera and understand that the various legal standards that must guide how patent infringement is analyzed. I have applied those standards in rendering my opinions expressed below in this Declaration.

21. I have been informed and understand that the “written specification” of a patent includes the written description of one or more preferred embodiments of the invention, including drawings or figures, and one or more claims that point out and distinctly claim the subject matter of the invention. It is the claims that define and measure the scope of the patent. A separate invention is defined by each claim.

22. I have been informed and understand that a dependent claim incorporates every element of the claim from which it depends.

23. I have been informed and understand that the determination of “infringement” is a two-step process in which the language of the claims asserted against a device or method accused of infringement (the “accused device” or “accused method”) is first construed by a court as a matter of law. Then the accused device or method is compared to the properly construed claim. Infringement is found by comparing the accused device or method to one or more of the claims of the patent, as properly construed.

24. I have been informed and understand that a claim term should be understood to have its plain and ordinary meaning as understood from the standpoint of a hypothetical person of ordinary skill in the art as of the invention date of the asserted claim in view of the intrinsic evidence, including the plain claim language, the specification, and the prosecution history. The specification and prosecution history often can inform the meaning of the claim language, if it differs from the plain and ordinary meaning as understood by one of ordinary skilled in the art, by demonstrating how the inventor understood the invention and whether the inventor limited the invention.

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

26. I have been informed and understand that patent claims can be infringed either literally or under the doctrine of equivalents. Literal infringement is found when each and every element of a claim is literally found in the accused device or practiced by the accused method.

27. My understanding of the patent law of infringement is that an accused structure or method infringes a claim if it contains all the elements of that claim, regardless of whether or not there are other additional elements in the accused structure or additional steps in the accused method.

28. While literal infringement requires each claim element to be literally present in the accused device or method, infringement under the doctrine of equivalents can be found when every claim element that is not literally present is nonetheless equivalently present.

29. I am informed and understand that, under the doctrine of equivalents, even if an accused system or method does not literally include one or more requirements of a claim of a patent, the accused system or method may still infringe that claim if the differences between the requirements of the accused system or method and the requirements of the claimed system or method are insubstantial.

30. I am further informed and understand that one test for the applicability of the doctrine of equivalents is that the accused system or method performs substantially the same function in substantially the same way to achieve substantially the same result as the claimed system or method.

31. I am further informed and understand that known interchangeability between structures or methods literally covered by the patent claim and structures and methods in the accused system or methods supports a finding of equivalence.

IV. LEVEL OF ORDINARY SKILL IN THE ART

32. In my opinion, based on the standards provided by counsel,⁴ a person of ordinary skill in the art (“POSA”),⁵ at or prior to 2010 and through to the present, would have at least a bachelor’s degree (with several additional years of laboratory experience) in a life or physical sciences discipline with applications in genomics. Through further education or graduate or postdoctoral experience, such a person would have obtained a thorough understanding of nucleic acid detection, quantification, and genotyping, and would have understood the basic underlying scientific principles of nucleic acid assay, detection, and genotyping techniques. One of ordinary skill in the art would also have had general knowledge of, and be able to read literature on, nucleic acid assay methods including, for example, amplification by PCR, nucleic acid sequencing including by multiplex or high-throughput sequencing, genotyping, and the detection and quantification of nucleic acids, including cell-free DNA, using techniques such as polymorphism-based genotyping. In determining the level of ordinary skill in the art, more education could compensate for less experience, and vice versa.

⁴ See *supra* at ¶ 25.

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

33. In or around 2014, I would have been at least one of ordinary skill in the art with respect to the subject matter of the '454 Patent. In or around 2010, I would have been at least one of ordinary skill in the art with respect to the subject matter of the '035 Patent.

V. TECHNOLOGY BACKGROUND

A. Minimal Residual Disease

34. Minimal residual disease refers to “a very small number of cancer cells that remain in the body during or after treatment.”⁶ Typically, when there is a positive response to cancer treatment, the presence of a tumor decreases within a patient, and the tumor eventually becomes undetectable in radiographic imaging or clinical examination. However, if tumor cells remain in the patient’s body after treatment, there is potential for cancer relapse, either locally or through distant metastases. A related concept is molecular residual disease (“MRD”), which refers to the presence of a small amount of tumor DNA molecules that remain in the body after treatment. The ability to detect MRD as early as possible is beneficial for patient outcomes and overall survival by enabling and informing further treatment decisions.

B. Liquid Biopsy

35. Liquid biopsy is a minimally invasive “test done on a sample of blood [] to look for cancer cells from a tumor or small pieces of DNA, RNA, or other molecules

⁶ See <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/minimal-residual-disease> (last visited July 4, 2023).

released by tumor cells” that are circulating in the blood.⁷ Compared to a solid tumor biopsy, a liquid biopsy has several advantages. First, it is usually less invasive, dangerous, and painful. Second, it may provide a more comprehensive molecular overview of tumor heterogeneity, possibly reflecting the characteristics of different cancer locations in a particular patient. For example, mutations may be considered “clonal” or “subclonal,”⁸ which can be observed or inferred by the comparison of matched tumor and plasma samples using the methods described in the Asserted Natera Patents.

36. A liquid biopsy may be used to specifically detect and quantify circulating tumor DNA (ctDNA) in a patient to determine MRD. ctDNA is a type of cell-free DNA (cfDNA) characterized by tumor-specific genomic and epigenomic alterations. cfDNA refers to short fragments of DNA found in blood as cells that typically shed their DNA into the bloodstream through certain biological process associated with cell death (*e.g.*, necrosis or apoptosis) or through active secretion. Tumor cells have a similar capacity to shed their DNA into the bloodstream compared to normal cells, albeit the former having a greater probability due to higher cellular turnover.⁹ DNA with tumor-specific alterations (*i.e.*, ctDNA) can thus be detected in blood to determine MRD.

⁷ See <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/liquid-biopsy> (last visited July 4, 2023).

⁸ See, *e.g.*, Ex. 2, Kirkizlar *et al.*, *Detection of clonal and subclonal copy-number variants in cell-free DNA from patients with breast cancer using a massively multiplexed PCR method*, TRANSLATIONAL ONCOLOGY 8:407-416 (2015) (“Kirkizlar (2015)”).

⁹ See Ex. 3, Leon *et al.* *Free DNA in the serum of cancer patients and the effect of therapy*. CANCER RESEARCH 37:646–650 (1977) (“Leon (1997)”).

C. The Accused RaDaR™ Assay

37. Like Signatera™ (described *infra*), the accused RaDaR™ assay is a personalized MRD assay. According to Defendant, RaDaR™ is “a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.”¹⁰ RaDaR™ uses a “tumor informed approach, unique and specific to each patient.”¹¹

38. According to Defendant, “RaDaR MRD testing is validated for use in multiple solid tumor cancers,” including “breast cancer, colorectal cancer, lung cancer, and head and neck cancer.”¹²

39. Defendant summarizes the workflow of RaDaR™ as follows:

The first step in using RaDaR™ is a process called whole exome sequencing (WES), which is performed on an individual’s tumor sample to identify mutations specific to that person.

Then, RaDaR™ next-generation sequencing (NGS) assay panels are designed and customized to each patient based on their identified mutations.

This state-of-the-art approach uses molecular fingerprints identified in the tumor to track up to 48 mutations in a blood sample. This tumor informed approach enables consistent identification of large numbers of patient specific mutations to track, therefore improving MRD sensitivity.

Following library preparation steps using the InVision® platform, deep sequencing is performed on the patient’s cfDNA, tumor DNA and leukocyte

¹⁰ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (last visited June 23, 2023).

¹¹ See <https://www.inivata.com/radar-technology-overview/> (last visited June 23, 2023).

¹² See <https://finditwithradar.com/> (last visited June 23, 2023).

DNA. Notably, the buffy coat fraction is sequenced to determine confounding signals derived from clonal hematopoiesis of indeterminate potential (CHIP).

A report is then generated to determine the MRD status of the blood samples, indicating whether tumor DNA is detected (ctDNA positive) or not detected (ctDNA negative). Changes to a patient's ctDNA level can also be monitored over time.¹³

40. It is my understanding that RaDaR™ has five basic steps, each of which is briefly described below.¹⁴

41. At Step 1, “[a] patient’s surgically resected tumor or biopsy sample undergoes whole-exome sequencing (WES) to identify tumor-specific variants.”¹⁵



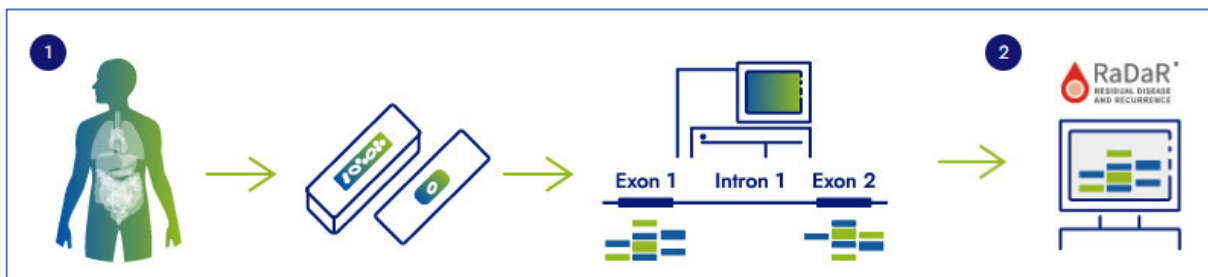
42. At Step 2, “[a] group of up to 48 variants are then selected using optimized algorithms. An assay targeting these mutations is customized to generate a personalized RaDaR™ panel.”¹⁶

¹³ See <https://www.inivata.com/radar-technology-overview/> (last visited June 23, 2023).

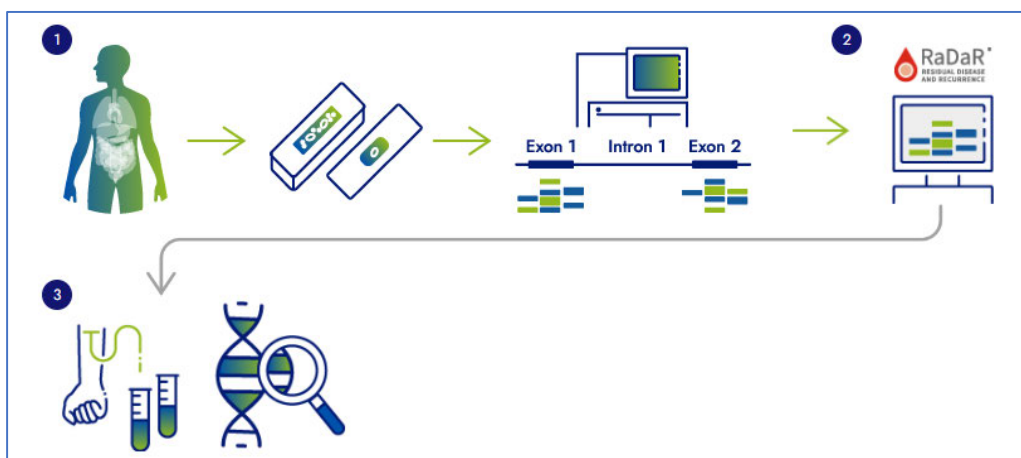
¹⁴ See Ex. 4, <https://finditwithradar.com/radar-technology> (last visited June 23, 2023).

¹⁵ *Id.*

¹⁶ *Id.*



43. At Step 3, “[u]sing the InVision® platform and the personalized assay, partition-based multiplex PCR (polymerase chain reaction) is performed. This generates sequencing libraries from the patient’s plasma cell-free DNA (cfDNA), tumor DNA and leukocyte DNA for the targeted regions.”¹⁷



44. Defendant’s InVision® liquid biopsy platform uses “an enhanced version of TAM-Seq [eTAM-Seq] technology to identify and quantify low frequency tumor-derived SNVs and indels in cfDNA.”¹⁸ InVision® relies on multiplex PCR and targeted next-

¹⁷ *Id.*

¹⁸ See Ex. 5, Gale *et al.*, *Development of a highly sensitive liquid biopsy platform to detect clinically-relevant cancer mutations at low allele fractions in cell-free DNA*, PLOS ONE (2018) 13(3):e0194630 pp. 1–18 (2018) (“Gale (2018)”) at 4; see also Ex. 6, Gale *et al.*,

generation sequencing (NGS).¹⁹ In an analytical validation study, Defendant describes the library preparation for multiplex PCR and NGS using eTAm-Seq technology as follows:

eTAm-Seq technology is based on methods previously described [22, 25,26], with an optimized assay workflow **utilizing multiplex PCR** to enable high-throughput library preparation without the use of microfluidics. **Next generation sequencing libraries were prepared using a two-step multiplex PCR amplification process** incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors. Different input amounts of DNA were used to assess the performance of the assay, using either 2,000 AC (low), 8,000 AC (medium) or 16,000 AC (high) input (~6.6ng to 53ng of amplifiable DNA). All regions were analysed multiple times using a fixed DNA input range for all samples to enable error correction [26]. As each sample is analysed multiple times, false positive and true positive calls can be readily identified, providing a robust analytical pipeline [22, 26]. After target enrichment, amplified regions were purified using SPRISelect beads (Beckman Coulter) following the manufacturer's protocol. Samples were quantified using the LabChip GX touch and DNA high sensitivity assay. Quantified samples were then pooled to generate a normalized library of 12 nM. This library was quantified using the Kapa Library Quantification Kit, and **1.8 pM libraries analysed on an Illumina NextSeq 500** (300 cycle PE) with 5% PhiX to monitor sequencing performance.²⁰

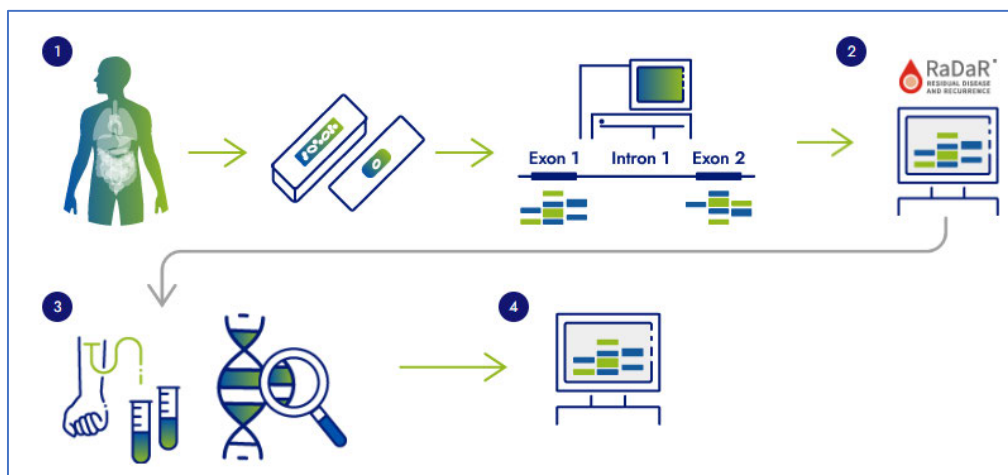
45. Continuing on with the RaDaR™ workflow, at Step 4, “[t]he patient’s plasma cfDNA and leukocyte DNA (from blood buffy coat) are now sequenced at a high depth. Tumor DNA is also sequenced to confirm the variants are tumor-specific. Leukocyte DNA high-depth sequencing allows for the identification and removal of

Analytical performance and validation of an enhanced TAm-Seq™ circulating tumor DNA sequencing assay, AACR Poster 3639 (2016) (“Gale (2016)”).

¹⁹ *Id.*

²⁰ See Gale (2018) at 4–5 (emphasis added) citing to reference [22]: Ex. 7, Forshew *et al.*, *Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA*, SCIENCE TRANSLATIONAL MEDICINE 4:136ra68 (2012) (“Forshew (2012)”) reference [25] to Gale (2016).

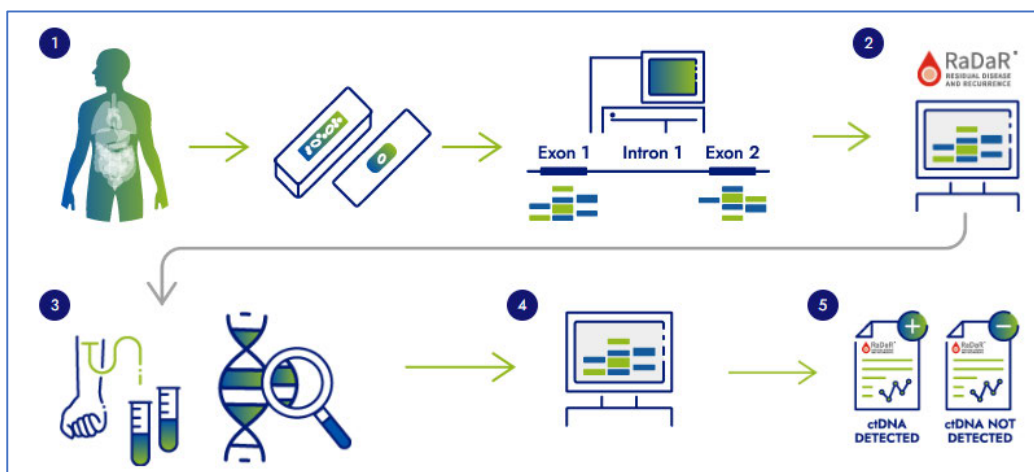
germline changes and confounding signals derived from clonal hematopoiesis of indeterminate potential (CHIP) (which may be present in the blood at low levels) from the analysis. This final process reduces false-positive results and contributes to high specificity and a high positive predictive value (PPV).”²¹



46. At Step 5, “NGS data are analyzed by combining results across all tumor-specific variants to determine the patient’s MRD status. A report is then generated indicating whether tumor DNA was “Detected” or “Not Detected” in the patient’s blood samples.”²²

²¹ See Ex. 4, <https://finditwithradar.com/radar-technology> (last visited June 23, 2023).

²² *Id.*



47. I understand that Defendant contends that RaDaR™ is 10x more sensitive than other MRD tests offered, which includes Signatera™. I am not aware of any head-to-head studies comparing the sensitivity and specificity of RaDaR™ against another MRD test. Moreover, I am not aware of any clinical evidence showing that RaDaR™ is 10x more sensitive than other MRD tests offered. Finally, I understand that Defendant’s “10x” claim about RaDaR appears to be based on an analytical validation using contrived cell line mixtures.²³ I am not aware of any evidence that the claimed analytical sensitivity is validated or supported by clinical data.

²³ See Ex. 10, Flach *et al.*, *Liquid BIOPsy for MiNimal RESidual DiSease Detection in Head and Neck Squamous Cell Carcinoma (LIONESS)—a personalized circulating tumour DNA analysis in head and neck squamous cell carcinoma*, BRITISH JOURNAL OF CANCER 126:1186–1195 (2022) (“Flach (2022)”) Supplementary Methods at 1-2.

VI. THE ASSERTED NATERA PATENTS

A. '454 Patent

48. The '454 Patent is titled “Detecting mutations and ploidy in chromosomal segments” and was issued on December 20, 2022.²⁴ The '454 Patent claims methods for preparing plasma samples to detect ploidy of chromosome segments and single nucleotide variant (“SNV”) mutations.²⁵ The claimed methods perform whole exome sequencing or whole genome sequencing of tumor samples and multiplex amplification of cell-free DNA isolated from plasma samples in order to detect SNV mutations.²⁶

49. The '454 Patent contains 28 claims. Independent Claim 1 is reproduced below.

Claim 1. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and

²⁴ See '454 Patent at cover page.

²⁵ *Id.*, e.g., at Abstract, Cols. 2:44–5:3, 6:1–5, 67:57–61, 147:45–48, 171:25–173:4 (Claims 1–6, 8, 10–13).

²⁶ *Id.* at Col. 171:25–173:4 (Claims 1–6, 8, 10–13).

sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.²⁷

50. Various dependent claims include limitations that the SNV mutations can be clonal or subclonal SNV mutations.²⁸ Other dependent claims include limitations that the cell free DNA can be circulating tumor DNA,²⁹ that the tumor sample is a tumor tissue sample,³⁰ and that the cancer can be colorectal cancer, lung cancer, bladder cancer, or breast cancer.³¹ Another dependent claim includes the limitation that specific numbers of target loci are amplified according to the methods.³² Another dependent claim recites detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.³³ Finally, some dependent claims include limitations directed to designing PCR primers or hybrid capture probes,³⁴ and performing barcoding PCR prior to sequencing.³⁵

²⁷ *Id.* at Col. 171:25–43.

²⁸ *Id.* at Col. 171:46–52 (Claims 3–5).

²⁹ *Id.* at Col. 171:44–45 (Claim 2).

³⁰ *Id.* at Col. 171:53–54 (Claim 6).

³¹ *Id.* at Col. 172:8–9 (Claim 13).

³² *Id.* at Col. 171:58–63 (Claim 8).

³³ *Id.* at Col. 172:4–7 (Claim 12).

³⁴ *Id.* at Col. 171:64–67 (Claim 10).

³⁵ *Id.* at Col. 172:1–3 (Claim 11).

B. '035 Patent

51. The '035 Patent is titled “Methods for Simultaneous Amplification of Target Loci” and issued on December 6, 2022.³⁶ The '035 Patent claims methods for amplifying and sequencing multiple nucleic acid regions of interest in one reaction volume.³⁷ The claimed methods include targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci associated with cancer on cell-free DNA in a single reaction volume and sequencing the plurality of SNP loci by conducting massively parallel sequencing.³⁸

52. The '035 Patent contains 15 claims, one of which is independent. Independent Claim 1 is reproduced below.

Claim 1. A method for amplifying and sequencing DNA, comprising:

tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;

amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and

sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.³⁹

³⁶ See '035 Patent at cover page.

³⁷ *Id.*, e.g., at Abstract, Cols. 2:59–61, 3:54–8:43, 21:54–24:4, 25:10–42, 27:43–28:31, 59:38–60:34, 76:15–77:63, 83:36–84:3, 84:66–85:29 (Claims 1, 12, 13).

³⁸ *Id.* at Col. 249:43–251:20 (Claims 1, 12, 13).

³⁹ *Id.* at Col. 249:44–62.

53. Various dependent claims are directed to universal tail adaptors. Specifically, some dependent claims contain limitations that one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor⁴⁰ and tagging cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.⁴¹

VII. CLAIM CONSTRUCTION

54. There should not be significant disputes as to the meanings of the claim terms of the Asserted Natera Patents because the claims are composed of terms known to one of ordinary skill in the art, who would have understood the meaning of the claim terms in light of the claims and the specification.

VIII. INFRINGEMENT ANALYSIS

55. Based on my below analysis, it is my opinion that the RaDaR™ assay infringes the Asserted Claims.

56. In forming the opinions set forth in this Declaration, I considered the '454 Patent, the '035 Patent, their file histories, and other documents cited in this Declaration, including the following documents:

- Gale (2018). (See Exhibit 5 attached hereto.)

⁴⁰ *Id.* at Col. 251:3–9 (Claim 12).

⁴¹ *Id.* at Col. 251:10–13 (Claim 13).

- Gale (2016). (See Exhibit 6 attached hereto.)
- Forshew (2012). (See Exhibit 7 attached hereto.)
- Heider (2020).⁴² (See Exhibit 8 attached hereto.)
- Lipsyc-Sharf (2022).⁴³ (See Exhibit 9 attached hereto.)
- Flach (2022). (See Exhibit 10 attached hereto.)
- Marsico (2020).⁴⁴ (See Exhibit 11 attached hereto.)
- Cutts (2021).⁴⁵ (See Exhibit 12 attached hereto.)
- Plagnol (2018).⁴⁶ (See Exhibit 13 attached hereto.)
- Russell (2023).⁴⁷ (See Exhibit 14 attached hereto.)
- Defendant's product description website for RaDaR™. (See Exhibit 4 attached hereto.)

I also relied upon my own education, experience, and knowledge of the field.

⁴² See Ex. 8, Heider *et al.*, *Sensitive detection of ctDNA in early-stage non-small cell lung cancer patients with a personalized sequencing assay*, AACR meeting, Poster 735 (2020) ("Heider (2020)").

⁴³ See Ex. 9, Lipsyc-Sharf *et al.*, *Circulating tumor DNA and late recurrence in high-risk hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer*, JOURNAL OF CLINICAL ONCOLOGY 40:2408-2419 (2022) ("Lipsyc-Sharf (2022)").

⁴⁴ See Ex. 11, Marsico *et al.*, *Analytical development of the RaDaR assay, as highly sensitive and specific assay for monitoring of minimal residual disease*, AACR meeting, Poster 3097 (2020) ("Marsico (2020)").

⁴⁵ See Ex. 12, Cutts *et al.*, *Molecular residual disease detection in early stage breast cancer with a personalized sequencing approach*, AACR meeting, Poster 536 (2021) ("Cutts (2021)").

⁴⁶ See Ex. 13, Plagnol *et al.* *Analytical validation of a next generation sequencing liquid biopsy assay for high sensitivity broad molecular profiling*. PLOS ONE 13:e0193802 pp. 1-18 (2018) ("Plagnol (2018)").

⁴⁷ See Ex. 14, Russell *et al.*, *Evaluation of a tumor informed MRD assay with contrived breast cancer samples*, AACR General Meeting, Poster 3384 (2023) ("Russell (2023)").

57. Below I explain why it is my opinion that the RaDaR™ assay infringes the Asserted Claims.

A. '454 Patent

1. Claim 1 of the '454 Patent is recited *infra*:

- (a) Claim 1[preamble]: *“A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:”*

58. To the extent the preamble is considered a claim limitation, the preamble is met by the Accused Assay. RaDaR™ includes methods for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample. For example, RaDaR™ may be used with “patients with high-risk [hormone receptor-positive breast cancers] at least 5 years after diagnosis.”⁴⁸ Defendant uses the following figure to demonstrate that a plasma sample is used with the RaDaR™ assay from a patient having cancer or suspected of having cancer.⁴⁹

⁴⁸ See Lipsyc-Sharf at 2409; see also *id.* at Abstract; Gale (2016); Plagnol (2018); Heider (2020); Marsico (2020); Cutts (2021); Flach (2022).

⁴⁹ See Lipsyc-Sharf (2022) at Appx. 1.

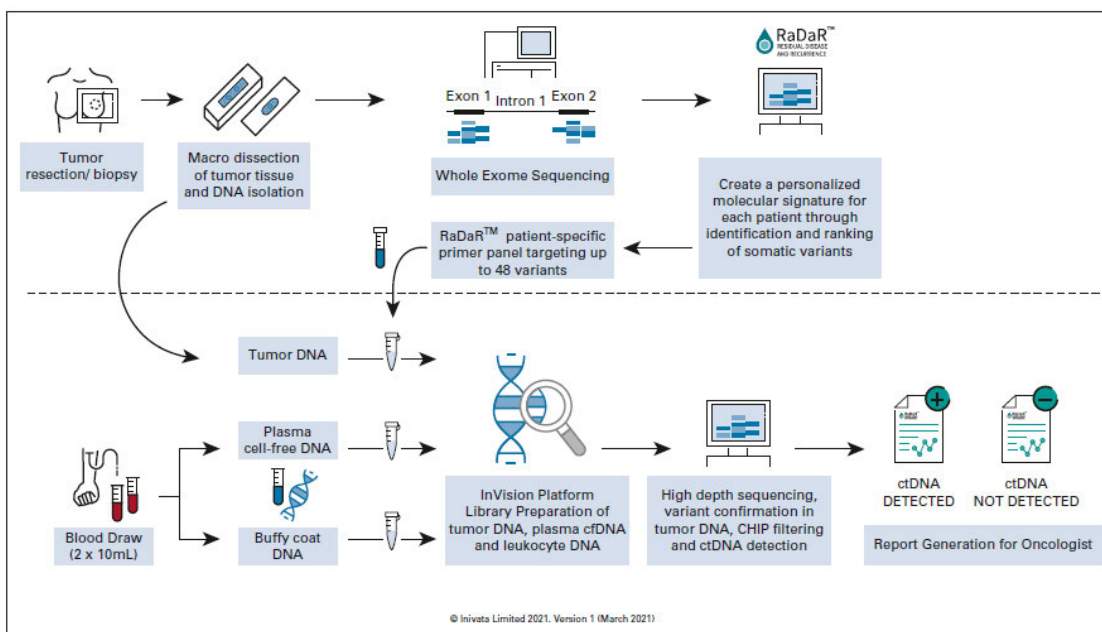


FIG A1. RaDaR assay workflow. Used with permission from Inivata, Inc.³⁸ cfDNA, cell-free DNA; CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating tumor DNA.

59. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. *RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.*”⁵⁰ Defendant also explains that “*RaDaR™ is a highly sensitive personalised sequencing ctDNA assay.* Tumor-specific variants are first identified by

⁵⁰ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (emphasis added) (last visited June 23, 2023).

exome sequencing of tumor tissue, followed by multiplex PCR and high-depth next generation sequencing to track low levels of ctDNA in patient plasma.”⁵¹

(b) Claim 1[a]: “*performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;*”

60. The Accused Assay satisfies this claim limitation. According to Defendant, “[t]he first step in using RaDaR™ is a process called whole exome sequencing (WES), which is performed on an individual’s tumor sample to identify mutations specific to that person. Then, RaDaR™ next-generation sequencing (NGS) assay panels are designed and customized to each patient based on their identified mutations. This state-of-the-art approach uses molecular fingerprints identified in the tumor to track up to 48 mutations in a blood sample. This tumor informed approach enables consistent identification of large numbers of patient specific mutations to track, therefore improving MRD sensitivity.”⁵²

61. In the Lipsyc-Sharf (2022) study, Defendant uses the figure below to demonstrate that RaDaR™ performs whole exome sequencing to detect somatic variants for patients:⁵³

⁵¹ See Heider (2020) (emphasis added).

⁵² See <https://www.inivata.com/radar-technology-overview/> (emphasis added) (last visited June 23, 2023).

⁵³ See Lipsyc-Sharf (2022) at Appx. 1; see also Heider (2020); Marsico (2020); Cutts (2021); Flach (2022) at 1187, Figure 1; Russell (2023).

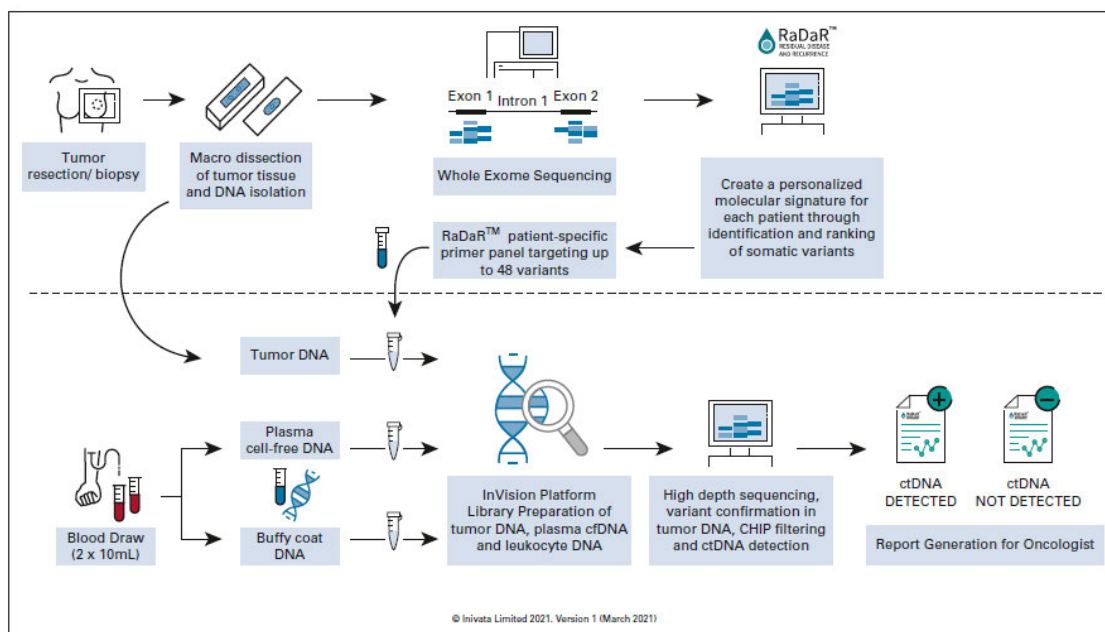


FIG A1. RaDaR assay workflow. Used with permission from Inivata, Inc.³⁸ cfDNA, cell-free DNA; CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating tumor DNA.

62. In the same study, Defendant further provides a table showing RaDaR's whole exome sequencing metrics, which is reproduced *infra*.⁵⁴

⁵⁴ See Lipsyc-Sharf (2022) at Supplemental Data.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Supplemental Table 1: Whole Exome Sequencing Metrics													
2	Project	Patient	Accession	Tumor	Reads M	Usable P	Usable Reads	Dup PCT	Aligned PCT	On Target PCT	Coverage	Panel	Total Variants	SNVs
3	DFCI_Bre	ONC100192	30005249	3E+09	197.29	75.111	148.1864919	18.904	99.777	92.755	100	30005249F	20	18
4	DFCI_Bre	ONC102380	30005235	3E+09	1355.55	11.274	152.824707	85.032	99.506	75.073	81	30005235F	50	42
5	DFCI_Bre	ONC105156	30005216	3E+09	201.72	77.363	156.0566436	17.287	99.711	93.699	115	30005216F	36	32
6	DFCI_Bre	ONC109055	30005256	3E+09	194.97	72.316	140.9945052	20.366	99.651	91.089	94	30005256F	40	40
7	DFCI_Bre	ONC109732	30005187	3E+09	280.14	77.619	217.4418666	16.513	99.803	93.001	159	30005187F	17	16
8	DFCI_Bre	ONC110363	30005241	3E+09	215.69	77.096	166.2883624	16.708	99.692	92.764	119	30005241F	16	13
9	DFCI_Bre	ONC112670	30005184	3E+09	264.6	76.496	202.408416	17.794	99.805	93.118	148	30005184F	28	28
10	DFCI_Bre	ONC113898	30005172	3E+09	235.16	80.086	188.3302376	14.123	99.778	93.369	138	30005172F	18	11
11	DFCI_Bre	ONC114585	30005182	3E+09	252.6	77.499	195.762474	16.779	99.782	93.214	141	30005182F	19	19
12	DFCI_Bre	ONC119801	30005237	3E+09	276.67	63.403	175.4170801	29.648	99.612	90.404	113	30005237F	29	29
13	DFCI_Bre	ONC120069	30005266	3E+09	241.77	66.333	160.3732941	27.541	99.659	91.797	114	30005266F	13	9
14	DFCI_Bre	ONC120664	30005176	3E+09	206.3	83.496	172.252248	10.409	99.818	93.215	131	30005176F	17	14
15	DFCI_Bre	ONC123954	30005240	3E+09	364.24	73.499	267.7127576	21.019	99.818	93.072	202	30005240F	21	19
16	DFCI_Bre	ONC124159	30005263	3E+09	275.02	77.818	214.0150636	15.367	99.767	92.041	160	30005263F	12	12
17	DFCI_Bre	ONC125115	30005268	3E+09	407.95	75.005	305.9828975	19.644	99.86	93.297	234	30005268F	24	19
18	DFCI_Bre	ONC125694	30005183	3E+09	341.91	67.41	230.481531	27.273	99.715	92.854	173	30005183F	50	45
19	DFCI_Bre	ONC127118	30005217	3E+09	406.37	70.69	287.262953	23.667	99.818	92.598	218	30005217F	48	45
20	DFCI_Bre	ONC127154	30005225	3E+09	427.27	72.465	309.6212055	22.659	99.873	93.668	228	30005225F	43	40
21	DFCI_Bre	ONC127410	30005254	3E+09	223.04	74.39	165.919456	19.933	99.674	93.154	121	30005254F	27	25
22	DFCI_Bre	ONC127559	30005252	3E+09	319.28	75.633	241.4810424	18.952	99.857	93.319	179	30005252F	39	38
23	DFCI_Bre	ONC129158	30005188	3E+09	407.65	76.199	310.6252235	18.599	99.884	93.575	243	30005188F	31	29
24	DFCI_Bre	ONC129398	30005261	3E+09	235.63	77.698	183.0797974	14.182	99.613	90.83	140	30005261F	45	41
25	DFCI_Bre	ONC130111	30005243	3E+09	360.52	73.957	266.6297764	20.84	99.789	93.472	205	30005243F	29	28
26	DFCI_Bre	ONC13031	30005269	3E+09	343.41	69.559	238.8725619	25.206	99.776	93.087	170	30005269F	48	48
27	DFCI_Bre	ONC131232	30005250	3E+09	387.31	75.17	291.140927	18.486	99.854	92.201	228	30005250F	45	38
28	DFCI_Bre	ONC132607	30005233	3E+09	400.91	73.142	293.2335922	20.514	99.869	91.911	222	30005233F	48	48
29	DFCI_Bre	ONC132921	30005212	3E+09	208.36	71.302	148.5648472	21.772	99.534	91.533	107	30005212F	16	16
30	DFCI_Bre	ONC13314	30005230	3E+09	309.83	66.125	204.8750875	28.043	99.785	91.99	149	30005230F	51	43
31	DFCI_Bre	ONC134863	30005191	3E+09	494.18	70.555	348.668699	23.738	99.821	92.519	269	30005191F	15	12
32	DFCI_Bre	ONC135623	30005236	3E+09	326.84	74.424	243.2474016	19.542	99.827	92.519	192	30005236F	48	41
33	DFCI_Bre	ONC136592	30005189	3E+09	348.59	58.885	205.2672215	34.823	99.731	90.485	144	30005189F	48	46
34	DFCI_Bre	ONC139563	30005224	3E+09	342.41	72.635	248.7095035	21.112	99.802	92.108	189	30005224F	30	28
35	DFCI_Bre	ONC139617	30005215	3E+09	453.22	70.995	321.763539	23.669	99.816	92.972	247	30005215F	35	33
36	DFCI_Bre	ONC140001	30005218	3E+09	401.75	68.061	273.4350675	26.472	99.782	92.642	195	30005218F	50	47

(c) Claim 1[b]: “performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and”

63. The Accused Assay satisfies this claim limitation. For example, RaDaR™ performs targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven

InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. ***RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy*** with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.”⁵⁵ Defendant also explains that “RaDaR™ is a highly sensitive personalised sequencing ctDNA assay. Tumor-specific variants are first identified by exome sequencing of tumor tissue, ***followed by multiplex PCR and high-depth next generation sequencing to track low levels of ctDNA in patient plasma.***”⁵⁶ Defendant further explains that “[w]e have developed the InVision™ liquid biopsy platform which utilizes enhanced TAm-Seq™ (eTAm-Seq™) technology, an amplicon-based next generation sequencing method for the identification of clinically-relevant somatic alterations at low frequency in ctDNA across a panel of 35 cancer-related genes.”⁵⁷ The eTAm-Seq technology “***utiliz[es] multiplex PCR to enable high-throughput library preparation.***”⁵⁸ Thus, one of ordinary skill in the art would have understood that the target loci are simultaneously amplified (*i.e.*, multiplexed).

⁵⁵ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (emphasis added) (last visited June 23, 2023).

⁵⁶ See Heider (2020) (emphasis added).

⁵⁷ See Gale (2018) at 1.

⁵⁸ *Id.* at 4 (emphasis added).

64. Lipsyc-Sharf (2022) described the workflow associated with RaDaR™ as including a “[*m*]multiplex PCR and [*s*]equencing” step.⁵⁹ Specifically, “[a]fter primer panel qualification and DNA extraction, ***RaDaR™ multiplex PCR was performed on cfDNA from plasma*** alongside a buffy coat DNA control sample[.]”⁶⁰

65. Gale (2018) explains that the InVision “panel was designed optimizing primers for ***amplification of fragmented DNA with amplicon sizes ranging from 72bp-154bp.***”⁶¹

66. According to Flach (2022), RaDaR uses up to 48 somatic variants, targeted by multiplex PCR:

RaDaR™ is a personalised ctDNA assay built on the InVision® platform [43], which ***utilises multiplex PCR and targeted NGS.*** Somatic variants identified in the tumour tissue by WES were prioritised using proprietary algorithms ***to build a patient-specific primer panel of up to 48 primer pairs capturing at least one somatic variant*** (Supplementary Table 2). The personalised primer panel was manufactured (IDT, Coralville, IA) and combined with a fixed primer panel of 21 common population-specific SNPs for quality control purposes during the NGS testing.⁶²

Following primer panel qualification, RaDaR™ assays were performed on cfDNA from plasma aliquots alongside a buffy coat DNA control sample, which was used for identification and removal of germline variants, the removal of variants due to clonal haematopoiesis of indeterminate potential (CHIP) from the analysis, and as a positive amplification control. ***Multiplex PCR was performed with input concentrations ranging from 1452 to***

⁵⁹ See Lipsyc-Sharf (2022) at Appx. 1 (emphasis added).

⁶⁰ *Id.* (emphasis added).

⁶¹ See Gale (2018) at 4 (emphasis added); see also Plagnol (2018) at 12, “an amplicon size distribution of 73bp-155bp (median = 112bp).”

⁶² See Flach (2022) at 1187 (emphases added).

20,000 amplifiable copies per sample, as measured by droplet digital PCR, with a median value of 14,550 copies.”⁶³

67. Defendant uses the figure below to demonstrate that the RaDaR™ assay uses cell-free DNA isolated from plasma sample to obtain amplicons in the same reaction volume.⁶⁴

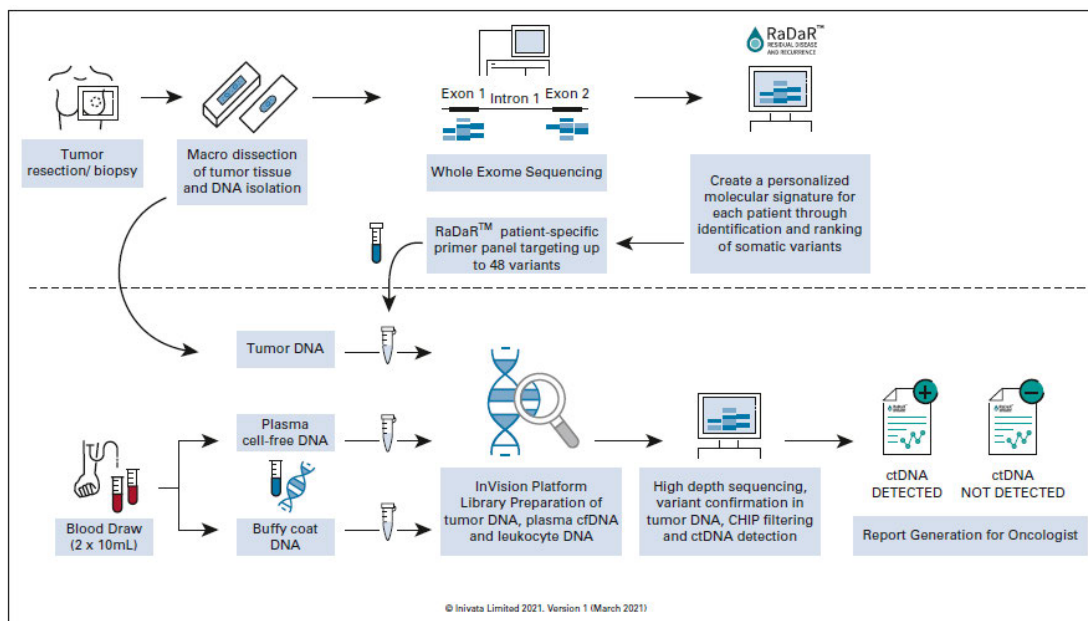


FIG A1. RaDaR assay workflow. Used with permission from Invitae, Inc.³⁹ cfDNA, cell-free DNA; CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating tumor DNA.

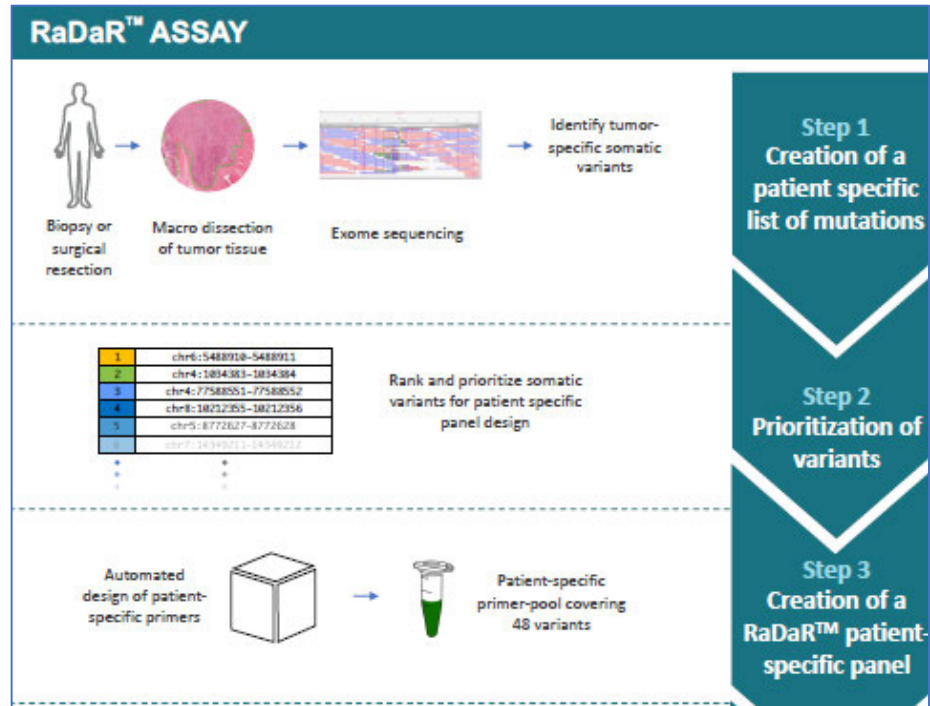
68. In the Marsico (2020) study, Defendant discloses that RaDaR™ includes amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume.⁶⁵ Defendant also

⁶³ *Id.* (emphasis added).

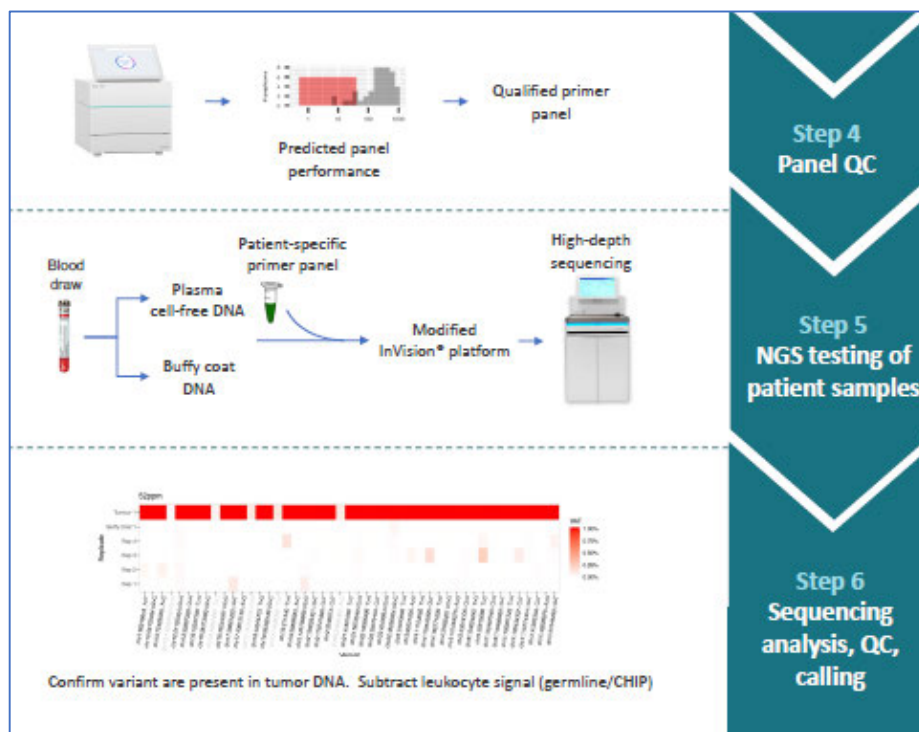
⁶⁴ See Lipsyc-Sharf (2022) at Appx. 1; see also Heider (2020); Marsico (2020); Cutts (2021); Flach (2022) at 1187, Figure 1; Russell (2023).

⁶⁵ See Marsico (2020).

explains its workflow as “multiplex PCR based on the InVision® platform” to amplify a patient-specific primer-pool covering 48 variants in the same reaction.⁶⁶



⁶⁶ *Id.*



- (d) Claim 1[c]: “sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.”

69. The Accused Assay satisfies this claim limitation. For example, RaDaR™ sequences the amplicons to obtain sequence reads and detects one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus. According to Defendant, “RaDaR is a personalized next generation sequencing (NGS) assay.”⁶⁷ The Defendant further explains that “[m]ultiplex polymerase chain reaction (PCR) and NGS were used to assess tumor variants in cfDNA[,]” and that “[a]fter multiplex PCR, reaction-specific index

⁶⁷ See Lipsyc-Sharf (2022) at Appx. 1.

barcode sequences were added to the amplified sequences using the NovaSeq 6000 system (Illumina Inc.).”⁶⁸ Defendant uses the figure below to demonstrate that the RaDaR™ assay uses high-depth sequencing to confirm variants in tumor DNA obtained from the plasma cell-free DNA.⁶⁹

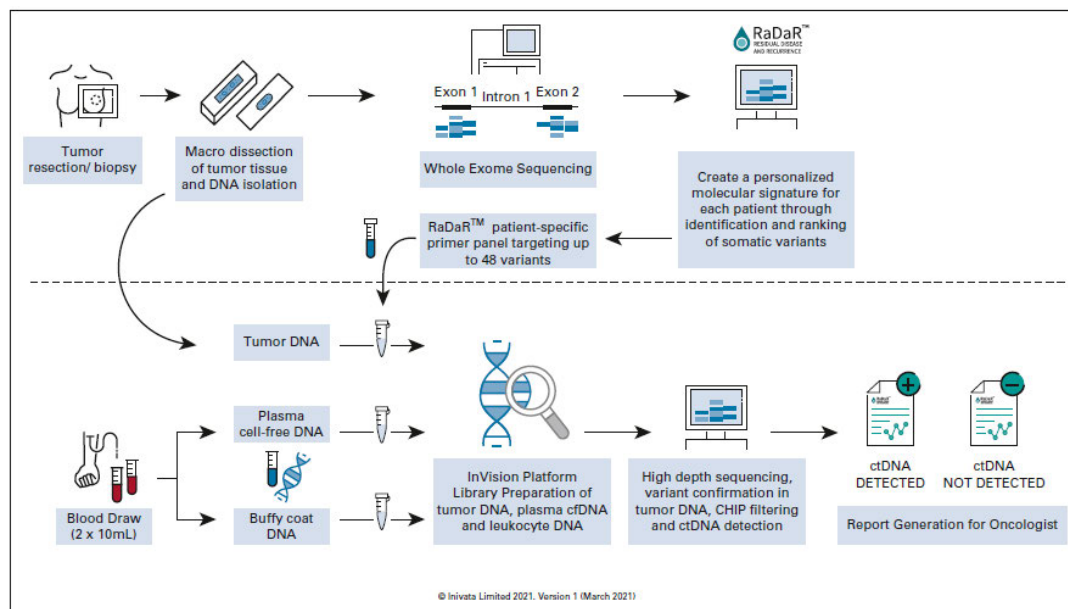


FIG A1. RaDaR assay workflow. Used with permission from Inivata, Inc.³⁸ cfDNA, cell-free DNA; CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating tumor DNA.

70. Defendant explains that “[w]e have developed the InVision™ liquid biopsy platform which utilizes enhanced TAm-Seq™ (eTAm-Seq™) technology, an amplicon-based next generation sequencing method for the identification of clinically-relevant somatic alterations at low frequency in ctDNA across a panel of 35 cancer-related genes.”⁷⁰ Defendant further asserts that the sequencing method of RaDaR™ uses “*Illumina*

⁶⁸ *Id.*

⁶⁹ *Id.*

⁷⁰ See Gale (2018) at 1.

NovaSeq flow cell with read depth > 100,000 reads per locus.”⁷¹ One of ordinary skill in the art would have understood that a read depth > 100,000 reads per locus would meet the claim limitation of a “depth of read of at least 50,000 per target locus.”

71. Cutts (2021) showed that as part of RaDaR™ assay workflow, “[c]ell free DNA was extracted from 147 plasma samples (median volume 4ml, range 0.5-5ml) and **sequenced with RaDaR assays, with 10-61 variants (median 41) per panel, to 100,000x per locus.**”⁷² One of ordinary skill in the art would have understood that sequencing to 100,000x per locus would meet the claim limitation of a “depth of read of at least 50,000 per target locus.”

2. Claim 2 of the ’454 Patent: *“The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.”*

72. The Accused Assay satisfies this claim limitation. For example, RaDaR™ includes cell-free DNA that comprises circulating tumor DNA. According to Defendant, “RaDaR sequencing assays monitor up to 48 tumor-specific mutations, unique to each patient’s tumor, to help detect **circulating tumor DNA (ctDNA).**”⁷³

3. Claims 3 of the ’454 Patent: *“The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.”*

73. The Accused Assay satisfies this claim limitation. RaDaR includes SNV mutations that comprise one or more clonal SNV mutations. Defendant have used RaDaR

⁷¹ See Marsico (2020) (emphasis added).

⁷² See Cutts (2021) (emphasis added).

⁷³ See <https://finditwithradar.com/> (emphasis added) (last visited June 23, 2023).

to track “clonal evolution in the MRD Setting with ctDNA.”⁷⁴ For example, Cutts (2021) showed that “[i]n 8/14 patients with multiple tumor samples sequenced, multiple clones (mean 3.4 clones/patient) were identified, with heterogeneous polyclonal relapse in 4/8 patients, and a single clone detectable in 4/8 patients.”⁷⁵ Cutts (2021) also reported for patient INVT-019 that the “ctDNA analysis shows persistent AR1D1 mutation” and for patient INVT-020 that the “ctDNA analysis shows multiple clusters in MRD, some below cut-off levels, present on relapse tissue sample.”⁷⁶ One of ordinary skill in the art would have understood that the data presented in Cutts (2021) would meet the claim limitation of “SNV mutations comprise one or more clonal SNV mutations.”

4. Claim 4 of the '454 Patent: *“The method of claim 1, wherein the SNV mutations comprise one or more subclonal SNV mutations.”*

74. The Accused Assay satisfies this claim limitation. RaDaR includes SNV mutations that comprise one or more subclonal SNV mutations. For example, Cutts (2021) reported for patient INVT-007 that the “ctDNA analysis shows clusters only found in the right breast sample.”⁷⁷ Flach (2022) also reported one or more subclonal SNV mutations in patient 14 from two tumor resections.⁷⁸ One of ordinary skill in the art would have

⁷⁴ See Cutts (2021).

⁷⁵ *Id.*

⁷⁶ *Id.*

⁷⁷ See Cutts (2021)

⁷⁸ See Flach (2022) at 1187-1188, Figure 4a.

understood that the data presented in Cutts (2021) and Flach (2022) would meet the claim limitation of “SNV mutations comprise one or more subclonal SNV mutations.”

5. Claim 5 of the '454 Patent: “*The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.*”

75. The Accused Assay satisfies this claim limitation. RaDaR includes SNV mutations that comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. Defendant have used RaDaR to track “clonal evolution in the MRD Setting with ctDNA.”⁷⁹ Cutts (2021) also showed that “[i]n 8/14 patients with multiple tumor samples sequenced, **multiple clones** (mean 3.4 clones/patient) were identified, with **heterogeneous polyclonal** relapse in 4/8 patients, and a single clone detectable in 4/8 patients”⁸⁰. Also, as described *supra* for Claims 3 and 4 of the '454 Patent, Cutts (2021) describes SNV mutations for several patients that comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.

6. Claim 6 of the '454 Patent: “*The method of claim 1, wherein the tumor sample of the subject is a tumor tissue sample.*”

76. The Accused Assay satisfies this claim limitation. RaDaR™ includes a tumor sample of the subject that is a tumor tissue sample.

77. For example, Flach (2022) explains that the tumor sample is a tumor tissue sample:

⁷⁹ See Cutts (2021)

⁸⁰ *Id.* (emphasis added).

RaDaR™ is a personalised ctDNA assay built on the InVision® platform [43], which utilises multiplex PCR and targeted NGS. ***Somatic variants identified in the tumour tissue by WES were prioritised using proprietary algorithms to build a patient-specific primer panel of up to 48 primer pairs capturing at least one somatic variant*** (Supplementary Table 2). The personalised primer panel was manufactured (IDT, Coralville, IA) and combined with a fixed primer panel of 21 common population-specific SNPs for quality control purposes during the NGS testing.⁸¹

7. Claim 8 of the '454 Patent: *“The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.”*

78. The Accused Assay satisfies this claim limitation. RaDaR™ includes targeted multiplex amplification that amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. RaDaR™ is a personalized assay that ***tracks a set of up to 48 tumor-specific variants*** in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.”⁸²

79. For example, Flach (2022) explains up to 48 targets containing tumor-specific somatic SNVs are amplified by targeted multiplex amplification:

RaDaR™ is a personalised ctDNA assay built on the InVision® platform [43], which utilises multiplex PCR and targeted NGS. ***Somatic variants***

⁸¹ See Flach (2022) at 1187 (emphasis added).

⁸² See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (emphasis added) (last visited June 23, 2023).

identified in the tumour tissue by WES were prioritised using proprietary algorithms to build a patient-specific primer panel of up to 48 primer pairs capturing at least one somatic variant (Supplementary Table 2). The personalised primer panel was manufactured (IDT, Coralville, IA) and combined with a fixed primer panel of 21 common population-specific SNPs for quality control purposes during the NGS testing.⁸³

8. Claim 10 of the '454 Patent: *“The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.”*

80. The Accused Assay satisfies this claim limitation. RaDaR™ includes designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. For example, Flach (2022) explains designing PCR primers to target a plurality of SNV mutations identified in the tumor sample:

RaDaR™ is a personalised ctDNA assay built on the InVision® platform [43], which utilises multiplex PCR and targeted NGS. Somatic variants identified in the tumour tissue by WES were prioritised using proprietary algorithms to build a **patient-specific primer panel of up to 48 primer pairs capturing at least one somatic variant (Supplementary Table 2). The personalised primer panel was manufactured (IDT, Coralville, IA) and combined with a fixed primer panel of 21 common population-specific SNPs for quality control purposes during the NGS testing.**⁸⁴

9. Claim 11 of the '454 Patent: *“The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.”*

81. The Accused Assay satisfies this claim limitation. RaDaR™ includes performing barcoding PCR prior to the sequencing. For example, Defendant explains that

⁸³ See Flach (2022) at 1187 (emphasis added).

⁸⁴ *Id.* (emphasis added).

“RaDaR is a personalized next generation sequencing (NGS) assay.”⁸⁵ The Defendant further explains that “[m]ultiplex polymerase chain reaction (PCR) and NGS were used to assess tumor variants in cfDNA[,]” and that “[a]fter multiplex PCR, reaction-specific index barcode sequences were added to the amplified sequences using the NovaSeq 6000 system (Illumina Inc.).”⁸⁶

82. Defendant describes the InVision liquid biopsy platform technology (used in the RaDaR assay), which includes performing barcoding PCR prior to the sequencing.⁸⁷ According to Defendant, Gale (2018) uses the eTAm-seq technology that employs barcoding PCR:

The development of next generation sequencing (NGS) has allowed for a broader application of ctDNA analysis. In 2012, Forshew et al. developed TAm-Seq technology, or tagged amplicon deep sequencing which, for the first time, enabled interrogation of 6 genes across a large genomic region spanning 5995 bases to detect low frequency mutations in cell-free DNA...Since this time, other NGS technologies have been implemented for analysis of ctDNA, including the use of hybrid capture and the introduction of panel assays that use molecular barcodes to enable error suppression...***Here we describe the development of the InVision liquid biopsy platform which utilizes enhanced TAm-Seq (eTAm-SeqTM) technology*** for the identification of low frequency mutations in ctDNA. The assay has been expanded to target hotspots and entire coding regions from 35 cancer-related genes, utilizing a primer design strategy that allows for amplification of highly fragmented DNA, typical of ctDNA. The calling algorithm has been revised, and in addition to improved detection of single

⁸⁵ See Lipsyc-Sharf (2022) at Appx. 1.

⁸⁶ *Id.*

⁸⁷ See Gale (2018).

nucleotide variants (SNVs) and short insertions/deletions (indels), it also identifies copy number variants (CNVs).⁸⁸

Library preparation using eTAm-Seq technology. eTAm-Seq technology is based on methods previously described [22, 25,26], with an optimized assay workflow utilizing multiplex PCR to enable high-throughput library preparation without the use of microfluidics. Next generation sequencing libraries were prepared using a ***two-step multiplex PCR amplification process incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors***.⁸⁹

10. Claim 12 of the '454 Patent: "*The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.*"

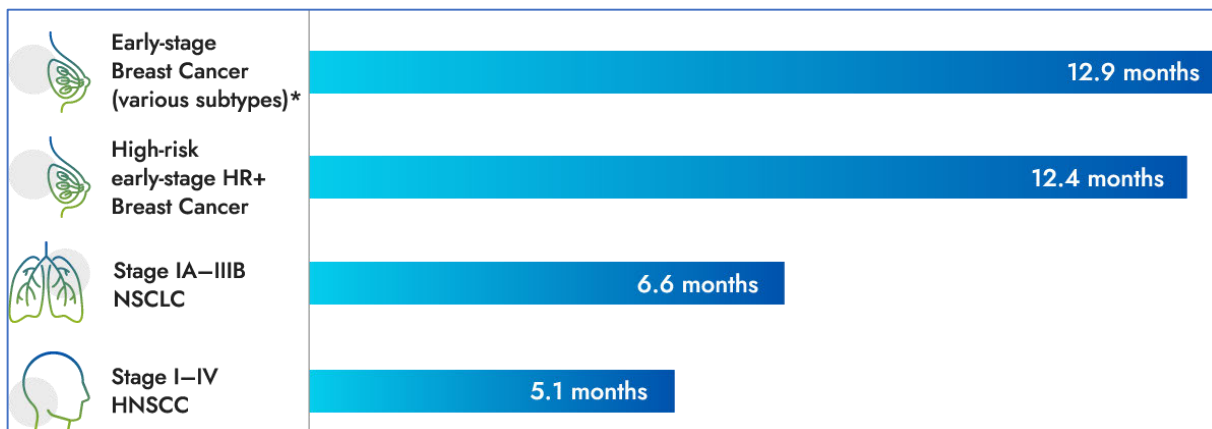
83. The Accused Assay satisfies this claim limitation. RaDaR™ includes detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. According to Defendant, "[t]he RaDaR™ assay is built on Inivata's proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both ***detection of residual disease*** following curative intent or definitive treatment and early detection of relapse."⁹⁰ Defendant uses the figure *infra* to explain that "[h]igh sensitivity ***enables longer lead times*** ahead of standard-of-care monitoring tools, such as

⁸⁸ *Id.* at 4 (emphasis added).

⁸⁹ *Id.* (emphasis added).

⁹⁰ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (emphasis added) (last visited June 23, 2023).

radiographic imaging. RaDaR’s high sensitivity allows for earlier identification of residual disease and opens up the possibility of intervention prior to overt metastatic disease.”⁹¹



11. Claim 13 of the ’454 Patent: *“The method of claim 1, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.”*

84. The Accused Assay satisfies this claim limitation. RaDaR™ includes assaying cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. According to Defendant, “RaDaR MRD testing is validated for use in multiple solid tumor cancers,” including “breast cancer, colorectal cancer, lung cancer, and head and neck cancer.”⁹²

⁹¹ See <https://finditwithradar.com/why-radar> (emphasis added) (last visited June 23, 2023).

⁹² See <https://finditwithradar.com/> (last visited June 23, 2023).

B. '035 Patent

1. Claim 1 of the '035 Patent is recited *infra*:

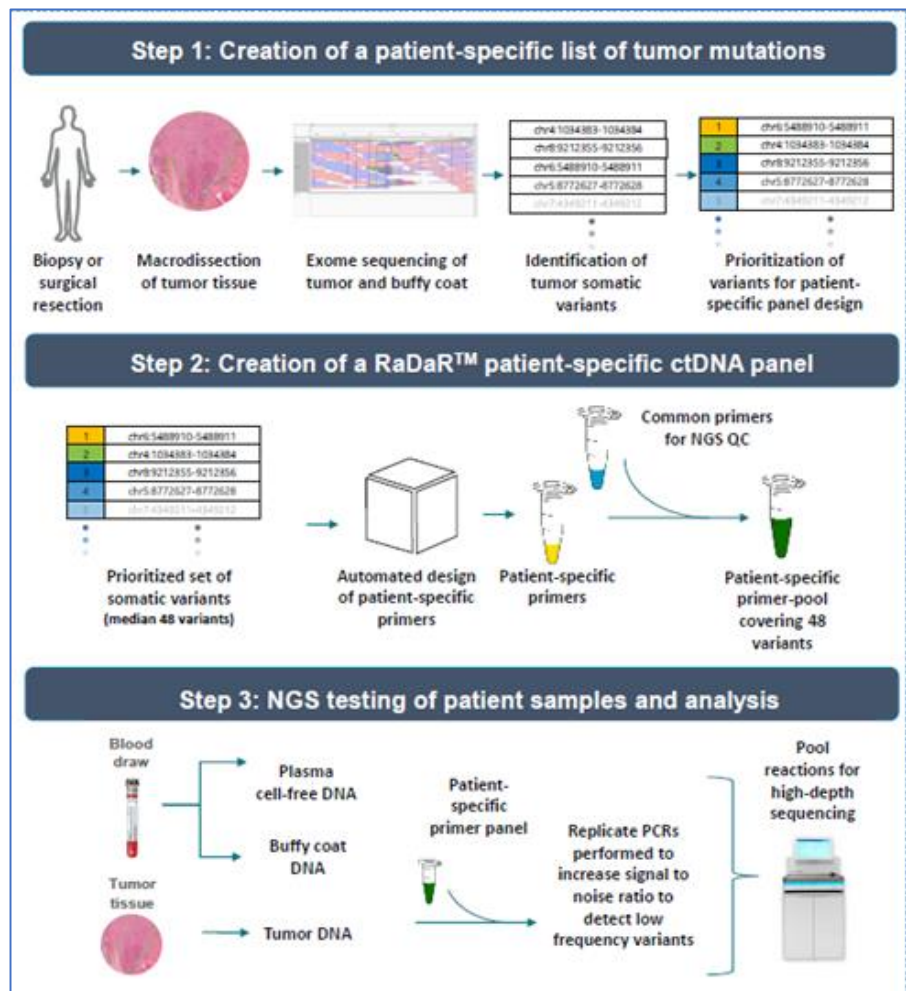
- (a) Claim 1[preamble]: “A method for amplifying and sequencing DNA, comprising:”

85. To the extent the preamble is considered a claim limitation, the preamble is met by the Accused Assay. For example, the RaDaR™ assay comprises methods for amplifying and sequencing DNA. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.”⁹³ Defendant also explains that “RaDaR™ is a highly sensitive personalised sequencing ctDNA assay. Tumor-specific variants are first identified by exome sequencing of tumor tissue, followed by multiplex PCR and high-depth next generation sequencing to track low levels of ctDNA in patient plasma.”⁹⁴ Defendant has used the figure *infra* to demonstrate that RaDaR™ includes the steps of amplifying and sequencing DNA.⁹⁵

⁹³ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (last visited June 23, 2023).

⁹⁴ See Heider (2020).

⁹⁵ *Id.*



86. In the study by Lipsyc-Sharf (2022), Defendant also included the figure reproduced *infra* showing the RaDaR assay includes amplifying and sequencing DNA.⁹⁶

⁹⁶ See Lipsyc-Sharf (2022) at Appx. 1.

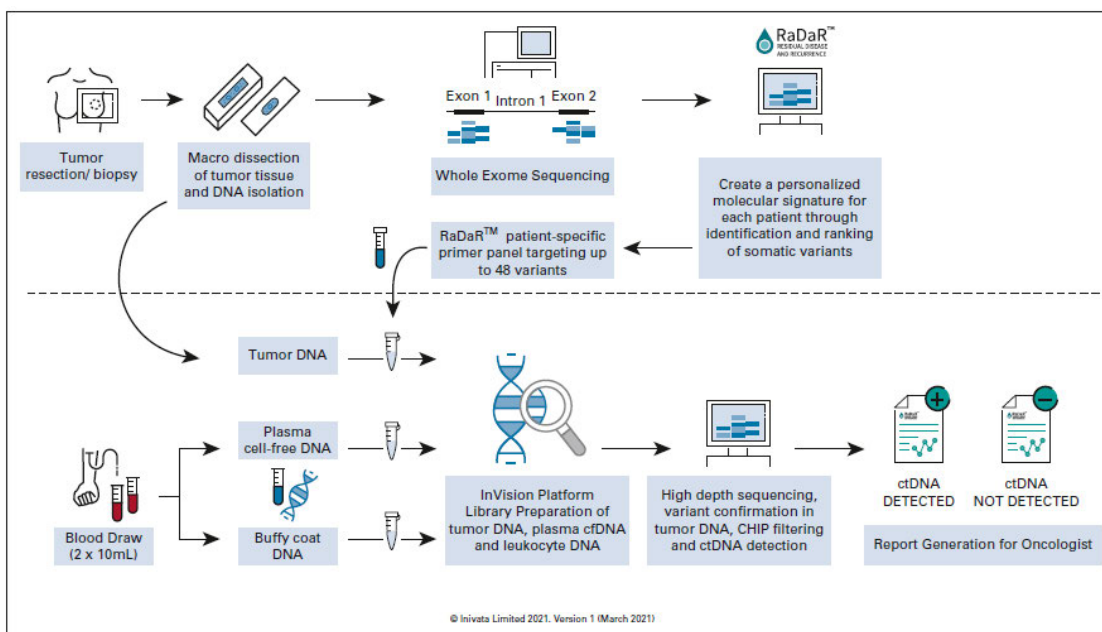


FIG A1. RaDaR assay workflow. Used with permission from Inivata, Inc.³⁸ cfDNA, cell-free DNA; CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating tumor DNA.

- (b) Claim 1[a]: “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant wom[a]n;”

87. The Accused Assay satisfies this claim limitation. The RaDaR assay includes tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early

detection of relapse.”⁹⁷ Gale (2018) described the InVision liquid biopsy platform technology (used in the RaDaR™ assay), which includes tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products.⁹⁸ According to Defendant, Gale (2018) uses the eTAm-seq technology that employs barcoding PCR:

The development of next generation sequencing (NGS) has allowed for a broader application of ctDNA analysis. In 2012, Forsheu et al. developed TAm-Seq technology, or tagged amplicon deep sequencing which, for the first time, enabled interrogation of 6 genes across a large genomic region spanning 5995 bases to detect low frequency mutations in cell-free DNA...Since this time, other NGS technologies have been implemented for analysis of ctDNA, including the use of hybrid capture and the introduction of panel assays that use molecular barcodes to enable error suppression...***Here we describe the development of the InVision liquid biopsy platform which utilizes enhanced TAm-Seq (eTAm-Seq™)*** technology for the identification of low frequency mutations in ctDNA. The assay has been expanded to target hotspots and entire coding regions from 35 cancer-related genes, utilizing a primer design strategy that allows for amplification of highly fragmented DNA, typical of ctDNA. The calling algorithm has been revised, and in addition to improved detection of single nucleotide variants (SNVs) and short insertions/deletions (indels), it also identifies copy number variants (CNVs).⁹⁹

Library preparation using eTAm-Seq technology. eTAm-Seq technology is based on methods previously described [22, 25,26], with an optimized assay workflow utilizing multiplex PCR to enable high-throughput library preparation without the use of microfluidics. Next generation sequencing libraries were prepared using a ***two-step multiplex PCR amplification process incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors.***¹⁰⁰

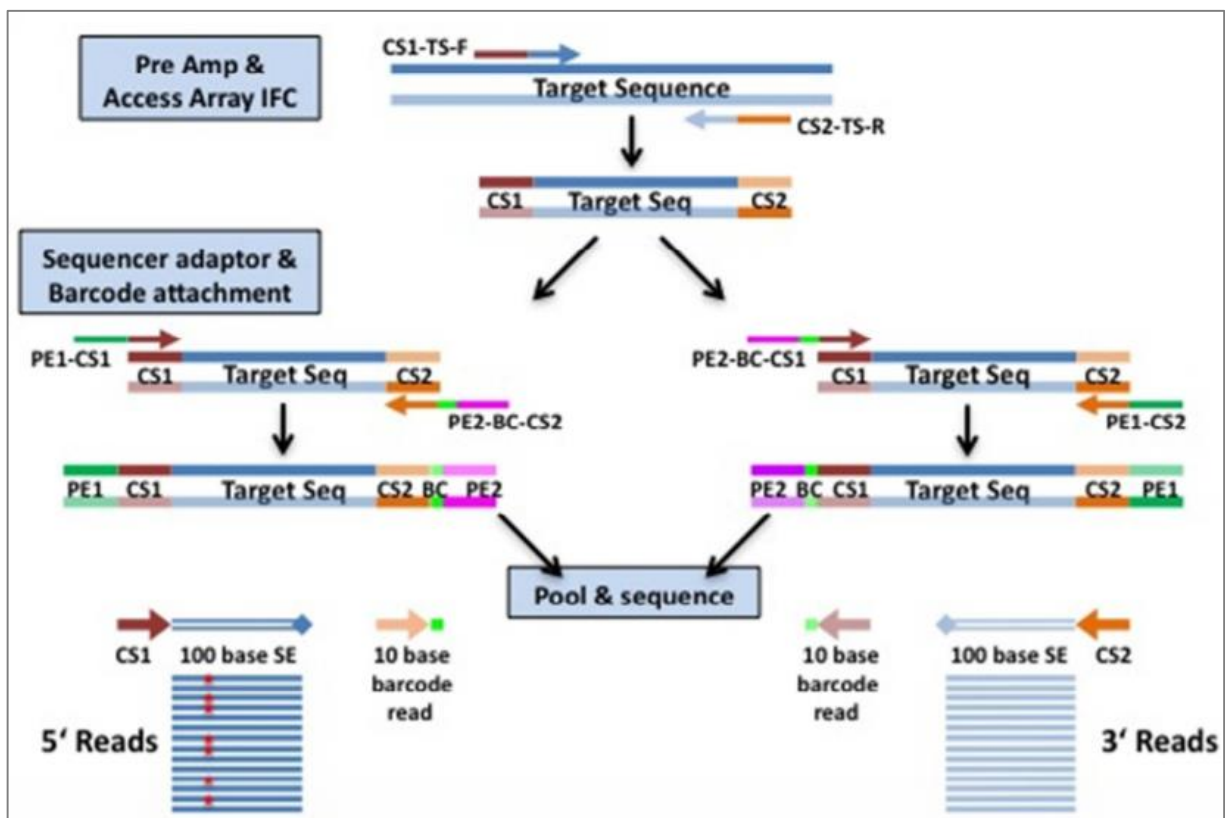
⁹⁷ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (last visited June 23, 2023).

⁹⁸ See Gale (2018).

⁹⁹ *Id.* at 3 (emphasis added).

¹⁰⁰ *Id.* at 4 (emphasis added).

88. Forshew (2012) illustrates that common tail sequences (*i.e.*, CS1 and CS2) are added to each end of a target sequence in a pre-amplification step via PCR to generate a tagged product, as reproduced in the figure *infra*.¹⁰¹ One of ordinary skill in the art would have understood that an adaptor with a common tail sequence is a universal tail adaptor.



89. Forshew (2012) summarizes its PCR strategy and primer design as follows.

Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal

¹⁰¹ See Forshew (2012) at Figure S1 (annotated).

tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced.¹⁰²

90. The RaDaR™ assay also includes isolating cell-free DNA from a blood sample. In the Lipsyc-Sharf (2022) study, Defendant describes the method as follows:

Blood samples were collected prospectively, spun, and stored at –80°C as plasma and buffy coat. Four milliliter of plasma and 2 mL of buffy coat samples were sent in batches to Inivata Inc. ***DNA was extracted from buffy coat,*** and circulating cell-free DNA was extracted from plasma as previously described. . . RaDaR assays were applied retrospectively in a research setting to identify plasma ctDNA (Appendix 1 and Appendix Figs A1 and A2, online only).¹⁰³

91. The workflow reproduced *infra* shows that RaDaR™ includes a blood draw, thereby isolating cell-free DNA from a blood sample.

¹⁰² See Forshew (2012), Supplementary Material at 5 (emphasis added).

¹⁰³ See Lipsyc-Sharf (2022) at 2401 (emphasis added).

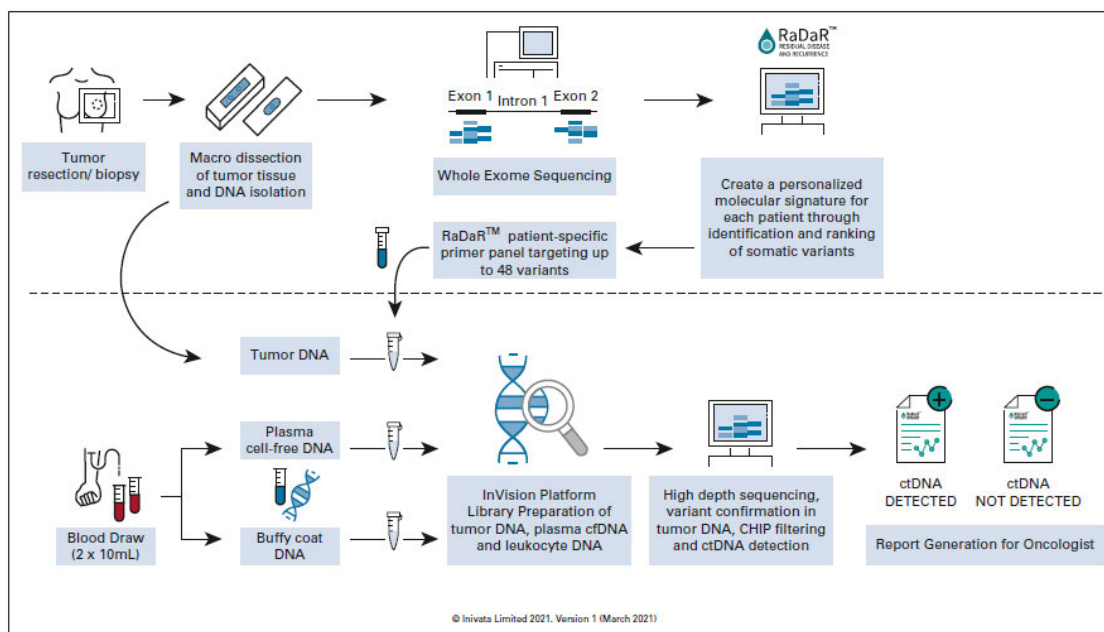
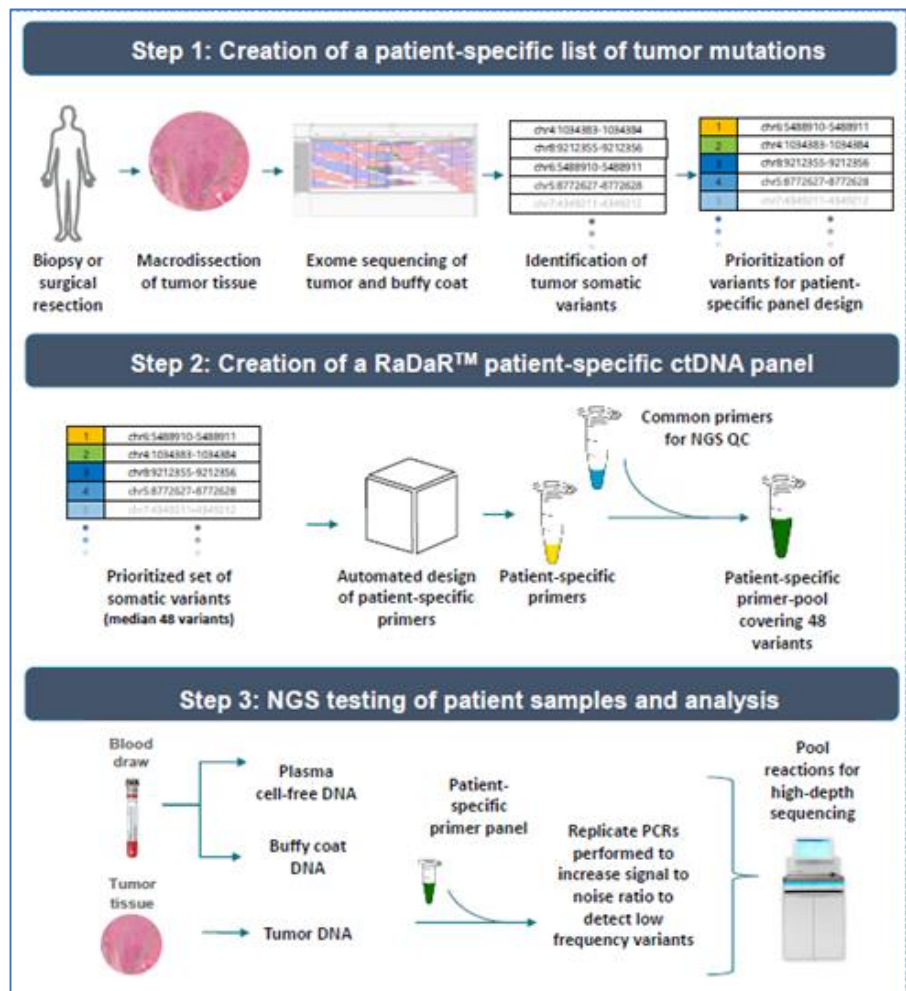


FIG A1. RaDaR assay workflow. Used with permission from Inivata, Inc.³⁸ cfDNA, cell-free DNA; CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating tumor DNA.

92. In the Heider (2020) study, Defendant explains that “RaDaR™ is a highly sensitive personalised sequencing ctDNA assay. Tumor-specific variants are first identified by exome sequencing of tumor tissue, followed by multiplex PCR and high-depth next generation sequencing to track low levels of ctDNA in patient plasma.”¹⁰⁴ The figure reproduced *infra* demonstrates that RaDaR™ includes a blood draw, thereby isolating cell-free DNA from a blood sample:¹⁰⁵

¹⁰⁴ See Heider (2020).

¹⁰⁵ *Id.*



93. Defendant's RaDaR™ assay also includes cell-free DNA isolated from a blood sample collected from a subject who is not a pregnant woman. According to Defendant, "RaDaR is used for patients with breast, colorectal, lung, or head and neck cancers. Following either surgery, chemotherapy or chemoradiation, RaDaR may be used as a tool to check for residual cancer or as a tool to monitor for recurrence over time."¹⁰⁶

¹⁰⁶ See Ex. 15, *NeoGenomics, RaDaR, Residual disease and recurrence, Understanding RaDaR, A guide to understanding minimal residual disease and recurrence* at 1.

As a contraindication, Defendant identifies that “[t]he RaDaR assay is not available to patients who are pregnant.”¹⁰⁷

- (c) Claim 1[b]: *“amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and”*

94. The Accused Assay satisfies this claim limitation. For example, the RaDaR™ assay includes amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. For example, Lipsyc-Sharf (2022) described the workflow associated with RaDaR as including a “[m]ultiplex PCR and [s]equencing” step.¹⁰⁸ Specifically, “[a]fter primer panel qualification and DNA extraction, RaDaR multiplex PCR was performed on cfDNA from plasma alongside a buffy coat DNA control sample[.]”¹⁰⁹

95. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific

¹⁰⁷ See Ex. 16, *NeoGenomics, RaDaR, Residual disease and recurrence, RaDaR assay and blood draw request form* at 2.

¹⁰⁸ See Lipsyc-Sharf (2022) at Appx. 1.

¹⁰⁹ *Id.*

variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.”¹¹⁰ In this case, a SNP (single nucleotide polymorphism) as used in the '035 Patent is synonymous with a SNV (single nucleotide variant).¹¹¹

96. Flach (2022) also demonstrated targeted amplification of a plurality of SNP loci:

RaDaRTM is a personalised ctDNA assay built on the InVision® platform [43], which utilises **multiplex PCR** and targeted NGS. Somatic variants identified in the tumour tissue by WES were prioritised using proprietary algorithms to build a patient-specific primer panel of **up to 48 primer pairs** capturing at least one somatic variant (Supplementary Table 2). The personalised primer panel was manufactured (IDT, Coralville, IA) and combined with a fixed primer panel of 21 common population-specific SNPs for quality control purposes during the NGS testing.¹¹²

Following primer panel qualification, RaDaRTM assays were performed on cfDNA from plasma aliquots alongside a buffy coat DNA control sample, which was used for identification and removal of germline variants, the removal of variants due to clonal haematopoiesis of indeterminate potential (CHIP) from the analysis, and as a positive amplification control. **Multiplex PCR** was performed with input concentrations ranging from 1452 to 20,000 amplifiable copies per sample, as measured by droplet digital PCR, with a median value of 14,550 copies.¹¹³

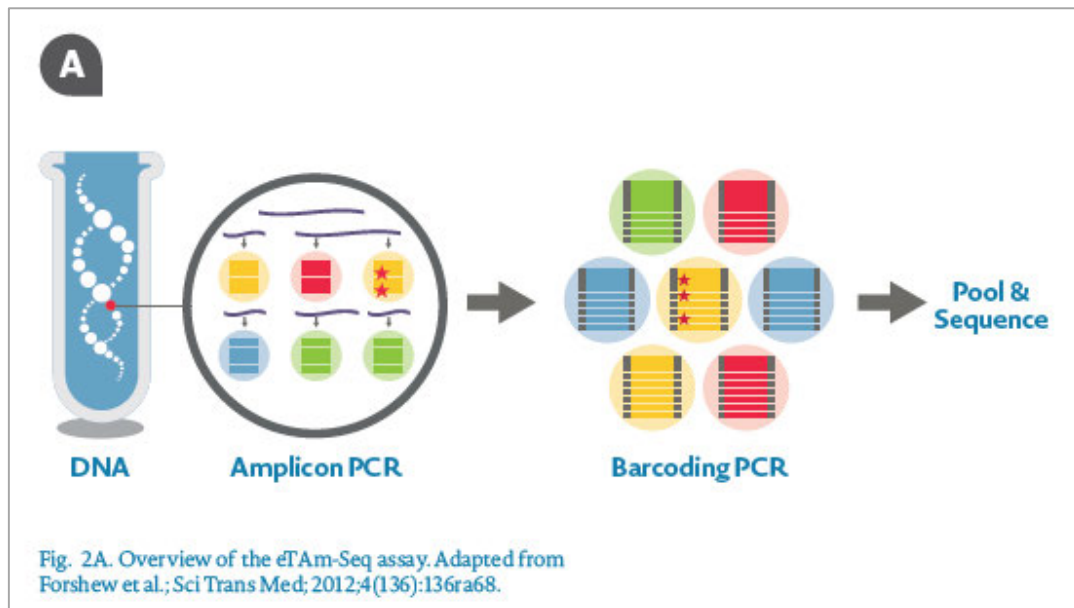
¹¹⁰ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (last visited June 23, 2023).

¹¹¹ See '035 Patent at 34:46-50.

¹¹² See Flach (2022) at 1187 (emphases added).

¹¹³ *Id.* (emphasis added).

97. Defendant uses the figure reproduced *infra* to demonstrate that the RaDaR™ assay uses multiplex PCR, which amplifies multiple targets in a single reaction volume and includes an amplification step to introduce barcodes.¹¹⁴

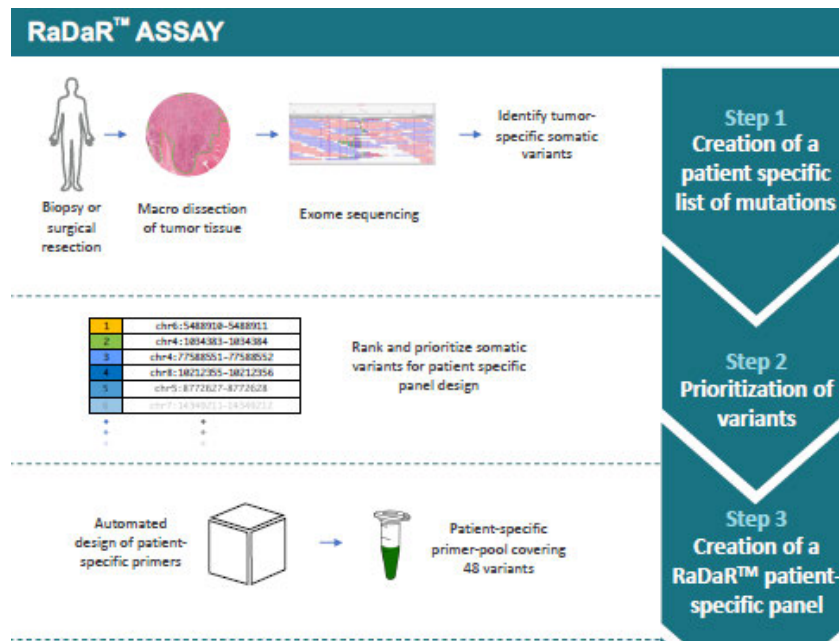


98. In the Marsico (2020) study, Defendant discloses that RaDaR includes amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume.¹¹⁵ Defendant includes a figure reproduced *infra* showing that targeted multiplex amplification occurs in the same reaction volume using a patient-specific primer-pool covering 48 variants:¹¹⁶

¹¹⁴ See Gale (2016).

¹¹⁵ See Marsico (2020).

¹¹⁶ *Id.*



99. Gale (2018) described the InVision liquid biopsy platform technology used in the RaDaR assay, which includes an amplifying step to introduces a barcode and one or more sequencing tags.¹¹⁷ According to Defendant, Gale (2018) uses the eTam-seq technology that employs barcoding PCR including adding sequencing tags:

The development of next generation sequencing (NGS) has allowed for a broader application of ctDNA analysis. In 2012, Forshew et al. developed Tam-Seq technology, or tagged amplicon deep sequencing which, for the first time, enabled interrogation of 6 genes across a large genomic region spanning 5995 bases to detect low frequency mutations in cell-free DNA...Since this time, other NGS technologies have been implemented for analysis of ctDNA, including the use of hybrid capture and the introduction of panel assays that use molecular barcodes to enable error suppression...*Here we describe the development of the InVision liquid biopsy platform which utilizes enhanced Tam-Seq (eTam-Seq™)* technology for the identification of low frequency mutations in ctDNA. The assay has been expanded to target hotspots and entire coding regions from 35 cancer-related genes, utilizing a primer design strategy that allows for amplification of highly fragmented DNA, typical of ctDNA. The calling

¹¹⁷ See Gale (2018).

algorithm has been revised, and in addition to improved detection of single nucleotide variants (SNVs) and short insertions/deletions (indels), it also identifies copy number variants (CNVs).¹¹⁸

Library preparation using eTAm-Seq technology. eTAm-Seq technology is based on methods previously described [22, 25,26], with an optimized assay workflow utilizing multiplex PCR to enable high-throughput library preparation without the use of microfluidics. ***Next generation sequencing libraries were prepared using a two-step multiplex PCR amplification process incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors.***¹¹⁹

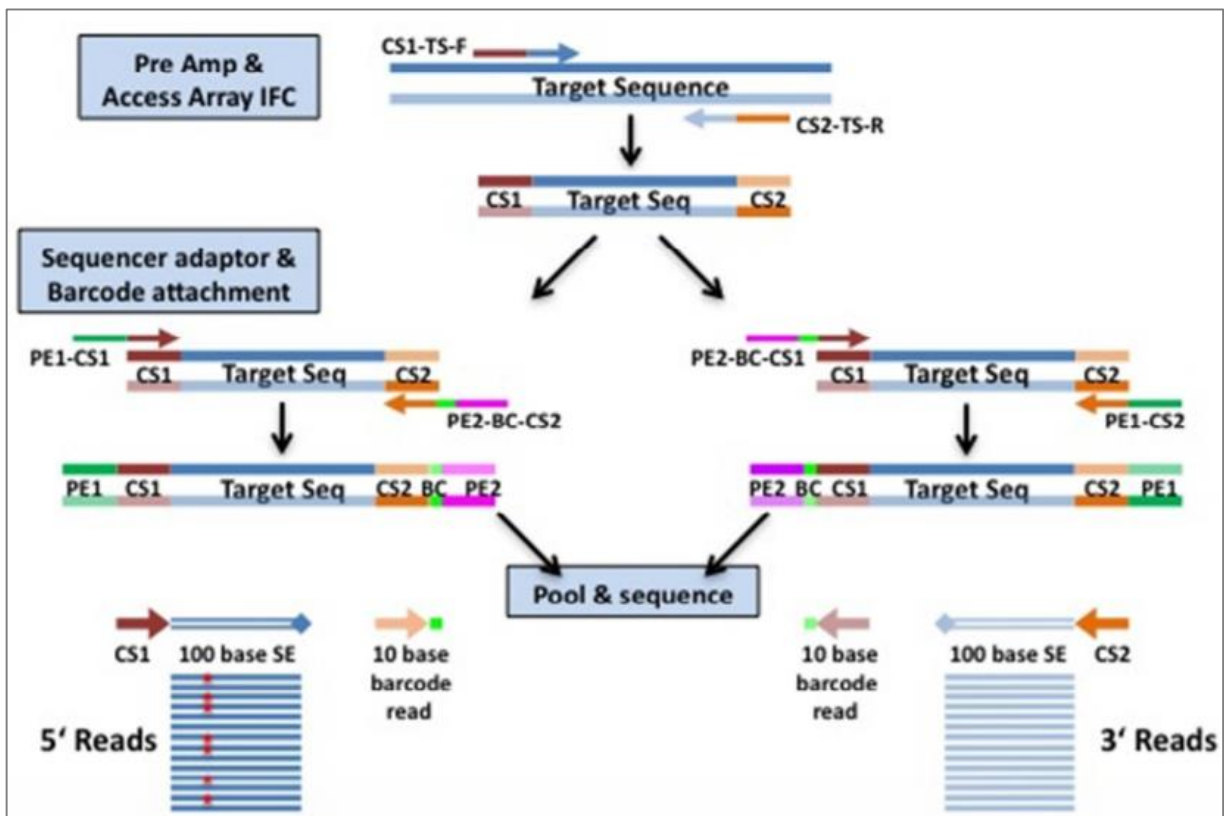
100. As shown in the “preamplification” stage in the figure *infra*,¹²⁰ Forshew (2012) illustrates in a first round of PCR amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. Moreover, as shown in “sequence adaptor & barcode attachment” stage of the same figure, Forshew (2012) illustrates in an additional round of PCR introducing a barcode and one or more sequencing tags.¹²¹

¹¹⁸ *Id.* at 3.

¹¹⁹ *Id.* at 4.

¹²⁰ *See* Forshew (2012) at Figure S1 (annotated).

¹²¹ *Id.*



101. Forshew (2012) summarizes its PCR strategy and primer design as follows.

Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. *Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR.* The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced.¹²²

¹²² See Forshew (2012) Supplementary Material at 5 (emphasis added).

- (d) Claim 1[c]: “sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.”

102. The Accused Assay satisfies this claim limitation. For example, the RaDaR™ assay includes sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer. Defendant explains that “[t]he RaDaR assay . . . tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity[.]”¹²³

103. In the Flach (2022) study, RaDaR™ is described as follows:

RaDaR™ is a personalised ctDNA assay built on the InVision® platform [43], which utilises multiplex PCR and **targeted NGS**. Somatic variants identified in the tumour tissue by WES [whole exome sequencing] were prioritised using proprietary algorithms to build a patient-specific primer panel of up to **48 primer pairs capturing at least one somatic variant** (Supplementary Table 2). The personalised primer panel was manufactured (IDT, Coralville, IA) and combined with a fixed primer panel of 21 common population-specific SNPs for quality control purposes during the NGS testing.¹²⁴

Following primer panel qualification, RaDaR™ assays were performed on cfDNA from plasma aliquots alongside a buffy coat DNA control sample, which was used for identification and removal of germline variants, the removal of variants due to clonal hematopoiesis of indeterminate potential (CHIP) from the analysis, and as a positive amplification control. **Multiplex PCR was performed** with input concentrations ranging from 1452 to 20,000

¹²³ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (last visited June 23, 2023).

¹²⁴ See Flach (2022) at 1187 (emphases added).

amplifiable copies per sample, as measured by droplet digital PCR, with a median value of 14,550 copies.¹²⁵

RaDaRTM libraries were sequenced using the Nova-Seq 6000 system (Illumina Inc., San Diego, USA) and sequencing data analysed in a multistep process: fastq files were demultiplexed using bcl2fastq, reads were then aligned using the *bwa mem* alignment software and processed using proprietary software to identify primer pairs and count mutant and reference bases.¹²⁶

104. Defendant uses the figure reproduced *infra* to demonstrate that the RaDaR assay includes sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.¹²⁷ Defendant explains that “RaDaR is a personalized next generation sequencing (NGS) assay.”¹²⁸ The Defendant further explains that “[m]ultiplex polymerase chain reaction (PCR) and NGS were used to assess tumor variants in cfDNA[,]” and that “[a]fter multiplex PCR, reaction-specific index barcode sequences were added to the amplified sequences using the NovaSeq 6000 system (Illumina Inc.).”¹²⁹

¹²⁵ *Id.* (emphasis added).

¹²⁶ *Id.* (emphasis added).

¹²⁷ See Lipsyc-Sharf (2022) at Appx. 1.

¹²⁸ *Id.*

¹²⁹ *Id.*

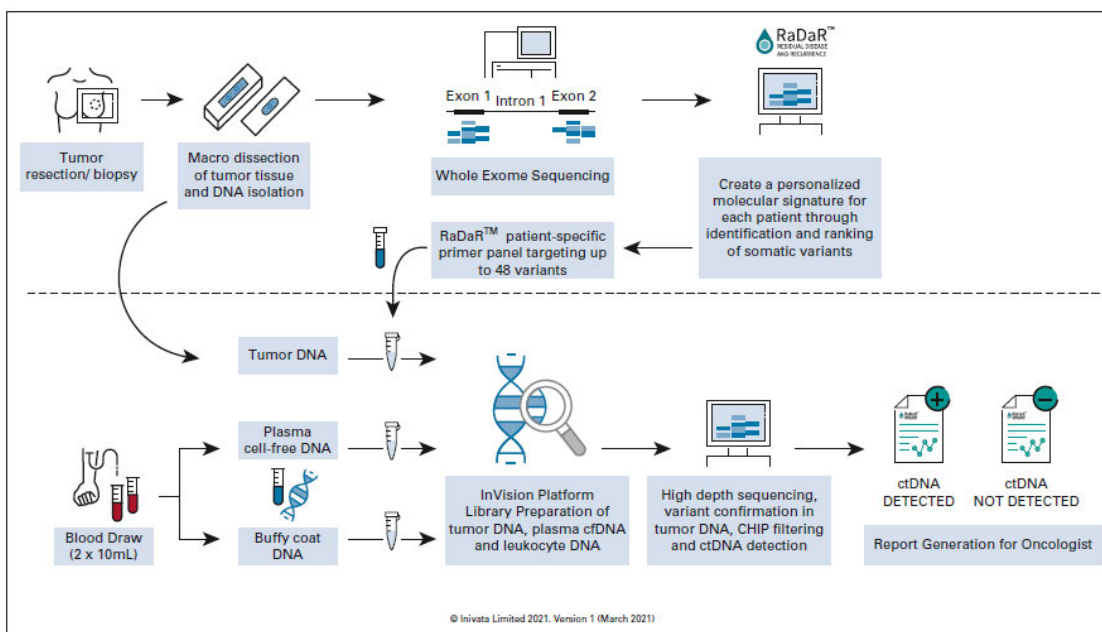


FIG A1. RaDaR assay workflow. Used with permission from Inivata, Inc.³⁸ cfDNA, cell-free DNA; CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating tumor DNA.

105. Gale (2018) described the InVision liquid biopsy platform technology used in the RaDaR™ assay, which includes eTAm-Seq technology, to sequence a plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing.¹³⁰

Library preparation using eTAm-Seq technology. eTAm-Seq technology is based on methods previously described [22, 25,26], with an optimized assay workflow utilizing multiplex PCR to enable high-throughput library preparation without the use of microfluidics. Next generation sequencing libraries were prepared using a ***two-step multiplex PCR amplification process incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors.***¹³¹

106. Cutts (2021) uses the RaDaR™ assay workflow wherein Defendant further explains that “cell free DNA was extracted from 147 plasma samples (median volume 4ml,

¹³⁰ See Gale (2018) at 4.

¹³¹ *Id.* (emphasis added).

range 0.5-5ml) and sequenced with RaDaR assays, with 10-61 variants (median 41) per panel, to 100,000x per locus.”¹³²

2. Claim 12 of the '035 Patent: *“The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.”*

107. The Accused Assay satisfies this claim limitation. The RaDaR™ assay includes one or more universal tail adaptors that comprise a first universal tail adaptor and a second universal tail adaptor.

108. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.”¹³³ Gale (2018) described the InVision liquid biopsy platform technology (used in the RaDaR™ assay), which includes tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products.¹³⁴ According to Defendant, Gale (2018) uses the eTAm-seq technology that employs barcoding PCR using universal tailed adaptors:

¹³² See Cutts (2021).

¹³³ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (last visited June 23, 2023).

¹³⁴ See Gale (2018).

The development of next generation sequencing (NGS) has allowed for a broader application of ctDNA analysis. In 2012, Forshew et al. developed TAm-Seq technology, or tagged amplicon deep sequencing which, for the first time, enabled interrogation of 6 genes across a large genomic region spanning 5995 bases to detect low frequency mutations in cell-free DNA...Since this time, other NGS technologies have been implemented for analysis of ctDNA, including the use of hybrid capture and the introduction of panel assays that use molecular barcodes to enable error suppression...**Here we describe the development of the InVision liquid biopsy platform which utilizes enhanced TAm-Seq (eTAm-Seq™)** technology for the identification of low frequency mutations in ctDNA. The assay has been expanded to target hotspots and entire coding regions from 35 cancer-related genes, utilizing a primer design strategy that allows for amplification of highly fragmented DNA, typical of ctDNA. The calling algorithm has been revised, and in addition to improved detection of single nucleotide variants (SNVs) and short insertions/deletions (indels), it also identifies copy number variants (CNVs).¹³⁵

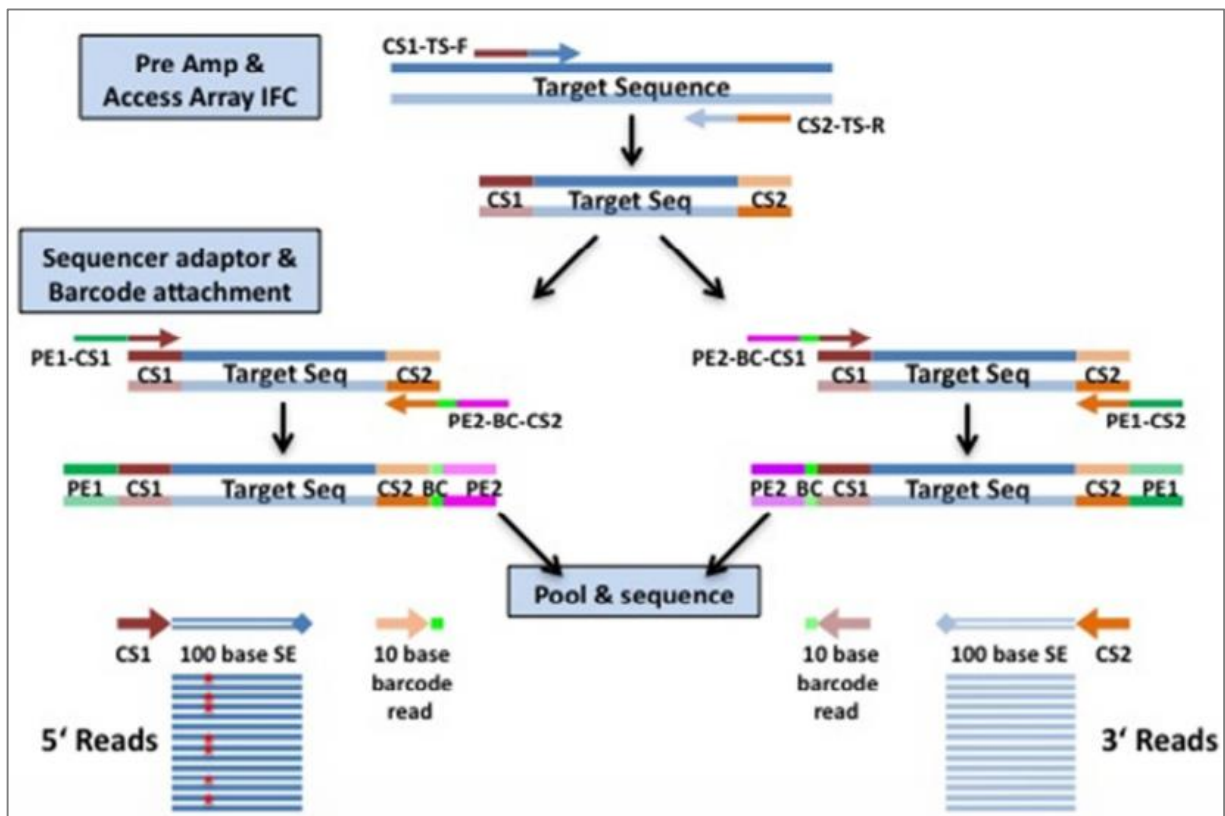
Library preparation using eTAm-Seq technology. eTAm-Seq technology is based on methods previously described [22, 25,26], with an optimized assay workflow utilizing multiplex PCR to enable high-throughput library preparation without the use of microfluidics. Next generation sequencing libraries were prepared using a **two-step multiplex PCR amplification process incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors**.¹³⁶

109. Forshew (2012) illustrates that one or more common adaptor sequences (*i.e.*, CS1 and CS2) are added to a target sequence in a pre-amplification step via PCR to generate a tagged product, as reproduced in the figure *infra*:¹³⁷

¹³⁵ *Id.* at 3 (emphasis added).

¹³⁶ *Id.* at 4 (emphasis added).

¹³⁷ See Forshew (2012) at Figure S1 (annotated).



110. Forshew (2012) summarizes its PCR strategy and primer design as follows.

Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2) . These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced.¹³⁸

¹³⁸ See Forshew (2012) Supplementary Material at 5 (emphasis added).

3. Claim 13 of the '035 Patent: *“The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.”*

111. The Accused Assay satisfies this claim limitation. The RaDaR™ assay includes tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.

112. According to Defendant, “[t]he RaDaR™ assay is built on Inivata’s proven InVision® liquid biopsy platform technology. This next-generation sequencing platform incorporates built-in controls and error-correction for highly sensitive and specific variant detection. RaDaR™ is a personalized assay that tracks a set of up to 48 tumor-specific variants in a patient using a liquid biopsy with exceptional sensitivity, allowing both detection of residual disease following curative intent or definitive treatment and early detection of relapse.”¹³⁹ Gale (2018) described the InVision liquid biopsy platform technology (used in the RaDaR™ assay), which includes tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products.¹⁴⁰ According to Defendant, Gale (2018) uses the eTAm-seq technology that employs barcoding PCR using universal tailed adaptors:

The development of next generation sequencing (NGS) has allowed for a broader application of ctDNA analysis. In 2012, Forsheew et al. developed

¹³⁹ See <https://www.inivata.com/radar-residual-disease-and-recurrence/> (last visited June 23, 2023).

¹⁴⁰ See Gale (2018).

TAm-Seq technology, or tagged amplicon deep sequencing which, for the first time, enabled interrogation of 6 genes across a large genomic region spanning 5995 bases to detect low frequency mutations in cell-free DNA...Since this time, other NGS technologies have been implemented for analysis of ctDNA, including the use of hybrid capture and the introduction of panel assays that use molecular barcodes to enable error suppression...**Here we describe the development of the InVision liquid biopsy platform which utilizes enhanced TAm-Seq (eTAm-Seq™)** technology for the identification of low frequency mutations in ctDNA. The assay has been expanded to target hotspots and entire coding regions from 35 cancer-related genes, utilizing a primer design strategy that allows for amplification of highly fragmented DNA, typical of ctDNA. The calling algorithm has been revised, and in addition to improved detection of single nucleotide variants (SNVs) and short insertions/deletions (indels), it also identifies copy number variants (CNVs).¹⁴¹

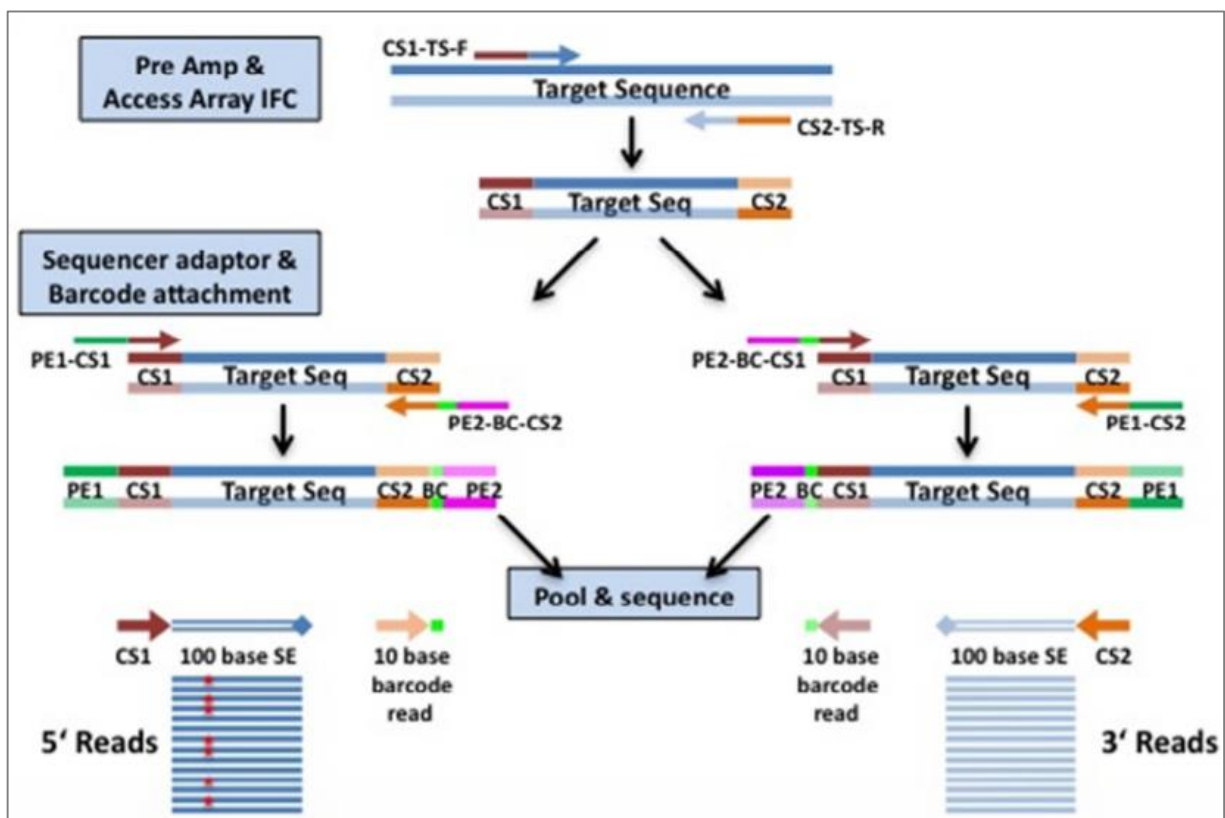
Library preparation using eTAm-Seq technology. eTAm-Seq technology is based on methods previously described [22, 25,26], with an optimized assay workflow utilizing multiplex PCR to enable high-throughput library preparation without the use of microfluidics. Next generation sequencing libraries were prepared using a **two-step multiplex PCR amplification process incorporating replicate and patient-specific barcodes and Illumina sequencing adaptors**.¹⁴²

113. Forshew (2012) illustrates that one or more common adaptor sequences (*i.e.*, CS1 and CS2) are added to a target sequence in a pre-amplification step via PCR to generate a tagged product, as reproduced in the figure *infra*:¹⁴³

¹⁴¹ *Id.* at 3 (emphasis added).

¹⁴² *Id.* at 4 (emphasis added).

¹⁴³ See Forshew (2012) at Figure S1 (annotated).



114. Forshew (2012) summarizes its PCR strategy and primer design as follows.

Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced.¹⁴⁴

¹⁴⁴ See Forshew (2012) Supplementary Material at 5 (emphasis added).

IX. NATERA'S SIGNATERA™ ASSAY

115. Signatera™ Molecular Residual Disease (MRD) Assay developed by Natera detects MRD and monitors for cancer recurrence using ctDNA.¹⁴⁵

116. According to Natera, Signatera™ is “[v]alidated to detect disease recurrence across multiple tumor types,” including breast cancer, colorectal cancer, lung cancer, and bladder cancer.¹⁴⁶ Natera summarizes the Signatera™ workflow as follows:

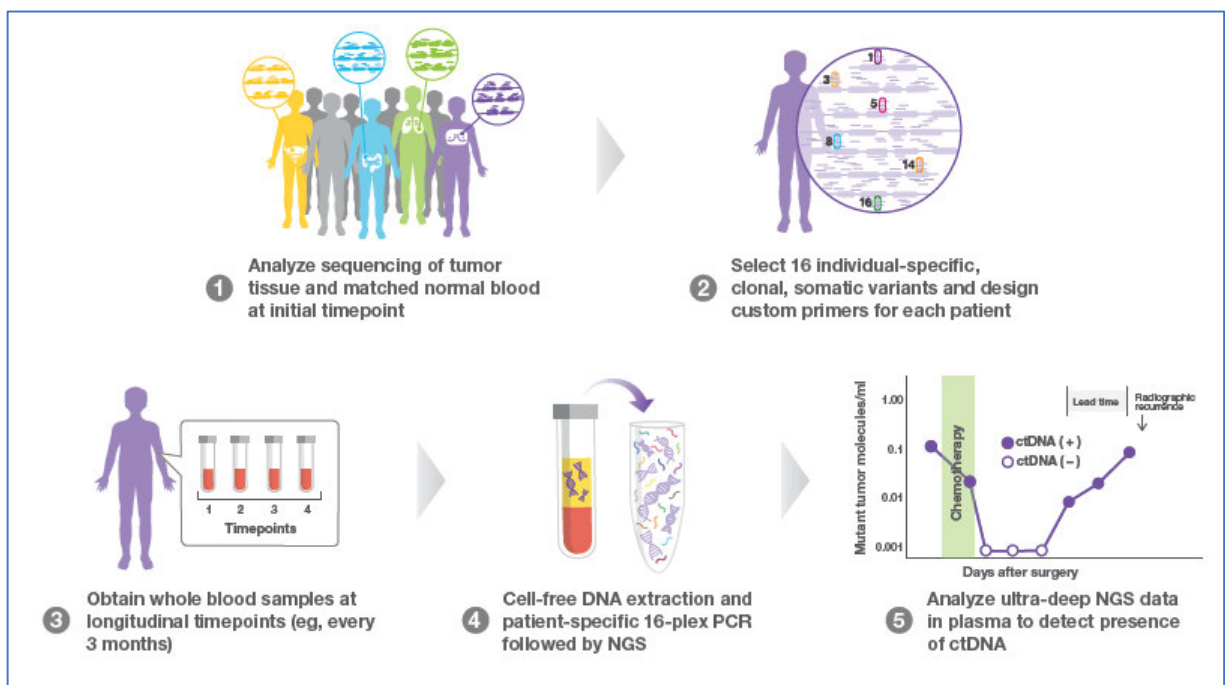
To monitor for cancer recurrence or to detect residual disease with ctDNA, the Signatera process starts with whole exome sequencing of the tumor tissue and the buffy coat from matched normal whole blood for each patient. Based on sequencing results, a list of somatic single-nucleotide and indel variants specific to each patient are bioinformatically identified and prioritized. Next, 16 somatic single-nucleotide and indel variants for multiplex PCR primer design are selected based on several factors, including the clonality,

¹⁴⁵ See Ex. 17, *Seeing beyond the limit: Detect residual disease and assess treatment response*, Signatera™ Residual disease test (MRD) (“Signatera White Paper”) at 2.

¹⁴⁶ See <https://www.natera.com/info/personalized-mrd-test/> (last visited July 4, 2023); Natera cites to five references in support of this statement: (i) Ex. 18, Reinert *et al.*, *Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer*, JAMA ONCOLOGY 5:1124-1131 (2019) (“Reinert (2019)”); (ii) Ex. 19, Coombes *et al.*, *Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence*, CLINICAL CANCER RESEARCH 25:4255–4263 (2019) (“Coombes (2019)”); (iii) Ex. 20, Abbosh *et al.*, *Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution*, NATURE 545:446-451 (2017) (“Abbosh (2017)”); (iv) Ex. 21, Christensen *et al.*, *Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma*, JOURNAL OF CLINICAL ONCOLOGY 37:1547-1557 (2019) (“Christensen (2019)”); (v) Kotaka *et al.*, *Association of circulating tumor DNA dynamics with clinical outcomes in the adjuvant setting for patients with colorectal cancer from an observational GALAXY study in CIRCULATE-Japan*, JOURNAL OF CLINICAL ONCOLOGY 40: Meeting Abstract, ASCO Gastrointestinal Cancers Symposium (2020) (“Kotaka (2020) Abstract”). This work is now published by Ex. 22, Kotani *et al.*, *Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer*, NATURE MEDICINE 29:127-134 (2023) (“Kotani (2023)”).

detectability, and frequency of the variants identified in the tumor tissue DNA. For longitudinal surveillance, cell free DNA (cfDNA) libraries are prepared from each blood sample, followed by patient-specific, 16-plex PCR. The amplicon products are tagged with sequencing barcodes and pooled for ultra-deep next generation sequencing, followed by data analysis to detect the presence or absence of ctDNA.¹⁴⁷

117. It is my understanding that Signatera™ has five steps, which are illustrated in the figure and subsequent description *infra*.¹⁴⁸



118. In the first step, “[p]rimary tumor tissue and matched normal blood are collected from each patient. Genomic DNA from tumor tissue and buffy coat are extracted, whole-exome sequenced, analyzed, and filtered for patient-specific somatic mutations.”¹⁴⁹

¹⁴⁷ See Signatera White Paper at 2.

¹⁴⁸ *Id.*

¹⁴⁹ *Id.*

119. In the second step, “[t]he top 16 somatic variants are selected based on clonality, detectability, and frequency of mutations. Multiple-PCR compatible primers are designed for each of the 16 somatic variants.”¹⁵⁰

120. In the third step, “[w]hole blood is collected at predefined time points for longitudinal surveillance.”¹⁵¹

121. In the fourth step, “[p]lasma are isolated and cfDNA extracted, followed by assaying with the patient-specific 16-plex PCR pool.”¹⁵²

122. In the fifth step, “[f]ollowing multiplex PCR amplification, ultradeep sequencing is performed. Next-generation sequencing data are analyzed to detect the presence of ctDNA.”¹⁵³

123. Signatera™ may be used in a clinical setting for various applications that include “predicting the likelihood of relapse at diagnosis, monitoring response to neoadjuvant treatment, detecting minimal residual disease, monitoring for recurrence after adjuvant treatment, or monitoring for treatment resistance.”¹⁵⁴ These applications are summarized in the table *infra*.¹⁵⁵

¹⁵⁰ *Id.*

¹⁵¹ *Id.*

¹⁵² *Id.*

¹⁵³ *Id.*

¹⁵⁴ *Id.* at 7.

¹⁵⁵ *Id.*

Applications	Signatera results	Implications	Trial designs	Example
Molecular residual disease after surgery	ctDNA-positive	Increased rate of relapse	Trials combining novel therapy and adjuvant therapy	Addition to SOC of anti-PD-1/PD-L1 vs placebo for NSCLC patients who are ctDNA-positive
	ctDNA-negative	Decreased rate of relapse	Trials to avoid or de-escalate treatment	
Recurrence monitoring	ctDNA-positive	Increased rate of relapse	Trials to increase surveillance during recurrence monitoring, showing correlation to outcomes	Imaging every 3 months vs 6 months in ctDNA-positive patients
Molecular relapse after adjuvant treatment	ctDNA-positive without evidence of clinical relapse	>98% certainty for clinical relapse without additional therapy	Trials to treat with additional therapy prior to clinical relapse	Extending duration of adjuvant therapy in patients who are persistently ctDNA-positive after finishing standard course of treatment
Treatment monitoring	ctDNA levels increasing or unchanged	Increased rate of therapy failure	Trials to correlate ctDNA-negative status after treatment with decrease in RFS or improvement in PFS	ctDNA to predict response to I-O therapy, distinguish pseudo-progression from true progression, and determine early trial readout
	ctDNA levels decreasing or undetectable	Decreased rate of therapy failure		

124. It is my opinion that Signatera™ practices the Asserted Natera Patents. For example, I explain *infra* how Signatera™ practices Claim 1 of the '454 Patent and Claim 1 of the '035 Patent.

1. Claim 1 of the '454 Patent is recited *infra*:

- (a) Claim 1[preamble]: “*A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:*”

125. To the extent the preamble is considered a claim limitation, the preamble is met by Signatera™. For example, Signatera™ includes a method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample. According to Natera, “Signatera™ is the first ctDNA assay that has been custom-built for detecting

molecular residual disease (MRD) and assessing treatment response.”¹⁵⁶ According to Natera, “Signatera has been analytically validated as a highly sensitive and specific platform for detecting and quantifying patient-specific, somatic SNVs and indels.”¹⁵⁷ In Signatera’s approach, “somatic variants are identified by whole–exome sequencing of the primary tumor and the matched normal (whole blood) sample. Following this, a bespoke assay of 16 clonal, somatic variants are generated for each patient. The resulting ‘tumor signature,’ individualized to each patient’s tumor, is monitored throughout the patient’s disease course to detect the presence of tumor DNA in the plasma.”¹⁵⁸

- (b) Claim 1[a]: “*performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;*”

126. Signatera™ satisfies this claim limitation. For example, in the first step of the Signatera™ assay, “[p]rimary tumor tissue and matched normal blood are collected from each patient. **Genomic DNA from tumor tissue** and buffy coat **are extracted, whole-exome sequenced, analyzed, and filtered for patient-specific somatic mutations.**”¹⁵⁹

- (c) Claim 1[b]: “*performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the*

¹⁵⁶ *Id.* at 1.

¹⁵⁷ *Id.* at 7.

¹⁵⁸ *Id.* at 1; Natera cites to the following references in support of this statement: (i) Abbosh (2017); (ii) Reinert (2019); and (iii) Christensen (2019).

¹⁵⁹ *Id.* at 2 (emphasis added).

*target loci are amplified together in the same reaction volume;
and”*

127. Signatera™ satisfies this claim limitation. According to Signatera’s workflow, “[t]he top 16 somatic variants are selected based on clonality, detectability, and frequency of mutations. Multiple-PCR compatible primers are designed for each of the 16 somatic variants.”¹⁶⁰ Next, “[w]hole blood is collected at predefined time points for longitudinal surveillance.”¹⁶¹ Subsequently, “[p]lasma are isolated and cfDNA extracted, followed by assaying with the patient-specific 16-plex PCR pool.”¹⁶² “**Following multiplex PCR amplification, ultra-deep sequencing is performed. Next-generation sequencing data are analyzed to detect the presence of ctDNA.**”¹⁶³

128. Coombes (2019), Christensen (2019), and Kotani (2023), which all used Signatera™, illustrate the presence of this claim limitation in Signatera™. For example, Coombes (2019), Christensen (2019), and Kotani (2023) performed targeted multiplex amplification in the same reaction volume to amplify 16 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample and sequencing the amplicons to generate sequence reads having a length of 50 bases.¹⁶⁴

¹⁶⁰ *Id.* (emphasis added).

¹⁶¹ *Id.*

¹⁶² *Id.* (emphasis added).

¹⁶³ *Id.* (emphasis added).

¹⁶⁴ See Coombes (2019) at Abstract, 4256-4258, 4261, Figure 1 and legend, Figure 2 and legend, Supplementary Data at 1-2; see also Christensen (2019) at 1548-1549, Figure 4 and legend, Data Supplement at 3; Kotani (2023) at Methods, citing to Reinert (2019); see Reinert (2019) at eMethods 6.

129. Kirkizlar (2015) explains that Signatera employs multiplex PCR in a single reaction volume to amplify a bespoke set of targets identified from a patient's tumor sample.¹⁶⁵ The bespoke amplification products from the multiplex PCR “were designed to have a maximum amplicon length of 75 bp” (i.e., 75 bases).¹⁶⁶

- (d) Claim 1[c]: “*sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.*”

130. Signatera™ satisfies this claim limitation. For example, Signatera™ includes sequencing the amplicons to obtain sequence reads wherein the sequencing has a depth of read of at least 50,000 per target locus. According to Natera, “[f]ollowing multiplex PCR amplification, ultra-deep sequencing is performed”¹⁶⁷ with “100,000X average depth of coverage.”¹⁶⁸ Signatera™ also includes detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads. According to Natera, “sample-level performance was derived by calculating a binomial probability for detecting at least two clonal mutations at a given ctDNA level, assuming that the majority of the custom panels have between 10 to 16 clonal variants.”¹⁶⁹

¹⁶⁵ See Kirkizlar (2015) at 409.

¹⁶⁶ *Id.*

¹⁶⁷ See Signatera White Paper at 2.

¹⁶⁸ *Id.* at 1.

¹⁶⁹ *Id.* at 3; see also *id.* at Table 1.

131. Additionally, Coombes (2019) performed “ultradeep sequencing of plasma cfDNA at extremely high depth (average of >100,000 reads per target),” meeting the limitation of sequencing the amplicons having a read depth at least 50,000 per target locus.¹⁷⁰ Christensen (2019) also explained that “[p]lasma samples were sequenced to a median target coverage of 105,000X.”¹⁷¹

2. Claim 1 of the '035 Patent is recited *infra*:

- (a) Claim 1[preamble]: “*A method for amplifying and sequencing DNA, comprising:*”

132. To the extent the preamble is considered a claim limitation, the preamble is met by Signatera™. For example, in Signatera™, “cell free DNA (cfDNA) libraries are prepared from each blood sample, [which is] followed by patient-specific, **multiplex PCR**.¹⁷² The amplicon products resulting from multiplex PCR “are tagged with sequencing barcodes and **pooled for ultra-deep next generation sequencing**, followed by data analysis to detect the presence or absence of ctDNA.”¹⁷³ Coombes (2019) also performed “**ultradeep sequencing of plasma cfDNA** at extremely high depth (average of >100,000

¹⁷⁰ See Coombes (2019) at 4261; see also *id.* at Abstract, Supplementary Data at 1-2, Figure S4 and legend.

¹⁷¹ See Christensen (2019) at 1549; see also *id.* at Abstract, Data Supplement at Figure A2 and legend; see also Kotani (2023), citing to Reinert (2019). “Ultradeep multiplex PCR–based NGS (**median target coverage, >105 000 reads**) was used to analyze and . . . quantify ctDNA in 795 plasma samples from 125 patients,” see Reinert (2019) at 1126 (emphasis added).

¹⁷² See Signatera White Paper at 2 (emphasis added).

¹⁷³ *Id.* (emphasis added).

reads per target),”¹⁷⁴ Christensen (2019) explained that “[p]lasma samples were sequenced to a median target coverage of 105,000x,”¹⁷⁵ and Kotani (2023) cites to Reinert (2019), which explains that “[u]ltradeep multiplex PCR-based NGS (median target coverage, >105 000 reads) was used.”¹⁷⁶ Coombes (2019), Christensen (2019) and Kotani (2023) all illustrate that Signatera meets the limitation of amplifying and sequencing DNA.¹⁷⁷

- (b) Claim 1[a]: “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant wom[a]n;”

133. Signatera™ satisfies this claim limitation. For longitudinal surveillance using the Signatera™ assay, “cell free DNA (cfDNA) libraries are prepared from each blood sample.”¹⁷⁸ Coombes (2019) also explains that “[t]he cfDNA was end-repaired, A-tailed, and **ligated with custom adapters**.”¹⁷⁹ Christensen (2019) also explains that “[a]fter the initial DNA extraction from plasma samples, the cfDNA was end-repaired, A-tailed, and **ligated with custom adapters**, as previously described.”¹⁸⁰ Kotani (2023) cites to

¹⁷⁴ See Coombes (2019) at 4261 (emphasis added); see also *id.* at Abstract, Supplementary Data at 1-2, Figure S4 and legend.

¹⁷⁵ See Christensen (2019) at 1549 (emphasis added); see also *id.* at Abstract, Data Supplement at Figure A2 and legend.

¹⁷⁶ See Kotani (2023) at Methods, citing to Reinert (2019) at 1126 (emphasis added).

¹⁷⁷ See Coombes (2019) at 4261; see also *id.* at Abstract, Supplementary Data at 1-2, Figure S4 and legend.

¹⁷⁸ See Signatera White Paper at 2 (emphasis added).

¹⁷⁹ See Coombes (2019) at Supplementary Data at 1-2 (emphasis added).

¹⁸⁰ See Christensen (2019) at Data Supplement at 3, citing the Abbosh (2017).

Reinert (2019), which explains that “[u]niversal libraries were created by end repair, A-tailing, and **ligation with custom adapters**, as previously described.”¹⁸¹ One of ordinary skill in the art would have understood that creating universal libraries using the custom adapters means that the custom adapters include universal tail sequences for universal amplification using universal primers.

134. Signatera™ also utilizes a blood sample collected from a subject who is not a pregnant woman. For example, Signatera is used “[t]o monitor for cancer recurrence or to detect residual disease with ctDNA” for all patients, including men and non-pregnant women.¹⁸²

- (c) Claim 1[b]: “*amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and*”

135. Signatera™ satisfies this claim limitation. For “longitudinal surveillance, cell free DNA (cfDNA) libraries are prepared from each blood sample,” which is followed by patient-specific multiplex PCR.¹⁸³ Coombes (2019) also explains that “[t]he cfDNA was end-repaired, A-tailed, and ligated with custom adapters. . . . **Samples were amplified using patient-specific assay and barcoded**, followed by pooling. **Sequencing was performed on an Illumina HiSeq 2500 Rapid Run** with 50 cycles of paired-end reads

¹⁸¹ See Kotani (2023) at Methods, citing to Reinert (2019) at 1125 (emphasis added).

¹⁸² See Signatera White Paper at 2, 5.

¹⁸³ *Id.* at 2.

using the Illumina Paired End v2 kit with an average read depth of >100,000X per amplicon.”¹⁸⁴ Christensen (2019) describes a similar workflow meeting the claim element:

After the initial DNA extraction from plasma samples, the cfDNA was end-repaired, A-tailed, and ligated with custom adapters, as previously described. **The purified ligation product was amplified for 20 cycles** and purified using Ampure XP beads (Agencourt/Beckman Coulter). An aliquot of each library was used as input into the associated patient-specific 16-plex PCR amplification. **Samples were amplified using the Signatera tumor-specific assay and barcoded, followed by pooling. Sequencing was performed on an Illumina HiSeq 2500 Rapid Run** with 50 cycles of paired-end reads using the Illumina Paired End v2 kit, as previously described.¹⁸⁵

136. In Signatera, one or more sequencing tags are added during one of the amplification steps that also introduces the barcode in the Signatera™ assay. Kirkizlar (2015) explains that Signatera employs multiplex PCR in a single reaction volume to amplify a bespoke set of targets identified from a patient’s tumor sample.¹⁸⁶ The bespoke amplification products are further amplified by a barcoding PCR reaction, which adds “sequencing tags and index sequences,” resulting in final barcoded products.¹⁸⁷

- (d) Claim 1[c]: “*sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.*”

137. Signatera™ satisfies this claim limitation. In the workflow for Signatera™, “[f]ollowing multiplex PCR amplification, ultradeep sequencing is performed. Next-

¹⁸⁴ See Coombes (2019) at Supplementary Data at 1-2 (emphases added).

¹⁸⁵ See Christensen (2019) at Data Supplement at 3, citing the Abbosh (2017) (emphases added); see also Kotani (2023) at Methods, citing to Reinert (2019) at 1125.

¹⁸⁶ See Kirkizlar (2015) at 409.

¹⁸⁷ *Id.*

generation sequencing data are analyzed to detect the presence of ctDNA.”¹⁸⁸ Coombes (2019) and Christensen (2019) also performed targeted multiplex amplification and sequencing of 16 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA.¹⁸⁹

138. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

¹⁸⁸ See Signatera White Paper at 2.

¹⁸⁹ See Coombes (2019) at Abstract, 4256-4258, 4261, Figure 1 and legend, Figure 2 and legend, Supplementary Data at 1-2; *see also* Christensen (2019) at 1548, 1549, Data Supplement at 3.

¹⁹⁰ [REDACTED]

[REDACTED]

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

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

Dated: July 29, 2023



Michael Metzker, Ph.D.