

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

NATERA, INC.,

Plaintiff,

v.

NEOGENOMICS LABORATORIES, INC.,
Defendant.

C.A. No. 1:23-cv-629

**CONTAINS HIGHLY CONFIDENTIAL
INFORMATION**

REPLY DECLARATION OF DR. MICHAEL METZKER

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I, Michael Metzker, declare as follows:

1. I previously submitted a declaration¹ in the above-captioned litigation with respect to the infringement of U.S. Patent Nos. 11,530,454² and 11,519,035³ (collectively, the “Natera Patents”). I incorporate that declaration herein by reference, including information regarding my qualifications and expertise, including my curriculum vitae, as well as my understanding of relevant legal principles.

2. I have been asked to consider the Declaration of NeoGenomics’ expert Dr. Brian Van Ness⁴ which I understand has been submitted in support of NeoGenomics’ opposition to Natera’s motion for a preliminary injunction regarding NeoGenomics’ RaDaR™ test. In the Van Ness Declaration, Dr. Van Ness has offered opinions regarding the invalidity and inventorship of the Natera Patents as well as opinions responding to my Opening Declaration. I have also considered his deposition testimony concerning the Van Ness Declaration (“Van Ness Tr.”).⁵ I respond and reply to his opinions herein.

¹ See Dkt 17, *Declaration of Dr. Michael L. Metzker in Support of Plaintiff Natera, Inc.’s Motion for a Preliminary Injunction*, dated July 29, 2023 (“Metzker Op. Decl.”).

² See Dkt. 1-1, NAT-NEO-00000001-00000222, U.S. Patent No. 11,530,454 to Babiarz *et al.*, (“Detecting mutations and ploidy in chromosomal segments”) (the “454 Patent”).

³ See Dkt 1-2, NAT-NEO-00000223-00000435, U.S. Patent No. 11,519,035 to Rabinowitz *et al.*, (“Methods for simultaneous amplification of target loci”) (the “035 Patent”).

⁴ See Dkt. 116, *Declaration of Dr. Brian Van Ness in Support of NeoGenomics Laboratories, Inc.’s Response in Opposition to Natera, Inc.’s Motion for a Preliminary Injunction*, dated October 18, 2023 (“Van Ness Declaration” or “Van Ness Decl.”).

⁵ See Ex. 1, Transcript of Deposition of Brian Van Ness, Ph.D., dated October 25, 2023 (“Van Ness Tr.”).

I. LEGAL STANDARDS

3. I am not an attorney and have no legal training. In this Reply Declaration, I have applied certain legal principles of which I have been informed by Natera's counsel as described in my Opening Declaration⁶ and *infra*.

A. Section 101

4. In evaluating whether patent claims are patent-eligible under 35 U.S.C. § 101, I have been informed and understand that a Court conducts a first inquiry to determine whether the claims at issue are directed to a patent-ineligible concept. I understand that, if this threshold determination at step one is met, the Court moves to the second step of the inquiry and considers the elements of each claim both individually and as an ordered combination to determine whether the additional elements transform the nature of the claim into a patent-eligible application.

5. I have been informed and understand that the “directed to” inquiry in step one of the eligibility analysis considered in light of the specification is based on whether the claims as a whole directed to excluded subject matter. I understand, for example, new and improved techniques for producing tangible and useful results fall outside the categories of inventions that are “directed to” patent-ineligible concepts. Moreover, I understand that the “directed to” inquiry cannot simply ask whether the claims involve a patent-ineligible concept, because essentially every routinely patent-eligible claim

⁶ See Metzker Op. Decl. § III.

involving physical products and actions involves a law of nature and/or natural phenomenon.

6. I have been informed and understand that step two of the patent-eligibility analysis has been described as a search for an inventive concept. At step two, I understand that more is required than well-understood, routine, conventional activity already engaged in by the scientific community, which fails to transform the claim into significantly more than a patent upon the ineligible concept itself. I understand that, at this step, claims that are “directed to” a patent-ineligible concept yet also improve an existing technological process are sufficient to transform the process into an inventive application of the patent-ineligible concept.

B. Section 103

7. I have been informed and understand that a patent is invalid for obviousness if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to one of ordinary skill in the art to which said subject matter pertains. I understand that obviousness is a question of law based on underlying findings of fact. An analysis of obviousness must be based on several factual inquiries: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art at the time the invention was made; and (4) objective evidence of nonobviousness, if any. I am informed that a party seeking to invalidate a patent as obvious generally must demonstrate by clear and convincing evidence that a skilled artisan would

have had reason to combine the teaching of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success from doing so.

8. I have been informed and understand that where the prior art gives only general guidance as to the particular form of the claimed invention or how to achieve it, relying on an obvious-to-try theory to support an obviousness finding is impermissible. Moreover, I understand that obviousness cannot be shown “by merely throwing metaphorical darts at a board” in hopes of arriving at a successful result when the prior art gives either no indication of which parameters are critical or no direction as to which of many possible choices is likely to be successful. Rather, I understand that courts should reject such “hindsight” claims of obviousness.

9. Moreover, I have been informed and understand that evidence of secondary considerations of non-obviousness must always be considered when present, for example, to guard against hindsight bias. I am informed that such evidence of secondary considerations may often be the “most probative and cogent” evidence in the record to establish that an invention appearing to have been obvious was not.

C. Section 112 – Written Description

10. I have been informed and understand that the test for sufficiency of a patent’s written description is whether the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date. I understand that this test requires an objective inquiry into the

four corners of the specification from the perspective of a person of ordinary skill in the art.

11. I have been informed and understand, based on that inquiry, the specification must describe an invention understandable to that skilled artisan and show that the inventor actually invented the invention claimed. I understand, however, that the description requirement does not demand any particular form of disclosure, and that the specification does not need to recite the claimed invention in the same words as recited in the claims.

II. TECHNICAL BACKGROUND

12. I identify certain technical principles that inform my opinions set forth in this Reply Declaration *infra*. In particular, I explain the challenges that one of ordinary skill in the art would have faced in working with cell-free DNA (cfDNA) that circulates in the body. Both Dr. Van Ness and I discuss cfDNA. In my view, the analysis in the Van Ness Declaration⁷ does not adequately describe the significant difficulties and challenges that faced one of ordinary skill in the art with respect to the use and manipulation of cfDNA to achieve the methods claimed in the Natera Patents, particularly in view of conflicting testimony Dr. Van Ness gave in his deposition. I also note Dr. Van Ness's admission that he has never published any work involving cfDNA and that his laboratory spent only about six months attempting to work with cfDNA in the early 2000s, and did not work on cfDNA

⁷ See Van Ness Decl. at ¶¶ 43-46.

again after the early 2000s. Nor has Dr. Van Ness published any work on tumor-specific genetic variants using cfDNA.

Q. You never worked on – you, yourself, in your lab never worked on cell-free DNA after the early 2000s, correct?

A. We never – after the early 2000s, we did not work on cell-free DNA, although all the methods of DNA extraction, multiple, and sequencing were routine in my lab. But we did not work on specifically cell-free DNA after the early 2000s.⁸

Q. Okay. I'll ask the question again. You and your lab have never published any work describing experiments using cell-free DNA, correct?

A. I have not. Only blood DNA we've worked on, but I have not looked at cell-free. We did not publish on cell-free.⁹

Q. You never published any work on tumor-specific SNPs or single nucleotide variants in cell-free DNA, correct?

A. We did publish on SNPs in tumor – unique to tumors. It wasn't from the cell-free DNA.¹⁰

A. Cell-Free DNA and Plasma Samples

13. Prior to 2014-2015, and even earlier, there were many obstacles to the assaying and analysis of cfDNA. These challenges were, and still are, greater than when working with DNA from tissue samples, such as samples of tumors. I discuss some of these challenges in this section. Dr. Van Ness agrees, as he testified in his deposition.

⁸ See Van Ness Tr. at 246:21-247:4 (objection omitted).

⁹ *Id.* at 247:15-22 (objection omitted).

¹⁰ *Id.* at 249:11-15.

Q. You and your lab were not consistently able to detect cell-free DNA with good sensitivity, correct?

A. In the early 2000s, we were all having some problems in the consistency of detecting cell-free DNA.

Q. And in fact –

A. Detecting the target of cell-free DNA we were looking for.¹¹

14. For example, Volik (2016)¹² provides a helpful summary of certain problems and difficulties facing scientists working with cfDNA. These challenges are also acknowledged in certain prior art references cited in the Van Ness Declaration, such as Forshew (2012).¹³ I discuss them *infra*.

15. As a general matter, cfDNA is difficult to biochemically process and analyze because it is relatively low-quality compared to DNA from other sources, such as tissue samples. In his deposition, Dr. Van Ness agreed that working with cfDNA posed technical challenges.

Q. Okay. Do you – you see here that Dr. Forshew, in that first sentence, says “Circulating DNA is fragmented to an average length of 140 to 170 base pairs and is present in only a few thousand amplifiable copies per milliliter of blood, of which only a fraction may be diagnostically

¹¹ *Id.* at 248:10-20 (objection omitted).

¹² See Ex. 2, Volik *et al.*, *Cell-free DNA (cfDNA): Clinical Significance and Utility in Cancer Shaped By Emerging Technologies*, 14 Mol. Cancer Res. 898 (2016) (“Volik (2016)”).

¹³ See Dkt. 1-16, NAT-NEO-00043262-00043275, Forshew *et al.*, *Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA*, SCIENCE TRANSLATIONAL MEDICINE 4:136ra68, pp. 1-12 (2012) (“Forshew (2012)”).

relevant”)? Do you disagree with Dr. Forshew’s statement?

A. I don’t.

Q. In the second sentence, a few lines down, it says, “But also the challenges involved in analysis of circulating tumor DNA, where mutated loci and AFs may be more variable.” Do you disagree with him that there are challenges involved in analysis of circulating tumor DNA because of those variable mutated loci and AFs?

A. He’s referring to past challenges that involve the analysis of circulating tumor DNA. ***I agree with that.***¹⁴

16. Here, I provide an example of one such problem. Consider a cfDNA molecule that contains a desired single nucleotide polymorphism (“SNP”), which is a genomic variant at a single base position in DNA. If the cfDNA is fragmented such that the cfDNA fragment does not contain sites for both primers of a primer pair to bind that primer pair will not be able to amplify that particular SNP successfully when PCR is performed.

17. As of 2014-2015, scientists were beginning to explore how to use cancer-associated cfDNA.

Known to be present in the blood of cancer patients for decades, cell-free DNA (cfDNA) is beginning to inform on tumor genetics, tumor burden, and mechanisms of progression and drug resistance.¹⁵

¹⁴ See Van Ness Tr. at 116:19-117:21 (emphasis added).

¹⁵ See Volik (2016) at Abstract.

18. Indeed, the art recognized that blood plasma contained a highly variable content of cfDNA. In the case of cancer patients, for example, the level of tumor-associated cfDNA (known as “circulating tumor DNA” or “ctDNA”) would vary, including relative to the normal cfDNA that represents the majority of cfDNA found in plasma.

The amount of cfDNA in cancer patients varies widely.¹⁶

Not unexpectedly, in addition to varying absolute levels of cfDNA, the fraction of DNA molecules in the circulation of cancer patients that can be recognized as being derived from tumor cells also varies.¹⁷

19. The art recognized that the fragmented nature of cfDNA made it difficult to manipulate through existing techniques such as PCR-based amplification and sequencing. Dr. Van Ness agrees that there are challenges in amplifying cfDNA, as he testified in his deposition.

Q. Is it your opinion that there are particular challenges in amplifying cell-free or plasma DNA that one doesn’t encounter in amplifying genomic DNA from tissue?

A. ***There are some challenges.*** And there are some challenges in whole genomic DNA amplification as well. Amplification was well known in the art, and, yes, there are challenges.¹⁸

Dr. Van Ness further acknowledged that “[t]here are challenges to amplifying genomic DNA. ***There are challenges to amplifying cell-free DNA.*** And are they the same? No,

¹⁶ *Id.* at 890.

¹⁷ *Id.*

¹⁸ See Van Ness Tr. at 109:25-110:7 (emphasis added).

the size of the fragments are different. The number of cycles you might want to do with cell-free DNA may be different than if you have a lot of genomic DNA from the tumor.”¹⁹

20. In addition, tumor genomes are genetically unstable and prone to mutate. A frequent consequence of cancer-associated genetic instability is that different populations of cells within the same tumor from a single patient can have different genetic mutations. When these different tumor cell populations release ctDNA into the plasma, one population’s ctDNA can have different sequences from other populations’ ctDNA. These different mutations among different cancer cells in a tumor are known as “subclonal” mutations. Biochemically processing and analyzing ctDNA sequences is therefore typically more challenging than simply collecting and processing DNA directly from healthy tissues and tumor tissues for study.

The fragmentation, low yield, and variable admixture of normal DNA present formidable technical challenges for realization of this potential.²⁰

[The] low amount, high degradation, and high admixture of normal DNA in cfDNA pose major challenges for the development of sensitive and robust detection pipelines. The fact that most (if not all) tumors are characterized by multiple subclonal populations with only a subset of somatic mutations shared among all cells (for review, see ref. 52) further complicates the issue.²¹

¹⁹ *Id.* at 113:4-10 (emphasis added).

²⁰ *See* Volik (2016) at Abstract.

²¹ *Id.* at 901.

21. Moreover, ctDNA is typically low-abundance in the plasma of a person with cancer relative to the levels of other cfDNA in the plasma.²² Specifically, the plasma is likely to contain far more cfDNA from non-cancer cells than ctDNA from tumor cells, which makes preparing preparations of the ctDNA more difficult (because it must be distinguished in some way from the non-tumor cfDNA).

Circulating tumor DNA (ctDNA) is typically so diluted by normal DNA that existing sequencing methods (e.g., Sanger sequencing) were not sufficiently sensitive to detect mutant DNA molecules. As a result, mutation-specific PCR was the only available technology that could provide sufficient specificity for detection of the weak tumor signal.²³

However, ***tumor-derived DNA usually constitutes only a small percentage of total cfDNA*** so the ability to detect rare genome aberrations is an essential requirement for cfDNA analysis pipelines.²⁴

22. In deposition, Dr. Van Ness acknowledged particular and “unique” challenges associated with multiplex amplification of cfDNA in the context of discussing amplification of different types of cfDNA (e.g., cfDNA from patients who received an organ transplant, or pregnant women, or subjects with cancer).

Q. I was asking about multiplex amplification, the actual multiplex PCR reaction. That – do you – is there something you have to do differently in performing multiplex PCR to amplify transplant cfDNA versus cancer-free [*sic*] DNA?

²² See Van Ness Tr. at 111:8-113:13.

²³ See Volik (2016) at 898 (emphasis added).

²⁴ *Id.* at 906(emphasis added).

- A. There may be differences because the multiplexing does involve sample preparation. It does involve understanding the limits of detection. How many cycles of multiplex PCR might be needed? ***So, yes, there are differences.***
- Q. Would you say those same differences would apply to performing multiplex PCR to amplify fetal cell-free DNA versus cancer cell-free DNA?
- A. ***I think every application has its unique challenges*** in terms of sample access, preparation that influence how the multiplex is being done.
- Q. So the answer's yes?
- A. ***I think all of those applications, yes,*** can have differences in protocols that might be required for successful multiplex amplification.
- Q. And by "applications," you mean cell-free DNA from a fetus versus cell-free DNA from an organ transplant versus cell-free DNA from a tumor?
- A. Recognize that the detection of cell-free DNA from each of those sources also requires access to those sources so that you know what you're looking for. So it's not sufficient simply to isolate cell-free DNA. You have to know the sequences of your target in order to be – to analyze that. And those target sequences are going to be present in different quantities in each of those applications.
- Q. I was just asking what you meant by the word "applications." You say, "I think all of those applications, yes, can have differences in protocols that might be required for successful multiplex amplifications." I'm just asking what you meant by "applications," that word.
- A. Okay.

Q. Are you referring to cell-free DNA of different sources?

A. *I'm referring to cell-free DNA as it is applied to different situations, of fetal DNA, cancer DNA, organ transplant DNA.*²⁵

III. SUMMARY OF OPINIONS

A. Claim Construction

23. It is my opinion that one of ordinary skill in the art would have understood each of the terms of the Asserted Claims of the Natera Patents to have their plain and ordinary meaning. In particular, I construe the terms “tagging isolated cell free DNA” (Claim 1 of the '035 Patent) and “amplifying the tagged products one or more times” (Claim 1 of the '035 Patent) to each have a plain and ordinary meaning, which are summarized *infra*.

24. It is my opinion that one of ordinary skill in the art would have understood that as of the priority date of the '035 Patent, and in light of the Specification, the plain meaning of “tagging isolated cell free DNA” encompasses adding one or more adaptor tags to isolated cell free DNA through primer extension or ligation. Dr. Van Ness agreed with this understanding during his deposition.²⁶

25. It is my opinion that one of ordinary skill in the art would have understood that as of the priority date of the '035 Patent, and in light of its specification, the plain

²⁵ See Van Ness Tr. at 64:4-66:6 (objections, court reporter clarification, and colloquy omitted, emphases added).

²⁶ *Id.* at 228:17-232:25.

meaning of “amplifying the tagged products one or more times” encompasses creating at least one copy of the tagged products. Dr. Van Ness agreed with this understanding during his deposition.²⁷ One of ordinary skill in the art would have readily recognized this occurs following at least one cycle of PCR.²⁸

B. Validity

26. As a preliminary matter, I note that Dr. Van Ness does not contend that any prior art reference discloses every limitation of the Natera Patent claims—*i.e.*, a “novelty” or “anticipation” challenge under Section 102 of the Patent Act.²⁹ Instead, Dr. Van Ness contends that several references, if modified and combined, render the claims obvious.

27. I disagree with Dr. Van Ness’s contention that the Natera Patents are invalid as obvious under Section 103.³⁰ As explained *infra*, Dr. Van Ness fails to account for technical limitations in prior art approaches and the many challenges inherent in manipulating cfDNA faced by skilled artisans.

28. Dr. Van Ness’s obviousness opinions are wholly conclusory. The Van Ness Declaration points to disparate teachings in the art or a skilled artisan’s knowledge that Dr. Van Ness believes remediate these deficiencies.

²⁷ *Id.*

²⁸ *Id.*

²⁹ *Id.* at 97:11-98:13; *see generally* Van Ness Decl.

³⁰ *See* Van Ness Decl. at ¶¶ 109-220 for the ’454 Patent and ¶¶ 221-303 for the ’035 Patent.

29. Dr. Van Ness also fails to explain why one of skill in the art would have been motivated to modify the known prior art approaches in the specific ways needed to achieve the claimed inventions. Just as critically, Dr. Van Ness fails to explain why one of skill in the art would have reasonably expected to succeed with any particular combined approach. In fact, success was unlikely or at the very least unpredictable in light of well-known obstacles, including using cfDNA, which is fragmented and challenging to manipulate even with known methods.³¹ It was particularly difficult to process samples in a manner that would permit analysis of genetic variants on low-abundance, unpredictable cancer-associated cfDNA. Dr. Van Ness fails to even contend, much less show, that a skilled artisan would have reasonably expected success in achieving the claimed methods given the art's unpredictability. His opinions are informed with the benefit of hindsight knowledge of Natera's claimed invention, which is I understand impermissible in an obviousness analysis.

30. I further disagree with Dr. Van Ness's contention that the Natera Patents are invalid as patent-ineligible under Section 101.³² The claims are directed to patent-eligible subject matter (methods of sample preparation), and recite specific concrete laboratory steps which generate DNA sequences not found in nature, and which require human effort to perform. The recited steps, when considered in an ordered combination as well as separately, are inventive and neither routine or conventional.

³¹ See Section II.

³² See Van Ness Decl. at ¶¶ 304-340 for the '454 Patent and ¶¶ 341-347 for the '035 Patent.

31. The Van Ness Declaration arrives at his patent-eligibility opinions by cherry-picking parts of the claims that Dr. Van Ness contends “involve” a natural phenomenon or abstract idea and disregarding all of the rest of the claims that does not support his opinions. Nor does Dr. Van Ness analyze whether the claimed steps are routine and conventional when they are considered in combination as recited in the claims.

32. In addition, I disagree with Dr. Van Ness’s contention that the Natera Patents are invalid as lacking adequate written description under Section 112.³³ The Natera Patents adequately describe the claimed inventions, and the Van Ness Declaration’s contrary opinions are in my opinion incorrect. Contrary to Dr. Van Ness’s opinion, the ’454 Patent explicitly describes the use of whole genome sequencing.³⁴ Dr. Van Ness opines otherwise only by looking outside the patent to other documents not incorporated into or cited in the patent in an attempt to interpret the patent, which I understand is impermissible in an analysis of adequacy of written description.

33. Dr. Van Ness also contends that the Natera Patents lack adequate description of the claimed methods, including approaches to multiplex PCR that do not require selecting primers to avoid primer dimers. I disagree. As explained *infra*, the Natera Patents adequately describe the claimed inventions and teach strategies for multiplex PCR that not only avoid primer dimers but also avoid other problems such as allelic bias.³⁵ In addition,

³³ *Id.* at ¶¶ 404-426 for both the ’454 Patent and ’035 Patent.

³⁴ *See, e.g.*, ’454 Patent at 112:50-66, 116:20-35.

³⁵ *See, e.g.*, ’035 Patent at 48:30-47.

the '035 Patent adequately describes the claimed invention, including the use of universal tail adaptors.³⁶

34. The Van Ness Declaration never asserts that Dr. Van Ness is offering an opinion on a substantial question of validity with respect to either the '454 or '035 Patents. I have considered the Van Ness Declaration, Dr. Van Ness's deposition transcript, and the cited materials. In my opinion, Dr. Van Ness has failed to identify a substantial question of validity for either Natera Patent. In fact, when properly considered, the evidence confirms the Natera Patents are valid.

C. Inventorship

35. I understand that Dr. Van Ness has opined on the issue of inventorship. I am informed by counsel that Dr. Van Ness's opinions are legally improper and inconsistent with the evidence in the record. I disagree with Dr. Van Ness's contention that the '454 Patent is invalid for improper inventorship.

D. Infringement

36. Based on my review of the materials referenced in my Opening Declaration³⁷, and my personal knowledge and experience in this field, it is my opinion that the accused RaDaR assay infringes, literally and under the doctrine of equivalents, the Asserted Claims of the Natera Patents. I respond to Dr. Van Ness's opinions regarding non-infringement in this Reply Declaration.

³⁶ See, e.g., *id.* at 95:16-23.

³⁷ See Metzker Op. Decl. at § VIII.

37. I disagree with the contention in Dr. Van Ness's Declaration that the accused RaDaR assay does not infringe the Asserted Claims of the Natera Patents.³⁸ As I explained in my Opening Declaration, the accused RaDaR assay literally infringes the Asserted Claims of the Natera Patents.³⁹ Dr. Van Ness's noninfringement theories require, as a foundation, claim constructions that deviate from the plain and ordinary meaning of the asserted claim terms. However, Dr. Van Ness has not offered an opinion on the construction of any claim terms except for the term "amplifying."⁴⁰ I have been informed and understand from counsel that Dr. Van Ness has thus waived an interpretation that differs from the plain and ordinary meaning of these claim terms. I also disagree with Dr. Van Ness's construction of "amplifying" because it directly contradicts the plain language of the claim. As explained *infra*, because the Van Ness Declaration's interpretation of these terms is incorrect, his opinions on noninfringement are likewise incorrect.

38. Even the Van Ness Declaration's interpretations of certain claim terms were adopted by the Court, it is my opinion that the accused RaDaR assay still infringes the Asserted Claims of the Natera Patents under the doctrine of equivalents.

E. Signatera

39. I disagree with Dr. Van Ness's contention that Natera's Signatera assay does not practice the Natera Patents. As I explained in my Opening Declaration, Signatera

³⁸ See Van Ness. Decl. at § IX.

³⁹ See Metzker Op. Decl. at § VIII.

⁴⁰ See Van Ness Decl. at § VII; see also § III.A *supra*.

practices at least Claim 1 of the '454 Patent and Claim 1 of the '035 Patent.⁴¹ My analysis is supported by Natera's internal technical documentation, which the Van Ness Declaration failed to consider. Moreover, Dr. Van Ness testified that he had not reviewed any of Natera's standard operating procedures.⁴² These materials, when properly considered, confirm that Signatera practices each of the Natera Patents. To the extent that Dr. Van Ness offers any additional opinions regarding Signatera, I reserve the right to supplement my analysis and opinions.

IV. NATERA PATENTS

40. I have previously described the Natera Patents in my Opening Declaration in this case⁴³ and incorporate that description again here in this Reply Declaration. For ease of reference, I include *infra* the claims of the Natera Patents challenged in the Van Ness Declaration.

A. U.S. Patent No. 11,530,454

41. Claim 1 of the '454 Patent recites:

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;

⁴¹ See Metzker Op. Decl. at ¶¶ 124-138.

⁴² See Van Ness Tr. at 246:11-20.

⁴³ See Metzker Op. Decl. at ¶¶ 48-53.

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

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.

42. Claim 8 of the '454 Patent recites:

The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.

43. Claim 11 of the '454 Patent recites:

The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.

B. U.S. Patent No. 11,519,035

44. Claim 1 of the '035 Patent recites:

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.

45. Claim 12 of the '035 Patent recites:

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.

46. Claim 13 of the '035 Patent recites:

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.

C. Priority Dates

47. Dr. Van Ness agrees with Natera's contention that the priority date for the '454 Patent is April 21, 2015 and that the priority date for the '035 Patent is May 18, 2011.⁴⁴

In light of this, and in order to respond to Dr. Van Ness's opinions, I apply the same priority dates for the respective Natera Patents in my analysis in this Reply Declaration, though I

⁴⁴ See Van Ness Decl. at ¶¶ 38-39.

reserve the right to offer opinions regarding different priority dates for the Natera Patents at another time.

D. Level of Ordinary Skill in the Art

48. Dr. Van Ness agrees with my prior definition of one of ordinary skill in the art with respect to the Natera Patents.⁴⁵ I apply that definition⁴⁶ again in my analysis in this Reply Declaration, and I reserve the right to offer another definition, for example, in response to any definition that any NeoGenomics expert may offer at another time.

V. CLAIM CONSTRUCTION

49. It is my opinion that one of ordinary skill in the art would have understood each of the terms of the Asserted Claims of the Natera Patents to have their plain and ordinary meaning. In particular, I construe the terms “tagging isolated cell free DNA” (Claim 1 of the ’035 Patent) and “amplifying the tagged products one or more times” (Claim 1 of the ’035 Patent) to each have a plain and ordinary meaning, which I explain in more detail *infra*.

A. “tagging isolated cell-free DNA” (Claims 1, 13 of the ’035 Patent)

(i) Intrinsic Evidence

50. It is my opinion that one of ordinary skill in the art would have understood that as of the priority date of the ’035 Patent, the plain and ordinary meaning of “tagging

⁴⁵ *Id.* at §IV.

⁴⁶ See Metzker Op. Decl. at § IV. Throughout this Reply Declaration, I also refer to a POSA as one of ordinary skill in the art.

isolated cell free DNA” encompasses adding one or more adaptor tags to isolated cell free DNA through primer extension or ligation. Dr. Van Ness agreed with this interpretation in his deposition.⁴⁷

51. The '035 Patent describes methods for preparing cell-free DNA samples. These methods include adding tags to the cfDNA, which the patent refers to as “tails.”⁴⁸ Specifically, in a section titled “Mini-PCR,” the '035 Patent describes that “[t]he following Mini-PCR method is desirable for samples containing short nucleic acids, digested nucleic acids, or fragmented nucleic acids, such as cfDNA.”⁴⁹ The '035 Patent describes amplifying cfDNA, which can include “polymorphic” sites of interest, using multiplex PCR with forward and reverse primers. For example, the '035 Patent explains that “[i]n an embodiment, the 3' end of the inner forward and reverse primers are designed to hybridize to a region of DNA upstream from the polymorphic site, and separated from the polymorphic site by a small number of bases.”⁵⁰ As part of this process, the '035 Patent explains that “tails with no homology to the target genome may also be added to the 3-prime or 5-prime end of any of the primers.”⁵¹ According to the '035 Patent, “[t]hese tails facilitate subsequent manipulations, procedures, or measurements.”⁵²

⁴⁷ See Van Ness Tr. at 228:17-232:25.

⁴⁸ See, e.g., '035 Patent at 90:38-91:18.

⁴⁹ *Id.* at 88:53-55.

⁵⁰ *Id.* at 89:39-43.

⁵¹ *Id.* at 90:38-40.

⁵² *Id.* at 90:40-41.

52. The '035 Patent describes the process of adding tails to DNA sequences using PCR. In the exemplary description *infra*, for example, the PCR primers contain a “tail” sequence that is not complementary to and therefore would not interact with the target sequence of interest. The addition of tags can be used to manipulate the cfDNA, for example by serving as a site for PCR amplification.

In an embodiment, the method as it pertains to a single target locus may comprise one or more of the following steps: (1) Designing a standard pair of oligomers for PCR amplification of a specific locus. (2) Adding, during synthesis, a sequence of specified bases with no or minimal complementarity to the target locus or genome to the 5' end of the one of the target specific oligomer. ***This sequence, termed the tail, is a known sequence, to be used for subsequent amplification, followed by a sequence of random nucleotides.***⁵³

53. As one of ordinary skill in the art would have understood from the '035 Patent, the description of “tails” includes adaptor tags. In its Definitions, the '035 Patent describes the use of adaptor tags, which is consistent with the '035 Patent's description of “tails.” As the '035 Patent clarifies, adaptor tags can be added to a DNA sequence (for example) either by using PCR or using ligation.

Universal Adapters, or 'ligation adaptors' or 'library tags' are DNA molecules containing a universal priming sequence that can be covalently linked to the 5-prime and 3-prime end of a population of target double stranded DNA molecules. The addition of the adapters provides universal priming sequences to the 5-prime and 3-prime end of the target population from which PCR amplification can take place,

⁵³ *Id.* at 118:14-23 (emphasis added).

amplifying all molecules from the target population, using a single pair of amplification primers.⁵⁴

Universal Priming Sequence refers to a ***DNA sequence that may be appended to a population of target DNA molecules, for example by ligation, PCR, or ligation mediated PCR.*** Once added to the population of target molecules, primers specific to the universal priming sequences can be used to amplify the target population using a single pair of amplification primers. Universal priming sequences are typically not related to the target sequences.⁵⁵

54. The '035 Patent explicitly describes adding adaptor tags to a DNA sequence of interest. These adaptors are added to the DNA as part of the process of preparing the DNA sample for subsequent sequencing and analysis. As the '035 Patent explains, these adaptors (*i.e.*, tails) can serve as a basis for (universal) amplification of the DNA sequence. In the exemplary disclosure *infra*, the '035 Patent describes adding the adaptors to the DNA using “target specific” primers that include the adaptor sequences, followed by PCR which attaches the adaptor sequence to the DNA through the amplification process.

In an embodiment, ***the targeted sequencing may involve*** one, a plurality, or all of the following steps. a) ***Generate and amplify a library with adaptor sequences on both ends of DNA fragments.*** b) Divide into multiple reactions after library amplification. c) ***Generate and optionally amplify a library with adaptor sequences on both ends of DNA fragments.*** d) ***Perform 1000- to 10,000-plex amplification of selected targets using*** one target specific “Forward” primer per target and ***one tag specific primer.*** e) ***Perform a second amplification from this product using*** “Reverse” target specific primers and ***one (or more) primer specific to a universal tag that was introduced as part of the target specific***

⁵⁴ *Id.* at 42:22-30 (emphases added).

⁵⁵ *Id.* at 42:14-21 (emphases added).

forward primers in the first round. f) Perform a 1000-plex preamplification of selected target for a limited number of cycles. g) Divide the product into multiple aliquots and amplify subpools of targets in individual reactions (for example, 50 to 500-plex, though this can be used all the way down to singleplex. h) Pool products of parallel subpools reactions. i) During these amplifications primers may carry sequencing compatible tags (partial or full length) such that the products can be sequenced.⁵⁶

55. The '120 Patent Pub. (Zimmermann),⁵⁷ which the '035 Patent incorporates by reference, contains additional description of this process, including on cfDNA-containing plasma samples.

The workflow may entail (1) extracting DNA such as plasma DNA, (2) preparing fragment library with universal adaptors on both ends of fragments, (3) amplifying the library using universal primers specific to the adaptors,⁵⁸ ... ('120 Pub. [0213])⁵⁹

56. Relatedly, the '035 Patent explains the usefulness of adding adaptor tags to cfDNA. For example, the '035 Patent describes that “primers are designed to anneal a site a greater distance from the polymorphism than is expected to be present among cell free fetal DNA fragments,” and that these “primers are tagged with a molecule or moiety that can allow selective recognition of the tagged pieces of DNA.”⁶⁰

⁵⁶ *Id.* at 85:47-67 (emphases added).

⁵⁷ See Zimmermann *et al.*, *Highly multiplex PCR methods and compositions*, U.S. Patent Application Publication No. US 2013/0123120 (2013) (the “'120 Patent Pub. (Zimmermann)”).

⁵⁸ See '035 Patent at 86:61-64.

⁵⁹ *Id.* at 250:9-11.

⁶⁰ *Id.* at 91:28-35.

57. Adding adaptor tags to cfDNA would facilitate sequencing the DNA, as “using forward and reverse tails corresponding to forward and reverse sequences required by any of the current sequencing platforms can enable direct sequencing following amplification.”⁶¹ The adaptor tags facilitate high-throughput sequencing in particular. As the ’035 Patent explains, “a 10,000-plex PCR assay pool is created such that forward and reverse primers have tails corresponding to the required forward and reverse sequences required by a high throughput sequencing instrument such as the HISEQ, GAIIX, or MYSEQ available from ILLUMINA.”⁶²

58. Additionally, consistent with the Definitions of adaptor sequences *supra*, the ’035 Patent teaches that “the tails can be used as common priming sites among all amplified targets that can be used to add other useful sequences,” such as a molecular barcode. To achieve this end, “the primer may contain a universal priming sequence designed to allow PCR amplification.”⁶³

59. I have reviewed the prosecution history for the ’035 Patent. From my review, the prosecution history is consistent with my opinion *supra* and I see nothing to indicate that the term “tagging isolated cell free DNA” should be construed to have a different meaning other than its plain and ordinary meaning that I describe *supra*. I see, for example,

⁶¹ *Id.* at 90:48-51.

⁶² *Id.* at 90:60-64.

⁶³ *Id.* at 90:51-59.

that this limitation was not amended and was present in the originally-filed claims (dated July 21, 2020), excerpted *infra*.⁶⁴

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;
amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and
sequencing a plurality of loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of loci comprises 25-2,000 loci.

(ii) Extrinsic Evidence

60. As further support for my opinions, one of ordinary skill in the art's understanding of adaptor tags and their addition to DNA sequences is shown in evidence extrinsic to the '035 Patent itself.

61. One of ordinary skill in the art would have readily recognized that for sequencing on most platforms, including some of the most widely-used models (*e.g.*, Illumina sequencers), sequencing adaptors are always required.⁶⁵ Briefly, adding these adaptors is necessary for sequencing DNA, which can include amplicons of cfDNA. Sequencers contain complementary sequences that recognize and bind the adaptor

⁶⁴ See NAT-NEO-00001599-NAT-NEO-00003251 (Prosecution History of the '035 Patent) at NAT-NEO-00001615.

⁶⁵ See Ex. 11, Meyer & Kircher, *Illumina sequencing library preparation for highly multiplexed target capture and sequencing*, COLD SPRING HARBOR PROTOCOLS, pdb.prot5448 pp. 1-10 (2010) ("Meyer (2010)").

sequences and hold them in place for the sequencing reactions to occur and be detected in the machine. When adaptor-tagged pieces of DNA (including amplicons of cfDNA) are introduced in the sequencer, the adaptors bind to complementary sequences in the instrument's *flow cell*. The pieces of DNA are held in place for sequencing and further analysis.

B. “amplifying the tagged products one or more times” (Claim 1 of the ’035 Patent)

(i) *Intrinsic Evidence*

62. It is my opinion that one of ordinary skill in the art would have understood that as of the priority date of the ’035 Patent, the plain meaning of “amplifying the tagged products one or more times” encompasses creating at least one copy of a tagged product, which one of ordinary skill in the art would have readily recognized occurs in multi-cycle PCR following the addition of a tag after the first cycle of PCR. Dr. Van Ness agreed with this in his deposition.⁶⁶

63. The ’035 Patent specification describes how DNA sequences of interest are tagged after a PCR cycle, and then those tagged products are amplified in one or more further PCR cycles. For context, consistent with one of ordinary skill in the art’s general understanding of the PCR reaction, the ’035 Patent describes that “[i]n some embodiments, most or all of the primers,” which are single-stranded DNA molecules that bind complementary sequences on the DNA sequence of interest, “are extended to form

⁶⁶ See Van Ness Tr. at 220:1-16.

amplified products” via the PCR method.⁶⁷ In consequence, the primers—including any tag sequences within the primer—are “consumed” via incorporation into the DNA strand being replicated during a PCR cycle. The ’035 Patent describes this process as “conversion” of the primer into the amplicon (which also contains the DNA sequence). “Having all the primers consumed in the PCR reaction increases the uniformity of amplification of the different target loci since the same or similar number of primer molecules are converted to target amplicons for each target loci.”⁶⁸

64. The ’035 Patent describes tagging DNA in a single PCR reaction in the context of explaining how to enrich the fraction of cell free DNA from a fetus isolated from a sample of plasma obtained from a pregnant woman. I note in this situation, the fetal cfDNA is typically present at relatively low levels and challenging to detect. One of ordinary skill in the art would have understood from the ’035 Patent’s written description that its methods regarding fetal cfDNA are applicable to other plasma samples containing cfDNA samples where the DNA sequences of interest are low-abundance (*e.g.*, as a “contaminant”). This could be in the case of a cancer subject’s plasma DNA that often contains tumor-associated cfDNA at low levels.⁶⁹

⁶⁷ See ’035 Patent at 83:8-9.

⁶⁸ *Id.* at 83:9-13.

⁶⁹ The ’035 Patent explain, for example, that “[t]he methods described herein may be used for a number of purposes where there is a target set of DNA that is mixed with an amount of contaminating DNA. In some embodiments, the target DNA and the contaminating DNA may be from individuals who are genetically related. For example, genetic abnormalities in a fetus (target) may be detected from maternal plasma which contains fetal

65. In the description of this enrichment process, the '035 Patent describes tagging DNA using tagged primers in a single cycle of PCR. Specifically, the '035 Patent explains that the primers used for this enrichment process “are designed to anneal [bind] a site a greater distance from the polymorphism than is expected to be present among cell free fetal DNA fragments.”⁷⁰ The '035 Patent explains that “[t]hese distal primers are tagged with a molecule or moiety that can allow selective recognition of the tagged pieces of DNA.”⁷¹ '035 Patent describes that “[t]hese primers may be used in a **one cycle** multiplex PCR reaction **prior to multiplex PCR** of the target polymorphic loci.”⁷² Accordingly, the '035 Patent teaches that, in the very first PCR cycle, tags on primers are appended to the DNA sequence being amplified. The tagged products are thereafter amplified one or more times in subsequent PCR cycles.

66. Although the process described *supra* relates to tagging DNA for removal from a sample, one of ordinary skill in the art would have understood that it applies equally

(target) DNA and also maternal (contaminating) DNA; the abnormalities include whole chromosome abnormalities (e.g. aneuploidy) partial chromosome abnormalities (e.g. deletions, duplications, inversions, and translocations), polynucleotide polymorphisms (e.g. STRs), single nucleotide polymorphisms, and/or other genetic abnormalities or differences. In some embodiments, the target and contaminating DNA may be from the same individual, but where the target and contaminating DNA are different by one or more mutations, for example in the case of cancer.” *Id.* at 92:16-32.

⁷⁰ *Id.* at 91:28-31.

⁷¹ *Id.* at 91:33-35.

⁷² *Id.* at 91:31-33 (emphasis added).

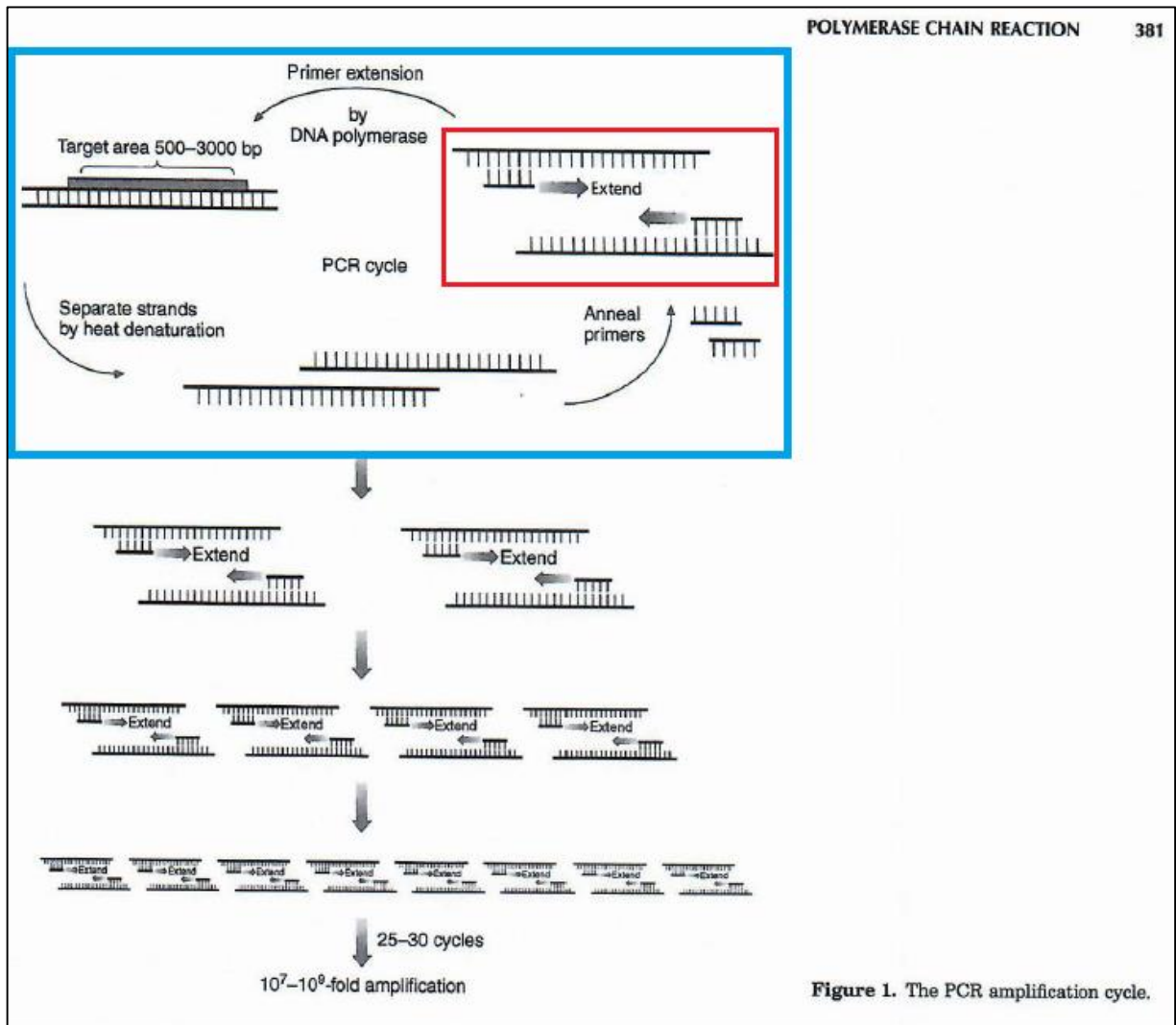
to tagging DNA that will be further manipulated. In either case, the addition of a tag to DNA via PCR occurs at the very first cycle that PCR is performed.

67. I have reviewed the prosecution history for the '035 Patent. Although I do not see anything specifically in the prosecution that addresses the term “amplifying the tagged products one or more times,” from my review, the prosecution history is consistent with my opinion. I see nothing to indicate that this term should be construed to have a different meaning other than its plain and ordinary meaning as described *supra*.

(ii) *Extrinsic Evidence*

68. One of ordinary skill in the art’s understanding of PCR and the addition of a tag to a DNA sequence of interest after a single cycle is also shown in evidence extrinsic to the '035 Patent itself. For example, I previously explained the fundamentals of PCR in a book chapter I authored, which was published in 2006 and discussed during my deposition.⁷³ The figure *infra* depicts how within a single cycle of PCR (blue outline), both a forward and reverse primer can hybridize to different ends of a template DNA molecule after it is denatured into two single strands. A DNA polymerase can then build two complementary strands (red outline). I explain this figure in greater detail *infra*.

⁷³ See Ex. 6, Metzker & Caskey, *Polymerase Chain Reaction*, In *ENCYCLOPEDIA OF MEDICAL DEVICES AND INSTRUMENTATION*, Second Edition, Volume 5, pp. 380-387 (2006) (“Metzker (2006)”) (cited as Ex. 7 in the September 26, 2023 Deposition of Michael Metzker Transcript (Dkt. 116-6)).



69. During the first cycle of PCR (blue outline), a template strand of DNA is denatured into two single strands by heat or chemical denaturant. At a different step in the same cycle, a forward primer will hybridize to the 5' end of one single stranded template DNA and DNA polymerase will build a complementary strand, extending from 5' to 3' (directionality is with reference to the template DNA strand). Also in the same cycle, a reverse primer will hybridize to the 3' end of the other single stranded template DNA and

DNA polymerase will build a complementary strand, extending from 5' to 3' (directionality is with reference to the newly synthesized complementary DNA strand).

70. Therefore, a single cycle of PCR can introduce up to **two tags** (one on each strand of the amplified DNA product). The subsequent cycles of PCR amplify the target region of interest, thereby performing targeted amplification of already tagged DNA.

(iii) *Dr. Van Ness' construction of "amplifying" contradicts the plain language of the claims*

71. The Declaration of Van Ness opines that the term "amplifying" in Claims 1 and 13 of the '035 Patent should be construed as "increasing the number copies of a molecule, such as a molecule of DNA."⁷⁴ I agree that this interpretation is consistent with the plain and ordinary meaning of "amplifying." I disagree with Dr. Van Ness's use of "copies," however, being "not just the copies that result from the initial copying of the original sample DNA strand, but also the copies of those copies and any subsequent copies."⁷⁵ In his interpretation, Dr. Van Ness treats "amplifying ... one or more times" as recited in the claim to mean a completed multi-cycle PCR process, overlooking the well-accepted understanding of one of ordinary skill in the art that PCR is a process comprised of iterative amplification cycles. Dr. Van Ness re-defines "amplifying ... one or more times" to mean completing all steps of a multi-cycle PCR. According to Dr. Van Ness,

⁷⁴ See Van Ness Decl. at ¶ 65.

⁷⁵ *Id.*

“copies”—which is a term not recited at all in the claim—do not result until the end of all amplification cycles of the *entire* PCR process. I disagree.

72. At the end of one amplification cycle in the PCR process, one of ordinary skill would have understood that the output (amplified products) is used as the input for the next amplification cycle, thus creating copies of these amplified products. Contrary to the opinion offered in the Van Ness Declaration, Dr. Van Ness in fact agreed with this basic understanding of how PCR works in his deposition.⁷⁶ This process is iterative throughout the PCR process such that copies are being generated at the end of each amplification cycle. Dr. Van Ness also agreed with this understanding of PCR in his deposition.⁷⁷

73. Dr. Van Ness adopts an overly narrow opinion and ignores the plain language of the claim itself, which as described *supra*, provides context for “amplifying” and **does not** recite the word “copies” that is the entire basis for Dr. Van Ness’s opinion. Claim 1 of the ’035 Patent recites “amplifying the tagged products *one or more times*.” One of ordinary skill in the art would have understood that once cell-free DNA has been tagged (as required earlier in the claim), the tagged cell-free DNA serves as a template for amplification and is amplified one or more times. Dr. Van Ness’s deposition testimony does not refute this understanding.⁷⁸ Even using Dr. Van Ness’s “copies” terminology,

⁷⁶ Van Ness Tr. at 220:1-16.

⁷⁷ *Id.* at 219:13-15.

⁷⁸ *Id.* at 228:17-232:25.

amplifying the tagged cell-free DNA one time—in one amplification cycle of the PCR—will generate a copy of the tagged cell-free DNA, satisfying the claim limitation. For this reason, I disagree with Dr. Van Ness’s overall construction of “amplifying” because it directly contradicts the plain language of the claim.

VI. THE NATERA PATENTS ARE NOT OBVIOUS UNDER SECTION 103

74. I have reviewed Dr. Van Ness’s opinions regarding purported obviousness of the Natera Patents under Section 103.⁷⁹ I disagree with his opinions including for all the reasons explained *infra*. In providing my opinions in reply, I focus my analysis on certain aspects of Dr. Van Ness’s contentions that in my opinion are incorrect or deficient and fail to support his conclusions. I reserve the right to offer additional opinions with respect to the Van Ness Declaration at another time. To be clear, the absence of any opinion expressed in this Reply Declaration should not be assumed to be an agreement with those opinions asserted in the Van Ness Declaration or a representation that I will not offer opinions in the larger case after this preliminary injunction proceeding.

75. **No motivation to combine or modify the cited references with a reasonable expectation of success in arriving at the claimed inventions.** As a preliminary matter, I have been informed and understand that an obviousness analysis requires an evaluation of whether one of ordinary skill in the art would have been motivated to combine or modify the cited references with a reasonable expectation of success in

⁷⁹ See Van Ness Decl. at ¶¶ 109-220 for the ’454 Patent and ¶¶ 221-303 for the ’035 Patent.

achieving the claimed invention, based on the teachings of the art in the cited references and the knowledge of a skilled artisan. This analysis of a motivation to combine or modify with a reasonable likelihood of success does not appear anywhere in the opinions asserted in the Van Ness Declaration—Dr. Van Ness does not address this legal requirement of obviousness at all. In consequence, Dr. Van Ness’s opinions are inadequately supported, at a minimum, because they fail to address all of the legal elements for establishing obviousness. Because a motivation to combine/modify with a reasonable expectation of success is nevertheless part of the obviousness analysis, I offer my opinions regarding why one of ordinary skill would not have been motivated to combine or modify the cited references and would not have reasonably expected success in achieving the Natera Patents’ inventive methods.

76. **No consideration of objective indicia of non-obviousness.** I also have been informed and understand that an obviousness analysis requires consideration of objective indicia of the claims’ non-obviousness when those indicia are present. Indeed, I understand that these objective indicia may be some of the most probative evidence of a patent’s claims’ non-obviousness. These objective indicia can include, for example, commercial success, unexpected results, failure of others, long felt but unmet need, and industry praise. Dr. Van Ness fails to analyze any objective indicia regarding the Natera Patents’ claims.

77. **Dr. Van Ness impermissibly relies on hindsight.** I have been informed and understand from counsel that the use of hindsight reconstruction of the claimed invention, by picking and choosing among isolated disclosures in the prior art, is impermissible in an

obviousness analysis. I have been further informed and understand that showing a teaching or motivation to combine prior art references is a defense to guard against the impermissible use of hindsight. I am also informed and understand that the consideration of objective indicia of non-obviousness is another way to avoid hindsight bias.

78. As explained further *infra*, Dr. Van Ness's opinions reflect the use of hindsight bias in reconstructing the Natera Patents' claimed inventions rather than the objective understanding of a skilled artisan guided only by the prior art and the then-accepted wisdom in the field. Specifically, Dr. Van Ness selects from among disparate disclosures in the prior art and the knowledge of one of ordinary skill in the art to support his opinions that the Natera Patents' claimed methods would have been obvious. However, the Van Ness Declaration does not articulate how, as of the patents' priority date(s), one of ordinary skill in the art would have been motivated to combine the teachings or understandings selected by Dr. Van Ness and reasonably expected to succeed in doing so in light of known challenges in the art. Moreover, as noted *supra*, the Van Ness Declaration's invalidity positions do not address objective indicia of non-obviousness.

79. I do not address the validity of the challenged dependent claims of the Natera Patents *infra* because they are not obvious at least for the reasons that the independent claims are not obvious. Dr. Van Ness has failed to establish that the independent claims would have been obvious for the reasons explained *infra*, and his opinions concerning the dependent claims are likewise flawed. I reserve the right to offer additional opinions concerning the dependent claims' validity at another time.

A. The '454 Patent Is Not Obvious

80. As Dr. Van Ness admitted in deposition, he is not opining that the '454 Patent is invalid under Section 102 of the Patent Act, which I understand and am informed by counsel precludes patentability if a prior art reference discloses each and every claim limitation, arranged as in the claim, either expressly or inherently.

Q. And you're – you don't offer any opinions that the '454 patent claims are invalid under Section 102 for anticipation, correct? All your prior art opinions are for obviousness under 103?

A. *That is correct. I'm offering invalidity based on obviousness.*⁸⁰

(i) Forshew (2012) Does Not Render the '454 Patent Claims Obvious

81. I disagree with Dr. Van Ness's opinions that the Asserted Claims of the '454 Patent are rendered obvious by Forshew (2012) in view of the skill and knowledge of a skilled artisan.⁸¹ At least for the reasons explained *infra*, Dr. Van Ness has failed to demonstrate that the '454 Patent claims are obvious over Forshew (2012), a reference already considered by the Patent Examiner during prosecution.

82. For the claims to be obvious, *inter alia*, it must have been obvious to one of ordinary skill in the art to have performed (ultra-deep) sequencing to “a depth of read of at least 50,000 per target locus,” as recited in Claim 1[c] of the '454 Patent,⁸² in the course of

⁸⁰ See Van Ness Tr. at 94:24-95:4 (emphasis added).

⁸¹ See Van Ness Decl. at § XA.

⁸² See '454 Patent at 171:39-43.

performing the other method steps. Dr. Van Ness's opinions regarding the value and utility of performing this sequencing are deficient at least because, as explained *infra*, one of ordinary skill in the art would not have understood the claimed ultra-deep $\geq 50,000$ sequencing depth to be something that would remediate the admitted limited sensitivity described in Forshew (2012)⁸³ and elsewhere.⁸⁴

83. One of ordinary skill in the art therefore would not have expected ultra deep sequencing to a depth of read of at least 50,000 per target locus to enable the Forshew (2012) method to analyze rare mutations in cfDNA. My analysis here focuses on Claim 1[c] of the '454 Patent. However, I reserve the right to offer additional opinions regarding the '454 Patent in view of Forshew (2012) alone or in combination with other references.

(a) Claim 1[c] of the '454 Patent

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.

A) Forshew (2012) Does Not Teach A Read Depth Greater Than 7,700 for Analyzing cfDNA

⁸³ See Forshew (2012) at 10. “**A current limitation of TAm-Seq is the detection limit** compared to assays that target individual loci (2, 3, 7, 40), which have been shown to detect two to three orders of magnitude lower frequencies.” (emphasis added).

⁸⁴ See Volik (2016) at 903. “[**The Tam-seq approach** afforded the opportunity to quantify mutant DNA at many loci in each patient but **had a relatively low sensitivity for mutations below approximately 1%–2%.**” (emphases added).

84. As a preliminary matter, I note that the Van Ness Declaration does not contend that Forshew (2012) teaches each and every limitation of the '454 Patent claims. In particular, the Van Ness Declaration does not dispute that Forshew (2012) does not explicitly teach achieving the ultra-deep sequencing to “a depth of read of at least 50,000 per target locus.”⁸⁵ In deposition, Dr. Van Ness confirmed that “I comment that Forshew had a depth of 15,400. So he did not directly meet that claim in his publication of 50,000.”⁸⁶

85. I agree that Forshew (2012) does not teach the claimed read depth of “at least 50,000 per target locus.” I disagree, however, that Forshew (2012) teaches a read depth of 15,400 in amplifying cfDNA. In fact, Forshew (2012) teaches amplifying cfDNA to an order of magnitude less—**no more than** a mean read depth of 7,700.⁸⁷

86. The Van Ness Declaration asserts that Forshew (2012) teaches sequencing cfDNA to a depth of 15,400 based on the statement that Forshew (2012) “amplified DNA from each sample in duplicate.”⁸⁸ Here, however, the Van Ness Declaration misinterprets disparate disclosures in Forshew (2012). In fact, the “duplicate” amplification suggested by Dr. Van Ness does not involve cfDNA. Rather, that passage from Forshew (2012)

⁸⁵ See Van Ness Decl. at ¶¶ 141-143.

⁸⁶ See Van Ness Tr. at 97:11-99:21, specifically citing 98:5-9 (emphasis added). However, Dr. Van Ness would not agree with the proposition that Forshew (2012) “doesn’t itself disclose all the claim elements” of the '454 Patent. Van Ness Tr. 95:17-23. To the extent Dr. Van Ness contends that Forshew (2012) discloses all the claim elements of the '454 Patent, I disagree as explained herein.

⁸⁷ See Forshew (2012) at Table 1, column titled “Mean depth (sequencing reads).”

⁸⁸ See Van Ness Decl. at ¶¶ 141-143; see also Forshew (2012) at 3.

involves genomic DNA (“gDNA”) isolated from formalin-fixed, paraffin-embedded (FFPE) tumor specimens, which one of ordinary skill in the art would have understood is *not* cfDNA. Unlike cfDNA, which is present in plasma, gDNA is isolated from intact solid tissues and has not undergone fragmentation and degradation.

87. Specifically, Forshew (2012) teaches the use of “TAm-Seq to sequence DNA extracted from 47 formalin-fixed, paraffin-embedded (FFPE) tumor specimens of ovarian cancers.”⁸⁹ Forshew (2012) further explains that “[w]e amplified DNA from each [ovarian tumor] sample in duplicate, tagging each replicate with a different barcode.”⁹⁰ This passage of Forshew (2012) does not teach duplicate amplification or sequencing those duplicated amplified samples using cfDNA isolated from plasma.

88. In fact, as reflected in Table 1 (reproduced *infra*), Forshew (2012) only teaches sequencing cfDNA ***from plasma samples*** to a maximum read depth of 7,700, as highlighted in the red box *infra*, with most of the applied read depths falling far lower (*e.g.*, 4,200).⁹¹

⁸⁹ See Forshew (2012) at 3, citing to Table S3, not Table 1 as suggested in the Van Ness Decl.

⁹⁰ *Id.*

⁹¹ *Id.* at Table 1 (red box added).

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

Specifically, Table 1's legend in Forshaw (2012) discloses "[m]utations identified by TAM-Seq in plasma samples from seven ovarian cancer patients," for which the highest mean depth of read is 7,700 (Patient ID 27). Forshaw (2012) does not provide any teachings or suggestion to increase the sequencing read depth for cfDNA samples to the claimed levels of 50,000 and, as explained *infra*, a skilled artisan would not have been motivated to duplicate or otherwise increase the read depth to detect rare genetic variants in plasma ctDNA as there would have been no reasonable expectation of success given the known technical limitations of Forshaw (2012)'s approach. If anything, Forshaw (2012) would encourage a skilled artisan to decrease read depth because the majority of read depths Forshaw (2012) applied were far below 7,700, going down to the low 4,000's.

B) It Would Not Have Been Obvious to Combine the Claimed Read Depth with Forshaw's (2012)'s Method

89. The Van Ness Declaration asserts that "one would have understood Forshaw (2012) to teach sequencing depths in excess of 15,400, including 50,000 and beyond."⁹²

⁹² See Van Ness Decl. at ¶ 142.

Relatedly, the Van Ness Declaration asserts that a “POSA would have understood that increasing sequencing depth increases sensitivity in detecting SNPs and it would be routine to increase sequence read depth to obtain greater sensitivity as required.”⁹³ I disagree with each of these contentions.

90. One of ordinary skill in the art would not have been motivated to combine the claimed depth of read with the other claimed method steps and have reasonably expected success sequencing to “a depth of read of at least 50,000 per target locus” (nearly 10x higher than Forshew (2012)’s mean read depth) to detect rare genetic variants such as SNVs, in light of the technical limitations of Forshew (2012)’s method. Specifically, sequencing to the claimed read depth—sometimes called “ultra-deep sequencing”—would not have remedied the limited sensitivity of Forshew (2012)’s approach to detect rare genetic variants, as explained further *infra*.

91. As an initial matter, Forshew (2012) admits the limited sensitivity of its method to detect rare genetic variants.

A current limitation of TAm-Seq is the detection limit compared to assays that target individual loci (2, 3, 7, 40), which have been shown to detect two to three orders of magnitude lower frequencies. Our approach may be sufficient for analyzing plasma from patients with certain advanced cancers, but further improvement may be necessary before this method can be more widely used in the clinic.⁹⁴

⁹³ *Id.* at ¶ 144.

⁹⁴ See Forshew (2012) at 10 (emphasis added).

In the context of plasma samples from cancer patients, at best, Forshew (2012) posits merely that its methods “may be sufficient” for use with patient samples containing abundant circulating tumor cfDNA (*e.g.*, patients with advanced cancers). But one of ordinary skill in the art would have understood from Forshew (2012) that its method would not be sensitive enough to detect genetic variants in low-abundance ctDNA in many other kinds of cancers (*e.g.*, in plasma from patients with early-stage cancers, or “minimal residual disease” in the case of patients whose cancers have been surgically excised or treated). Thus, the limitations of Forshew (2012)’s approach would have impaired the ability of a skilled artisan to identify rare tumor-associated SNPs in cfDNA isolated from a plasma sample, as Forshew (2012) itself recognizes.

92. In order for the ’454 Patent claims to be obvious, *inter alia*, it must have been obvious to one of ordinary skill in the art that the claimed ultra-deep sequencing—*i.e.*, the specific $\geq 50,000$ level recited—would have improved Forshew (2012)’s approach and permit detection of rare mutations. But the Forshew (2012) approach alone would not be effective on either score, as one of ordinary skill in the art would have recognized, and therefore the skilled artisan would not have reasonably expected success with the Forshew (2012) strategy.

93. Specifically, the limitations of Forshew (2012)’s approach arise because the errors associated with Forshew (2012)’s sample library preparation and sequencing workflow exceed the technique’s ability to detect rare genetic DNA variants, as explained

in greater detail *infra*. Under the Forshew (2012) approach, the rare variants are lost in the “noise” associated with the abundant errors found in the sequence read data.

1) Ultra-Deep Sequencing Does Not Enable Detection of Rare Genetic Variants that Fall Below the Sequencing Workflow Error Rate

94. Here, I provide background and context for my opinions regarding Forshew (2012). Prior to 2015, there were many challenges in using next-generation sequencing (“NGS”), sometimes also referred to as “massively parallel sequencing,” to process samples and facilitate the analysis of tumor-associated genetic variations including single nucleotide variants (“SNVs”), copy number variations (“CNVs”), and chromosomal rearrangements. Even relatively high-fidelity sequencing platforms were known to introduce sequencing errors at a rate of $\geq 0.1\%$.⁹⁵

95. Additional erroneous nucleotide changes could also be introduced during the PCR amplification steps of sequencing library preparations. For example, Schmitt (2012) explains that base-specific mutagenic DNA damage followed by polymerase misincorporation against such damaged base contributes the error rate of the sequencing process.

Base-specific mutagenic DNA damage is a likely explanation of these imbalances. Excess G→T mutations are consistent with the oxidative product 8-oxo-guanine (8-oxo-G) causing first round PCR errors and artifactual G→T mutations. DNA polymerases, including those commonly used in PCR, have a strong tendency to ***insert adenine opposite 8-oxo-G*** (35, 36),

⁹⁵ See Ex. 3, Glenn, *Field guide to next-generation DNA sequencers*, MOLECULAR ECOLOGY RESOURCES 11:759–769 (2011) (“Glenn (2011)”) at Table 3.

and misinsertion of A opposite 8-oxo-G would result in ***erroneous scoring of a G→T mutation***. Likewise, the excess C→T mutations are consistent with ***spontaneous deamination of cytosine to uracil*** (37), a particularly common DNA damage event that results in ***insertion during PCR of adenine opposite uracil and erroneous scoring of a C→T mutation***.⁹⁶

Such accumulation of potential false positive errors by both PCR and sequencing was known to limit the reliable analysis of true variants that occur with <1% frequency.

96. These technical limitations were recognized in the art. For example, Kinde (2011) explains that massively parallel sequencing (sometimes also referred to as “next generation sequencing” or “NGS”) could not have been used generally to analyze rare variants because of high error rates associated with the sequencing workflow:

[M]assively parallel sequencing cannot generally be used to detect rare variants because of the high error rate associated with the sequencing process. For example, with the commonly used Illumina sequencing instruments, this error rate varies from ~1% (31, 32) to ~0.05% (33, 34), depending on factors such as the read length (35), use of improved base-calling algorithms (36–38), and the type of variants detected (39). Some of these errors presumably result from mutations introduced during template preparation, during the preamplification steps required for library preparation, and during further solid-phase amplification on the instrument itself. Other errors are due to base misincorporation during sequencing and base calling errors. Advances in base calling can enhance confidence (e.g., refs. 36–39), but instrument-

⁹⁶ See Ex. 4, Schmitt *et al.*, *Detection of ultra-rare mutations by next-generation sequencing*, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES 109: 14508-14513 (2012) (“Schmitt (2012)”) at 14510 (emphases added).

based errors are still limiting, particularly in clinical samples wherein the mutation prevalence can be $\leq 0.01\%$ (11).⁹⁷

97. Additionally, prior to the Natera Patents, skilled artisans understood that although increasing read depth⁹⁸ could potentially increase sensitivity, it would not increase the sensitivity beyond the limit of detection based on the error rate. For that reason, “ultra-deep” sequencing of 30,000 reads or more per locus was uncommon and unnecessary for most sequencing efforts. Ultra-deep sequencing was also generally undesirable as it was recognized as expensive and not cost-effective for routine use.

98. Accordingly, one of ordinary skill in the art would have recognized that the sensitivity of preparing samples of and analyzing variants associated with a patient’s cancer, particularly when using cfDNA as source material, would have been hampered by errors introduced during the sample preparation process as well as by the fragmented and variable nature of the cfDNA itself. Such errors could be introduced at both the amplification and sequencing steps of preparing cfDNA preparations.

99. Even after 2014-2015, the field continued to recognize that methods of sample processing and analyzing cancer-associated variant loci were hampered by the lack

⁹⁷ See Ex. 5, Kinde et al., *Detection and quantification of rare mutations with massively parallel sequencing*, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES 108:9530-9535 (2011) (“Kinde”) at 9530 (emphasis added).

⁹⁸ The ’454 Patent explains, for example, that “the depth of read is the number of sequencing reads that map to a given locus” and, “for the depth of read of a locus, the depth of read is the number of reads measured by the sequencer mapping to that locus. In general, the greater the depth of read of a locus, the closer the ratio of alleles at the locus tend to be to the ratio of alleles in the original sample of DNA.” ’454 Patent at 99:66-100:9.

of sensitivity of the available techniques to analyze variants in cfDNA. By way of example, Volik (2016) provides:

Broadly, current approaches for detection of tumor aberrations in cfDNA can be divided into two categories: methods targeting specific changes and methods allowing detection of all possible aberrations in DNA (including targeted and whole exome/genome sequencing). The latter next-generation sequencing (NGS)-based options offer numerous potential benefits for observing clonal differences in tumor cell populations, an advantage that until very recently ***was offset by more limited sensitivity and specificity.***⁹⁹

While these early observations highlighted many possibilities for using ctDNA as a noninvasive approach to analyze tumor genomes, ***sufficiently sensitive and specific laboratory techniques to fully leverage this potential were not yet developed.***¹⁰⁰

As we have demonstrated earlier, the mutant VAF in patients' cfDNA can go as low as 0.01%. Clearly, any strategy to suppress sources of errors thereby increasing accuracy in detecting mutant DNA molecules is important for cfDNA analysis. ***This problem is equivalent to an extreme case of identifying low-abundance mutations in tissue samples, which has also proven difficult for NGS methods.***¹⁰¹

While targeted assays can be fruitful in the clinical setting, sequence-based approaches offer clear advantages in terms of flexibility of coverage and the ability to detect a wide range of aberrations in tumor genomes. This flexibility will be especially important for managing metastasis and resistance to therapy; widely recognized to be among the most important problems in cancer management. Resistance to therapy can be driven by a wide range of genomic aberrations such as point mutations and copy number aberrations. Moreover, resistant

⁹⁹ See Volik (2016) at 901 (emphasis added).

¹⁰⁰ *Id.* at 898 (emphasis added).

¹⁰¹ *Id.* at 905 (emphasis added).

subclones can constitute a very small proportion of the tumors total clonal population until the selective pressure of therapy leads to their rapid expansion. Clearly, the early detection of resistant clones requires sensitivity to detect such events. However, ***this requires minimizing noise in the ctDNA analyses and pushing the sensitivity of detection to the theoretical limits imposed by the plasma levels of ctDNA.***¹⁰²

100. Regarding Forshew (2012), Volik (2016) explains that Forshew (2012) had “relatively low sensitivity” for mutations below approximately 1%-2%. Volik (2016) summarizes Forshew (2012)’s approach as follows:

An early example of a PCR-based strategy for analysis of mutations found in individual patients involved the design of a set of tailed site-specific primers followed by multiplex PCR (for preamplification) and subsequent uniplex PCR using each individual primer pair. The second PCR, in which locus-specific primers are applied individually, is accomplished using a Fluidigm AccessArray system and the entire procedure was named TAm-Seq (Tagged Amplicon Sequencing; ref. 75).¹⁰³

This approach afforded the opportunity to quantify mutant DNA at many loci in each patient ***but had a relatively low sensitivity for mutations below approximately 1%–2%.***¹⁰⁴

101. The lack of sensitivity of Forshew (2012)’s approach was significant because, among other things, the highly fragmented nature of cfDNA, and the low abundance of ctDNA in blood plasma, presented significant challenges in the biochemical preparation and analysis of cancer-associated genetic variants.

¹⁰² *Id.* at 906 (emphasis added).

¹⁰³ *Id.* at 903.

¹⁰⁴ *Id.* (emphasis added).

102. I also note that, in my experience, ultra-deep sequencing was not a common approach in 2014-2015.¹⁰⁵ Achieving this depth of read by sequencing tens or hundreds or thousands of times in the same region is typically unnecessary to achieve research goals. At the time, most researchers were not trying to find the “needle in the haystack” that this ultra-deep sequencing can accomplish. This approach would have been comparatively (and undesirably) expensive to the extent even available, which would have discouraged one of ordinary skill in the art from its use.

2) Ultra-Deep Sequencing Would Not Have Been An Obvious Modification of Forshew (2012) In Light of the Error Rate of Forshew (2012)’s Method

103. As noted *supra*, I disagree with Dr. Van Ness’s opinions that the ’454 Patent claims are obvious over Forshew (2012).¹⁰⁶ The consequence of the sequencing workflow errors described *supra* is that, following Forshew (2012)’s approach, rare genetic variants—*e.g.*, SNVs on tumor cfDNA present in the plasma at low levels—are not likely to be detectable below the “noise” of the error rate even if amplified 50,000 times or more at each locus. Dr. Van Ness does not address this problem, nor explain why a one of skill in the art would have been motivated to sequence to the claimed depth of read to remediate Forshew (2012)’s limited sensitivity, or have reasonably expected success in doing so.

¹⁰⁵ See, *e.g.*, Volik (2016), discussed *supra* in Section II.

¹⁰⁶ See Van Ness Decl. at ¶¶ 142, 144.

104. Specifically, as a skilled artisan would have recognized, genetic variants in tumor-associated cfDNA are likely to be low-abundance or variable in patient plasma and consequently rare and difficult to target.¹⁰⁷ Forshew (2012) itself explicitly describes very similar challenges in working with cfDNA. For example, Forshew (2012) acknowledges that cfDNA is highly fragmented.

Circulating DNA is fragmented to an average length of 140 to 170 base pairs (bp) and is present in only a few thousand amplifiable copies per milliliter of blood, of which only a fraction may be diagnostically relevant (2, 3, 23–25). Recent advances in noninvasive prenatal diagnostics highlight the clinical potential of circulating DNA (25–28), but also ***the challenges involved in analysis of circulating tumor DNA (ctDNA), where mutated loci and AFs may be more variable***. Various methods have been optimized to detect extremely rare alleles (1, 2, 6, 7, 29–31), and can assay for predefined or hotspot mutations. ***These methods, however, interrogate individual or few loci and have limited ability to identify mutations in genes that lack mutation hotspots***, such as the TP53 and PTEN tumor suppressor genes (32). In patients with more advanced cancers, ctDNA can comprise as much as 1% to 10% or more of circulating DNA (2), presenting an opportunity for more extensive genomic analysis. Targeted resequencing has been recently used to identify mutations in selected genes at AFs as low as 5% (33–35). However, ***identifying mutations across sizeable genomic regions spanning entire genes at an AF as low as 2%, or in few nanograms of fragmented template from circulating DNA, has been more challenging***.¹⁰⁸

105. It would have been necessary to detect and correct these sequencing workflow errors in order to sequence deeply enough to practice the claimed depth of read

¹⁰⁷ See Section II.

¹⁰⁸ See Forshew (2012) at 1 (emphases added).

in a plasma sample of cancer patient DNA. This is because SNVs associated with a particular cancer are present at relatively low levels in ctDNA, and, consequently, these sequencing workflow errors need to be detected and corrected to identify rare genetic variants at the targeted loci. Without such modifications, one of ordinary skill in the art would have understood that repeatedly sequencing a locus up to 50,000 times or more—which in any event was not taught or suggested by Forshew (2012)—would not have improved detection of rare genetic variants. Indeed, such repeated (and costly) sequencing would merely propagate the “noise” of the sequencing workflow errors without identifying the targeted rare genetic variant.

106. On this critical point, the Van Ness Declaration’s analysis is profoundly deficient. Dr. Van Ness fails to explain why one of ordinary skill in the art would have reasonably expected success in improving the sensitivity of Forshew (2012)’s method to detect rare variants specifically simply by increasing the sequencing depth of read to 50,000 or more per locus. In fact, doing so would not have remediated the deficiencies of Forshew (2012) because, as explained *supra*, performing deep sequencing to a read depth of 50,000 or more will simply increase the sequencing workflow noise. A substantial increase in the sequencing workflow noise would further obfuscate the detection of rare mutations, making it much harder to identify such mutations. In opining that it would have been obvious to increase the depth of sequencing read, Dr. Van Ness fails to account for any of these challenges that one of ordinary skill in the art would have confronted in 2014-2015.

107. Accordingly, contrary to the Van Ness Declaration's contention¹⁰⁹, the parameter of sequencing read depth would **not** merely be "routinely adjusted to achieve whatever sensitivity was required." In fact, it would have been nonsensical to undertake the time, effort, and expense of sequencing many thousands of times if doing so would not have been predicted to identify rare variants above the background "noise" (error rate) of the sequencing approach. A skilled artisan would not have been motivated to pursue this strategy, and would not have reasonably expected success doing so.

108. Other rationale provided in the Van Ness Declaration fails to support a conclusion that the '454 Patent claims would have been obvious over Forshew (2012). The Van Ness Declaration's reliance on the '189 Patent Pub. (Bentley) reference¹¹⁰ does not cure the deficiencies in Dr. Van Ness's rationale identified *supra*. The Van Ness Declaration relies on the '189 Patent Pub. (Bentley) for the general proposition that increasing sequencing depth increases sensitivity,¹¹¹ but fails to explain how the '189 Patent Pub. (Bentley) addresses the technical considerations I identified *supra*. To support the Van Ness Declaration's proposition "that increasing sequencing depth increases sensitivity, Dr. Van Ness relies on Table 2 in the '189 Patent Pub. (Bentley).¹¹² The Van

¹⁰⁹ See Van Ness Decl. at ¶ 143.

¹¹⁰ See Dkt. 118-9, U.S. Patent Application Publication No. US 2010/0261189) to Bentley *et al.* ("System and method for detection of HLA variants") (the "'189 Patent Pub. (Bentley)").

¹¹¹ See Van Ness Decl. at ¶ 144.

¹¹² *Id.*

Ness Declaration fails to appreciate that the '189 Patent Pub. (Bentley) is describing this relationship using error-free reads.

For example, based on binomial statistics the lower limit of detection (i.e., one event) for a fully loaded 60 mmx60 mm PicoTiterPlate (***2x10⁶ high quality bases, comprised of 200,000x100 base reads***) with 95% confidence, is for a population with allelic frequency of at least 0.002%, and with 99% confidence for a population with allelic frequency of at least 0.003% (it will also be appreciated that a 70x75 mm PicoTiterPlate could be employed as described above, which allows for an even greater number of reads and thus increased sensitivity).¹¹³

109. One of ordinary skill in the art would have understood that the “high quality bases” described in '189 Patent Pub. (Bentley) are error-free reads. As such, I do not see such a solution to the sequencing workflow errors described *supra* from my review of the '189 Patent Pub. (Bentley). Dr. Van Ness’s citation of prior testimony by Natera witnesses, including myself, also fails to account for the importance of sequencing workflow errors in the detection of rare variants for the same reason.¹¹⁴

110. Moreover, consistent with the Van Ness Declaration, Dr. Van Ness’s deposition testimony (excerpted *infra*) fails to address how one of ordinary skill in the art would have overcome the challenges Forshew (2012) presents. Dr. Van Ness opined that the NGS platforms are “very accurate,” which is an oversimplification of the technology, particularly because—as explained *infra*—the Examiner distinguished Forshew (2012)

¹¹³ See '189 Patent Pub. (Bentley) at [0085] (emphasis added).

¹¹⁴ See Van Ness Decl. at ¶¶ 145-150.

from the '454 Patent claims on the basis of sensitivity. And in deposition, Dr. Van Ness did not explain how the conventional NGS technology of Forshew (2012) could be used to identify rare variants that occur below these platforms' known error rate. Dr. Van Ness's testimony merely confirms that more information is obtained with each read—but, given the error rate, it would have been challenging if not impossible to determine or distinguish between information arising from accurately sequencing a rare genetic variant and information arising from an error in the preparation or sequencing process.

Q. Is... Are you aware of any problems that occur -- technical problems that occur by increasing depth of read?

A. You know, anytime you do an experiment and you extend the experiment, you have to weigh the pluses and minuses. ***So I think, are there opportunities for errors that increase with sequencing depth?*** Maybe. But you get more information. So there's much more benefit and enhanced sensitivity particularly because the platforms are very accurate. And so I think with the Illumina platforms -- I mean, the whole reason for doing 50,000 is because you could have an error of 5 or 6 base calls, but because you did 50,000, you're -- you're looking at a very large sampling so that you are more and more assured that the sequences that you're getting are correct.¹¹⁵

111. In sum, Forshew (2012) identifies a significant problem—the desirability of identifying of rare genetic variants in tumor-associated cfDNA—without providing any solution. It would not have been obvious to remediate this deficiency by combining the

¹¹⁵ See Van Ness Tr. at 102:2-103:14, specifically citing 102:2-21 (objection omitted, emphasis added).

claimed depth of read with Forshew (2012)’s method for reasons explained *supra*. Dr. Van Ness’s analysis does not support a different conclusion because the Van Ness Declaration does not articulate how one of ordinary skill in the art would have reasonably expected to use sequencing to “a depth of read of at least 50,000 per target locus” effectively to identify rare mutations in light of the known limitations of the available equipment and approaches. Moreover, as discussed further *infra*, Forshew (2012) does not identify a specific technique that will work for this purpose.

112. The lack of an obvious solution is further reflected in the Editor’s Summary accompanying Forshew (2012)’s publication. The Summary notes (emphasis added) that “TAm-Seq ***will need to achieve a more sensitive detection limit*** (<2% allele frequency) to identify mutations in the plasma of patients with less advanced cancers”¹¹⁶—*e.g.*, patients with less tumor-associated cfDNA present in plasma and, consequently, more rare tumor-associated genetic variations. The Editor’s Summary continues that “***once optimized***, this ‘liquid biopsy’ approach will be amenable to personalized genomics.”¹¹⁷ Accordingly, the Editor’s Summary is further evidence of how one of ordinary skill in the art would not have reasonably expected success in achieving the claimed methods following the teachings of Forshew (2012) based on the approach identified in the Van Ness Declaration.

¹¹⁶ See NAT-NEO-00043262-00043275 (Editor’s Summary to Forshew (2012)) at NAT-NEO-00043262 (emphasis added).

¹¹⁷ *Id.*

3) ForsheW (2012) Does Not Teach that Ultra-Deep Sequencing Would Have Improved the Sensitivity of Its Approach

113. The Van Ness Declaration suggests ForsheW (2012) itself teaches sequencing more deeply to achieve the methods claimed in the '454 Patent.¹¹⁸ I disagree that ForsheW (2012) proposed possibilities that could be tried to improve the sensitivity of detection of TAm-Seq.

114. First, ForsheW (2012) specifically would not have motivated one of ordinary skill in the art to achieve the claimed read depth because ForsheW (2012) was not trying to find a rare genetic variant at a sensitivity of 0.1%, and ForsheW (2012) does not teach that this particular goal would have been desirable to achieve.

115. Second, ForsheW (2012) would not have motivated one of ordinary skill in the art to achieve the claimed read depth because it does not teach that this solution would remediate the admitted limitations of ForsheW (2012)'s approach. At page 10 (reproduced *infra*), ForsheW (2012) merely references "higher read depth" in the context of four other suggestions—higher fidelity, additional replicates, improve algorithms, and rare allele enrichment. None of these suggest increasing the read depth to the claimed levels.

Higher read depth or fidelity, additional replicates, or improved algorithms **could allow** for enhanced mutation detection without change to protocols. An alternative strategy is through rare allele enrichment, for example, by combining

¹¹⁸ See Van Ness Decl. at ¶ 142.

TAm-Seq with protocols such as COLD-PCR (co-amplification at lower denaturation temperature PCR).¹¹⁹

116. Notably, Forshew (2012) does not state that any of these strategies, including higher read depth, would necessarily solve its issues with detecting rare mutations, only that they “could”—hypothetically—“allow for enhanced mutation detection.” Forshew (2012) thus speculates about what other approaches “could allow” rather than teach (much less test) how to address its shortcomings.

117. Consistent with my opinions, Dr. Van Ness acknowledged that there were “challenges” in Forshew (2012)’s strategy.

Q. In discussing targeted resequencing, Dr. Forshew says that, quote, “However, identifying mutations across sizable genomic regions spanning entire genes at an AF as low as 2%, or in few nanograms of fragmented template from circulating DNA, has been more challenging.” So that – so according to Dr. Forshew, the targeted resequencing has not solved that particular challenge, right?

A. *Again, it’s more challenging.* Didn’t say it wasn’t doable. He just said it’s more challenging.

Q. Okay. And do you agree with him? Or disagree with him?

A. *No, I agree that at the time he was sort of justifying his publication, that he recognized that there were challenges,* not impossibilities, but there were challenges that could bear improvement.¹²⁰

¹¹⁹ See Forshew (2012) at 10 (emphases added). The underlined portions represent the other possibilities suggested by Forshew (2012) that the Van Ness Decl. failed to disclose.

¹²⁰ See Van Ness Tr. at 120:20-121:16 (objection and stricken question omitted, emphases added).

118. I am informed and understand from counsel that an invention is not obvious merely because there are a finite number of options available for one of ordinary skill in the art to pursue—rather, the skilled artisan must also have had a reasonable expectation of success that a particular solution will work. Here, one of ordinary skill in the art would not have reasonably expected success with any particular idea proposed by Forshew (2012) *supra*, including increasing sequencing read depth. In fact, Forshew (2012)’s speculative suggestion of different possible approaches, all stated generically without any implementation details, and with no further reason given to select one or another, indicates that Forshew (2012) did not know which approach, if any, would have been effective to improve the sensitivity of the disclosed TAm-Seq approach.

C) Forshew (2012) Was Overcome During Prosecution

119. I understand that Forshew (2012) was before the Examiner during prosecution of the ’454 Patent. I have been informed and understand from counsel that, when prior art is before the Examiner, the Examiner is presumed to have properly considered the prior art. I have reviewed the ’454 Patent application’s prosecution history and I see no basis to believe that the Examiner misunderstood the teachings of Forshew (2012) or other teachings or knowledge in the art, or otherwise erred in allowing the ’454 Patent claims.

120. As the Van Ness Declaration acknowledges, the Examiner of the '454 Patent application actively considered Forshew (2012) during prosecution of the '454 Patent application and determined that the pending claims were allowable over Forshew (2012).¹²¹

121. The Van Ness Declaration nevertheless substitutes Dr. Van Ness's judgment for that of the Examiner and asserts that the Examiner accepted "erroneous arguments" by Natera in allowing the '454 Patent claims over Forshew (2012).¹²² I disagree. As excerpted from Forshew (2012) *infra*, Forshew (2012) teaches, among other things, the use of targeted single-plex amplification, as Natera rightly pointed out, and Natera accurately quotes Forshew (2012) regarding potential problems associated with multiplexing.¹²³ There is no evidence that the Examiner did not independently consider the entirety of Forshew (2012)'s disclosure in finding the '454 Patent claims patentable over its teachings.

Performing single-plex amplification with each of these primer pairs would require dispersing the initial sample into many separate reactions, considerably increasing the probability of sampling errors and allelic loss. ***Multiplex amplification using a large set of primers could result in nonspecific amplification products and biased coverage.*** We therefore applied a two-step amplification process: a limited-cycle preamplification step where all primer sets were used together to capture the starting molecules present in the template, ***followed by individual amplification to purify and select for intended targets*** (Fig. 1B) (Supplementary Methods).¹²⁴

¹²¹ See Van Ness Decl. at ¶¶ 166-176.

¹²² *Id.* at ¶¶ 168-169; *see also* Dkt. 118-10 (Remarks to an Office Action Dated October 7, 2022) & Dkt. 118-11 (Notice of Allowance Dated November 8, 2022).

¹²³ *Id.* at ¶ 168.

¹²⁴ See Forshew (2012) at 3 (emphases added),

122. Moreover, the '454 Patent claims recite more than just multiplex amplification—for example, as discussed *supra* they recite an ultra-deep depth of read of 50,000 or more, which it is undisputed Forshew (2012) does not disclose. The Examiner specifically pointed to this limitation—**not** multiplex amplification—as a basis for allowing the claims. Specifically, on page 5 of the '454 Patent application's notice of allowance¹²⁵, the Examiner stated:

Reasons for Allowance

19. The following is an examiner's statement of reasons for allowance: The prior art does not show the limitation in independent claim 1 of a sequencing read depth of at least 50,000 per target locus in the context of the claimed subject matter. The prior art does not show the limitation in independent claim 15 of detecting a single nucleotide variant mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the single nucleotide variant locus in the context of the claimed subject matter.

123. The Van Ness Declaration contends, however, that it “seems likely” that the Examiner overlooked the obviousness of increasing sequencing depth.¹²⁶ This contention is contradicted by Dr. Van Ness's assertion that increasing sequencing depth was a well-known approach to improve sensitivity, as evidenced (and Dr. Van Ness contends) by the '189 Patent Pub. (Bentley).¹²⁷

¹²⁵ See Dkt. 118-11 (Notice of Allowance Dated November 8, 2022) at 5.

¹²⁶ See Van Ness Decl. at ¶ 144.

¹²⁷ *Id.* at ¶¶ 170-175.

124. Additionally, the Van Ness Declaration identifies no factual basis to indicate that the Examiner did not consider all claim limitations, including sequencing to a read depth of 50,000 or more per target locus. Indeed, if anything, it seems unlikely that the Examiner would not have considered the arguments by Natera given that Forshew (2012) was explicitly identified during prosecution by the Examiner and then subsequently addressed in substance by Natera. In addition, Dr. Van Ness does not address the points I raise *supra*—namely, that sequencing to the claimed read depth would not have been obvious because one of ordinary skill in the art would not have expected or predicted this strategy to be effective to identify rare variants when using Forshew (2012)’s approach. Thus, the allowance of the claims is consistent with my opinions that such a sequencing strategy would not have been obvious to one of ordinary skill in the art.¹²⁸

(ii) *Bashashati (2013) Does Not Render the ’454 Patent Claims Obvious*

125. At least for the reasons explained *infra*, the Van Ness Declaration has failed to show that the ’454 Patent claims are obvious over Bashashati (2013).¹²⁹ As described *infra*, I provide my reasons and bases for why I disagree with Dr. Van Ness’s opinions regarding Bashashati (2013).¹³⁰

¹²⁸ See Dkt. 118-11 (Notice of Allowance Dated November 8, 2022).

¹²⁹ See Dkts. 118-14, 118-15, Bashashati *et al.*, *Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling*, JOURNAL OF PATHOLOGY 231:21-34 (2013), including the Supplementary Appendix (“Bashashati (2013)”).

¹³⁰ Dr. Van Ness admitted in deposition that he did not include a specific section in the Van Ness Declaration analyzing the combination of Forshew (2012) and Bashashati (2013). See Van Ness Tr. at 106:7-13.

126. For the claims to be obvious, *inter alia*, I have been informed and understand that it must have been obvious to one of ordinary skill in the art to perform “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” in the course of performing the other method steps. Bashashati (2013) does not perform this approach, as Dr. Van Ness acknowledges,¹³¹ and my analysis here focuses primarily on ’454 Patent Claim 1[b].

127. Dr. Van Ness’s contrary opinions conflate disparate teachings in Bashashati (2013) without articulating how one of ordinary skill in the art would have been motivated to practice the claimed methods or would have reasonably expected success in doing so, particularly given the difficulties in multiplexing many target loci in a single reaction volume. The results of this multiplexing strategy would have been unpredictable in light of the technical challenges in its implementation, as discussed further *infra*. I reserve the right to offer additional opinions regarding the ’454 Patent in view of Bashashati (2013) alone or in combination with other references.

(a) Claim 1[b] of the ’454 Patent

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

¹³¹ See, e.g., Van Ness Decl. at ¶ 190.

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

128. The '454 Patent claims require “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.”¹³² I refer to this limitation as claim 1[b] of the '454 Patent.

129. The Van Ness Declaration does not opine that Bashashati (2013) teaches this approach. Instead, in arguing this limitation is obvious, Dr. Van Ness points to Bashashati (2013)’s description of using target-specific primers to amplify tumor DNA and then using those same primers to amplify ctDNA.¹³³ For both Bashashati (2013)’s tumor and ctDNA samples, however, individual primer pairs were used, not multiplex PCR as required by Claim 1 of the '454 Patent.

130. Regarding the tumor DNA analysis, Bashashati (2013) explains that “DNA primers were synthesized in 96-well plates ... Polymerase cycling reactions were set up in 96-well plates and comprised of 0.5 uM forward primer, 0.5 uM reverse primer, 1-2 ng of gDNA... Reaction plates were cycled ... PCR reactions were visualized on 3% agarose

¹³² See '454 Patent at 171:32-38.

¹³³ See, e.g., Van Ness Decl. at ¶ 189.

... Successful reactions were manually pooled.”¹³⁴ Similarly, regarding the ctDNA analysis, Bashashati (2013) explains that “[t]he primers used and PCR cycling conditions were the same as in the previous section. Circulating DNA was used as PCR templates at 0.1 ng per reaction.”¹³⁵

131. From these descriptions, the Van Ness Declaration can only say that “[t]hese sections teach the use of targeted PCR to amplify target loci encompassing different SNV.”¹³⁶ Dr. Van Ness cannot and does not say that Bashashati (2013) teaches targeted multiplex PCR on ctDNA from plasma samples.

132. Indeed, the Van Ness Declaration acknowledges that in Bashashati (2013), “targeted amplification of SVNs from ctDNA was conducted using *single primer pairs per reaction*.”¹³⁷ I agree with the Van Ness Declaration’s implicit acknowledgement that Bashashati (2013) does not disclose claim 1[b] of the ’454 Patent. Dr. Van Ness in deposition agreed that Bashashati (2013) does not teach targeted multiplex amplification of 10 to up to 500 targets in one reaction volume.¹³⁸

133. To argue the ’454 Patent claims are obvious, the Van Ness Declaration attempts to cure this deficiency in Bashashati (2013)’s disclosure in four ways, discussed

¹³⁴ See Bashashati (2013) at S6-S7.

¹³⁵ *Id.* at S7.

¹³⁶ See Van Ness Decl. at ¶ 190.

¹³⁷ *Id.* at ¶ 195 (emphasis added).

¹³⁸ See Van Ness Tr. at 134:2-13, particularly 134:11-13 (“I don’t know of an example of that, although there are other references that I referred to that have done it. This one I have not seen it.”).

in turn *infra*. None of these points are successful because Dr. Van Ness fails to explain how one of ordinary skill in the art would have been motivated to modify Bashashati (2013), much less with any reasonable expectation of success.

134. First, Dr. Van Ness states in a single, unsupported sentence that a “POSA also would have been motivated to do so [amplifying target loci in multiplex reactions in the same reaction volume] for the benefit of requiring fewer separate reactions.”¹³⁹ I disagree. Dr. Van Ness identifies no actual or theoretical benefit that fewer separate reactions would confer in the performance of the methods taught in Bashashati (2013). In fact, combining multiple PCR-based amplification reactions—especially up to 150 such reactions—in one volume was associated with known significant technical hurdles and, consequently, would have been an undesirable strategy for one of ordinary skill in the art to have pursued in the context of the claimed methods.

135. The ’454 Patent itself explains some of those technical hurdles. In light of these challenges, the outcome of multiplexing many primer pairs in a single reaction volume would have been unpredictable to a skilled artisan.

[H]ighly multiplexed PCR can often result in the production of ***a very high proportion of product DNA that results from unproductive side reactions such as primer dimer formation.*** In an embodiment, the particular primers that are most likely to cause unproductive side reactions may be removed from the primer library to give a primer library that will result in a greater proportion of amplified DNA that maps to the genome.¹⁴⁰

¹³⁹ *Id.*

¹⁴⁰ See ’454 Patent at 105:51-58 (emphasis added).

Multiplexing large numbers of primers imposes considerable constraint on the assays that can be included. Assays that unintentionally interact result in spurious amplification products.¹⁴¹

Such challenges were known even earlier, as disclosed in Natera's '035 Patent.¹⁴² One of ordinary skill in the art would not have reasonably expected success in increasing the multiplexing of any of Bashashati (2013)'s PCR reactions in a single volume. The Van Ness Declaration offers no basis to believe otherwise. Rather, one of ordinary skill in the art would have viewed consolidating multiple reactions into a single volume as potentially introducing complications to the reactions, as opposed to facilitating them. Accordingly, it would not have been obvious to one of ordinary skill in the art to perform the claimed targeted multiplex amplification of tumor-associated cfDNA in a single read volume.

136. Second, the Van Ness Declaration argues that one of ordinary skill in the art could have looked to a different example in Bashashati (2013) that used the Ion Torrent PGM platform to perform targeted multiplex PCR to validate SNVs.¹⁴³ I disagree that this is support for obviousness for several reasons. The Ion Torrent example was performed on tumor tissue samples, not on ctDNA isolated from plasma.¹⁴⁴ Indeed, the portion of

¹⁴¹ *Id.* at 107:65-108:1 (emphasis added).

¹⁴² *See, e.g.*, '035 Patent at 48:7-14, 54:26-29.

¹⁴³ *See* Van Ness Decl. at ¶ 195.

¹⁴⁴ *See* Bashashati (2013) at 25. "Forty-three mutations present in the right ovary (mixed histology) were not present in the samples from the left (all HGSCs; Figure 1 B [case 4]). As the index exome for [case 4] was restricted to the right set of samples, we subjected all samples to the PGM Ion AmpliSeq Cancer panel."

Bashashati (2013) quoted in the Van Ness Declaration¹⁴⁵ asserts that Bashashati (2013) performed the PGM Ion AmpliSeq Cancer panel on “primary ovarian cancer samples.”

137. Furthermore, the Van Ness Declaration fails to explain why one of ordinary skill in the art would have been motivated to use the Ion Torrent-based approach, as previously applied merely for the purpose of validating candidate SNVs in DNA from tumor samples. In tumor samples, tumor-associated DNA is not rare. The Van Ness Declaration fails to explain why the use of tumor tissue samples would render obvious the claimed methods using cfDNA. As explained *supra*,¹⁴⁶ working with cfDNA from plasma samples was challenging and unpredictable for many reasons, such as cfDNA fragmentation. Identifying genetic mutations in tumor-associated cfDNA would have been even more difficult, given that tumor-associated cfDNA is relatively low-abundance and highly variable within a person’s cfDNA. The Van Ness Declaration also fails to explain how one of ordinary skill in the art would have reasonably expected success with the Ion Torrent-based approach in light of these technical challenges. Accordingly, it would not have been obvious to use a method used only for validating SNVs in tissue-derived DNA in order to perform the claimed targeted multiplexing of cfDNA in a single reaction volume.

138. Third, the Van Ness Declaration points to Forshew (2012) because Dr. Van Ness contends Bashashati (2013) cites Forshew (2012) as support for the proposition that

¹⁴⁵ See Van Ness Decl. at ¶ 195.

¹⁴⁶ See *supra* at Section II.

“sequencing of cell-free circulating tumour DNA (ctDNA) extracted from plasma has been demonstrated to be an effective non-invasive tool for monitoring tumour burden.”¹⁴⁷ This citation, however, is not relevant to obviousness here because it says nothing relating to the claimed targeted multiplex amplification in a single reaction volume. In fact, this citation indicates that Bashashati (2013) was aware of Forshew (2012) but **chose not to follow** Forshew (2012)’s approach to the extent Forshew (2012) teaches multiplex amplification of multiple loci in a single reaction volume.

139. Consistent with my opinions, Forshew (2012) recognized the technical challenges associated with multiplexing many reactions in a single volume. Forshew (2012) explains that “[m]ultiplex amplification using a large set of primers could result in nonspecific amplification products and biased coverage.”¹⁴⁸ As a consequence of those admitted technical challenges, the Forshew (2012) strategy required a singleton amplification step, which one of ordinary skill in the art would have recognized from reading Forshew (2012). In deposition, Dr. Van Ness confirmed that Forshew (2012)’s strategy did not involve multiplexing independent of single-plex amplification. Dr. Van Ness stated that “Dr. Forshew incorporated the multiplex reaction in his series of steps. I didn’t see anywhere where he did just the multiplex and analyzed it. But he incorporated it into all of his steps.”¹⁴⁹ Thus, these limitations in Forshew (2012) would have further

¹⁴⁷ See Van Ness Decl. at ¶ 196.

¹⁴⁸ See Forshew (2012) at 3 (emphasis added).

¹⁴⁹ See Van Ness Tr. at 129:24-130:11 (objection omitted).

discouraged one of ordinary skill in the art from modifying Bashashati (2013) to perform targeted multiplex amplification of cfDNA in a single volume.

140. Fourth, the Van Ness Declaration contends that the “ability to use greater multiplex targeted amplification would also have been obvious to one of ordinary skill in the art prior to the priority date for the claim because even significantly greater multiplex amplification of DNA was already well established.”¹⁵⁰ I disagree. The teachings that Dr. Van Ness relies on do not teach greater multiplexing in a single reaction volume. In fact, they teach the opposite, particularly in light of known challenges in multiplexing as discussed *supra*.¹⁵¹

141. For example, the Van Ness Declaration relies on the '038 Patent Pub. (Mir) (*i.e.*, Fluidigm system).¹⁵² Similar to Forshew (2012), Fluidigm uses a preamplification step using multiplex PCR followed by singleton PCR (each reaction in its own reaction volume) to generate individual target-specific amplicons.¹⁵³ In fact, Forshew (2012) uses

¹⁵⁰ See Van Ness Decl. at ¶¶ 197-199.

¹⁵¹ See, *e.g.*, '454 Patent at 105:51-58, 107:65-108:1.

¹⁵² See Dkt. 116-13, Mir et al., *Assay methods for increased throughput of samples and/or targets*, U.S. Patent Application Publication No. US 2010/0120038 (2010) (the “'038 Patent Pub. (Mir)”).

¹⁵³ See '038 Patent Pub. (Mir) at [0119] (“In illustrative embodiments, the encoding reaction can be a preamplification reaction, which may be carried out on a microfluidic device. To increase target nucleic acid concentration prior to encoding, an optional pre-preamplification reaction can be carried out before the encoding preamplification reaction. The pre-preamplification can be carried out in multiplex [*sic*]. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture. This mixture can then be divided into R=96 aliquots and each aliquot subjected to an encoding preamplification reaction on a microfluidic device, using T=96 different primer pairs that

the Fluidigm system in its singleton PCR step.¹⁵⁴ Dr. Van Ness's citation to a previous, unrelated declaration I submitted in an *inter partes* review¹⁵⁵ ignores the context I provided, which explains the preamplification reaction is then aliquoted into individual wells to perform singleton PCR,¹⁵⁶ just as that described in Forshew (2012).

142. As explained *supra*, Dr. Van Ness fails to show that one of ordinary skill in the art would have been motivated to modify Bashashati (2013)'s approach to perform "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," or that one of ordinary skill in the art would have reasonably expected to succeed in doing so given

add 96 different nucleotide tag pairs to the target nucleotide sequences in each of the 96 aliquots."); *id.* at [0120] ("After the encoding preamplification reaction, amplification can be carried out in separate chambers of a microfluidic device. For example, each of the 96 aliquots produced upon encoding preamplification can be loaded into individual sample lines of a matrix-type microfluidic device, and each of 96 different tag-specific primer combinations can be loaded into individual assay columns. Each different of the 96 primer combination can amplify a different target nucleic acid in each of the 96 aliquots. The resulting 9216 reaction chambers (sub-aliquots) can then be subjected to amplification, followed by detection of amplification product(s), which can be carried out by any suitable means, including SYBR Green, universal probe library, use of one probe per tag combination (e.g., wherein probe sequences are introduced into nucleotide tags), use of fluorescent primers to add nucleotide tags.").

¹⁵⁴ See Forshew (2012) at S3 ("Individual primer pairs were loaded into the primer inlets of the Access Array IFC (Fluidigm).").

¹⁵⁵ See Dkt. 116-12, *Declaration of Michael L. Metzker, Ph.D.*; IPR2018-01317, dated June 29, 2018 ("Metzker IPR Decl.").

¹⁵⁶ *Id.* at ¶ 100.

the well-known technical difficulties in multiplexing many targets in one reaction volume, as recognized in Forshew (2012), for example.

143. Consistent with my opinions, Dr. Van Ness admitted in deposition that multiplexing many targets in one reaction volume could lead to undesirable consequences.

Q. What kinds of challenges are involved in amplifying multiple targets together in one reaction volume that are not challenges in amplifying multiple targets individually in separate reaction volumes?

A. ***Primarily, I think one of the challenges is primer interactions.*** Because you have multiple primers, you have ***potential for primer interactions that could interfere with the amplification of the targets*** you're after.

Q. Is another problem – or another challenge – allelic bias?

A. ***It can be.***¹⁵⁷

144. I disagree with Dr. Van Ness's evaluation of Bashashati (2013) and the opinions expressed in the Van Ness Declaration on the '454 Patent claims' obviousness in other respects. For example, the Van Ness Declaration asserts that the use of an amplicon length of 50-150 base pairs "would have been obvious, particularly in the context of cell-free DNA."¹⁵⁸ I disagree. The Van Ness Declaration provides no reasons or bases to support this opinion. In fact, Bashashati (2013) uses Illumina's MiSeq system to sequence the amplicons from ctDNA isolated from plasma using "paired-end 250 bp [base pair]

¹⁵⁷ See Van Ness Tr. at 132:13-133:3 (emphases added).

¹⁵⁸ See Van Ness Decl. at ¶¶ 192-193.

reads.”¹⁵⁹ These sequence reads are anywhere from 100 to 200 bp longer than amplicon length on which Dr. Van Ness opines. The Van Ness Declaration provides no explanation why one of ordinary skill in the art would have deviated from a sequencing system that provided 250 base pair reads to achieve an amplicon length of 50-150 bases. Bashashati (2013)’s use of MiSeq would in fact inform the one of ordinary skill in the art that the amplicon lengths would have been closer to 250 base pairs to maximize the performance of the Illumina MiSeq system.¹⁶⁰

(b) Claim 1[c] of the ’454 Patent

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

145. The Van Ness Declaration’s opinions regarding the ’454 Patent’s sequencing step are also deficient or factually incorrect. Dr. Van Ness asserts, for example, that “Bashashati [] teaches sequencing amplicons on an Illumina GAII.”¹⁶¹ But Bashashati (2013) uses the GAII to sequence amplicons from “tumour and normal DNA templates,”¹⁶² not ctDNA obtained from plasma samples.

¹⁵⁹ See Bashashati (2013) at S7.

¹⁶⁰ *Id.* at S6.

¹⁶¹ See Van Ness Decl. at ¶ 201.

¹⁶² See Bashashati (2013) at S6.

146. The Van Ness Declaration also fails to establish that one of ordinary skill in the art would have had a reasonable expectation of success in achieving the methods claimed in the '454 Patent, *e.g.*, to the extent one of ordinary skill in the art would have been motivated to perform ultra-deep sequencing to “a depth of read of at least 50,000 per target locus.” I discuss deficiencies in Dr. Van Ness’s reasoning *infra*.

147. First, as explained *supra* with respect to Forshew (2012), as of 2014-2015, ultra-deep sequencing would not have been useful to identify rare genetic variants which are buried in the “noise” of sequencing workflow errors associated with sample preparation and next-generation sequencing technology. Dr. Van Ness fails to account for these technical limitations in his assessment of Bashashati (2013). For example, one of ordinary skill in the art would have understood that the 454 Life Sciences’ PicoTiterPlate format, used in Bashashati (2013), has an error rate of ~1%.¹⁶³ As the error rate dictates the frequency at which SNVs can be identified, Table 2 in the '189 Patent Pub. (Bentley) (cited by Dr. Van Ness) suggests that read depths between 400 and 1,000 would have been sufficient to identify an SNP at the 1% level, as shown *infra* (red box added to highlight the number of reads to an SNP at ~1% level).

¹⁶³ See Ex. 7, Gilles *et al.*, *Accuracy and quality assessment of 454 GS-FLX Titanium pyrosequencing*, BMC GENOMICS 12:245 pp. 1-11 (2011) (“Gilles (2011)”) at Abstract: “We obtained a mean error rate for 454 sequences of 1.07%.”

TABLE 2

SNP Classes	Number of Reads	Minimum frequency of SNP in population detectable with 95% confidence	Minimum frequency of SNP in population detectable with 99% confidence
1	200000	0.002%	0.003%
2	100000	0.005%	0.007%
5	40000	0.014%	0.018%
10	20000	0.028%	0.037%
50	4000	0.14%	0.18%
100	2000	0.28%	0.37%
200	1000	0.55%	0.74%
500	400	1.39%	1.85%
1000	200	2.76%	3.64%

Higher read depth would result in the detection of noise (*i.e.*, sequencing workflow errors).

148. Thus, in light of known sequencing error rates for all sequencing platforms discussed *supra*, I disagree with Dr. Van Ness that “sequencing depth is a parameter that would be routinely adjusted to achieve whatever sensitivity was required.”¹⁶⁴ In reality, some sensitivity levels would not have been achievable due to technical limitations of equipment or experimental design (*e.g.*, sample preparation), no matter how much sequencing depth was increased because of fundamental issues (*e.g.*, noise levels) with the system.

149. Second, and relatedly, the Van Ness Declaration points to the ’454 Patent’s disclosure for the propositions that “the ’454 patent does not identify any particular sequencing depth as being inventive or special” and “sequencing depth is a parameter that

¹⁶⁴ See Van Ness Decl. at ¶ 208.

would be routinely adjusted to achieve whatever sensitivity was required.”¹⁶⁵ I disagree with this reading of the patent and the premise. As explained *supra*, simply repeatedly sequencing a target locus will not improve the sensitivity of detection beyond the method’s inherent error rate and will not identify rare variants that fall below that threshold. In fact, the ’454 Patent describes the advantages of its inventive methods for this purpose.

In another aspect, the present invention generally relates, at least in part, to improved methods of detecting single nucleotide variations (SNVs). These improved methods include improved analytical methods, improved bioassay methods, and improved methods that use a combination of improved analytical and bioassay methods. ***The methods in certain illustrative embodiments are used to detect, diagnose, monitor, or stage cancer, for example in samples where the SNV is present at very low concentrations***, for example less than 10%, 5%, 4%, 3%, 2.5%, 2%, 1%, 0.5%, 0.25%, or 0.1% relative to the total number of normal copies of the SNV locus, such as circulating free DNA samples. That is, ***these methods in certain illustrative embodiments are particularly well suited for samples where there is a relatively low percentage of a mutation or variant relative to the normal polymorphic alleles present for that genetic loci***.¹⁶⁶

150. The Van Ness Declaration’s citations to my and others’ testimony¹⁶⁷ likewise fail to explain how one of ordinary skill in the art would have reasonably expected success in overcoming the technical challenges by modifying the Bashashati (2013) approach as explained *supra*. Dr. Van Ness also fails to explain why one of ordinary skill in the art

¹⁶⁵ *Id.* at ¶ 209.

¹⁶⁶ See ’454 Patent at 44:6-21 (under “Detailed Description of the Invention”) (emphases added).

¹⁶⁷ See Van Ness Decl. at ¶¶ 210-215.

would have been motivated to do so with respect to Bashashati (2013), and those citations are therefore irrelevant.

151. Third, the Van Ness Declaration also fails to show that Bashashati (2013) achieves the claimed sequencing depth of read as Dr. Van Ness misreads Bashashati (2013). In paragraphs 204-205 of the Van Ness Declaration, Dr. Van Ness identifies 17 rows from Table S2 of Bashashati (2013) (“lines 48, 54, 82, 91, 98, 111, 121, 124, 129, 130, 144, 151-153, 156, 170, and 173”) as support for the proposition that “[a] POSA would understand that these positive resequencing read values for tumor-specific SNVs indicate that Bashashati detected tumor-specific SNV mutations present in the cell-free DNA from the sequence reads.”¹⁶⁸ But none of these rows support his assertion and I therefore disagree. Dr. Van Ness is not reading Bashashati (2013) Table S2 correctly.

152. In particular, Column V of Bashashati (2013) (*i.e.*, Plasma BinomExact_adjustedpVal) is used to perform “detecting one or more of the tumor-specific SNV mutations.” Bashashati (2013) explains that “[t]he number of mutations based on positions with coverage (c), no coverage (nc) and those with coverage that were significantly (s) detected, based on the binomial extract test (adjusted $p < 0.05$).”¹⁶⁹ In contrast, for the rows cited *supra* in the Van Ness Declaration, only two rows, 98 and 156 have p values of < 0.05 —namely, 1.36×10^{-7} and 4.04×10^{-7} , respectively. The total number of reads for rows 98 and 156 are well below “a depth of read of at least 50,000 per

¹⁶⁸ *Id.* at ¶ 205.

¹⁶⁹ See Bashashati (2013) at Fig. 2 legend.

target loci.” For example, the “refcountplasma” (column T) and “altcountplasma” (column U) for row 98 is 8,190 and 105, which totals 8,295 reads. The “refcountplasma” (column T) and “altcountplasma” (column U) for row 156 is 78 and 5, which totals 83 reads. None of this comes anywhere close to the recited “of at least 50,000 per target locus.”

153. Dr. Van Ness also argues that “Bashashati teaches that the sequencing has a depth of read of at least 50,000” by pointing to row 276 showing a value of 969,361 in the “refcountplasma” (column T).¹⁷⁰ I disagree as, again, Dr. Van Ness is misreading Table S2 and again fails to demonstrate that Bashashati (2013) discloses this limitation. Column V (*i.e.*, Plasma BinomExact_adjustedpVal) of row 276 has a $p = 1$, which would fail the binomial exact test and therefore not have been identified as a SNV mutation.

B. The '035 Patent Is Not Obvious

(i) *Kaper (2010) Poster Does Not Render the '035 Patent Claims Obvious*

154. At least for the reasons explained *infra*, Dr. Van Ness has failed to show that the '035 Patent claims are obvious over the teachings of the Kaper (2010) Poster.¹⁷¹ I disagree with Dr. Van Ness’s opinions regarding the Kaper (2010) Poster.

¹⁷⁰ See Van Ness Decl. at ¶ 207.

¹⁷¹ See Dkt. 116-15, Kaper *et al.*, *Parallel preparation of targeted resequencing libraries from 480 genomic regions using multiplex PCR on the Access Array system*, PROCEEDINGS OF THE 101ST ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH (AACR), Poster (2010) (“Kaper (2010) Poster”).

155. For the claims to be obvious, *inter alia*, I have been informed and understand it must have been obvious to one of ordinary skill in the art the following claim elements in the course of performing the other method steps:

- Claim 1[a]: using “isolated cell-free DNA” that “is isolated from a blood sample”
- Claim 1[b]: to perform “targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume”
- Claim 1[c]: to perform “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”

Dr. Van Ness’s opinions regarding these individual limitations, separately and in combination, are deficient because nothing in the Kaper (2010) Poster would have motivated one of ordinary skill in the art to pursue the claimed combination. Dr. Van Ness’s opinions regarding these individual limitations, separately and in combination, are deficient because the skilled artisan would not have reasonably expected success in modifying Kaper to achieve them as Dr. Van Ness proposes, at least in light of the unpredictability and technical challenges in the art involved in manipulating cfDNA.¹⁷² I reserve the right to offer additional opinions regarding the ’035 Patent in view of the Kaper (2010) Poster alone or in combination with other references.

¹⁷² See Section II.A, *supra*.

(a) Claim 1[a] of the '035 Patent

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

156. The Van Ness Declaration asserts that “Kaper renders this claim limitation obvious.”¹⁷³ I disagree.¹⁷⁴ As a preliminary matter, the Kaper (2010) Poster does not teach or suggest amplifying or sequencing cfDNA. Rather, the samples used in Kaper (2010) Poster are obtained from tumor tissue samples or control (healthy) DNA from a HapMap sample (human genome DNA isolated from a cell line). The Van Ness Declaration does not contend otherwise.

157. Rather, Dr. Van Ness opines that “Kaper teaches applications of its technique to cancer” and from this leaps to the conclusion that “it would have been natural and obvious to apply the method to cell-free DNA.”¹⁷⁵ I disagree. It is true that cfDNA, including tumor-associated cfDNA, was known to exist.¹⁷⁶ However, the use of tissue

¹⁷³ See Van Ness Decl. at ¶ 237.

¹⁷⁴ Additionally, Dr. Van Ness mischaracterizes my deposition testimony, which he attempts to use to interpret the '035 Patent claims. Dr. Van Ness stated that, “[a]ccording to [me], the first element of the claim, 1[a] is limited only to the first cycle of amplification.” Van Ness Decl. at ¶ 250. This statement is incorrect as I did not testify that claim 1[a] of the '035 Patent is limited to the first PCR cycle only. In fact, my testimony was “[w]ell, I think as I’ve testified, it does happen in the first cycle; but it can also happen in subsequent cycles.” See Dkt. 116-6 at 103:17-104:24.

¹⁷⁵ See Van Ness Decl. at ¶ 240.

¹⁷⁶ *Id.* at ¶¶ 241-247. Dr. Van Ness cites certain references for the proposition that “use of cell-free DNA in cancer monitoring and detection was well-known,” without explaining how it would have been obvious to modify Kaper’s disclosure using such references. See

sources of tumor-associated DNA would not render obvious the use of cfDNA in the claimed method because, for example, as explained in greater detail *supra*,¹⁷⁷ tumor tissue samples contain tumor-associated DNA at a high abundance in contrast to cfDNA.

158. The Van Ness Declaration fails to support a contrary conclusion for several reasons. First, as Dr. Van Ness admitted in deposition, Kaper (2010) Poster itself does not “describe how to modify multiplex amplification in order to address problems that are particular to cell-free DNA as opposed to genomic DNA.”¹⁷⁸ Nor does the Van Ness Declaration identify any specific teaching in other prior art references that could be used to modify the Kaper (2010) Poster’s approach for non-cfDNA successfully with respect to tumor-associated SNPs, or why a skilled artisan would have been motivated to do so. Second, the Van Ness Declaration does not explain why one of ordinary skill in the art would have reasonably expected success in arriving at the claimed combination in light of the technical challenges associated with manipulating cfDNA.

159. These known difficulties in manipulating cfDNA are consistent with the limitations of the Fluidigm Access Array that are specifically reported in the ’035 Patent (quoted *infra*), and which would have been before the Examiner during prosecution of the ’035 Patent application. Despite these obstacles to success specifically described in the

id. at ¶¶ 241 (Cantor), ¶ 242 (Ehrich), ¶ 243 (Lo). In deposition, for example, Dr. Van Ness could not recall whether those references taught multiplex amplification. Van Ness Tr. 151:8-152:23.

¹⁷⁷ See Section II.

¹⁷⁸ See Van Ness Tr. at 149:6-22.

'035 Patent, the Van Ness Declaration does not address the '035 Patent's disclosure or explain how one of ordinary skill in the art would have reasonably expected to succeed in using the Kaper (2010) Poster's specific approach to amplify tumor-associated cfDNA (ctDNA) or what modifications would have been necessary to achieve that particular goal in light of the unpredictability and technical challenges in working with ctDNA.

160. Specifically, the '035 Patent explicitly distinguishes its invention from the use of the Fluidigm Access Array equipment that is described in the Kaper (2010) Poster. The inventors explain that the Access Array approach is *not* desirable for amplifying samples that only contain “a limited amount of DNA.” As explained *supra*,¹⁷⁹ tumor-associated cfDNA is relatively low-abundance among the other cfDNA (from healthy cells) in a person's plasma sample, and the levels of ctDNA in a plasma sample are also variable. As one of ordinary skill in the art would have recognized, the results from using such low-abundance samples will be biased if the Access Array's uniplex reactions does not contain the target DNA that is the subject of the amplification. This problem is compounded when the approach is intended to amplify multiple loci that have different sequences (“polymorphic” loci), which can be present in tumor DNA sequences as those sequences are frequently mutated relative to healthy DNA. The '035 Patent recognizes these limitations with use of the Access Array, and the unpredictability in the art of using cfDNA and ctDNA as a source material for amplification.

¹⁷⁹ See Section II.

In general, to perform targeted sequencing of multiple (n) targets of a sample (greater than 50, greater than 100, greater than 500, or greater than 1,000), ***one can split the sample into a number of parallel reactions that amplify one individual target. This has been performed in PCR multiwell plates or can be done in commercial platforms such as the FLUIDIGM ACCESS ARRAY*** (48 reactions per sample in microfluidic chips) or DROPLET PCR by RAIN DANCE TECHNOLOGY (100 s to a few thousands of targets). Unfortunately, ***these split-and-pool methods are problematic for samples with a limited amount of DNA***, as there is often not enough copies of the genome to ensure that there is one copy of each region of the genome in each well.

This is an especially severe problem when polymorphic loci are targeted, and the relative proportions of the alleles at the polymorphic loci are needed, as the stochastic noise introduced by the splitting and pooling will cause very poorly accurate measurements of the proportions of the alleles that were present in the original sample of DNA.

Described here is a method to effectively and efficiently amplify many PCR reactions that is applicable to cases where only a limited amount of DNA is available. In an embodiment, the method may be applied for analysis of single cells, body fluids, mixtures of DNA such as the free floating DNA found in maternal plasma, biopsies, environmental and/or forensic samples.¹⁸⁰

In acknowledging these problems, the '035 Patent reports that its methods are comparatively advantageous when amplifying low-abundance DNA, including “free floating” cfDNA found in plasma.

161. The Van Ness Declaration does not address the '035 Patent's description of its methods' advantages over the Access Array, as the use of that equipment is described

¹⁸⁰ See '035 Patent at 85:21-46 (emphases added).

in the Kaper (2010) Poster. Nor does Dr. Van Ness identify with any specificity how one of ordinary skill in the art would modify the Kaper (2010) Poster's approach to achieve the claimed inventions.

162. The Van Ness Declaration also does not explain how one of ordinary skill in the art would have reasonably expected to succeed in doing so. As explained *supra*, these modifications would have been challenging to one of ordinary skill in the art at least because manipulating tumor-associated cfDNA would have been relatively difficult due, for example, to its fragmented nature and low abundance in plasma samples. Instead of explaining how one of ordinary skill in the art would have overcome these challenges and why one of ordinary skill in the art would have been motivated to do so, Dr. Van Ness combines disparate, unrelated teachings in the prior art without explaining why one of ordinary skill in the art would have been motivated to combine those particular teachings as opposed to the myriad other disclosures available to one of ordinary skill in the art. Dr. Van Ness's approach is therefore more consistent with the impermissible use of hindsight rather than what would have been obvious to a skilled artisan considering the Kaper (2010) Poster without the benefit of the '035 Patent's disclosure.

163. There are additional reasons why the use of cancer gene exons in the Kaper (2010) Poster does not render obvious the use of cell-free DNA as well. Setting aside the use of the problematic Fluidigm approach, the PCR data in Figure 3 of the Kaper (2010) Poster further teaches away from the use of cell-free DNA. The Kaper (2010) Poster's PCR product sizes, based on the figure's size markers, indicate that 46 of the 48 (96%) are

equal to or greater than 200 base pairs and that 16 of the 48 (33%) are equal to or greater than 300 bp. In contrast, it was known the cell-free DNA has a typical size range of 140-170 base pairs.¹⁸¹ Simply put, the PCR product sizes, and therefore the distance of the primer pair sets used in the Kaper (2010) Poster, are too large for use with cfDNA and would have required a complete overhaul and redesign of the Kaper (2010) Poster approach to manipulate cfDNA—something not taught in any reference cited by Dr. Van Ness.

(b) Claim 1[b]-[c] of the '035 Patent

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.

164. The Van Ness Declaration contends that the Kaper (2010) Poster renders obvious the claim 1[b] limitation.¹⁸² I disagree. To begin with, the Kaper (2010) Poster provides no disclosure whatsoever of targeted amplification of SNPs, much less SNPs associated with cfDNA.

¹⁸¹ See, e.g., Forshew (2012) at 1 (“Circulating DNA is fragmented to an average length of 140 to 170 base pairs (bp).”).

¹⁸² See Van Ness Decl. at ¶ 249.

165. Indeed, the Van Ness Declaration acknowledges that the Kaper (2010) Poster does not disclose the limitation “wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume.”¹⁸³ I agree with this assessment: the Kaper (2010) Poster does not teach amplifying a plurality of SNP loci in a single reaction volume, particularly using cfDNA containing such SNPs which would have been challenging as explained *supra*.

166. The primers used in the Kaper (2010) Poster are used to identify the presence of certain genes in tumor tissue samples (“cancer gene exons”). It might be theoretically feasible to use the Kaper (2010) Poster’s Access Array to amplify DNA sequences containing SNP loci instead, but this is not taught or suggested and doing so would require substantial effort to redesign the Kaper (2010) Poster’s approach. For example, to identify SNPs, one of ordinary skill in the art would have needed to redesign the Kaper (2010) Poster’s primers to target SNP loci. The Van Ness Declaration does not explain why one of ordinary skill in the art, even if they had such an idea, would have reasonably expected to succeed in pursuing any particular approach to modifying the Fluidigm Access Array’s design and methods to achieve this goal.

167. The Van Ness Declaration’s citations to general teachings in the art or by other witnesses fail to establish a different conclusion.¹⁸⁴ For instance, those citations do

¹⁸³ *Id.* at ¶ 252.

¹⁸⁴ *Id.*, *e.g.*, at ¶¶ 253-256. Dr. Van Ness argues that “SNPs were well-known in the art” (*id.* at ¶¶ 253-254), also citing to Ehrich & Van der Boom, *Restriction endonuclease*

not address the unpredictability and known technical challenges in the art associated with manipulating cfDNA, much less teach anything specific about (or even related to) modifying the Kaper (2010) Poster approach. Therefore, these sources do not support a finding that one of ordinary skill in the art would have reasonably expected success in modifying the Kaper (2010) Poster to achieve the methods recited in the '035 Patent claims.

168. For essentially the same reasons regarding claim 1[b], I disagree with Dr. Van Ness's opinions that the Kaper (2010) Poster renders obvious claim 1[c] of the '035 Patent,¹⁸⁵ which recites sequencing SNPs, including 25-2,000 SNPs as required by the '035 Patent claims.¹⁸⁶ Again, Dr. Van Ness fails to show that the Kaper (2010) Poster teaches this limitation (as it does not) and instead suggests that it would have been obvious to do so. I disagree. The Kaper (2010) Poster's disclosure of sequencing 480 cancer gene exons,

enhanced polymorphic sequence detection, PCT International Patent Publication No. WO 2008/118988 (2008) (the "PCT '988 Appl. (Ehrich)") (*id.* at ¶ 254), Cantor, *Method for non-invasive prenatal diagnosis*, PCT International Patent Publication No. WO 2005/023091 (2005) (the "PCT '091 Appl. (Cantor)") (*id.* at ¶ 255), and Dr. Quackenbush's testimony in the *CareDx* trial (*id.* at ¶ 256).

¹⁸⁵ See Van Ness Decl. at ¶ 260.

¹⁸⁶ I understand that although the Court has not construed the '035 Patent claims, the claims recite that "one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume" and, in a subsequent step, that "the plurality of SNP loci comprises 25-2,000 loci associated with cancer." To the extent that "the plurality of SNP loci" refers to "a plurality of single nucleotide polymorphism (SNP) loci" recited in the amplification step, Kaper (2010) Poster does not teach or suggest this method. Kaper (2010) Poster teaches only multiplex amplification of 10 targets (not SNPs) in a single reaction volume in a preamplification step. Performing a greater number of multiplex amplification reactions in a single volume would therefore require redesigning the Kaper (2010) Poster approach.

absent more, would not render obvious an approach of sequencing 25-2,000 SNP loci in particular, especially SNP loci present on cfDNA. Indeed, other well-known gene alterations exist in cancers including, but not limited to, structural variants and copy-number variants.

169. Relatedly, the Van Ness Declaration contends that “higher degrees of multiplex were commonplace in the art.”¹⁸⁷ Even assuming this were true, Dr. Van Ness does not explain why it would have been obvious to modify the Kaper (2010) Poster approach using the (problematic) Fluidigm Access Array in order to achieve increased multiplexing capability, much less for the purpose of performing targeted amplification of a plurality of SNP loci in a single reaction volume. I disagree that any of these references support Dr. Van Ness’s obviousness opinions.

(ii) *The Combination of ’183 Patent Pub. (Han) and Wang (2010) (“ARM-PCR”) Does Not Render the ’035 Patent Claims Obvious*

170. At least for the reasons explained *infra*, Dr. Van Ness has failed to show that the ’035 Patent claims are obvious over the teachings of the combination of the ’183 Patent

¹⁸⁷ See Van Ness Decl. at ¶ 263. In support of this opinion, Dr. Van Ness also cites to Varley & Mitra, *Nested patch PCR enables highly multiplexed mutation discovery in candidate genes*, GENOME RESEARCH 18:1844-1850 (2008) (“Varley (2008)”) at ¶ 264; cites to ’038 Patent Pub. (Mir) (*i.e.*, Fluidigm system) at ¶ 265; cites to Broude, *Novel compositions and methods for carrying out multiplex PCR reactions on a single sample*, U.S. Patent Application Publication No. US 2004/0126760 (2004) (the “’760 Patent Pub. (Broude)”) at ¶ 266; cites to Lyamichev *et al.*, *Amplification methods and compositions*, U.S. Patent No. 7,790,393 (2010) (the “’393 Patent (Lyamichev)”) at ¶ 267; and cites to Shoemaker *et al.*, *Rare cell analysis using sample splitting and DNA tags*, PCT International Patent Publication No. WO 2007/147079 (2007) (the “PCT ’079 Appl. (Shoemaker)”) at ¶ 268. Dr. Van Ness noted in deposition that Varley (2008) did not use cfDNA. Van Ness Tr. at 138:137:20-138:9.

Pub. (Han)¹⁸⁸ and Wang (2010).¹⁸⁹ I disagree with Dr. Van Ness's opinions regarding their combination.

171. Dr Van Ness's attempt to combine the teachings of '183 Patent Pub. (Han) and Wang (2010) is flawed in multiple respects. Neither reference teaches or suggests all limitations of the '035 Patent's claimed methods, which Dr. Van Ness does not dispute. This alone renders the combination insufficient.

172. Nevertheless Dr. Van Ness purports to combine these references in an attempt to use the teachings of one to remediate the gaps in the other's disclosure. As explained *infra*, Dr. Van Ness's analysis fails because the teachings of the two references do not complement each other in such a way that one of ordinary skill in the art would have either been motivated to achieve the claimed invention or would have reasonably expected to succeed in doing so, including given the unpredictability and known technical challenges in the art in manipulating ctDNA. I reserve the right to offer additional opinions regarding the '035 Patent in view of these references, including in combination with any other art.

¹⁸⁸ See Dkt. 116-16, Han, *Amplicon rescue multiplex polymerase chain reaction for amplification of multiple targets*, U.S. Patent Publication No. US 2009/0253183 (2009) (the "'183 Patent Pub. (Han)").

¹⁸⁹ See Dkt. 117-4, Wang *et al.*, *High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets*, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES 107:1518-1523 (2010) ("Wang (2010)").

(a) Claim 1[a] of the '035 Patent

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

173. I disagree with Dr. Van Ness's opinion that the combination of '183 Patent Pub. (Han) and Wang (2010) render obvious Claim 1[a] of the '035 Patent.¹⁹⁰ As a preliminary matter, '183 Patent Pub. (Han) does not describe the performance of its methods on cfDNA. Rather, '183 Patent Pub. (Han) teaches that ARM-PCR can be useful to amplify DNA from certain specific cellular and viral sources that do not include cfDNA or even DNA from plasma samples.

The inventor has developed a new method for amplifying nucleic acids that may be used to detect the presence, and relative amounts present, of **nucleic acids from viruses, bacteria, fungi, plant and/or animal cells** for the evaluation of medical, environmental, food, and other samples to identify microorganisms and other agents within those samples.¹⁹¹

174. Manipulating cfDNA—and particularly low abundance and highly variable ctDNA, an unpredictable source material for amplification—presented significant technical difficulties, as described *supra*.¹⁹² The '183 Patent Pub. (Han) does not teach or explain how its methods can be used successfully for any purpose with respect to cfDNA, and Dr. Van Ness does not contend otherwise.

¹⁹⁰ See Van Ness Decl. at ¶ 278.

¹⁹¹ See '183 Patent Pub. (Han) at [0009] (emphasis added).

¹⁹² See Section II.

175. The Van Ness Declaration also does not explain why one of ordinary skill in the art would have been motivated to use cfDNA in '183 Patent Pub. (Han)'s methods, assuming it would even be feasible to do so. Rather, to overcome '183 Patent Pub. (Han)'s lack of disclosure of cfDNA, Dr. Van Ness points to phrases such as “clinical diagnosis” (mentioned once) and “detection of genetic disorder, and/or the detection of disease conditions” to render obvious the use of cfDNA.¹⁹³ But these generalized statements in '183 Patent Pub. (Han) do not refer to cfDNA, nor suggest its use. Wang (2010) likewise also does not teach or suggest using cfDNA and Dr. Van Ness does not contend otherwise.¹⁹⁴

176. The Van Ness Declaration attempts to overcome these undisputed deficiencies, arguing once more that that “the use of cell-free DNA in cancer monitoring and detection was well-known.”¹⁹⁵ These general citations do not rescue the Van Ness Declaration's faulty opinions regarding the '035 Patent claims' obviousness because Dr. Van Ness does not explain—for any cited reference—why one of ordinary skill in the art would have been motivated to use cfDNA in '183 Patent Pub. (Han)'s method, how Han's method would need to be modified to do so, or why one of ordinary skill in the art would have reasonably expected success in making the claimed combination given the known

¹⁹³ See Van Ness Decl. at ¶ 280.

¹⁹⁴ *Id.* at ¶¶ 278-280.

¹⁹⁵ *Id.* at ¶ 280, citing to ¶¶ 241-248 in the Van Ness Decl. that refer to Cantor, (¶ 241), Ehrlich (¶ 242), Lo (¶ 243), myself (¶ 244), Natera (¶ 245), and Quackenbush (¶¶ 246-247)).

technical challenges in working with tumor-associated cfDNA and unpredictability of results of such approaches, as discussed *supra*.

(b) Claim 1[b] of the '035 Patent

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

177. I disagree with Dr. Van Ness's assertion that "Han teaches amplifying the products one or more times wherein one of the steps comprises targeted amplification of SNP loci in a single reaction volume."¹⁹⁶ For one thing, '183 Patent Pub. (Han) does not mention the terms "single nucleotide polymorphism" or SNP even once.¹⁹⁷

178. Moreover, in my opinion one of ordinary skill in the art would not have found it obvious to modify '183 Patent Pub. (Han)'s approach to amplify or identify SNPs. In addition to the well-known challenges in manipulating SNP-containing cfDNA explained *supra*, '183 Patent Pub. (Han) teaches the use of ARM-PCR for the purpose of improving molecular differential diagnostic (MDD) assays.¹⁹⁸ As taught in '183 Patent Pub. (Han), this approach represents a binary assay that reads out either the presence or absence of

¹⁹⁶ *Id.* at ¶¶ 282-283.

¹⁹⁷ *See* '183 Patent Pub. (Han) throughout.

¹⁹⁸ *Id.* at [0003]-[0004].

targeted sample types (*e.g.*, the cells and viruses listed *supra*, not SNPs). The examples provided in '183 Patent Pub. (Han) illustrate the results of the ARM-PCR assay.¹⁹⁹

179. In contrast, targeting SNPs does not involve a binary assay. For example, there may be any of four base pairs at a given SNP locus. The Van Ness Declaration does not explain how the '183 Patent Pub. (Han)'s binary method (merely detecting presence versus absence) could be used with respect to targeting SNPs (which have specific sequences). Dr. Van Ness also does not explain how one of ordinary skill in the art would have reasonably expected success in modifying it for this purpose, and, accordingly, the Van Ness Declaration has also failed to demonstrate that '183 Patent Pub. (Han) discloses this limitation.

180. Although Dr. Van Ness asserts that “Wang also teaches this limitation,”²⁰⁰ I disagree. Like '183 Patent Pub. (Han), Wang (2010) does not once mention the terms “single nucleotide polymorphism” or SNP,²⁰¹ much less in the combination of the claimed methods. Rather, Wang (2010) teaches the use of ARM-PCR as a semiquantitative PCR amplification method to maintain the representation of CDR3 sequences obtained from different subsets of T-cells.²⁰² One of ordinary skill in the art would have understood that Wang (2010) was attempting to amplify the same region (the CDR3 sequence) in each of

¹⁹⁹ *Id.* at [0026], [0029]-[0052].

²⁰⁰ *See* Van Ness Decl. at ¶ 284.

²⁰¹ *See* Wang (2010) throughout.

²⁰² *Id.* at 1518, 1520.

the T-cell population subsets. The consequence of Wang (2010)’s approach is that one of ordinary skill in the art would have understood Wang (2010) to teach only using a *single pair of PCR primers*—*e.g.*, a forward primer and a reverse primer that can be used to amplify the same CDR3 region on the different subsets of T-cells—*not* a “targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume” as claimed. The latter approach would require *different* primer pairs to amplify *different* SNP loci (*i.e.*, a plurality of these loci), which Wang does not teach or suggest (and in fact teaches away from).

181. Thus, Dr. Van Ness has not demonstrated that Wang (2010)’s disclosure suggests, much less discloses, this limitation. Dr. Van Ness’s citation to the knowledge of one of ordinary skill in the art to further remediate this deficiency²⁰³ further fails at least for the same reasons explained *supra*—*e.g.*, Dr. Van Ness does not explain how one of ordinary skill in the art would have reasonably expected success doing so given the known technical challenges in working with tumor-associated cfDNA.

²⁰³ See Van Ness Decl. at ¶ 285.

(c) Claim 1[c] of the '035 Patent

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

182. Dr. Van Ness opines that this limitation is obvious as Wang (2010) teaches “sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products.”²⁰⁴ I disagree.

183. As a preliminary matter, '183 Patent Pub. (Han) does not disclose sequencing, and Dr. Van Ness does not contend otherwise. The Van Ness Declaration does not acknowledge this deficiency besides combining '183 Patent Pub. (Han) with Wang (2010), which does disclose sequencing. However, one of ordinary skill in the art would not have been motivated to modify '183 Patent Pub. (Han)'s approach with Wang (2010)'s methods to achieve the claimed invention, and would not have reasonably expected to succeed in doing so, for the reasons explained *supra* with respect to Claim 1[b]—namely, Wang (2010) teaches amplification of *one* locus (CDR3) in different cell populations and does not suggest, much less disclose, “targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume.”

184. Dr. Van Ness points to the '183 Patent Pub. (Han) for the proposition that the '183 Patent Pub. (Han) teaches “that the plurality of SNP loci comprises 25-2,000 loci associated with cancer”—specifically, that Table 2 of Han demonstrates that this technique

²⁰⁴ *Id.* at ¶¶ 295-296.

is capable of the degree of multiplex recited in the claim.”²⁰⁵ According to Dr. Van Ness, this “Table displays 72 forward and reverse primers which when run in a single volume reaction would be within the multiplex range recited in this claim.” I disagree as Dr. Van Ness is misreading the teachings of the ’183 Patent Pub. (Han). Specifically, Figure 1 of ’183 Patent Pub. (Han) shows that each locus requires six primers—three forward primers, and three reverse primers. Applying this teaching, ’183 Patent Pub. (Han)’s approach teaches a maximum of targeting 12 loci, not 72 as Dr. Van Ness contends (six primers per loci x 12 loci = 72 total primers). And neither ’183 Patent Pub. (Han) nor Wang (2010) teaches or suggests targeting any higher number of loci.

VII. THE NATERA PATENTS ARE VALID UNDER SECTION 101

185. Dr. Van Ness opines that the claims of the ’454 and ’035 Patents are all directed to patent-ineligible subject matter.²⁰⁶ I disagree.

186. As a preliminary matter, Dr. Van Ness’s opinions are conclusory and based on a misreading of the claims—indeed, Dr. Van Ness’s opinions ignore the claims’ plain language. The claims on their face say they are directed to methods of preparing non-natural preparations, which I understand is patentable subject matter under controlling Federal Circuit precedent. The Van Ness Declaration’s analysis is also flawed in that it is based on limited sets of information. Dr. Van Ness relies on out of context positions taken by Natera in other proceedings, regarding irrelevant CareDx/Stanford patents that are not

²⁰⁵ *Id.* at ¶ 298.

²⁰⁶ *See* Van Ness Decl. at § XI.

related to the patents at issue, with claims that are very different, and cherry-picked statements in the patents themselves.²⁰⁷ I disagree with Dr. Van Ness's approach and his conclusions. For example, statements Natera purportedly made about individual techniques made in other proceedings are not relevant at least to the extent that, as I understand from counsel, the character of the claims as a whole—*i.e.*, the use of techniques in the specific context recited in the claims—must be considered in determining eligibility.

187. As discussed *infra*, the Asserted Claims of the '454 and '035 Patents represent new method of preparation methods for achieving useful results. Similarly, these claimed methods are not routine, conventional, or well-understood, at least when they are considered in the recited ordered combinations.²⁰⁸ The Van Ness Declaration fails to evaluate the claimed methods according to the appropriate standards, which I have been informed and understand represent the proper framework for the eligibility analysis.

188. I provide my specific opinions *infra* regarding patent-eligibility of the Asserted Claims of the Natera Patents at step one and (if the Court reaches step two) at step two of the patent-eligibility analysis. I reserve the right to offer additional opinions at another time.

²⁰⁷ *Id.* throughout.

²⁰⁸ As noted *supra*, Dr. Van Ness has not located any prior art reference that even arguably discloses all of the claimed limitation of any claim, and thus is not alleging any claim is anticipated under Section 102 of the Patent Act.

A. The '454 Patent Claims Are Patent-Eligible

(i) The '454 Patent Claims Are Directed to Patent-Eligible Subject Matter

189. Dr. Van Ness opines that the '454 Patent claims are “methods of detection” claims.²⁰⁹ I disagree. As a preliminary matter, I am unaware that patent claims for methods of preparation useful for detecting SNVs are necessarily patent-ineligible and the Van Ness Declaration does not identify such a principle.

190. Regardless, from my understanding of principles governing the “directed to” analysis, I believe Dr. Van Ness’s analysis is flawed. It appears that Dr. Van Ness reaches these opinions by focusing on certain claim limitations and excluding others. I am not aware of a basis to exclude claim limitations in determining what claims are directed to. Rather, I understand from counsel that the “directed to” analysis requires consideration of the character of the claims as a whole. I note that the Van Ness Declaration does not assert that Dr. Van Ness is considering the character of the claims as a whole. Additionally, I understand from Dr. Van Ness’s testimony that he believes the Natera Patent claims are ineligible because they “involve[]” detecting a natural phenomenon, such as DNA.

Q. And in determining, for your opinions in this case, whether the Natera patents claims are directed to a natural phenomenon, did you simply look as to whether the claims involved natural phenomenon? Is that how you did it?

A. That was one of the ways that I looked to see to see if it was directed to a natural product. The claims define the scope of the invention, and ***the scope of the invention***

²⁰⁹ See Van Ness Decl. at ¶¶ 305-310.

is related to whether that is involved in detecting a natural product. So, yes, I did look at the claims to help inform that decision.²¹⁰

I am informed by counsel that the patent-eligibility determination requires more than determining if the claims “involve” an ineligible concept. Rather, claims may be eligible for patenting if they recite a method for achieving a new and useful result.

191. In my opinion, the Asserted Claims of the '454 Patent are directed not to a natural phenomenon, but rather to an inventive combination of specific laboratory techniques, each of which requires significant human effort to perform. The combination of techniques recited in the Asserted Claims of the '454 Patent represents a novel and innovative improvement over earlier methods of sample preparation, and which achieves superior results. I discuss the advantages of the claimed methods in greater detail *infra* with respect to step two of the eligibility analysis.

192. Notably, when performed in combination as recited in Asserted Claims of the '454 Patent, the claimed techniques recite methods for preparing both tumor DNA samples and cfDNA samples in specific ways. This claimed method therefore represents a new and useful method of sample preparation that can be used to yield a tangible and useful result. This result is only achieved by the combination of specific laboratory techniques that, together, represent a new and useful sample preparation method. The Van Ness Declaration does not address the new and useful result achieved by the claimed

²¹⁰ See Van Ness Tr. at 59:10-23 (emphasis added).

combination of steps. Nor does this section of the Van Ness Declaration discuss certain specific limitations recited in the claims, which I consider *infra*.

193. First, the Van Ness Declaration does not address that, in the '454 Patent preamble, the Asserted Claims expressly recite that they involve “[a] method of preparing a sample.”²¹¹ I believe this limitation should be considered at least because, indeed, the Asserted Claims of the '454 Patent recite a specific protocol for preparing a sample and that is what they are directed to. I discuss its steps in turn *infra*. One of the method’s steps is a detecting step, but that is not the only step. Indeed, detection is only possible because of the performance of the other steps of the claimed method that precede detection. Dr. Van Ness acknowledged at his deposition that the '454 Patent claims recite methods of DNA manipulation. Dr. Van Ness also admitted that the '454 Patent claims recite amplifying specific numbers of SNV loci.

Q. Is it your contention that – I’m not asking you whether you think they’re routine and conventional, just yes or no whether they’re there. Is it your contention that Claim 1 of the '454 patent does not recite any methods for manipulating DNA, amplifying and sequencing?

A. Claim 1 does discuss manipulating and performing procedures on DNA and amplifying.²¹²

Q. Okay. Do you disagree that the claims recite a specific number of loci that are amplified?

A. ***It provides a range of 10 to 500.***²¹³

²¹¹ See Van Ness Decl. at ¶¶ 314-317.

²¹² See Van Ness Tr. at 88:25-89:11 (objection and colloquy omitted).

²¹³ *Id.* at 91:10-13 (emphasis added).

194. Second, the first-recited step of the Asserted Claims of the '454 Patent requires performing one of two specific sequencing techniques—whole genome sequencing or whole exome sequencing—on a subject's tumor sample in order to identify a plurality of tumor-specific SNV mutations. Both techniques require significant human effort and intervention to achieve. For example, I reproduce information *infra* about these methods from a 2010 review²¹⁴ I published regarding the use of next-generation sequencing platforms to accomplish these techniques—a significant technical endeavor. One of ordinary skill in the art would have understood that performance of these specific sequencing techniques achieves a new and useful result—namely, the identification of a cancer patient's genomic sequences, either throughout the whole genome or in the entire coding regions of the genome (*i.e.*, all of the exons). Dr. Van Ness agrees, as he testified at his deposition.

Q. Thank you. And do you – strike that. You would agree that whole genome sequencing or whole exome sequencing of DNA requires human intervention and effort to perform?

A. DNA doesn't sequence itself. ***It does take somebody to do the process of the sequencing.***²¹⁵

195. As the Asserted Claims of the '454 Patent recite, these comprehensive sequencing approaches (compared to more limited approaches, *e.g.*, identifying a few

²¹⁴ See Ex. 8, Metzker, *Sequencing technologies — the next generation*, NATURE REVIEWS GENETICS, 11: 31-46 (2010) (“Metzker 2010”).

²¹⁵ See Van Ness Tr. at 89:14-21 (objection omitted, emphasis added).

cancer-associated biomarkers) permit identification of multiple tumor-associated SNVs which will be unique for each subject. Thus, the claims do not involve merely “sequencing,” as Dr. Van Ness suggests.²¹⁶ “Sequencing” alone would not have been adequate to provide new and useful comprehensive information about a subject’s genome. Rather, the ’454 Patent’s method—all of the recited steps—must be performed using the specific recited techniques in order to obtain the genomic information needed to achieve a new and useful result.

196. As referenced *supra*, my 2010 review exemplifies how the use of next-generation sequencing (also known as massively parallel sequencing) platforms to accomplish whole genome sequencing and whole exome sequencing involved significant effort to design and implement—far more than merely “sequencing.”

The inexpensive production of large volumes of sequence data is the primary advantage over conventional methods. Here, I present a technical review of template preparation, sequencing and imaging, genome alignment and assembly approaches, and recent advances in current and near-term commercially available NGS instruments.²¹⁷

The ability to sequence the whole genome of many related organisms has allowed large-scale comparative and evolutionary studies to be performed that were unimaginable just a few years ago.²¹⁸

Here, I present a technical review of template preparation, sequencing and imaging, genome alignment and assembly, and current NGS platform performance to provide guidance on

²¹⁶ *Id.*, *e.g.*, at ¶¶ 318-328.

²¹⁷ See Metzker 2010 at Abstract.

²¹⁸ *Id.* at 31.

how these technologies work and how they may be applied to important biological questions. I highlight the applications of human genome resequencing using targeted and whole-genome approaches, and discuss the progress and limitations of these methods, as well as upcoming advances and the impact they are expected to have over the next few years.²¹⁹

Despite the substantial cost reductions associated with NGS technologies in comparison with the automated Sanger method, whole-genome sequencing is still an expensive endeavour (see below). An interim solution to this problem may be to use NGS platforms to target specific regions of interest. This strategy can be used to examine all of the exons in the genome, specific gene families that constitute known drug targets or megabase-size regions that are implicated in disease or pharmacogenetics effects through genome-wide association studies. Although any of the NGS platforms can perform the sequencing phase of targeted capture, some may be better suited than others (Table 1).²²⁰

197. In addition to misapprehending the nature of the Asserted Claims, the Van Ness Declaration's analysis is deficient in that Dr. Van Ness does not address whole genome sequencing or whole exome sequencing in his analysis of what the Asserted Claims of the '454 Patent are directed to. Nor does Dr. Van Ness explain his reasoning for ignoring these claim limitations and others.

198. Third, beyond sequencing, the Asserted Claims of the '454 Patent additionally recite a specific protocol for amplifying cfDNA.²²¹ This step requires one of ordinary skill in the art to perform at least five specific activities, each of which requires

²¹⁹ *Id.*

²²⁰ *Id.* at 41 (internal citations omitted).

²²¹ *See* '454 Patent at 171:32-38.

significant human effort to design and implement. Specifically, the Asserted Claims of the '454 Patent require [1] performing targeted multiplex amplification [2] which has been designed to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA [3] isolated from a plasma sample of the subject or DNA derived therefrom [4] to obtain amplicons having a length of 50-150 bases, [5] wherein the target loci are amplified together in the same reaction volume.²²²

199. Notably, targeted amplification in one reaction volume is one way to amplify DNA (using human effort), but not the only way, as Dr. Van Ness acknowledged in his deposition.

Q. Thank you. You would agree that targeted multiplex amplification to amplify 10 to 500 target loci, each encompassing a different tumor-specific SNV mutation, requires human intervention and effort to perform, correct?

A. Yes, there are a lot of humans that can do that, sure.

Q. Okay. Do you disagree that claim 1 of the '454 patent recites a specific kind of amplification, which is targeted multiplex amplification?

A. It – it states that “Wherein” – “the target loci are amplified together in the same reaction.” So there is a statement there of amplifying target loci.²²³

Q. Right. But targeted amplification is not the only way to amplify DNA, right? Are there other ways to do it?

²²² *Id.*

²²³ See Van Ness Tr. at 89:22-90:6 (objection and clarification by court reporter omitted).

A. ***Sure.***²²⁴

Q. And there are ways of – of amplifying multiple targets without amplifying them in the same reaction volume, right?

A. You can amplify a target, one in every individual tube. ***Certainly there are ways to amplify a single target in one tube and a different target in another tube.***²²⁵

200. In deposition, as previously discussed (Section II, *supra*), Dr. Van Ness acknowledged particular and “unique” challenges associated with multiplex amplification of cfDNA, in discussing amplification of different types of cfDNA (*e.g.*, cfDNA from patients who received an organ transplant, or pregnant women, or subjects with cancer).²²⁶ Dr. Van Ness admitted, for example, that “it’s not sufficient simply to isolate cell-free DNA” in order to perform multiplex amplification in light of the challenges of working with cfDNA.²²⁷ As Dr. Van Ness acknowledged, depending on the context of working with particular cfDNA, different protocols “might be required for successful multiplex amplification.”²²⁸

201. In combination, these approaches achieve a new and useful result of efficiently amplifying a subject’s tumor-associated SNVs together. Notably, the targeted amplification process generates DNA that did not previously exist in nature; that is, the

²²⁴ *Id.* at 91:3-9 (objection omitted, emphasis added).

²²⁵ *Id.* at 92:16-23 (objection omitted, emphasis added).

²²⁶ *Id.* at 64:4-66:6 (objections, court reporter clarification, and colloquy omitted).

²²⁷ *Id.* at 65:14-15.

²²⁸ *Id.* at 65:4-6.

amplicons which are modified with additional DNA sequences necessary for the following sequencing step. As explained *infra*, the specific claimed approach is also a material improvement over prior art techniques because it avoids dividing the plasma sample into individual reactions, an approach which would require more cfDNA starting material, and may lead to biased results when the sample contains very low, and/or variable levels of DNA, as in the case of ctDNA.

202. Fourth, the Asserted Claims of the '454 Patent recite ultra-deep sequencing—*i.e.*, sequencing to a depth of read of 50,000 or more for each target SNV locus.²²⁹ As explained *supra*, it was not common to sequence to this depth of read for a variety of reasons. Doing so was expensive and time-consuming and, although increasing read depth could under some conditions could improve sensitivity, ultra-deep sequencing would not necessarily be useful for any particular purpose. I discuss certain limitations of ultra-deep sequencing *supra* with respect to Forshew (2012). Thus, the combination of sequencing to the claimed depth of read with the other steps of the method represented a new and useful technique for sample preparation.

203. Accordingly, from my review of the Asserted Claims of the '454 Patent, in my opinion, their character as a whole is directed to eligible subject matter—a new and useful method for sample preparation using a combination of specific laboratory techniques. They are not, as Dr. Van Ness opines, directed to natural phenomenon or other

²²⁹ *Id.* at 171:39-43.

ineligible subject matter.²³⁰ Moreover, as explained *supra*, the “detecting” step is performed on DNA that did not exist in nature—*i.e.*, amplicons from isolated cfDNA.

204. In reaching his contrary conclusions, Dr. Van Ness discusses other patents from separate proceedings.²³¹ I disagree with his assessment of these non-asserted patents at least because those patents recite different limitations. The Van Ness Declaration does not offer any reasoned basis to equate their recited combination to the combination recited in the Asserted Claims of the ’454 Patent.

205. For all the aforementioned reasons, in my opinion that the Asserted Claims of the ’454 Patent, when considered as a whole, are directed to patent-eligible subject matter and step two of the patent-eligibility analysis is unnecessary. Even under an analysis of step two, the Asserted Claims of the ’454 Patent should still be found patent-eligible, as explained *infra*.

(ii) The ’454 Patent Claims, Considered Individually and In Combination, Are Not Routine or Conventional

206. The Van Ness Declaration opines that certain aspects of the claimed methods of the ’454 Patent—sample preparation, amplification, and sequencing—were routine and conventional.²³² I agree that, considered independently and in isolation, these general techniques were known in the art as of 2014-2015. This is not to say, however, that these methods were routine or conventional, particularly in the context of working with tumor-

²³⁰ See Van Ness Decl. at § XI.

²³¹ *Id.* at ¶ 310.

²³² *Id.* at ¶¶ 312-338.

associated cfDNA. In fact, as explained *supra*,²³³ this approach was challenging in many respects even after the '454 Patent's priority date due in a large part to the difficulties in identifying rare genetic variants over the background "noise" (errors) of the sample preparation and sequencing techniques.

207. In any event, in my understanding, even if the recited limitations were routine, conventional, or well-understood, that would not be sufficient to show patent ineligibility, as the claim limitations must be considered both individually and as their ordered combination. The Van Ness Declaration fails to consider the latter—whether the '454 Patent limitations are well-understood, routine, or conventional in their ordered combinations. In my opinion, these claim limitations when considered in their ordered combination are not well-understood, routine, or conventional. Thus, the Asserted Claims of the '454 Patent should be deemed patent-eligible if the Court reaches step two of the eligibility analysis.

208. When considered as an ordered combination, the method recited in the Asserted Claims of the '454 Patent is not routine or conventional at least because the method's ordered steps recite improved, inventive processes for sample preparation. The ordered combination of steps improves upon prior art approaches in several respects, as described *infra*.

²³³ See Section II.

209. First, the claimed methods recited in the Asserted Claims of the '454 Patent require use of the specific laboratory techniques whole genome sequencing or whole exome sequencing. This step provides holistic information about a specific person's genetic background—and not merely “sequencing,” which does not itself provide this information.

210. Second, the methods can be used to prepare small amounts of cancer-associated cfDNA, which are challenging to manipulate.²³⁴ The '454 Patent specification explains the inventiveness of the claimed approach:

In another aspect, the present invention generally relates, at least in part, to improved methods of detecting single nucleotide variations (SNVs). These improved methods include improved analytical methods, improved bioassay methods, and improved methods that use a combination of improved analytical and bioassay methods. ***The methods in certain illustrative embodiments are used to detect, diagnose, monitor, or stage cancer, for example in samples where the SNV is present at very low concentrations ... such as circulating free DNA samples.*** That is, these methods in certain illustrative embodiments are particularly well suited for samples where there is a relatively low percentage of a mutation or variant relative to the normal polymorphic alleles present for that genetic loci.²³⁵

In certain preferred embodiments the sample is a single cell sample or a plasma sample suspected of containing circulating tumor DNA. These embodiments take advantage of the discovery that by ***interrogating DNA samples from single cells or plasma for CNVs and SNVs using the highly sensitive multiplex PCR methods disclosed herein, improved cancer detection can be achieved,*** versus interrogating for

²³⁴ See Section II.

²³⁵ See '454 Patent at 44:6-21 (emphasis added).

either CNVs or SNVs alone ... The methods provided herein for detecting CNVs and/or SNVs in plasma of subjects ... provide the advantage of detecting CNVs and/or SNVs from tumors that often are composed of heterogeneous cancer cell populations in terms of genetic compositions. Thus, traditional methods, which focus on analyzing only certain regions of the tumors can often miss CNVs or SNVs that are present in cells in other regions of the tumor.²³⁶

211. Third, the specific claimed approach recited in the Asserted Claims of the '454 Patent is also an improvement over prior art techniques because amplifying the targeted loci together, when done successfully as the '454 Patent describes, is efficient. This approach has the additional benefit of avoiding dividing the plasma sample into individual reactions which may require more cfDNA starting material, lead to biased results when the sample contains very low, and/or variable levels of tumor-associated cfDNA.

Improved PCR amplification methods have also been developed that minimize or prevent interference due to the amplification of nearby or adjacent target loci in the same reaction volume (such as part of the sample multiplex PCR reaction that simultaneously amplifies all the target loci). These methods can be used to simultaneously amplify nearby or adjacent target loci, which is faster and cheaper than having to separate nearby target loci into different reaction volumes so that they can be amplified separately to avoid interference.²³⁷

The multiplex reaction methods provided herein, such as the massive multiplex PCR disclosed herein provide ***an exemplary process for carrying out the amplification reaction to help***

²³⁶ *Id.* at 147:22-45 (emphases added).

²³⁷ *Id.* at 103:2-11 (emphasis added).

attain improved multiplexing and therefore, sensitivity levels.²³⁸

In an embodiment, the particular primers that are most likely to cause unproductive side reactions may be removed from the primer library to give a primer library that will result in a greater proportion of amplified DNA that maps to the genome. ***The step of removing problematic primers, that is, those primers that are particularly likely to form [sic] dimers has unexpectedly enabled extremely high PCR multiplexing levels*** for subsequent analysis by sequencing.²³⁹

212. Fourth, the claimed methods recited in the Asserted Claims of the '454 Patent use the uncommon and expensive method of sequencing to a depth of read of 50,000 or more.

In certain aspects, the present invention provides a method for detecting cancer. The sample, it will be understood can be a tumor sample or a liquid sample, such as plasma, from an individual suspected of having cancer. ***The methods are especially effective at detecting genetic mutations such as single nucleotide alterations such as SNVs, or copy number alterations, such as CNVs in samples with low levels of these genetic alterations as a fraction of the total DNA in a sample.*** Thus the sensitivity for detecting DNA or RNA from a cancer in samples is exceptional. The methods can combine any or all of the improvements provided herein for detecting CNV and SNV to achieve this exceptional sensitivity.²⁴⁰

213. In addition to my opinions *supra*, I further note that the prior art cited by Dr. Van Ness under Section 103 does not show that any reference teaches each and every limitation of the claims, as arranged in the Asserted Claims of the '454 Patent. The fact

²³⁸ *Id.* at 64:58-62 (emphasis added).

²³⁹ *Id.* at 105:54-61 (emphasis added).

²⁴⁰ *Id.* at 63:53-65 (emphasis added).

that even combinations of references do not teach or suggest the claimed methods as recited in the Asserted Claims of the '454 Patent further supports my opinion that the ordered combination of the claims was not routine, conventional, or well-understood in the art.

B. The '035 Patent Is Patent-Eligible

(i) *The '035 Patent Claims Are Directed to Patent-Eligible Subject Matter*

214. Dr. Van Ness opines that the '035 Patent claims are “methods of detection” claims.²⁴¹ I disagree. “Detecting” is not even a recited step in the methods claimed in the '035 Patent.²⁴²

215. Regardless, from my understanding of principles governing the “directed to” analysis, I believe the Van Ness Decl.’s analysis is flawed in several respects. Dr. Van Ness reaches his opinion by focusing on certain claim limitations and excluding others. I am unaware of a basis to exclude claim limitations in determining what claims are directed to. Rather, I have been informed and I understand from counsel that the “directed to” analysis requires consideration of the character of the claims as a whole, which the Van Ness Decl. does not address. Indeed, Dr. Van Ness analyzes portions of the claims individually. Additionally, I understand from Dr. Van Ness’s testimony²⁴³ that Dr. Van Ness believes that the Asserted Claims of the '035 Patent are ineligible because they

²⁴¹ See Van Ness Decl. at ¶¶ 341-347.

²⁴² See, e.g., '035 Patent at 249:44-62.

²⁴³ See Van Ness Tr. at 59:10-23, shown *supra*.

involve detecting a natural phenomenon, such as DNA or SNPs associated with cancer.²⁴⁴

I am informed by counsel that the patent-eligibility determination requires more than determining if the claims “involve” an ineligible concept. Rather, claims may be eligible for patenting if they recite a method for achieving a new and useful result.

216. In my opinion, the Asserted Claims of the '035 Patent are directed not to a natural phenomenon, but rather to an inventive combination of specific laboratory techniques, each of which requires significant human effort to perform and which involve modifying natural-occurring products as part of the process. Although these techniques were previously known in the art, their combination as recited in the claims represents a novel and innovative improvement over earlier methods of sample preparation that achieve superior results. I discuss the advantages of the claimed methods in greater detail *infra* with respect to step two of the eligibility analysis.

217. Notably, when performed in combination as recited in the Asserted Claims of the '035 Patent, the claimed techniques permit identification of 25-2,000 SNPs associated with cancer using cfDNA, a DNA source that the art recognized as highly challenging to manipulate successfully.²⁴⁵ This claimed method therefore represents a new and useful method of sample preparation that can be used to yield a tangible and useful result. This result is only achieved by the combination of specific laboratory techniques that, together, represent a new and useful method of preparing cfDNA. The Van Ness

²⁴⁴ See Van Ness Decl. at ¶¶ 341-347.

²⁴⁵ See Section II.

Decl. does not address the new, useful result achieved by the claimed combination. Nor does Dr. Van Ness discuss certain specific limitations recited in the claims, which I consider *infra*.

218. First, the Van Ness Declaration does not address that, in the '035 Patent preamble, the Asserted Claims of the '035 Patent expressly recite that they involve “[a] method for amplifying and sequencing DNA,” not “detecting.”²⁴⁶ I believe this limitation should be considered at least because the Asserted Claims recite these specific techniques in order to prepare the cfDNA sample. Detection is not a recited step anywhere in the claimed methods. I discuss the claimed steps in turn *infra*.

219. Second, the first-recited step of the Asserted Claims of the '035 Patent requires performing two different steps, each of which depends upon human intervention and effort. Specifically, it recites [1] tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, wherein [2] the isolated cell-free DNA is isolated from a blood sample.²⁴⁷ To perform the method, cfDNA must be isolated and then tagged with one or more universal tail adaptors. This approach generates tagged products that are not found anywhere in nature.

220. Third, the Asserted Claim of the '035 Patent recite a specific protocol for amplifying cfDNA. This step requires specific technical approaches, each of which requires significant human effort to design and implement. Specifically, the claims require

²⁴⁶ See Van Ness Decl. at ¶¶ 341-382, where the preamble is not mentioned at all.

²⁴⁷ See '035 Patent at 249:44-49.

[1] amplifying the tagged products one or more times to generate final amplification products, wherein [2] one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, [3] wherein one of the amplifying steps introduces a barcode and one or more sequencing tags.²⁴⁸ The products of this amplification step, which are barcoded and contain sequencing tag(s), are not found anywhere in nature.

221. Fourth, the Asserted Claims of the '035 Patent recite sequencing the plurality of SNP loci on cfDNA by conducting massively parallel sequencing on the final amplification products. Like the other recited preparation steps, the massively parallel sequencing step requires significant human effort to implement and does not occur in nature. Nor is massively parallel sequencing simply “sequencing,” as described *supra*. It requires certain sequencing equipment to perform and entails sequencing on a very large scale—millions or billions of reads per each run of the instrument.

222. Accordingly, from my review of the Asserted Claims of the '035 Patent, in my opinion, their character as a whole is directed to eligible subject matter—a new and useful method for sample preparation by amplifying and sequencing cfDNA using a combination of specific laboratory techniques. Manipulating SNP-containing tumor-associated cfDNA in this way would have been particularly challenging for reasons explained *supra*.²⁴⁹ The combination of recited techniques transform DNA into artificial

²⁴⁸ *Id.* at 249:50-57.

²⁴⁹ *See* Sections II.

products with at least one universal tail adaptor, a barcode, and one or more sequencing tags—products that are not found anywhere in nature. They are not, as Dr. Van Ness opines, directed to natural phenomenon or any other ineligible subject matter.²⁵⁰

223. In reaching his contrary conclusions, Dr. Van Ness discusses other patents from separate proceedings.²⁵¹ I disagree with his assessment of these non-asserted patents at least because those patents recite different limitations.. The Van Ness Declaration fails to offer any reasoned basis to equate their recited combination to the combination recited in the Asserted Claims of the '035 Patent. Dr. Van Ness also discusses a claim amendment during prosecution concerning sequencing SNPs “associated with cancer.”²⁵² This does not change my view as, *e.g.*, sequencing cancer-associated SNPs represents a new and useful result that Dr. Van Ness acknowledges was not found in the prior art [*e.g.*, Chuu (2012)²⁵³] as the '035 Patent claims were allowable over Chuu (2012).

224. For all the aforementioned reasons, in my opinion the Asserted Claims of the '035 Patent, when considered as a whole, are directed to patent-eligible subject matter and step two of the patent-eligibility analysis is unnecessary. Even under an analysis of step two, the Asserted Claims of the '035 Patent should still be found patent-eligible, as explained *infra*.

²⁵⁰ See Van Ness Decl. at § XI.

²⁵¹ *Id.* at ¶¶ 345-346.

²⁵² *Id.* at ¶ 344.

²⁵³ See Chuu & Rava, Methods of fetal abnormality detection, U.S. Patent 8,318,430 (2012) (“Chuu (2010)”).

(ii) *The '035 Patent Claims, Considered Individually and In Combination, Are Not Routine or Conventional*

225. The Van Ness Declaration opines that certain aspects of the claimed methods of the '035 Patent—tagging, amplification, and sequencing—were routine and conventional.²⁵⁴ Even if, considered independently and in isolation, these general techniques may have been known in the art as of 2011, this is not to say, however, that these methods were routine or conventional, particularly in the context of working with ctDNA. They were not, as explained *supra*.²⁵⁵

226. In any event, in my understanding, even if the recited limitations were routine, conventional, or well-understood that would not be sufficient to show patent ineligibility, as the claim limitations must be considered both individually and as their ordered combination. The Van Ness Declaration fails to consider the latter—whether the '035 Patent limitations are well-understood, routine, or conventional in their ordered combinations. In my opinion, these claim limitations when considered in their ordered combination are not well-understood, routine, or conventional. In consequence, the Asserted Claims of the '035 Patent should be deemed patent-eligible if the Court reaches step two of the eligibility analysis.

227. When considered as an ordered combination, the method recited in the Asserted Claims of in the '035 Patent is not routine or conventional at least because the

²⁵⁴ See Van Ness Decl. at ¶¶ 348-381.

²⁵⁵ See Section II.

method's ordered steps recite improved, inventive processes for sample preparation. This ordered combination improves upon prior art approaches in several respects, as described *infra*.

228. First, as the '035 Patent explains, certain problems in multiplex amplification were not addressed by the prior art. These problems become more pronounced when the number of reactions are performed in a single volume increases. One example is the formation of "primer-dimers"—PCR primers that bind to each other rather than their targets—which may reduce the amplification of certain targets. Another example is "allelic bias," where some targets are amplified more efficiently than others, skewing the representative amounts of cancer-associated versus non-cancer-related cfDNA present in the sample. As exemplified *infra*, the '035 Patent identifies various limitations in prior art techniques.

Use of multiplex PCR can significantly simplify experimental procedures and shorten the time required for nucleic acid analysis and detection. ***However, when multiple pairs are added to the same PCR reaction, non-target amplification products may be generated, such as amplified primer dimers.*** The risk of generating such products increases as the number of primers increases. These non-target amplicons significantly limit the use of the amplified products for further analysis and/or assays. ***Thus, improved methods are needed to reduce the formation of non-target amplicons during multiplex PCR.***²⁵⁶

Currently, performing multiplex PCR reactions of more than 5 to 10 targets presents a major challenge and is often hindered by primer side products, such as primer dimers, and

²⁵⁶ See '035 Patent at 3:4-14 (emphases added).

other artifacts. When detecting target sequences using microarrays with hybridization probes, primer dimers and other artifacts may be ignored, as these are not detected. **However, when using sequencing as a method of detection, the vast majority of the sequencing reads would sequence such artifacts and not the desired target sequences in a sample.** Methods described in the prior art used to multiplex more than 50 or 100 reactions in one reaction volume followed by sequencing will typically result in more than 20%, and often more than 50%, in many cases more than 80% and in some cases more than 90% off-target sequence reads.²⁵⁷

In general, to perform targeted sequencing of multiple (n) targets of a sample (greater than 50, greater than 100, greater than 500, or greater than 1,000), **one can split the sample into a number of parallel reactions that amplify one individual target.** This has been performed in PCR multiwell plates or can be done in commercial platforms such as the FLUIDIGM ACCESS ARRAY (48 reactions per sample in microfluidic chips) or DROPLET PCR by RAIN DANCE TECHNOLOGY (100 s to a few thousands of targets). Unfortunately, these split-and-pool methods are problematic for samples with a limited amount of DNA, as there is often not enough copies of the genome to ensure that there is one copy of each region of the genome in each well.²⁵⁸

Disclosed herein are methods that permit the targeted amplification of over a hundred to tens of thousands of target sequences (e.g., SNP loci) from a nucleic acid sample such as genomic DNA obtained from plasma. **The amplified sample may be relatively free of primer dimer products and have low allelic bias at target loci.** If during or after amplification the products are appended with sequencing compatible adaptors, analysis of these products can be performed by sequencing.²⁵⁹

²⁵⁷ *Id.* at 85:5-20 (emphases added).

²⁵⁸ *Id.* at 85:21-33 (emphasis added).

²⁵⁹ *Id.* at 86:2-10 (emphasis added).

229. The claimed methods of the '035 Patent address these problems in inventive ways that were not routine or conventional in 2011. Notably, the claimed approach recited in the Asserted Claims of the '035 Patent would have been technically challenging as of the priority date. As one of ordinary skill in the art would have recognized, a mixture containing cfDNA derived from both tumor cells and non-cancerous cells presents particular challenges in multiplex amplification suitable for subsequent massively parallel sequencing. This is due at least in part to the scarcity of tumor-derived cfDNA in the sample. These challenges are magnified as the number of SNPs amplified within the single reaction mixture increases. In overcoming these technical challenges, the '035 Patent teaches that its methods can be used to prepare small amounts of cancer-associated cfDNA, which are challenging to manipulate, for example, because they are fragmented.²⁶⁰

Primer tails may improve the detection of fragmented DNA from universally tagged libraries. If the library tag and the primer-tails contain a homologous sequence, hybridization can be improved (for example, melting temperature (T_M) is lowered) and primers can be extended if only a portion of the primer target sequence is in the sample DNA fragment.²⁶¹

230. Second, the specific claimed approach recited in the Asserted Claims of the '035 Patent also represents an improvement over prior art techniques because amplifying multiple targeted loci together, when done successfully as the '035 Patent describes, is efficient and provides more comprehensive genetic information than amplifying only one

²⁶⁰ See Section II, *supra*.

²⁶¹ See '035 Patent at 94:51-57 (emphasis added).

or a few targets. It reduces the time and operational complexity in a method that requires processing and sequencing up to thousands of SNPs.

Improved PCR amplification methods have also been developed that minimize or prevent interference due to the amplification of nearby or adjacent target loci in the same reaction volume (such as part of the sample multiplex PCR reaction that simultaneously amplifies all the target loci) ... ***These methods can be used to simultaneously amplify nearby or adjacent target loci, which is faster and cheaper than having to separate nearby target loci into different reaction volumes*** so that they can be amplified separately to avoid interference.²⁶²

Disclosed herein are methods that permit the targeted amplification of over a hundred to tens of thousands of target sequences (e.g., SNP loci) from a nucleic acid sample such as genomic DNA obtained from plasma. ***The amplified sample may be relatively free of primer dimer products and have low allelic bias at target loci.*** If during or after amplification the products are appended with sequencing compatible adaptors, analysis of these products can be performed by sequencing.²⁶³

The use of tags on the primers may reduce amplification and sequencing of primer dimer products.²⁶⁴

In an embodiment, a 10,000-plex PCR assay pool is created such that reverse primers have tails corresponding to the required reverse sequences required by a high throughput sequencing instrument. After amplification with the first 10,000-plex assay, a subsequent PCR amplification may be performed using a another 10,000-plex pool having partly nested forward primers (e.g. 6-bases nested) for all targets and a reverse primer corresponding to the reverse sequencing tail included in the first round. ***This subsequent round of partly nested amplification with just one target specific primer and***

²⁶² *Id.* at 101:29-41 (emphasis added).

²⁶³ *Id.* at 86:2-10 (emphasis added).

²⁶⁴ *Id.* at 55:11-12 (emphasis added).

a universal primer limits the required size of the assay, reducing sampling noise, but greatly reduces the number of spurious amplicons. The sequencing tags can be added to appended ligation adaptors and/or as part of PCR probes, such that the tag is part of the final amplicon.²⁶⁵

231. Third, the claimed approach recited in the Asserted Claims of the '035 Patent is also advantageous because it has the additional benefit of avoiding dividing the plasma sample into individual reactions. Dividing a cfDNA sample may lead to biased results when, for example, the sample contains very low and/or variable levels of tumor-associated cfDNA.

For samples where either the overall sample or some subpopulation of DNA molecules is limited, splitting the sample would introduce statistical noise. In an embodiment, a small or limited quantity of DNA may refer to an amount below 10 µg, between 10 and 100 µg, between 100 µg and 1 ng, between 1 and 10 ng, or between 10 and 100 ng. Note that while this method is particularly useful on small amounts of DNA where other methods that involve splitting into multiple pools can cause significant problems related to introduced stochastic noise, this method still provides the benefit of minimizing bias when it is run on samples of any quantity of DNA.²⁶⁶

Note that this approach could be used to perform targeted amplification in a manner that would result in low levels of allelic bias for 50-500 loci, for 500 to 5,000 loci, for 5,000 to 50,000 loci, or even for 50,000 to 500,000 loci. In an embodiment, the primers carry partial or full length sequencing compatible tags.²⁶⁷

²⁶⁵ *Id.* at 91:4-18 (emphasis added).

²⁶⁶ *Id.* at 84:51-62 (emphasis added).

²⁶⁷ *Id.* at 86:54-60 (emphasis added).

232. In addition to my opinions *supra*, I further note that the prior art cited by Dr. Van Ness under Section 103 does not show that any reference teaches each and every limitation of the claims, arranged as in the claim. The fact that even combinations of references do not teach or suggest the claimed methods as recited further supports my opinion that the ordered combination of the claims was not routine, conventional, or well-understood in the art.

VIII. THE NATERA PATENTS ARE VALID UNDER SECTION 112

233. The Van Ness Declaration additionally opines that the Natera Patents are invalid under Section 112 for lack of adequate written description.²⁶⁸ I disagree with the bases Dr. Van Ness contends are invalidating at least for the reasons explained *infra*. I reserve the right to offer additional opinions at another time.

234. Notably, the Van Ness Declaration does not raise any challenge to the Asserted Claims under either an indefiniteness or lack of enablement theory under Section 112. I therefore do not address these grounds herein. To the extent Dr. Van Ness raises such a challenge in the future, I reserve the right to offer opinions in rebuttal.

A. The '454 Patent Adequately Describes Whole Genome Sequencing

235. The Van Ness Declaration contends that there is no description of “whole genome sequencing” in the '454 Patent.²⁶⁹ This is incorrect and I disagree with Dr. Van

²⁶⁸ See Van Ness Decl. at ¶¶ 404-426.

²⁶⁹ *Id.* at ¶¶ 405-407.

Ness. Further, the Van Ness Declaration's analysis is flawed in several respects, as described *infra*.

236. First, I note that his belief is informed by his review of Jamal-Hanjani Thesis (2015)²⁷⁰ and the testimony of a Natera witness, Bernhard Zimmermann.²⁷¹ It is my understanding from counsel that the adequacy of a patent's written description is determined based on the written description within the four corners of that patent, and from the perspective of one of ordinary skill in the art. The Jamal-Hanjani Thesis (2015) is a separate document and not part of the patent's four corners—for example, not incorporated by reference. The Van Ness Declaration does not contend otherwise. Dr. Zimmermann's testimony is also not part of the patent disclosure. Therefore, to the extent that the opinions expressed in the Van Ness Declaration are founded on these extrinsic sources of information, it is my understanding that Dr. Van Ness has misapplied the relevant legal principles governing written description.

237. Second, the '454 Patent explicitly uses the words “whole genome sequencing” to describe experimental results reported in the '454 Patent. I reproduce this teaching *infra*. In the accompanying Figure 51B, the '454 Patent reports data on certain

²⁷⁰ See Dkt. 118-1, Jamal-Hanjani, *The role of intratumour heterogeneity and chromosomal instability in cancer*, University College London, PhD Supervisor: Professor Charles Swanton; A thesis submitted for the degree of Doctor of Philosophy, University College London (“Jamal-Hanjani Thesis (2015)”).

²⁷¹ See Van Ness Decl. at ¶¶ 406-407.

genes, which could be captured either by whole genome sequencing or by whole exome sequencing.

FIG. 51A represents the histological finding/history for primary lung tumors analyzed for clonal and subclonal tumor heterogeneity. FIG. 51B is a table of the VAF identities of the biopsied lung tumors by **whole genome sequencing** and assaying by AmpliSEQ.²⁷²

Sample	Gene	Chr	ChrStart	VAF R1	VAF R2	VAF R3
L12	BRIP1	chr17	59924572	14	6	8
L12	CARS	chr11	3062181	22	11	16
L12	CIC	chr19	42797381	0	0	7
L12	CYFIP1	chr15	22940733	0	6	0
L12	FAT1	chr4	187519147	8	4	0
L12	KDM6A	chrX	44921898	10	5	0
L12	MLLT4	chr6	168347475	9	3	0
L12	NFE2L2	chr2	178098801	69	31	50
L12	RASA1	chr5	86642517	7	0	0
L12	TP53	chr17	7578406	23	9	17
L12	TP53	chr17	7578190	22	8	16
L13	EGFR	chr7	55241708	21	25	55
L13	EGFR	chr7	55242511	20	17	48
L13	HERC4	chr10	69793756	0	3	6
L13	JAK2	chr9	5022084	0	11	7
L13	KMT2C	chr7	151947008	0	0	4
L13	MSH2	chr2	47693816	5	0	11
L13	MTOR	chr1	11292495	2	0	3
L13	PLCG2	chr16	81942036	5	0	6
L13	TP53	chr17	7579509	7	4	16
L15	ALK	chr2	29940530	6	2	
L15	GABRG1	chr4	46060315	13	0	
L15	KDM6A	chrX	44922755	18	6	
L15	MLL2	chr12	49443815	5	0	
L15	ROS1	chr6	117687379	10	3	
L15	SLC39A4	chr8	145638322	14	0	
L15	TP53	chr17	7578254	19	3	
L15	ZFX4	chr8	77776735	13	5	
L15	ZMYM4	chr1	35827319	14	2	
L17	BRCA2	chr13	32914959	16	28	0
L17	KRAS	chr12	25398284	13	22	0
L17	NF1	chr17	29653134	20	25	0
L17	NF1	chr17	29528088	0	0	20
L17	PAX8	chr2	113984793	9	0	0
L17	TP53	chr17	7577610	0	0	16
L17	TP53	chr17	7577079	20	35	0
L17	TRIM67	chr1	231299607	0	50	0
L17	TRIP11	chr14	92471631	7	0	0

FIG. 51B

238. Separately, the '454 Patent also refers to whole exome sequencing in Example 13, but it does not state that only whole exome sequencing was performed. At most, the '454 Patent's disclosures reflect an ambiguity or typographical error (*e.g.*, referring to whole exome when whole genome was actually performed).

²⁷² See '454 Patent at 41:57-61 (emphasis added).

239. In any event, this ambiguity would not have taught one of ordinary skill in the art that the inventors did not possess the use of whole genome sequencing in the context of the claimed methods. To the contrary, whole exome sequencing is a modification of whole genome sequencing, in which specific portions of the genome (the protein-coding portions, called exons) are targeted and enriched. Thus, by the '454 Patent's plain language, one of ordinary skill in the art would have understood that the named inventors possessed the use of both whole genome sequencing and whole exome sequencing to achieve the claimed methods.

240. Third, the Van Ness Declaration does not dispute that whole genome sequencing was well-known in the art in 2014-2015. On the contrary, in the Van Ness Declaration's opinions regarding Section 101, Dr. Van Ness asserts that "[w]hole genome sequencing and whole exome sequencing were well known to a POSA."²⁷³ Dr. Van Ness points to multiple sources for the proposition that whole genome sequencing was a routine and conventional technique in the 2014-2015 time period and acknowledges that "the '454 Patent cites to a wealth of prior art publications describing routine, standard, gene sequencing techniques known in the art, including whole genome sequencing."²⁷⁴

241. As to the general technique of whole genome sequencing, I agree with the Van Ness Declaration that whole genome sequencing was well known in the art before 2014-2015. As Dr. Van Ness admits, multiple prior art references cited in the '454 Patent

²⁷³ See Van Ness Decl. at ¶ 318.

²⁷⁴ *Id.* at ¶ 320.

describe the use of this technique for various purposes.²⁷⁵ Even looking beyond the '454 Patent, other prior art describes the use of this technique, further establishing that it was known in the art. As one example, in 2010, I published Metzker (2010), which is a review discussing the use of whole genome sequencing for various purposes. I excerpt *infra* Table 1²⁷⁶ from that review that illustrates certain early “biological applications” that could be achieved using whole genome sequencing and whole exome sequencing approaches. These biological applications had been performed using commercially-available next-generation sequencing technologies.

²⁷⁵ *Id.*

²⁷⁶ See Metzker (2010) at Table 1.

Table 1 Comparison of next-generation sequencing platforms									
Platform	Library/ template preparation	NGS chemistry	Read length (bases)	Run time (days)	Gb per run	Machine cost (US\$)	Pros	Cons	Biological applications
Roche/454's GS FLX Titanium	Frag, MP/ emPCR	PS	330*	0.35	0.45	500,000	Longer reads improve mapping in repetitive regions; fast run times	High reagent cost; high error rates in homo- polymer repeats	Bacterial and insect genome <i>de novo</i> assemblies; medium scale (<3 Mb) exome capture; 16S in metagenomics
Illumina/ Solexa's GA _{II}	Frag, MP/ solid-phase	RTs	75 or 100	4 [†] , 9 [‡]	18 [†] , 35 [‡]	540,000	Currently the most widely used platform in the field	Low multiplexing capability of samples	Variant discovery by whole-genome resequencing or whole-exome capture; gene discovery in metagenomics
Life/APG's SOLID 3	Frag, MP/ emPCR	Cleavable probe SBL	50	7 [†] , 14 [‡]	30 [†] , 50 [‡]	595,000	Two-base encoding provides inherent error correction	Long run times	Variant discovery by whole-genome resequencing or whole-exome capture; gene discovery in metagenomics
Polonator G.007	MP only/ emPCR	Non- cleavable probe SBL	26	5 [‡]	12 [‡]	170,000	Least expensive platform; open source to adapt alternative NGS chemistries	Users are required to maintain and quality control reagents; shortest NGS read lengths	Bacterial genome resequencing for variant discovery
Helicos BioSciences HeliScope	Frag, MP/ single molecule	RTs	32*	8 [†]	37 [†]	999,000	Non-bias representation of templates for genome and seq-based applications	High error rates compared with other reversible terminator chemistries	Seq-based methods
Pacific Biosciences (target release: 2010)	Frag only/ single molecule	Real-time	964*	N/A	N/A	N/A	Has the greatest potential for reads exceeding 1 kb	Highest error rates compared with other NGS chemistries	Full-length transcriptome sequencing; complements other resequencing efforts in discovering large structural variants and haplotype blocks

242. Fourth, it is my understanding that a patent does not need to describe an invention in the exact same words as recited in the claim as long as one of ordinary skill in the art would have understood that the named inventors were in possession of that aspect of the invention. From my review of the '454 Patent, it describes the use of whole genome sequencing in different words than simply calling out the technique's specific name. In other words, the '454 Patent describes familiar applications of whole genome sequencing which one of ordinary skill in the art would have recognized as such despite not referring to those applications as involving whole genome sequencing.

243. For example, the '454 Patent describes a technique called “long read sequencing.”²⁷⁷ As one application of this technique, the '454 Patent describes “phasing,” which is a method to sequence long stretches of DNA on the same chromosome.²⁷⁸ “Phasing refers to the act of determining the haplotypic genetic data of an individual given unordered, diploid (or polyploidy) genetic data.”²⁷⁹ The '454 Patent describes “Exemplary Methods for Phasing Genetic Data” across several columns.²⁸⁰ These techniques form “long haplotype contigs” of DNA—*i.e.*, reassembling the genome from the phased sequencing data. “After sequencing, the computational steps involve ... assembling the fragments at their overlapping heterozygous SNVs into haplotype blocks, and phasing the blocks statistically based on a phased reference panel and producing long haplotype contigs.”²⁸¹ Such approaches could be performed, for example, using Illumina’s Moleculo Technology described in the ‘454 Patent, which one of ordinary skill in the art would have

²⁷⁷ See '454 Patent at 116:20-35; 112:50-56; *see also* Ex. 9, Kuleshov *et al.*, *Whole-genome haplotyping using long reads and statistical methods*, NATURE BIOTECHNOLOGY 32:261-266 (2014) (“Kuleshov (2014)”) (“***Here we describe statistically aided, long-read haplotyping (SLRH), ...*** For a human sample, as little as 30 Gbp of additional sequencing data are needed to phase genotypes identified by ***50× coverage whole-genome sequencing***”) at Abstract (emphases added); *see also* Ex. 10, GenomeWeb article dated March 5, 2014 describing that “Stanford University was an early-access user of Moleculo technology, and researchers there detailed in a Nature Biotechnology publication last week ... Volodymyr Kuleshov, a former consultant with Moleculo, now a member of Mike Snyder’s Stanford laboratory and also the lead author of the paper.”

²⁷⁸ *Id.* at 115:7-34; 116:20-123:3.

²⁷⁹ *Id.* at 32:35-37.

²⁸⁰ *Id.* at 123:3-127:46.

²⁸¹ *Id.* at 116:20-35.

understood can perform whole genome sequencing.²⁸² “In some embodiments, a haplotype of an individual is determined by long read sequencing, such as by using the Molecule Technology developed by Illumina.”²⁸³

244. The '454 Patent describes other techniques that could perform whole genome sequencing, including whole genome amplification (WGA) methods.

In some embodiments, whole genome application [*sic*] (WGA) is used to amplify a nucleic acid sample. There are a number of methods available for WGA: ligation-mediated PCR (LM-PCR), degenerate oligonucleotide primer PCR (DOP-PCR), and multiple displacement amplification (MDA).²⁸⁴

One of ordinary skill in the art would have understood that these descriptions implicate the use of whole genome sequencing, despite not being described using that specific phrase.

245. I understand from his deposition that Dr. Van Ness did not consider the '454 Patent's disclosure of long-read sequencing, described *supra*, in rendering his opinions. I note Dr. Van Ness agrees with me that one of ordinary skill in the art would have understood that long-read sequencing can achieve whole genome sequencing.

Q. Okay. Thank you. Have you heard the concept of long-read sequencing?

A. **Yes.**

Q. You don't discuss long-read sequencing in your declaration, correct?

²⁸² See Kuleshov (2014) at Abstract.

²⁸³ '454 Patent at 116:20-22.

²⁸⁴ *Id.* at 96:30-34.

A. I don't see any reference to long-read – I'm not sure if I saw a reference to long-read sequencing. I did not discuss it. I discuss next-gen sequencing. Long-read sequences -- sequencing is one of the concepts of next-gen sequencing.

Q. You agree that persons of skill in the art would understand that long-read sequencing can achieve whole genome sequencing?

A. ***It can achieve it.***

Q. And do you have any understanding of whether the '454 patent describes long-read sequencing? Did you consider that?

A. ***I did not consider that.***²⁸⁵

246. Dr. Van Ness also agreed that with my opinions regarding phasing and determining a haplotype, but did not consider the '454 Patent's disclosures of those techniques in rendering his opinions.

Q. You would agree that phasing is performed using whole genome sequencing?

A. It's one of the components of analyzing the data and coming up with a whole genome sequence.

Q. And do you have any understanding of whether the '454 patent describes phasing?

A. I don't recall.

Q. Did you consider any discussion in the '454 patent of phasing in forming your opinions regarding written description of whole genome sequencing?

²⁸⁵ See Van Ness Tr. at 269:17-270:14 (emphases added).

A. ***I did not consider that.***

Q. Did you consider any discussion in the '454 patent of phasing in forming your opinions regarding written description of whole genome sequencing?

A. ***I did not consider that.***

Q. Determining a haplotype of an individual is a way of -- involves whole genome sequencing, correct?

A. It can. It's not the only way.

Q. But a person of skill in the art would understand that whole genome sequencing can be used to determine the haplotype of an individual, correct?

A. It can.

Q. And you -- you didn't consider determining haplotypes of individuals in formulating your written description opinions regarding the '454 patent?

A. I -- it was not an issue of -- that I considered to reflect any kind of lack of description, so I -- ***I did not consider it.***²⁸⁶

247. From these disclosures, one of ordinary skill in the art would have recognized that the named inventors were in possession of whole genome sequencing to perform the claimed methods. This is in addition to the explicit reference to "whole genome sequencing" in the '454 Patent. as described *supra*.

²⁸⁶ *Id.* at 271:5-272:11 (emphases added).

B. The '454 and '035 Patents Adequately Describe the Claimed Methods

248. The Van Ness Declaration asserts that the Natera Patent claims are also invalid for failure to describe techniques of PCR amplification without the use of techniques for selecting primers, *e.g.*, to avoid primer dimers.²⁸⁷ Relatedly, the Van Ness Declaration contends that Dr. Van Ness does not “believe there is any embodiment that discloses the elements of the claimed inventions of the '454 or '035 Patents as arranged in the claims.”²⁸⁸ I disagree with his contentions as explained *supra*. In my opinion, one of ordinary skill in the art would have recognized that the named inventors of the Natera Patents were in possession of the claimed methods as of the Natera Patents’ respective priority dates.

(i) The Patents Adequately Describe Methods that Do Not Require Selecting Primers to Avoid Primer Dimers

249. As an initial matter, I note that it is technically feasible to perform multiplex PCR without taking steps to avoid primer dimers, as usable sequencing data can be obtained even if primer dimers are generated. As the '035 Patent notes, only a small number of “bad” primers are responsible for forming the majority of the observed primer dimers in any reaction—thus, most amplifications will avoid primer dimer formation without involving primer selection approaches.²⁸⁹ “The present invention is based in part

²⁸⁷ A “primer dimer” occurs when primers intended to amplify a target DNA sequence instead bind and amplify each other; *see also* Van Ness Decl. at ¶¶ 409-426.

²⁸⁸ *Id.* at ¶ 414.

²⁸⁹ *See* '035 Patent at 48:30-47.

on the surprising discovery that ***often only a relatively small number of primers*** in a library of primers ***are responsible for a substantial amount of the amplified primer dimers*** that form during multiplex PCR reactions.”²⁹⁰

250. Even in the situation where the correctly-amplified (targeted) product represents a minor fraction of the amplification and the (off-target) primer dimers represent the major fraction, next-generation sequencing technology permits excluding primer dimers by size selection in the library preparation workflow. For example, the primer dimers are comparatively small relative to the targeted amplicon, they can be excluded from analysis on the basis of size. One relatively simple method for doing this that was known to one of ordinary skill in the art was to gel-purify or SPRI purify the amplicons.²⁹¹

251. Indeed, the ’454 Patent explains that avoiding primer dimers is merely an optional component of the claimed methods, albeit an advantageous one.

Exemplary Primer Design Methods

If desired, multiplex PCR may be performed using primers with a decreased likelihood of forming primer dimers. In particular, highly multiplexed PCR can often result in the production of a very high proportion of product DNA that results from unproductive side reactions such as primer dimer formation. ***In an embodiment, the particular primers that are most likely to cause unproductive side reactions may be removed from the primer library*** to give a primer library that

²⁹⁰ *Id.* at 46:37-41 (emphases added).

²⁹¹ See Meyer (2010) at 5 “Carboxyl-coated magnetic beads (SPRI beads) are ideally suited for reaction purification in a 96-well plate setup. However, under the conditions described here, ***SPRI purification does not retain molecules shorter than 100-150 bp.***” (emphasis added.)

will result in a greater proportion of amplified DNA that maps to the genome.²⁹²

252. Similarly, one of ordinary skill in the art would have understood that the '035 Patent teaches that primers do not need to be designed to avoid primer dimers, although it could be beneficial in some instances to do so. The '035 Patent explains, for example that “[i]n **some embodiments**, the test primers are selected from a library of candidate primers based at least in part on the ability of the candidate primers to form primer dimers.”²⁹³ “The amplified sample **may** be relatively free of primer dimer products and have low allelic bias at target loci.”²⁹⁴

253. Consistent with this statement, the '035 Patent describes methods of performing PCR that do not include designing primers to avoid the formation of primer dimers although it would be desirable and standard practice in the art to do so.²⁹⁵ For example, the '035 Patent describes that “using lower primer concentrations and much longer annealing times than normal increases the likelihood that the primers hybridize to the target loci instead of hybridizing to each other and forming primer dimers.”²⁹⁶ In another amplification strategy, the primers’ “annealing temperature for the reaction

²⁹² See '454 Patent at 105:48-58 (emphases added).

²⁹³ See '035 Patent at 11:29-31.

²⁹⁴ *Id.* at 86:5-7 (emphasis added).

²⁹⁵ *Id.* at 48:30-47.

²⁹⁶ *Id.* at 46:51-55.

conditions is greater than a melting temperature.”²⁹⁷ The ’035 Patent also teaches other, different methods of improving the quality of its amplification results, for example, by avoiding allelic bias.²⁹⁸ “A number of methods are described herein that may be used to preferentially enrich a sample of DNA at a plurality of loci in a way that minimizes allelic bias.”²⁹⁹ Dr. Van Ness also testified that it would be feasible to avoid allelic bias.

Q. What if you know that there are certain target loci that lead to allelic bias and you design your PCR multiplex assay to avoid those loci? Would that be one way to avoid allelic bias?

A. Yeah, that is sort of a – I mean, hypothetically, sure, if you wanted to eliminate certain targeted sequences ***because you couldn’t accurately develop the allelic representation, you could just avoid them just by not including primers to amplify those particular sequences.*** Sure, hypothetically.³⁰⁰

254. The Natera Patents therefore contemplate that primer dimers can be present at some level. This is also reflected in the following disclosures.

In various embodiments, less than 60, 50, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.05% of the amplified products are primer dimers.³⁰¹

In some embodiments, (i) less than 60% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (ii) less than 40% of the amplified products are primer dimers and at least 60% of the

²⁹⁷ *Id.* at 4:30-31.

²⁹⁸ *Id.* at 86:2-10.

²⁹⁹ *Id.* at 197:52-54.

³⁰⁰ *See* Van Ness Tr. at 276:3-15 (objection omitted, emphasis added).

³⁰¹ *See* ’454 Patent at 98:18-21.

amplified products are target amplicons, (iii) less than 20% of the amplified products are primer dimers and at least 80% of the amplified products are target amplicons, (iv) less than 10% of the amplified products are primer dimers and at least 90% of the amplified products are target amplicons, or (v) less than 5% of the amplified products are primer dimers and at least 95% of the amplified products are target amplicons.³⁰²

255. Moreover, any avoidance of primer dimers during the 2011-2015 time period was not novel or inventive. By 2011, one of ordinary of skill in the art would have been familiar with multiplex PCR amplification. Even in well-functioning assays at that time, primer dimers would have been likely to occur. Although it would not have been necessary to design primers to avoid primer dimers, there were known methods to do so.

(ii) *The Natera Patents Contain Examples of the Claimed Methods*

256. I am informed by counsel that the adequacy of written description does not require any particular form of disclosure, and that, for example, examples are not required. The Natera Patents adequately describe the claimed methods, including with the use of Examples. For example, the Asserted Claims of the '454 Patent recite the following.

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

³⁰² See '035 Patent at 22:3-14.

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

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

Claim 11: The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.

257. Such limitations are described throughout the '454 Patent, including the legend of Fig. 51B described *supra*. Exemplary disclosures from the specification are reproduced *infra*, including from the '454 Patent's description of "Exemplary Sample Preparation Methods."

Exemplary Sample Preparation Methods

In some embodiments, the method includes isolating or purifying the DNA and/or RNA.³⁰³

In some embodiments, whole genome application (WGA) is used to amplify a nucleic acid sample. There are a number of methods available for WGA: ligation-mediated PCR (LM-PCR), degenerate oligonucleotide primer PCR (DOP-PCR), and multiple displacement amplification (MDA). In LM-PCR, short DNA sequences called adapters are ligated to blunt ends of DNA.³⁰⁴

In some embodiments, PCR (referred to as mini-PCR) is used to generate very short amplicons (U.S. application Ser. No. 13/683,604, filed Nov. 21, 2012, U.S. Publication No.

³⁰³ See '454 Patent at 95:41-43.

³⁰⁴ *Id.* at 96:30-36.

2013/0123120, U.S. application Ser. No. 13/300,235, filed Nov. 18, 2011, U.S. Publication No 2012/0270212, filed Nov. 18, 2011, and U.S. Ser. No. 61/994,791, filed May 16, 2014, which are each hereby incorporated by reference in its entirety).³⁰⁵

In some embodiments, multiplex PCR is used. In some embodiments, the method of amplifying target loci in a nucleic acid sample involves (i) contacting the nucleic acid sample with a library of primers that simultaneously hybridize to least 100; 200; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 20,000; 25,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture; and (ii) subjecting the reaction mixture to primer extension reaction conditions (such as PCR conditions) to produce amplified products that include target amplicons. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the targeted loci are amplified. In various embodiments, less than 60, 50, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.05% of the amplified products are primer dimers.³⁰⁶

The selective enrichment and/or amplification may involve tagging each individual molecule with different tags, molecular barcodes, tags for amplification, and/or tags for sequencing. In some embodiments, the amplified products are analyzed by sequencing (such as by high throughput sequencing) or by hybridization to an array, such as a SNP array, the ILLUMINA INFINIUM array, or the AFFYMETRIX gene chip.³⁰⁷

258. The '454 Patent also teaches that “[i]n certain embodiments, a depth of read of greater than ... 50,000, or 100,000 on the low end of the range and ... 50,000, 100,000, 250,000 or 500,000 reads on the high end, is attained in the sequencing run for each single

³⁰⁵ *Id.* at 97:13-20.

³⁰⁶ *Id.* at 98:7-21.

³⁰⁷ *Id.* at 98:35-42.

nucleotide variant position in the set of single nucleotide variant positions.”³⁰⁸ In other examples of amplifying SNV loci, for example, the inventors “observed improved DOR efficiency with increasing cycles,” and “[t]he limit of detection decreased (i.e. SNV sensitivity increased) with increasing depth of read.”³⁰⁹ Moreover, the ’454 Patent describes “exemplary methods for calculating the limit of detection for any of the methods of the invention,” including methods “used to calculate the limit of detection for single nucleotide variants (SNVs) in a tumor biopsy.”³¹⁰

259. The ’035 Patent claims are also adequately described. The Asserted Claims of the ’035 Patent recite the following.

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.

³⁰⁸ *Id.* at 68:33-39.

³⁰⁹ *Id.* at 169:57-62, citing to Figs. 55-61.

³¹⁰ *Id.* at 157:51-55, citing to Example 6.

Claim 12: 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.

Claim 13: 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.

260. Such limitations are adequately described at, *e.g.*, the following disclosures in the '035 Patent:

Disclosed herein are methods that permit the targeted amplification of over a hundred to tens of thousands of target sequences (e.g., SNP loci) from a nucleic acid sample such as genomic DNA obtained from plasma.³¹¹

The workflow may entail (1) extracting DNA such as plasma DNA, (2) preparing fragment library with universal adaptors on both ends of fragments, (3) amplifying the library using universal primers specific to the adaptors, (4) dividing the amplified sample “library” into multiple aliquots, (5) performing multiplex (e.g. about 100-plex, 1,000, or 10,000-plex with one target specific primer per target and a tag-specific primer) amplifications on aliquots, (6) pooling aliquots of one sample, (7) barcoding the sample, (8) mixing the samples and adjusting the concentration, (9) sequencing the sample. The workflow may comprise multiple sub-steps that contain one of the listed steps (e.g. step (2) of preparing the library step could entail three enzymatic steps (blunt ending, dA tailing and adaptor ligation) and three purification steps). Steps of the workflow may be combined, divided up or performed in different order (e.g. bar coding and pooling of samples).³¹²

³¹¹ See '035 Patent at 86:2-5.

³¹² *Id.* at 86:61-87:10.

261. The '035 Patent further describes that its methods “are especially useful when the sample of DNA is particularly small, or when it is a sample of DNA where the DNA originates from more than one individual.”³¹³ The '035 Patent further describes that its methods may be “used in the case where cells of different genetic constitution may be present in a single individual, such as with cancer.”³¹⁴

(iii) The '035 Patent Adequately Describes Use of Universal Tail Adaptors

262. Relatedly, Dr. Van Ness opines in conclusory fashion that the '035 Patent does not adequately describe the use of universal tail adaptors.³¹⁵ I disagree as there is adequate written description of the use of such adaptors in the '035 Patent specification. In addition to the disclosure *supra*, the '035 Patent describes these adaptors as described *infra*.

Universal Adapters, or ‘ligation adaptors’ or ‘library tags’ are DNA molecules containing a universal priming sequence that can be covalently linked to the 5-prime and 3-prime end of a population of target double stranded DNA molecules. The addition of the adapters provides universal priming sequences to the 5-prime and 3-prime end of the target population from which PCR amplification can take place, amplifying all molecules from the target population, using a single pair of amplification primers.³¹⁶

³¹³ *Id.* at 95:25-27.

³¹⁴ *Id.* at 95:32-34.

³¹⁵ *See* Van Ness Decl. at ¶ 425.

³¹⁶ *See* '035 Patent at 42:23-30.

263. In terms of using the adaptors, the '035 Patent specification teaches that “preparing the sample (e.g., the first sample) further includes appending **universal adapters** to the DNA in the sample (e.g., the first sample) and amplifying the DNA in the sample (e.g., the first sample) using the polymerase chain reaction.”³¹⁷ The universal adapters, as described, are covalently linked to the ends of the DNA strands (through known means), and as such are considered “tail” adaptors.

264. For all of these reasons, it is my opinion that one of ordinary skill in the art would have recognized that the Natera Patent inventors possessed the claimed methods, including all of the recited limitations, as of the Natera Patents’ respective priority dates. Consequently, the Asserted Claims of the Natera Patents are not invalid under Section 112 for lack of written description.

IX. INFRINGEMENT OF THE NATERA PATENTS

A. '454 Patent

265. As explained *infra*, I disagree with Dr. Van Ness’s contention that the accused RaDaR assay does not infringe the Asserted Claims of the '454 Patent.

266. As an initial matter, Dr. Van Ness’s noninfringement theory is predicated on a claim construction that deviates from the plain and ordinary meaning of the claim language. With one exception (“amplifying,” discussed *infra*)³¹⁸, Dr. Van Ness has not offered opinions on the construction of any claim terms that underlie NeoGenomics’

³¹⁷ *Id.* at 32:19-23 (emphasis added).

³¹⁸ *See* Van Ness Decl. at § VII; *see also id.* at § III.A *supra*.

noninfringement position. I have been informed and understand from counsel that Dr. Van Ness has thus waived an interpretation on these terms that differs from their plain and ordinary meaning. If the Court were nevertheless to permit Dr. Van Ness to offer additional opinions on claim construction for those terms Dr. Van Ness has not purported to construe, I reserve the right to respond and amend or supplement my opinions.

267. With regard to Dr. Van Ness's construction of "amplifying," I disagree with Dr. Van Ness's construction because it contradicts the plain language of the claim as explained *supra*.³¹⁹ Moreover, it is premised on a re-writing of the claim to inject the term "directly" where it does not exist. Claim 1 of the '454 Patent recites "comprising," which has an open-ended plain meaning and allows for other unrecited steps or elements to be present during the infringing method. Because Dr. Van Ness's interpretation of these terms is incorrect, his opinions on noninfringement are likewise incorrect.

268. As I explained in my Opening Declaration, the Asserted Claims of the Natera Patents are composed of terms the plain and ordinary meaning of which would have been known to one of ordinary skill in the art.³²⁰ I therefore used the plain meaning of the terms for my infringement analysis.³²¹ It is my opinion that the accused RaDaR assay infringes the Asserted Claims of the Natera Patents and the presence of a universal PCR reaction after the targeted multiplex reaction does not alter my conclusion.

³¹⁹ See Section **Error! Reference source not found.**

³²⁰ See Metzker Op. Decl. ¶¶24, 54.

³²¹ *Id.* § VIII

(ii) Dr. Van Ness's Reliance on "Amplifying"

269. Dr. Van Ness opines that the accused RaDaR assay does not infringe because, in his view, it does not "directly" sequence the amplicons from the targeted multiplex reaction.³²² I disagree.

270. First, the claim language itself does not require "direct" sequencing of anything, including any amplified DNA product. That term does not appear anywhere in claim 1. The claim states "sequencing the amplicons to obtain sequence reads," without any reference to that sequencing being "direct." In my opinion, the limitation "sequencing the amplicons to obtain sequence reads" of claim 1 is met by the accused RaDaR assay by its sequencing step. While the amplified products of the targeted PCR reaction are further amplified to add sequencing-related sequences (adaptors and barcodes), the same sequence generated by the targeted multiplex reaction (50-150 bp comprising the target loci) is subjected to sequencing, and sequence reads for that sequence are obtained.

271. Second, as mentioned *supra*, this noninfringement opinion relies on a flawed interpretation of the claims, including the term "the amplicon." Under a plain and ordinary understanding, Claim 1 is an open-ended claim by virtue of its use of the term "comprising" in the preamble. I understand from counsel that "comprising" claims can be infringed even in the presence of unrecited steps or elements, so long as the other elements of the claims are met. Claim 1 recites "performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free

³²² See Van Ness Decl. at ¶¶79-80, 87; Van Ness Tr. at 235:5-236:25.

DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases” and is open-ended by the use of “comprising” recited earlier in Claim 1 of the ’454 Patent. One of ordinary skill in the art would have understood from the claim language itself that the sole restriction on “amplicons” is based on a length of 50-150 base pairs.

272. One of ordinary skill in the art would have readily understood that the “comprising” language allows for subsequent amplification(s) following targeted multiplex PCR and prior to sequencing. A subsequent amplification may be, for example, a universal PCR reaction to amplify those tags added to the ends of DNA and/or to attach additional sequencing adaptors for downstream sequencing. Subsequent amplification may also be a universal PCR to attach sequencing adaptors together with sample barcodes.

273. Third, dependent claim 11 of the ’454 Patent confirms that subsequent amplification(s) following targeted multiplex PCR and prior to sequencing are not excluded from Claim 1 of the ’454 Patent, as Dr. Van Ness argues. Claim 11 of the ’454 Patent recites “performing barcoding PCR prior to sequencing.”³²³ This barcoding PCR is a separate, intermediate PCR reaction from the targeted multiplex PCR and serves to add so-called “barcode” sequences so that different populations of DNA can be identified after sequencing. I am informed by counsel that Claim 1 of the ’454 Patent, as the independent claim from which Claim 11 depends, is presumed to be broader and to encompass the scope

³²³ See ’454 Patent at 172:1-3.

of Claim 11. Accordingly, Claim 1 of the '454 Patent permits, but does not require, additional PCR reactions for sequencing.

274. It is my opinion that Claim 1 of the '454 Patent encompasses a possible universal PCR step to add sequencing adaptors (or other sequencing-related sequences like barcodes) following targeted multiplex PCR in a single reaction volume, which one of ordinary skill in the art would have understood to be necessary for sequencing on certain instruments, including routinely and commonly-used Illumina sequencers which are mentioned repeatedly in the '454 Patent specification.³²⁴

275. For all these reasons, Dr. Van Ness's opinion is incorrect to the extent it excludes a step to add sequencing adaptors for sequencing (e.g., by universal PCR), conflicting with the plain and ordinary meaning of the claim language. This is made clear by his reading of the claim language that appears to inject the term "directly" where it does not exist.

(iii) Dr. Van Ness's Opinions on Natera's '008 Patent

276. In support of his non-infringement opinion, Dr. Van Ness also purports to rely on a separate, unasserted Natera patent—namely, the '008 Patent.³²⁵ I disagree that Natera's statements regarding the '008 Patent, which is not at issue in this case and claims different inventions, have any bearing on how the '454 Patent claims should be interpreted.

³²⁴ See, e.g., Meyer (2010).

³²⁵ See Van Ness Decl. at ¶¶79-80, 87.

277. First, the Van Ness Declaration refers to the '008 Patent as being “directly related to the '454 Patent” but fails to acknowledge that the two patents are part of separate lineages descending from the '703 Patent Appl.³²⁶ The '454 Patent claims priority to and is a great-granddaughter of the '703 Appl.³²⁷ The '703 Appl. also has **a separate lineage** of descendants, including a daughter '008 Patent.³²⁸

278. Second, while the '454 and '008 Patents share the same specification, their claims differ in several material ways, as shown *infra*:

US 11,530,454	US 11,486,008
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	A method for detecting one or more mutations or genetic variations in a biological sample of a subject having cancer or suspected of having cancer, the method comprising: identifying a plurality of tumor-specific mutations or genetic variations in a cancer sample of the subject by sequencing, wherein the mutations or genetic variations comprise one or more single nucleotide variant (SNV) mutations; collecting a biological sample from the subject, and isolating cell-free DNA from the biological sample; performing multiplex targeted amplification to amplify at least 100 target loci corresponding to the tumor-specific mutations or genetic variations from the isolated cell-free DNA to obtain amplicons, wherein the

³²⁶ See Babiarz *et al.*, *Detecting mutations and ploidy in chromosomal segments*, U.S. Patent Application 14/692,703 (2015) (the “'703 Patent Appl.”).

³²⁷ See '454 Patent at cover page.

³²⁸ See Dkt. 108-1 ('008 Patent) at cover page.

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.	target loci are amplified together in the same reaction volume and not in separate reaction volumes; performing high-throughput sequencing to sequence the amplicons obtained in the multiplex targeted amplification reaction to obtain sequence reads; and detecting one or more of the mutations or genetic variations present in the cell-free DNA from the sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of cell-free DNA molecules having the SNV locus in the biological sample is detected.
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279. Notably, Claim 1 of the '454 Patent recites “**sequencing** the amplicons to obtain sequence reads” whereas Claim 1 of the '008 Patent refers to “**performing high-throughput sequencing** to sequence the amplicons obtained in the multiplex targeted amplification reaction to obtain sequence reads.” The underlined language is not present in any claim of the '454 Patent, evincing a difference in scope. The scope of Claim 1 of the '008 Patent is narrower than Claim 1 of the '454 Patent since Claim 1 of the '008 Patent requires that sequencing is performed on the amplicons directly resulting from multiplex targeted amplification reaction. Moreover, to the extent that Dr. Van Ness contends the dependent claims of the '008 Patent essentially cover the same claim scope as that of Claim 1 of the '454 Patent,³²⁹ I disagree. Because independent Claim 1 of the '008 Patent has a scope that is narrower compared to Claim 1 of the '454 Patent, the addition of any

³²⁹ See Van Ness Tr. at 212:24-215:4.

dependent claims will only further narrow the scope of claims in the '008 Patent, not broaden their scope to create overlap with that of the '454 Patent claims.

280. Third, Dr. Van Ness does not accurately describe Natera's back and forth with the patent examiner, which was based on a combination of prior art (Forsheaw (2012) and Takano (2005)) that was not raised against the '454 Patent (Forsheaw (2012) and Benesova (2013)).³³⁰ Dr. Van Ness asserts that Natera's assertions "require that any amplicons generated by performing a targeted multiplex amplification must be **directly** sequenced without any intervening step."³³¹ But this is not what Natera told the Examiner. Natera never referred to "direct" sequencing as Dr. Van Ness suggests. In distinguishing Forsheaw (2012), Natera referred specifically to the presence of "a subsequent and separate **targeted** amplification"³³²—not a **untargeted**, universal amplification to, for example, add sequencing adaptors and/or barcodes.

281. Fourth, Dr. Van Ness points to statements by Natera employee Dr. Bernard Zimmermann, an inventor on both the '454 and '008 Patents³³³, but again fails to note that these are statements about the '**008 Patent** claimed inventions, not the '454 Patent. The

³³⁰ Compare Dkt. 118-12 with Dkt. 116-5; compare Dkt. 118-10 with 116-9; see also Takano *et al.*, *Epidermal growth factor receptor gene mutations and increase copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer*, JOURNAL OF CLINICAL ONCOLOGY 28:6829-6837 (2005) ("Takano (2005)"); Benesova *et al.*, *Mutation-based detection and monitoring of cell-free tumor DNA in peripheral blood of cancer patients*, Analytical Biochemistry 433:227-234 (2013) ("Benesova (2013)").

³³¹ See Van Ness Decl. at ¶ 80 (emphasis in original).

³³² See Dkt. 116-9 at 4 (emphasis added).

³³³ See Van Ness Decl. at ¶ 80.

'454 and '008 Patent inventions are separate and distinct, and the '008 Patent claims are in response to rejections not made against the '454 Patent claims. Dr. Zimmermann also did not say anything about universal PCR to add sequencing adaptors. In my opinion, Dr. Zimmermann's statements do not support Dr. Van Ness's opinions about the '454 Patent claims.

(iv) [REDACTED]
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282. [REDACTED]

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[REDACTED] [REDACTED]
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³³⁴ See '454 Patent at 171:40-41.

³³⁵ See Dkt. 107 at 6.

³³⁶ See Van Ness Tr. at 10:17-24.

283. [REDACTED]

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³³⁷ See Dkt. No. 112, *Declaration of Vishal Sikri in Support of Neogenomics Laboratories, Inc.’s Opposition to Natera, Inc.’s Motion for Preliminary Injunction*, dated October 18, 2023 (“Sikri Decl.”).

³³⁸ *Id.* at ¶ 49.

³³⁹ *Id.*

³⁴⁰ *Id.* at ¶¶ 50-51.

³⁴¹ See Dkt. 139-7 (Sikri Tr.) at 74:17-21.

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³⁴² *Id.* at 75:3-9.

[REDACTED]

(v) *RaDaR infringes under the Doctrine of Equivalents*

288. As discussed *supra*, the accused RaDaR assay literally infringes the Asserted Claims of the '454 Patent. However, even if Dr. Van Ness's interpretations of certain claim terms were adopted by the Court, it is my opinion that RaDaR would still infringe the Asserted Claims of the '454 Patent under the doctrine of equivalents.

289. I understand from counsel that one way to evaluate infringement under the Doctrine of Equivalents is to determine whether an accused method performs substantially the same function in substantially the same way to achieve substantially the same result as the claimed method. Here, the incorporation of a routine and conventional universal PCR step after targeted multiplex amplification in a single reaction volume does not alter the functionality or result of the claimed method (*i.e.*, detecting one or more of the tumor-specific SNV mutations). It is also my opinion that the incorporation of a universal PCR

³⁴³ See Ex. 12 (NeoGenomics' Medicare Announcement dated July 27, 2023).

step after targeted multiplex amplification in a single reaction volume does not substantially alter the way the claimed method is performed.

290. For example, one of ordinary skill in the art would have understood that sequencing amplicons could require universal amplification to add sequencing adaptors (*e.g.*, sequencing adaptors are required for sequencing on an Illumina sequencer, which the '454 Patent teaches can be used for the sequencing step). I disagree with Dr. Van Ness's contention that this universal PCR step will be so indiscriminate such that "the relative amounts of mutations present in the original sample will be lost."³⁴⁴ Universal PCR generally uses common primer sequences, but will preserve the relative quantities of mutations present. One of ordinary skill in the art would have recognized that the relative quantities of mutations present can be preserved in a subsequent PCR by controlling the number of cycles performed. If the number of cycles remains within the exponential amplification phase, the relative abundance of mutations will not be diluted. Additionally, Dr. Van Ness fails to describe that in RaDaR, only the amplicons having the CS1/CS2 common tail adaptor sequences (first introduced through tagging) will be amplified in a universal PCR reaction since the universal PCR primers will hybridize to the common tail sequences. Therefore, universal PCR will amplify the final products from the targeted multiplex PCR step.

³⁴⁴ See Van Ness Decl. at ¶ 87.

291. I understand that Dr. Van Ness further contends that an additional PCR would necessarily introduce further polymerase error that would have materially impacted the results.³⁴⁵ I disagree. One of ordinary skill in the art would have readily recognized that an additional PCR could be performed using a proofreading polymerase (as opposed to *Taq* polymerase), such that there is no substantial increase in error.

292. I further understand that Dr. Van Ness contends that Natera may not assert a Doctrine of Equivalents theory for “sequencing the amplicons” based on an alleged disclaimer during prosecution of the ’008 Patent. I disagree that there has been any statement in the ’454 Patent prosecution that could be a disclaimer. Dr. Van Ness does not identify one.

293. To the extent that the Court permits Dr. Van Ness to supplement his noninfringement position regarding the ’454 Patent, I reserve the right to respond and supplement or amend my opinions.

B. ’035 Patent

294. As explained *infra*, I disagree with Dr. Van Ness’s contention that the accused RaDaR assay does not infringe the Asserted Claims of the ’035 patent.

295. Contrary to Dr. Van Ness’s assertions,³⁴⁶ RaDaR performs targeted amplification of ***already*** tagged DNA as part of its 15-cycle PCR. As I explained during

³⁴⁵ *Id.*

³⁴⁶ *Id.* at ¶¶ 89-104.

my deposition, the first cycle of the pre-amplification step disclosed by Forshew (2012) tags cell-free DNA with universal tail adaptors CS1 and CS2.

296. Dr. Van Ness contends that tagging “cannot be satisfied by the first step of PCR because tagged products with both first and second universal tail adaptors are only generated in cycles after a first PCR cycle. During the first cycle a first tail adaptor is introduced. It is only after the first PCR cycle, at which point a second primer with a second tail adapter hybridizes to a copy of the original molecule, that products with the second tail adaptor are generated.”³⁴⁷ I disagree with Dr. Van Ness’s opinion and understanding of the foundations of the principles of PCR.

297. As explained *supra*,³⁴⁸ a single cycle of PCR can introduce **two tags** (one on each strand of the amplified DNA product). The subsequent cycles of PCR amplify the target region of interest, thereby performing targeted amplification of **already tagged** DNA. In mapping this scientific theory to the accused RaDaR assay, the first cycle of RaDaR’s preamplification PCR step introduces **two tags** (one on each strand of the amplified DNA product), which I have explained in my Opening Declaration.³⁴⁹ The subsequent cycles of PCR amplify the target region of interest, thereby performing targeted amplification of already tagged DNA.³⁵⁰ In my opinion, Dr. Van Ness’s theory that tagging requires two

³⁴⁷ *Id.* at ¶101.

³⁴⁸ See Section V.B(ii).

³⁴⁹ See Metzker Op. Decl. at ¶¶ 87-101.

³⁵⁰ See *id.*

cycles of PCR is inconsistent with the fundamental principles of PCR. Regardless, Dr. Van Ness's assessment does not change my opinion because even if Dr. Van Ness were correct about the two tags being added over two PCR cycles (not one), tagged products are still being subsequently amplified afterwards in every remaining PCR cycle.

298. Support for my opinion further derives from Claim 12, which recites: "the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor." Therefore, even under the Van Ness Declaration's view of the second tag being added to an amplicon and not cell-free DNA, Dr. Van Ness does not deny that the first tagging step adds "one or more adaptors."³⁵¹

299. I also disagree with Dr. Van Ness's reliance on a dependent claim (Claim 13)³⁵² to attempt to narrow the scope of independent Claim 1 of the '035 Patent, which contradicts the plain meaning of Claim 1 as described *supra*.³⁵³

300. Therefore, it is my opinion that the first cycle of RaDaR's preamplification step satisfies the "tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products" limitation of Claim 1 of the '035 Patent. Additionally, the subsequent cycles of RaDaR's preamplification step satisfy the "amplifying the tagged products one or more times." For this reason, it is my opinion that the accused RaDaR assays infringes the Asserted Claims of the '035 Patent.

³⁵¹ See Van Ness Tr. at 228:17-232:25.

³⁵² See Van Ness Decl. at ¶¶ 100-101.

³⁵³ See Section **Error! Reference source not found.**

(ii) *RaDaR infringes under the Doctrine of Equivalents*

301. As discussed *supra*, the accused RaDaR assay literally infringes the Asserted Claims of the '035 Patent. However, even if Dr. Van Ness's interpretations of certain claim terms were adopted by the Court, it is my opinion that RaDaR would still infringe under the Doctrine of Equivalents.

302. I understand that one way to evaluate infringement under the Doctrine of Equivalents is to determine whether an accused method performs substantially the same function in substantially the same way to achieve substantially the same result as the claimed method. Here, the incorporation of a universal PCR step in the accused RaDaR assay after targeted multiplex amplification in a single reaction volume does not substantially alter the functionality, the way, or result of the claimed method because the claimed method already includes "wherein one of the amplifying steps introduces a barcode and one or more sequencing tags," which one of ordinary skill in the art would have understood may be done through universal PCR.

303. For example, universal PCR generally uses common primer sequences, but will preserve the relative quantities of mutations present. One of ordinary skill in the art would have recognized that the relative quantities of mutations present can be preserved in a subsequent PCR by controlling the number of cycles performed. If the number of cycles remains within the exponential amplification phase, the relative abundance of mutations will not be diluted. Additionally, Dr. Van Ness fails to describe that in RaDaR, only the amplicons having the CS1/CS2 common tail adaptor sequences (first introduced through

tagging) will be amplified in a universal PCR reaction since the universal PCR primers will hybridize to the common tail sequences. Therefore, universal PCR will amplify the final products from the targeted multiplex PCR step.

304. I understand that Dr. Van Ness further would necessarily introduce further polymerase error that would have materially impacted the results. I disagree. One of ordinary skill in the art would have readily recognized that an additional PCR could be performed using a proofreading polymerase (as opposed to *Taq* polymerase), such that there is no substantial increase in error.

305. To the extent that the Court permits Dr. Van Ness to supplement his noninfringement position regarding the '035 Patent, I reserve the right to respond and supplement or amend my opinions.

X. SIGNATERA PRACTICES THE NATERA PATENT CLAIMS

306. I previously described how Signatera practices Claim 1 of each of the Natera Patents in my Opening Declaration.³⁵⁴ I understand that Dr. Van Ness opines that Signatera does not practice Claim 1 of the '454 Patent nor Claim 1 of the '035 Patent. Dr. Van Ness testified during his deposition, however, that he did not review any of Natera's technical documents that have been produced to date in reaching this alleged conclusion.³⁵⁵ I disagree with Dr. Van Ness's opinions as described *infra*.

³⁵⁴ See Metzker Op. Decl. at § IX.

³⁵⁵ See Van Ness Tr. at 246:4-20.

A. '454 Patent

307. With respect to the '454 Patent, Dr. Van Ness opines that Signatera does not practice the limitation of “amplicons having a length of 50-150 bases.”³⁵⁶ I disagree with Dr. Van Ness. One of Natera’s technical documents states the following:

The prioritized list of variants is used to design PCR amplicons based on optimized design parameters, ensuring uniqueness of the amplicon sequences in the human genome and efficiency of the amplicons. ***Each amplicon can be designed to amplify sequences of up to 70 base pair length*** and is designed to contain one base pair that differs between the tumor and somatic genotypes.³⁵⁷

308. Therefore, it is my opinion that Signatera does practice the aforementioned limitation of Claim 1 of the '454 Patent.

B. '035 Patent

309. With respect to the '035 Patent, the Declaration of Dr. Van Ness opines that Signatera does not practice the limitation of “amplifying the tagged products one or more times to generate final amplification products . . . wherein one of the amplifying steps introduces a barcode and one or more sequencing tags.”³⁵⁸ I disagree with Dr. Van Ness’s opinions.

310. In my Opening Declaration, I describe how Signatera practices “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged

³⁵⁶ See Van Ness Decl. at ¶¶ 428-438.

³⁵⁷ See Van Ness Tr. at Ex. 30, NAT-NEO-00625926-00625931 (“Device description for Signatera”) at NAT-NEO-00625928 (emphasis added).

³⁵⁸ See Van Ness Decl. at ¶¶ 439-442.

products.”³⁵⁹ I relied upon Coombes (2019),³⁶⁰ Christensen (2019),³⁶¹ and Kotani (2023)³⁶² as support.³⁶³ Dr. Van Ness does not refute my opinion that Signatera practices this step. My opinion is further supported by Natera’s internal technical documentation, which states, “[t]he cfDNA is end-repaired, A-tailed, and ligated with custom adapters, as previously described in Abbosh *et al.* 2017. The purified ligation product is amplified.”³⁶⁴

311. Dr. Van Ness argues that Signatera does not practice the limitation of “amplifying the tagged products one or more times to generate final amplification products. . . wherein one of the amplifying steps introduces a barcode and one or more sequencing tags”³⁶⁵ even though the references on which I rely (*e.g.*, Coombes (2019) and Christensen (2019)) show how Signatera practices this limitation, as explained in my Opening Declaration.³⁶⁶ My opinion is further supported by Natera’s internal technical

³⁵⁹ See Metzker Op. Decl. at ¶¶ 133-134.

³⁶⁰ See Dkt. 17-19, Coombes *et al.*, *Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence*, CLINICAL CANCER RESEARCH 25:4255–4263 (2019) (“Coombes (2019)”).

³⁶¹ See Dkt. 17-21, 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)”);

³⁶² See Dkt. 17-22, Kotani *et al.*, *Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer*, NATURE MEDICINE 29:127-134 (2023) (“Kotani (2023)”).

³⁶³ See Metzker Op. Decl. at ¶¶ 133-134.

³⁶⁴ See Device description for Signatera at NAT-NEO-00625929.

³⁶⁵ See Van Ness Decl. at ¶¶ 439-442.

³⁶⁶ See Metzker Op. Decl. at ¶ 135.

documentation.³⁶⁷ Dr. Van Ness does not address the disclosure of Coombes (2019) and Christensen (2019) nor any of Natera's technical documents in forming his opinions. Dr. Van Ness's opinions regarding Signatera are thus flawed.

312. Therefore, it is my opinion that Signatera does practice the aforementioned limitation of Claim 1 of the '035 Patent.

³⁶⁷ See Device description for Signatera at NAT-NEO-00625930.

I confirm that the contents of this Reply 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.

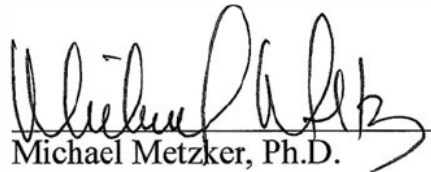
Dated: November 6, 2023

Michael Metzker, Ph.D.

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: November 6, 2023



Michael Metzker, Ph.D.