

**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-CCE-JLW

**DECLARATION OF DAVID I. SMITH, PH.D. ON CLAIM
CONSTRUCTION**

I, David I. Smith, declare as follows:

I. QUALIFICATIONS AND EXPERIENCE

1. I received a Bachelor of Science degree in Molecular Biology in 1974, and a Ph.D in Biochemistry in 1978, both from University of Wisconsin-Madison. I discovered two important restriction endonucleases during my Ph.D research, PstI and KpnI, which played important roles in the early work of genomic cloning.

2. From 1978 to 1980, I was a postdoctoral fellow at Albert Einstein College of Medicine. From 1980 to 1982, I was a Senior Research Scientist at Enzo Biochem. From 1982 to 1985, I was a postdoctoral fellow at University of California at Irvine. I started my work on the physical characterization and mapping of human chromosome 3, which was one of the foundational works leading up to the eventual mapping and sequencing of the human genome.

3. In 1985, I joined the faculty of the Department of Molecular Biology and Genetics at Wayne State University School of Medicine as an Assistant Professor. I was the Head of the Recombinant DNA Laboratory from 1986 to 1989. I was promoted to Associate Professor in 1993, and then to a full Professor in 1995.

4. In 1996, I moved to the Department of Laboratory Medicine and Pathology at Mayo Clinic as a Professor and Consultant.

5. From 1998 to 2010, I was Director of the Gene Identification Laboratory as part of the NIH-funded Comprehensive Cancer Center. The Gene Identification Laboratory at the Mayo Clinic provided expertise in DNA and molecular genetic testing

for congenital and inherited cancer syndromes. As Director, my responsibilities included providing expertise on the state-of-the-art molecular technologies to both laboratory and clinical scientists. I also provided training in my laboratory to research fellows, many of whom have since become successful independent researchers.

6. From 2009 to 2018, I was the Chairman of the Technology Assessment Group of the Center of Individualized Medicine at Mayo Clinic. As Chairman, I led the efforts to evaluate new technologies that could have an important impact on both research and translation to clinical practice.

7. From 1998 to 2008, I was also Director of the Cancer Genetics Program. My responsibilities included assisting members of the Mayo Clinic Comprehensive Cancer Center in the newest molecular technology development, as well as research that provided better understanding of inherited and sporadic cancers.

8. From 1999 to 2005, I also served as Co-Director of the Ovarian Cancer Program of the Mayo Clinic Comprehensive Cancer Center. From 2001 to 2005, I was the Principal Investigator of a Department of Defense-funded Program Project analyzing the genetics of ovarian cancer.

9. At Mayo Clinic, I helped to develop their next generation sequencing capabilities and obtained funding from the Minnesota Partnership to purchase the first two next generation sequencing machines at the Mayo Clinic.

10. As early as 2013, I worked on liquid biopsy technologies for the monitoring of patients with oropharyngeal squamous cell carcinoma (OPSCC). We performed

targeted sequencing on patient tumor biopsies to identify SNVs and optimized strategies to best detect the identified SNVs in the same patient's blood. Our goal was to develop assays with the highest sensitivity so that they could be useful for the highest proportion of patients, not just those with more advanced cancers.

11. I also worked with the group that was developing technologies that eventually became part of the testing of stool samples for the early detection of colorectal cancers. That work eventually became part of a Mayo Clinic partnership with Exact Sciences that resulted in the development of the highly successful Cologuard test for colorectal cancer screening.

12. In 2020, I became a Professor Emeritus in the Department of Laboratory Medicine and Pathology at Mayo Clinic.

13. I have almost 40 years of teaching experience as a faculty member at Wayne State University and then at Mayo Clinic. The courses I developed and taught include Genome Analysis, Cancer Genetics, Molecular Biology and Genetics, and Cancer Biology.

14. Over the course of my four-decade-long career, I have presented at numerous scientific and industry conferences both domestically and internationally. The topics of these presentations include DNA sequencing, next generation sequencing, genome mapping, and positional cloning to identify important cancer-related genes, with particular applications in cancer diagnostics. I have also served as organizer and/or chairperson for more than ten conferences.

15. I supervised about over 30 graduate students as a Professor at Wayne State University and then at Mayo Clinic. Many of my graduate students' dissertations focused on studies with DNA sequencing and next generation sequencing.

16. I am the author or co-author of more than 400 journal publications and meeting abstracts relating to DNA sequencing, cancer diagnostics and detection. I have also published about eight books and book chapters covering topics such as cloning and characterizing genes related to cancer detection. I have also served as an editorial reviewer or on the editorial board of over 30 leading journals in the field of genetics and cancer research.

17. I have been recognized for my work in the field of DNA sequencing and its use for cancer detection by my peers and received numerous accolades and awards for my research. These include the National Institutes of Health Senior Postdoctoral Fellowship and Basil O'Conner Starter Research Fellowship from the March of Dimes Birth Defects Foundation. I also received the Innovator Award from the Mayo Clinic for my work in developing next generation sequencing technologies.

18. The full details of my education, employment, and other achievements are given in my CV attached as Exhibit 1.

II. ASSIGNMENT

19. I have been asked by counsel for Defendant to opine on how a person of ordinary skill in the art would interpret the following claim terms in the context of U.S. Patent No. 11,530,454 (“the ’454 Patent”) as of its priority date¹:

- **“clonal SNV mutations”** as recited in claims 3 and 5 of the ’454 Patent;
- **“subclonal SNV mutations”** as recited in claims 4 and 5 of the ’454 Patent;
- **“wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus”** as recited in claim 14 of the ’454 Patent; and
- **“wherein the method is capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus”** as recited in claim 28 of the ’454 Patent.

III. TECHNICAL BACKGROUND

A. Single Nucleotide Variants (SNVs) and Variant Allele Frequency (VAF)

20. Cancer is associated with mutations in DNA. These mutations can affect large or small portions of DNA. Mutations that affect a single nucleotide are called single nucleotide variants (“SNVs”). Variant allele frequency (VAF) describes the fraction of sequencing reads observed for a particular variant (*e.g.*, an SNV) over the total number of reads at the same location.

¹ I understand Natera asserts the ’454 Patent is entitled to a priority date of April 21, 2015 and possibly April 21, 2014. I have not analyzed Natera’s asserted dates, but for purposes of this declaration, I have treated those dates as the applicable dates. My opinions herein apply equally to both dates.

B. Clonal and Subclonal Tumor Mutations

21. Cancer tumors are made up of populations of many cells. Tumors may be homogenous, meaning all of the cells have the same genetic make-up. While tumors may initially be relatively homogeneous, one of the key features that furthers cancer development is an increase in overall genomic instability which then further leads to additional mutations and other alterations, which further promotes growth, metastasis, and evasion from immune processes within the body. *See, e.g., Ex. 2, Roberts et al., A comparative analysis of algorithms for somatic SNV detection in cancer*, 29 Bioinformatics, Review 18, 2223-2230 (Jul. 9, 2013). Mutations that are shared by all cells in a tumor are referred to as clonal mutations.

22. By contrast, heterogeneous tumors include cells with different genetic make-ups, caused by continuing mutations as the cancer cells propagate. For instance, mutations that confer a selective advantage tend to form subpopulations with the same subclonal mutations. *Id.* Mutations that differ from the shared mutations of the clonal population are referred to as subclonal mutations. Heterogeneous tumors will then contain a mixture of cells—cells with the original clonal SNV mutations, but also distinct subclonal SNV mutation(s) that were not present in the original clonal tumor cells.

C. Tumor Biopsies and Liquid Biopsies

23. A tumor biopsy is a tissue sample taken from a tumor. Clinical tumor biopsies are comprised of a mix of cancer cells as well as other material. For instance, a biopsy sample might contain cancer cells as well as a mixture of lymph cells, stromal

cells, necrotic cells, and healthy cells, among other material. *See* Ex. 3, Liu et al., *MixHMM: Inferring Copy Number Variation and Allelic Imbalance Using SNP Arrays and Tumor Samples Mixed with Stromal Cells*, 5 PLoS One 6, 1-2 (June 2010) (“heterogeneity in clinical samples, due to stromal contamination and somatic alterations, complicates analysis and interpretation of these data”). This issue of contamination is dependent on the type of cancer and is especially prevalent in certain types, such as brain cancer and pancreatic cancer, known to be diffuse even at their initial site. The issue is even more prevalent in samples taken from a site of metastasis. There are dramatic differences in what is contained within a single biopsy sample, for example depending upon which type of cancer a particular patient has and how advanced their cancer is.

24. Liquid biopsies are utilized as a way to monitor cancer development or response to therapy without the invasive procedures required to obtain tissue biopsies. Liquid biopsies have long been known to be used for minimal residual disease (“MRD”) monitoring of cancer. MRD is the monitoring of residual cancer that remains after a patient has undergone treatment such as surgery, radiation, or chemotherapy. *See, e.g.,* Ex. 4, Chan et al., *Scanning for Cancer Genomic Changes in Plasma: Toward an Era of Personalized Blood-Based Tumor Markers*, 59 Clinical Chemistry 11, 1553 (July 2013) (“The analysis of circulating cell-free tumor DNA has remarkable potential for the detection and monitoring of cancers. Genetic alterations are present in virtually all types of cancers, allowing broad applicability of this approach regardless of the primary organ and histologic type of the cancer.”).

D. Limit of Detection

25. An important metric for assessing the quality of a laboratory test is its ability to detect low amounts of cancer. Sensitivity, Analytical Sensitivity, Functional Sensitivity, Lower Limit of Detection, Limit of Blank (LOB), Limit of Detection (LOD), and Limit of Quantitation (LOQ) are all terms used to describe the smallest unit of a measurand that can be reliably measured by an analytical method. Ex. 5, Armbruster et al., *Limit of Blank, Limit of Detection and Limit of Quantitation*, 29 Clinical Biochem Review, S49-S52 (August 2008) at S49. A measurand is a quantity or property intended to be measured. Ex. 6, Arora et al., *Tissue Engineering Measurands*, ACS Biomaterials Science & Engineering 6, 5368-5376 (2020). <https://doi.org/10.1021/acsbiomaterials.0c00475> (citing Ex. 7, International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM), 3rd Edition. Joint Committee for Guides in Metrology (JCGM) 200:2012). For instance, LOB is “the highest *apparent* analyte concentration expected to be found when replicates of a sample containing no analyte are tested.” *Id.* (emphasis in original). LOD is “the lowest analyte concentration likely to be reliably distinguished from the LOB and at which detection is feasible.” *Id.* at S50.

26. There has been a lack of agreement in the clinical laboratory field about the terminology best suited to describe the capability of a method to reliably measure a measurand (such as an SNV). *See id.* at S49. Furthermore, various methods are used for estimating detection capability parameters such as LOD. *Id.* In the context of SNV

detection in cell-free DNA, LOD is a parameter used to describe the capability of a method to detect an SNV present in a certain percentage of cell-free DNA comprising the SNV locus. In other words, LOD indicates the lowest concentration of variants that a sequencing method is capable of identifying consistently.

IV. LEGAL STANDARDS

27. While I am neither a patent lawyer nor an expert in patent law, I have been informed of the applicable legal standards to claim construction and patent invalidity (and specifically indefiniteness). I have relied upon these legal principles, as explained to me by Counsel, in forming my opinions as set forth in my declaration.

A. Person of Ordinary Skill in the Art

28. I was asked to provide testimony on how a person of ordinary skill in the art (a “skilled person”) would understand certain terms in the patents in suit. I understand that a skilled person is a hypothetical person considered to have normal skills and knowledge in the field to which the patents in suit relate at the time the invention was made.

29. I have been informed and understand that factors that may be considered in determining the level of ordinary skill include: (1) the type of problems encountered in the art; (2) prior art solutions to those problems; (3) rapidity with which innovations are made; (4) sophistication of the technology; and (5) education level of active workers in the relevant field. I have been further informed and understand that a person of ordinary skill is also a person of ordinary creativity. In view of the state of the art and the subject

matter of the '454 patent, it is my opinion that a person of ordinary skill in the field of the '454 patents would have had, by the priority date, at least (1) a Master's Degree and/or Ph.D. in biochemistry, molecular genetics, pharmacology, or an equivalent discipline with applications in genomics and (2) at least five years of academic or industrial laboratory experience. As of the April 21, 2015 or April 21, 2014 priority dates asserted by Natera, I exceed this definition of a skilled person.

30. A skilled person in the field of the '454 Patent would have had a deep understanding of genomics, including nucleic acid detection, quantification, and genotyping and would understand the underlying scientific principles to such. A skilled person also would have been apprised of then current literature on genomic assays, such as multiplex PCR, NGS techniques, and the use of cfDNA in genotyping. A skilled person would also have generally understood the processes behind the development of DNA mutation-based illnesses, such as cancers, and would have been apprised of the recent literature in those fields.

B. Claim Construction

31. I understand that claim construction is a question of law for the Court alone, and that claims are generally given their ordinary and customary meaning, which is the meaning that the term would have to a skilled person at the time of the invention in view of the entire patent. I further understand that courts interpret disputed terms in light of the claims themselves, the patent specification, and the prosecution history. I also understand that extrinsic evidence consists of all other evidence external to the patent and

prosecution history, and that it is relevant to a skilled person's understanding of the plain and ordinary meaning of a term.

C. Indefiniteness

32. I understand that the patent laws include a requirement for definiteness. I understand that patent claims must particularly point out and distinctly claim the subject matter that the applicant regards as the invention. I understand that claims of a patent are invalid for indefiniteness if the claims “when read in light of the specification delineating the patent, and the and prosecution history, fail to inform, with reasonable certainty, those skilled in the art about the scope of the invention.” *Nautilus, Inc. v. Biosig Instruments, Inc.*, 572 U.S. 898, 901 (2014). I understand that a “patentee need not define his invention with mathematical precision,” but “there must be objective boundaries.” *Niazi Licensing Corp. v. St. Jude Medical S.C., Inc.*, 30 F.4th 1339, 1347 (Fed. Cir. 2022). I understand that where a phrase is “too vague” to “give the scope of the claims meaning,” the claim is indefinite. *See Aubin Industries, Inc. v. Caster Concepts, Inc.*, No. 2:14-cv-02082, 2017 WL 4284715, at *7-*8 (E.D. Cal. Sept. 27, 2017). I also understand that claim terms may be indefinite where the distinction between them is ambiguous. *See Icon Health & Fitness, Inc. v. Polar Electro Oy*, 656 Fed. App'x 1008, 1016 (Fed. Cir. 2016) (“Polar argues that the ambiguous nature of the distinction between the two claim terms renders them incapable of construction. We agree. . . ‘In-band’ cannot provide a basis, therefore to determine which communications are ‘out-of-band’ if the terms are a moving target that may change over time.”).

33. It is also my understanding that where multiple tests for a claimed property are disclosed, but those “tests do not provide consistent results” such that a skilled person “would not know under what standard to evaluate” the claimed property, the term is indefinite. *HZNP Medicines LLC v. Actavis Lab’ys UT, Inc.*, 940 F.3d 680, 696–99 (Fed. Cir. 2019). I understand “a claim may be invalid as indefinite when (1) different known methods exist for calculating a claimed parameter, (2) nothing in the record suggests using one method in particular, and (3) application of the different methods result in materially different outcomes for the claim's scope.” *Saso Golf, Inc. v. Nike, Inc.*, 843 Fed. Appx. 291, 296 (Fed. Cir. 2021) (citation omitted). I understand that “a claim is indefinite if its language might mean several different things and no informed and confident choice is available among the contending definitions.” *Media Rights Techs., Inc. v. Capital One Fin. Corp.*, 800 F.3d 1366, 1371 (Fed. Cir. 2015) (internal quotations marks omitted).

IV. INDEFINITE TERMS IN THE ’454 PATENT

34. I have been asked by Counsel to provide my opinions about how a person of ordinary skill in the art would have understood certain terms of the ’454 Patent applying the appropriate legal standard for claim construction as explained to me. My opinions are further explained below.

A. “clonal SNV mutations” (claims 3 and 5 of the ’454 Patent) and “subclonal SNV mutations” (claims 4 and 5 of the ’454 Patent)

35. It is my opinion that the terms “clonal SNV mutations” and “subclonal SNV mutations” are indefinite because a skilled person reading the specification would not be able to ascertain the scope of these terms with reasonable certainty.

36. A skilled person reading the ’454 Patent would understand that the difference in claim scope between the terms “clonal” and “subclonal” are inherently vague when applied to real systems such as in the clinic or the laboratory. As of Natera’s claimed priority dates, a skilled person would have been familiar with the well-known concept of the “illusion of clonality,” which refers to the phenomenon where mutations that appear clonal using one frame of reference might also appear to be subclonal when using a different frame of reference. *See* Ex. 8, Gerlinger et al., *Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing*, Europe PMC Funders Group, Nature Genetics-Authors Manuscript, PMC, 1-24 (November 6, 2015) at 6 (“These findings show that the detection of a clonally dominant driver in one tumor region does not predict clonal dominance in the entire tumor.”); *see also* Ex. 9, de Bruin et al., *Spatial and temporal diversity in genomic instability processes defines liver cancer evolution*, Europe PMC Funders Group, Science-Authors Manuscript, PMC, 1-12 (November 6, 2015) (“many heterogeneous mutations gave the ‘illusion of clonality,’ as they are present in all cells from certain regions but undetectable within other regions.”). Based on the method recited at ’454 Patent at 168:20-26, for example, a mutation might be classified as “clonal” if it were present in two out of three tumor sites,

and only the two sites with the mutation were sampled. The same mutations in the same patient would be classified as “subclonal” if the same patient were examined, but a biopsy happened to include the third tumor site that lacked the mutation. Thus, the analysis of the first two tumor sites is an example of the illusion of clonality, and the “illusion” is confirmed by the third sample. One would run into the same issue if only a portion of a single site were sampled that was not representative of the entire site. Said another way, the only way for a skilled person to know if a mutation is in fact clonal is to sample and analyze the entire tumor. This is not practical, and the amount of sampling necessary to make a call as to whether a mutation is clonal is subjective to the skilled person designing the analysis or the experiment. *See, e.g., id.* (“Notably, although our multiregional sampling approach allowed us to evaluate spatial heterogeneity, only a small part of the entire tumor was sampled (on average <5%), which indicates that we might be underestimating the full extent of heterogeneity in these tumors.”).

37. The specification of the ’454 Patent does not provide any guidance as to how to choose the frame of reference to determine whether a mutation is clonal or subclonal within the meaning of the claims. It does not describe how many samples should be taken, where those samples should be taken from, or what proportion of those samples must share the mutation for it to be deemed clonal. For instance, McGranahan describes a statistical method for determining whether mutations are “clonal” or “subclonal” based on the percent of “cancer cells” that contain the mutation in a single biopsy. Ex. 10, McGranahan et al., *Clonal status of actionable driver events and the timing*

mutational processes in cancer evolution, Europe PMC Funders Group, Science Translation Medicine-Authors Manuscript, PMC, 1-22 (November 6, 2015) at 3. Specifically, McGranahan describes calling mutations clonal “if the upper band of the 95% cancer cell fraction confidence interval was ≥ 1 ,” and subclonal otherwise. *Id.* By contrast, Gerlinger “calls into question” the ability to accurately measure clonality using a “single biopsy-based” analysis. Gerlinger describes collecting tissue from “multiple tumor regions, representing the spatial extent and macroscopic heterogeneity of the primary tumors” and constructing “phylogenetic trees” to show a “branched evolutionary pattern.” Ex. 8 (Gerlinger) at 5. *See also id.* at 4 (discussing how to resolve intraregional subclones); 6 (describing criteria for excluding regions). The ’454 Patent does not instruct which method—for example, a method like Gerlinger, a method like McGranahan, or another method altogether—should be used or any of the parameters necessary to classify mutations as clonal or subclonal.

38. Importantly, the specification also does not provide any guidance as to how one should handle contamination in determining clonality. As discussed above, it is a known problem in the field that biopsy samples are inevitably comprised of a mix of cancer cells as well as other material. For instance, a biopsy sample might contain cancer cells as well as a mixture of lymph cells, necrotic cells, and healthy cells, among other material. The presence of non-cancer cells in tumor biopsies can dramatically change calls of clonality. For example, an SNV detected in only 40% of the total sample cells might be classified as subclonal when it might actually be clonal amongst the cancer cells

in that sample. This issue of contamination is especially prevalent in certain types of cancers, such as brain cancer and pancreatic cancer, which are known to be diffuse even at their initial site, meaning they tend to be spread out and mixed with other types of cells, rather than packed tightly together and confined. The '454 Patent and prosecution history do not provide any guidance regarding how a skilled person would determine whether a particular mutation is clonal or subclonal when inevitably faced with contamination.

39. The Examiner's Non-Final Rejection dated July 7, 2022 (Ex. C) stated that "the terms 'clonal SNV mutations' and 'subclonal SNV mutations' are interpreted as discussed in the specification at paragraphs 111 and 748." In my opinion, those paragraphs do not fix the issue of indefiniteness. Paragraph [0111] (now 31:17-24 of the '454 Patent) uses an altogether different meaning of the term "clonal." It does not refer to clonality in terms of whether tumor cells share genetic makeup with other tumor cells. Rather, it refers to using a "clonal sequencing method" for the production of "clones," or copies, of an original DNA molecule. '454 Patent at 31:17-24 ("Clonal sequencing may produce sequence data representing single, or clones, or clusters of one original DNA molecule."). Paragraph [0111] thus would not have provided any guidance to a skilled person as to the scope of claims 3-5.

40. Paragraph [0748] is similarly unhelpful. Paragraph [0748] (now 168:20-26 of the '454 Patent) states that "[c]lonal populations had VAF [variant allele frequency] allele calls in all biopsied samples assayed and in plasma, while subclonal populations

had VAF allele calls in at least one biopsy sample, but not all biopsy samples.” ’454 patent at 168:20-26. As discussed above, this high-level description alone suffers from the same issues discussed above. For instance, it describes determining clonality based on the biopsy samples but does not describe how many samples were taken from each tumor, where those samples were taken, what was included and/or excluded from those samples, or how issues of contamination were addressed, if at all. Paragraph [0748] does not provide sufficient guidance to a skilled person regarding how to determine whether a variant a “clonal SNV mutation” or a “subclonal SNV mutation.”

41. Because a skilled person would not be able to determine with reasonable certainty whether a mutation is “clonal” or “subclonal,” a skilled person would not understand the metes and bounds of the claimed limitations.

B. “wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus” (Claim 14 of the ’454 Patent)

42. It is my opinion that this term is indefinite because a skilled person would not be able to ascertain the scope of this term with reasonable certainty. Claim 14 recites “wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.” But claim 14 does not specify any particular method to use to determine the capability of a method to detect an SNV mutation present in a given percentage of cell-free DNA comprising the SNV locus. Nor do the specification or prosecution history provide sufficient guidance on how

to measure the claimed capability for “detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.”

43. The specification does describe measuring a “limit of detection” (LOD), which a skilled person would understand describes the lowest concentration of variants that a sequencing method is capable of identifying consistently. *See, e.g.*, ’454 Patent at 6:19–21. In my opinion, the claims are still indefinite in view of those disclosures.

44. First, the ’454 Patent describes three different methods for determining LOD, each of which gives different results. *See, e.g.*, ’454 Patent at 40:25–30, 157:51–158:36, Figs. 34–35.

45. For instance, Example 6 describes a first method for calculating the LOD of a method (denoted “LOD-mr5” in FIGS. 34 and 35) by “calculat[ing] the limit of detection based on a minimum of 5 reads being chosen as the minimum number of times a SNV is observed in the sequencing data to have sufficient confidence the SNV is actually present.” ’454 Patent at 157:57-60. In this method, the “limit of detection is based on whether the observed the depth of read (DOR) is above this minimum of 5.” *Id.* at 157:60-62.

46. As described in Example 6, the second method (denoted “LOD-zs5.0” in FIGS. 34 and 35) “calculates the limit of detection based on the z-score,” which is “the number of standard deviations an observed error percentage is away from the background mean error.” *Id.* at 158:1-5. The ’454 Patent states that for the “LOD-zs5.0” method, “[if] desired, outliers can be removed and the z-score can be recalculated and this process

can be repeated.” *Id.* at 158:5-6. In this case, “[the] final weighted mean and the standard deviation of the error rate are used to calculate the z-score.” *Id.* at 158:7-8. Because the step of discarding outliers is optional and no specific parameters are required for selecting outliers, a skilled person would not be able to determine the scope of the claimed limitation even if only the “LOD-zs5.0” method were used to calculate LOD.

47. Finally, the ’454 Patent states that the third method (denoted “LOD-zs5.0-mr5” in FIGS. 34 and 35) calculates the limit of detection based on the maximum value of the above two metrics. Although the specification states that “in some embodiments, the ‘LOD-zs5.0-mr5’ method of Example 6 is used,” ’454 Patent at 93:35-36, I understand the claims are not limited to any particular embodiment.

48. The differences between the three described methods are illustrated in FIG. 35. *Id.* at 40:28–30 (describing FIG. 35 as “a graph showing the limit of detection for single nucleotide variants in a plasma sample using three different methods described in Example 6.”). As shown in FIG. 35, the three different methods for calculating LOD give significantly different results.

49. For illustration purposes, FIG. 35 is reproduced below with added annotations. The x-axis and y-axis of the graph are not labeled in the figure. However, based on the mean and median LOD values described in Example 6, the y-axis appears to be the calculated LOD, and the x-axis appears to represent different SNVs assayed. As shown in FIG. 35, the thick solid line represents “LOD-zs5.0-mr5,” which calculates the limit of detection based on the maximum value of the other two methods. A thin dashed

line below the thicker “LOD-zs5.0-mr5” line indicates that LOD-mr5 (thin dashed line) gave a lower LOD value than LOD-zs5.0-mr5 in the experiment—at this point, LOD-zs5.0-mr5 gives the maximum value. In contrast, a thin solid line below the thicker “LOD-zs5.0-mr5” line indicates that LOD- zs5.0-mr5 (thin solid line) gave a lower LOD value than LOD-mr5 in the experiment—at this point, LOD-mr5 gives the maximum value.

50. The annotated FIG. 35 below highlights examples of data points where the different methods of measuring LOD give different results. As one example, the blue arrow points to a measurement for which the LOD-mr5 method appears to result in an LOD between 0.01% and 0.1%, while the LOD-z5.0 and LOD-zs5.0-mr5 method appear to result an LOD between 0.1% and 1%. In contrast, the pink arrow points to a measurement for which the LOD-z5.0 method appears to result in an LOD less than 0.01%, and the LOD-mr5 and LOD-zs5.0-mr5 methods appear to result in an LOD greater than 0.1%. FIG. 35 accordingly demonstrates multiple instances of a ten-fold difference between the different methods of determining the LOD.

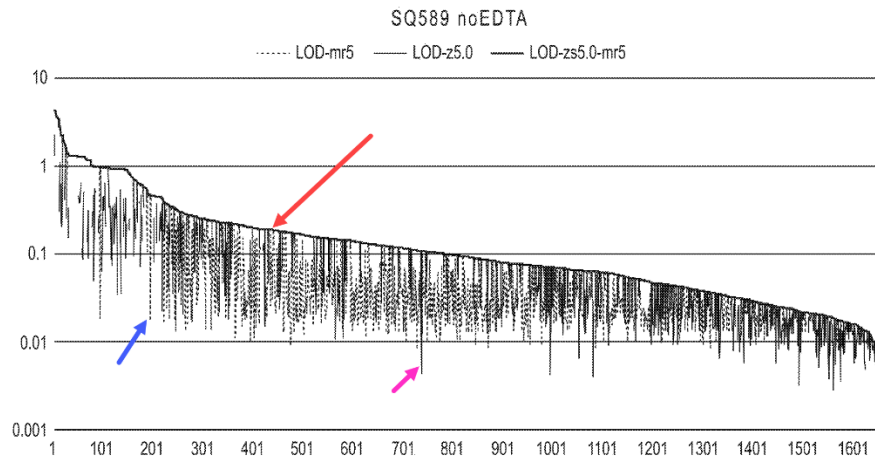


FIG. 35

51. Second, the '454 Patent and prosecution history do not provide sufficient guidance regarding whether the mean, median, or some other statistic should be used to determine whether the method is “capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.” Example 6 itself acknowledges that different statistical representations of LOD can be used, which also yield different results. For instance, Example 6 describes mean and median LODs that differ by more than a factor of 2. '454 Patent at 32-34 (“For the analysis of cDNA in a plasma sample shown in FIG. 35, the mean limit of detection was 0.24%, and the median limit of detection was 0.09%.”).

52. A skilled person would need to know at least the method for calculating LOD, the parameters used in the method for calculating LOD (e.g., whether and how to discard outliers), and what representation of LOD (e.g., mean or median) to use to determine whether a method is “capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.” For the

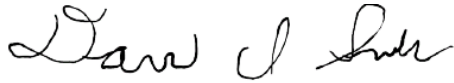
reasons discussed above, the specification and claims do not provide adequate guidance in these respects. It is my opinion that a skilled person reading the specification would not know under what standard to evaluate the claimed capability and would not be able to make an informed and confident choice as to how to determine whether a method falls within the scope of the claims.

C. “wherein the method is capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus” (claim 28 of the ’454 Patent)

53. For the same reasons discussed above regarding claim 14, this term is indefinite because a skilled person would not be able to ascertain the scope of this term with reasonable certainty. Particularly, a skilled person would not be able to determine whether a particular method satisfies this claim limitation because the claims, specification, and prosecution histories do not provide any clear guidance on how to measure the claimed capability for detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus. Claim 28, like claim 14, does not recite how to determine the capability of a method to detect an SNV mutation present in a given percentage of cell-free DNA comprising the SNV locus. Accordingly, a skilled person would not be able to determine with reasonable certainty whether the claimed limitation is met for a given method.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on 4/6/2024

A handwritten signature in cursive script, appearing to read "David I. Smith".

David I. Smith, Ph.D.