

**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 NIAL L LENNON, PH.D., ON CLAIM
CONSTRUCTION**

I, Niall Lennon, declare as follows:

I. QUALIFICATIONS AND EXPERIENCE

1. I am currently part of the Institute Leadership team at the Broad Institute of MIT and Harvard, one of the largest genome sequencing centers in the world. I am also currently the Senior Director of the Genomics Platform, an Institute Scientist, and an Associate Director of the Gerstner Center for Cancer Diagnostics. Specifically, at the Gerstner Center, I evaluate new and upcoming techniques with respect to liquid biopsies. As part of my role, I am responsible for the technology and clinical development strategy for the Genomics Platform at the Broad. I am directly responsible for the evaluation, optimization, and application of Next-Generation Sequencing (“NGS”) for clinical trials, research, and diagnostics. I also oversee custom method development for use on NGS platforms, such as Illumina’s line of sequencing instruments. I am also the founder, Chief Scientific Officer, and current Chairman of the Board of Broad Clinical Laboratories, a CLIA/CAP clinical laboratory that performs cancer and inherited disease molecular assays.

2. Prior to my current position, I was the Senior Director of Translational Genomics at the Broad. Before that I was the Director of Technology and Clinical Development at the Broad. Before that I was a Research Scientist for the Genome Sequencing Platform of the Broad. Prior to joining the Broad I was a Postdoctoral Research Fellow at Harvard Medical School. I have over 19 years of experience studying

and researching molecular biotechnology, including nucleic acid sequencing and its application to cancer, germline, and viral genomics.

3. I have authored over 80 peer reviewed articles, including articles directed to genomic technologies and the application of sequencing technologies to cancer and viral genomics.

4. I currently serve or have served on technical advisory boards for a number of NGS companies, including Genturi Inc., Swift Biosciences, and FYR Diagnostics. I have been a member of a Key Opinion Leader panel for New England Biolabs. I am an Adjunct Assistant Professor in the Department of Biomedical Engineering at Tufts University, where I teach Molecular Biotechnology to graduate students.

5. In addition to membership of advisory groups for NGS reagent companies (NEB and Swift), I have extensive experience collaborating with, evaluating, and implementing novel reagent formulations and kits for sample preparation and sequencing. I have worked closely over the years with the R&D teams of several NGS kit and instrumentation companies including Illumina, Kapa Biosystems (now part of Roche), NEB, Swift Biosciences, Watchmaker Genomics, IDT, Twist Biosciences, Ion Torrent, and others. In many cases the purpose of the collaboration is to provide feedback and work with the product development teams who are hoping to bring new methods and kits to the market to support a particular aspect of NGS sample preparation.

6. A copy of my curriculum vitae is attached as Ex. 1.

II. ASSIGNMENT

7. I understand that Natera and NeoGenomics dispute the scope of the term “wherein an SNV mutation . . . is detected from the sequence reads,” which appears in asserted claim 1 of U.S. Patent No. 11,319,596 (“the ’596 patent”). Claim 1 is shown below, with the disputed term discussed in this declaration emphasized.

A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

(b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, ***wherein an SNV mutation*** that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus ***is detected from the sequence reads.***

8. It is my understanding that the parties' proposals are:

Natera Construction	NeoGenomics Construction
Plain and ordinary meaning, which means that the terms are part of the “performing high-throughput sequencing . . . to obtain sequence reads” step and the “wherein” clause informs the mechanics of how the high-throughput sequencing is performed.	Plain and ordinary meaning, which is wherein an SNV mutation is determined to be present from the sequence reads.

9. I understand that the parties dispute whether the phrase “wherein an SNV mutation . . . is detected from the sequence reads,” requires that an SNV mutation is determined to be present from the sequence reads.

10. I have been asked by Counsel to provide my opinions about how a person of ordinary skill in the art would have understood the above claim term in the context of the '596 patent as of its priority date.¹

III. LEGAL STANDARDS

11. 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. I have relied upon these legal principles, as explained to me by Counsel, in forming my opinions as set forth in my declaration.

¹ I understand Natera asserts the '596 patent is entitled to a priority date that “is no later than” April 21, 2015. I further understand that the face of the '596 patent identifies a provisional patent application (No. 61/982,245) filed on April 21, 2014. Although I am informed that Natera has not claimed priority to this provisional application's filing date, for purposes of this declaration only, I have treated those dates as the applicable dates. My opinions herein apply equally to both dates.

A. Person of Ordinary Skill in the Art

12. 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.

13. 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 ’596 patent, it is my opinion that a person of ordinary skill in the field of the ’596 patent would have had 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.

14. In my opinion, a skilled person in the field of the ’596 patent would have a deep understanding of genomics, including nucleic acid detection, quantification, and genotyping and would understand the underlying scientific principles to such.

15. In my opinion, a skilled person would stay apprised of recent literature on genomic assays, such as multiplex PCR, next-generation nucleic acid sequencing techniques, methods for detecting SNVs, and the use of cfDNA in genotyping. A skilled person would also generally understand the processes behind the development of DNA mutation-based illnesses, such as cancers, and would stay apprised of the recent literature in those fields.

B. Claim Construction

16. 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 priority date in view of the entire patent. The entire patent includes the claim language, 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.

IV. TECHNICAL BACKGROUND

A. Single Nucleotide Variants (SNVs)

17. DNA is a double-stranded polymer comprised of nucleotides. Each nucleotide comprises a base. There are four types of bases that make up DNA: adenine ("A"), thymine ("T"), cytosine ("C"), and guanine ("G"). Each base has a complementary base—A is complementary to T, while C is complementary to G.

18. 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”). Cancer mutations can be detected in circulating tumor DNA (ctDNA) present in a patient’s blood to monitor cancer progression and to detect recurrence or metastases. Circulating tumor DNA—fragments of DNA shed from a tumor—is present at low levels compared to normal DNA in the blood. For this reason, when scientists perform sequencing to detect ctDNA, they often combine ctDNA isolated from multiple blood samples in a single tube for assaying and sequencing. This was true in 2014 and 2015, and is still true today.

B. High-Throughput Sequencing

The ’596 patent identifies high-throughput sequencers that were commercially available as of the asserted priority dates of the patent. Examples include the Illumina HiSeq and MiSeq sequencers, ’596 patent at 169:39-40, the Illumina GAIIX sequencer, *id.* at 152:63-65, and the Ion Torrent PGM sequencer, *id.* at 169:40-41. The basic operation of these high-throughput sequencers would have been well-understood by a skilled person in 2014 and 2015, as summarized below.

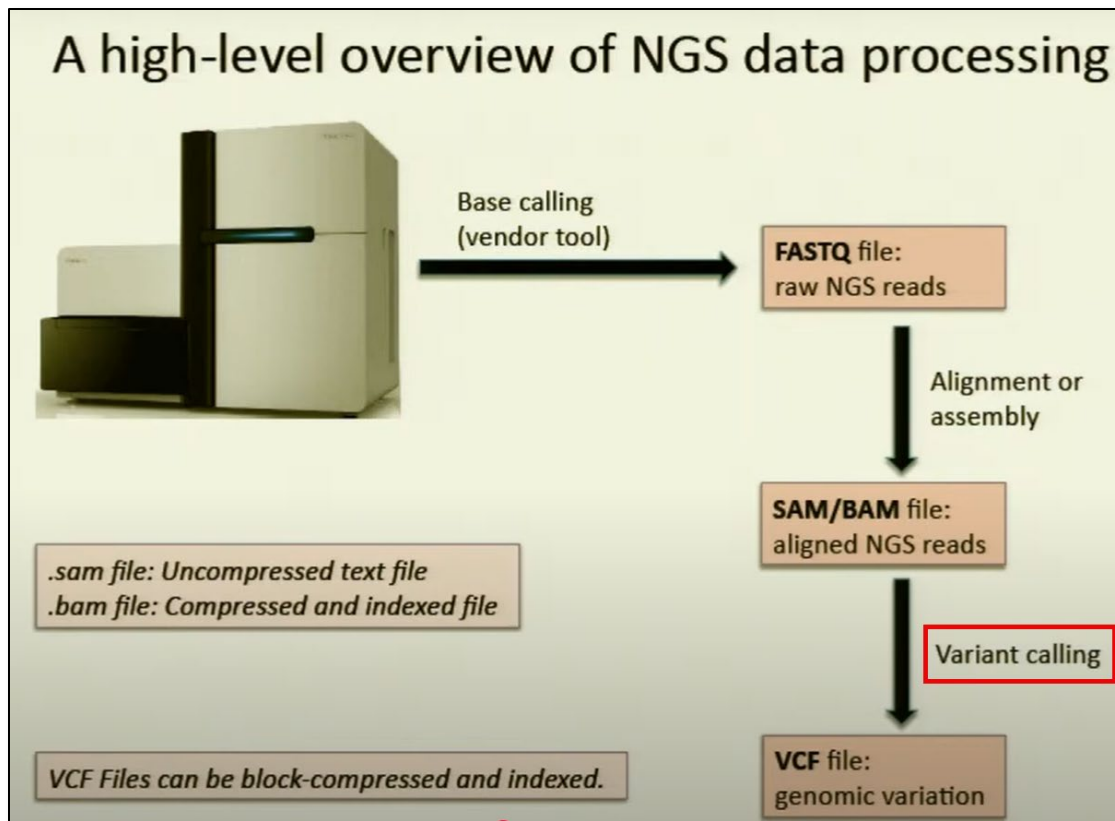
19. ***Obtaining sequence reads:*** High-throughput sequencers “read” the sequences of the DNA in the sample (e.g., A, T, G, C) by adding complementary bases (e.g., T, A, C, G) and measuring a signal for each complementary base as it is added. For Illumina sequencers such as those described in the ’596 patent, the signal is a different

fluorescent signal for each base, which appears as a color corresponding to a particular base.

20. The resulting sequence reads are short strings of the different bases called by the sequencer. These short reads correspond to places that are spread throughout the subject's genome, which is billions of bases long. The process of obtaining the sequence reads does not identify what portion of the genome is being read. Without this location information, one cannot tell from the sequence read alone whether it contains any alterations or mutations at genomic base locations of interest.

21. Because a sequencer cannot make a base call with 100% certainty, the process of obtaining sequence reads typically also involves generating a quality score that indicates the probability that a base is called incorrectly by the sequencer. Ex. 2, Illumina Technical Note: Sequencing, *Quality Scores for Next-Generation Sequencing* (2011). For example, a quality score of 30 (Q30) for a base call indicates a 0.1% chance that the base was called incorrectly. *Id.* The quality scores generated while obtaining sequence reads only indicate the quality of the sequence reads. A quality score does not identify the portion of the genome that was read or inform whether a mutation at that portion of the genome is present.

22. Accordingly, a series of steps after obtaining sequence reads are required to detect sequence variants from the sequence reads. An overview of these steps that follow obtaining sequence reads is illustrated in the below slide from a Broad Institute presentation delivered in 2012 and uploaded to YouTube in 2013:

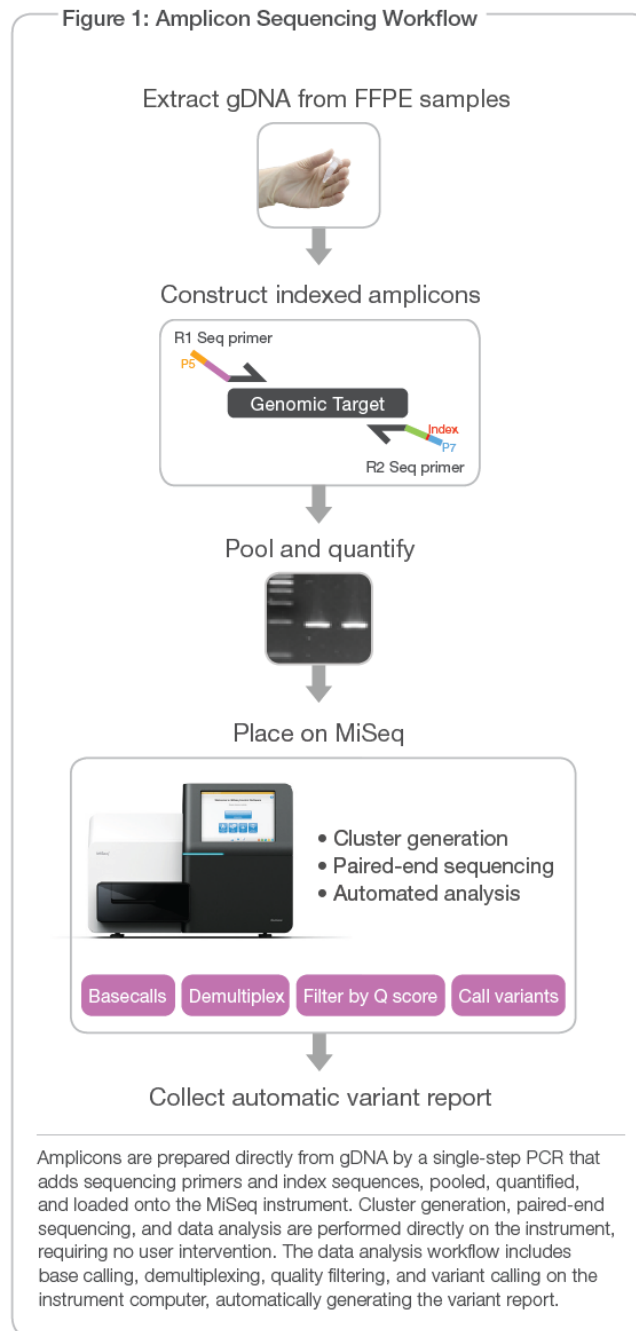


Ex. 3, Broad Institute, *BroadE: Introduction to data processing and variant detection for next-generation DNA sequencing*, YouTube (March 4, 2013),

<https://www.youtube.com/watch?v=1m0ZiEvzDKI> (“Broad Institute Presentation”), at 12:44-13:29 (cropped and annotated with a red box to highlight the variant calling step)

(explaining that variant calling is performed after read alignment to generate a file containing the variants identified). This presentation from the Broad Institute uses the term “Variant calling” to refer to the process of determining whether an SNV is present.

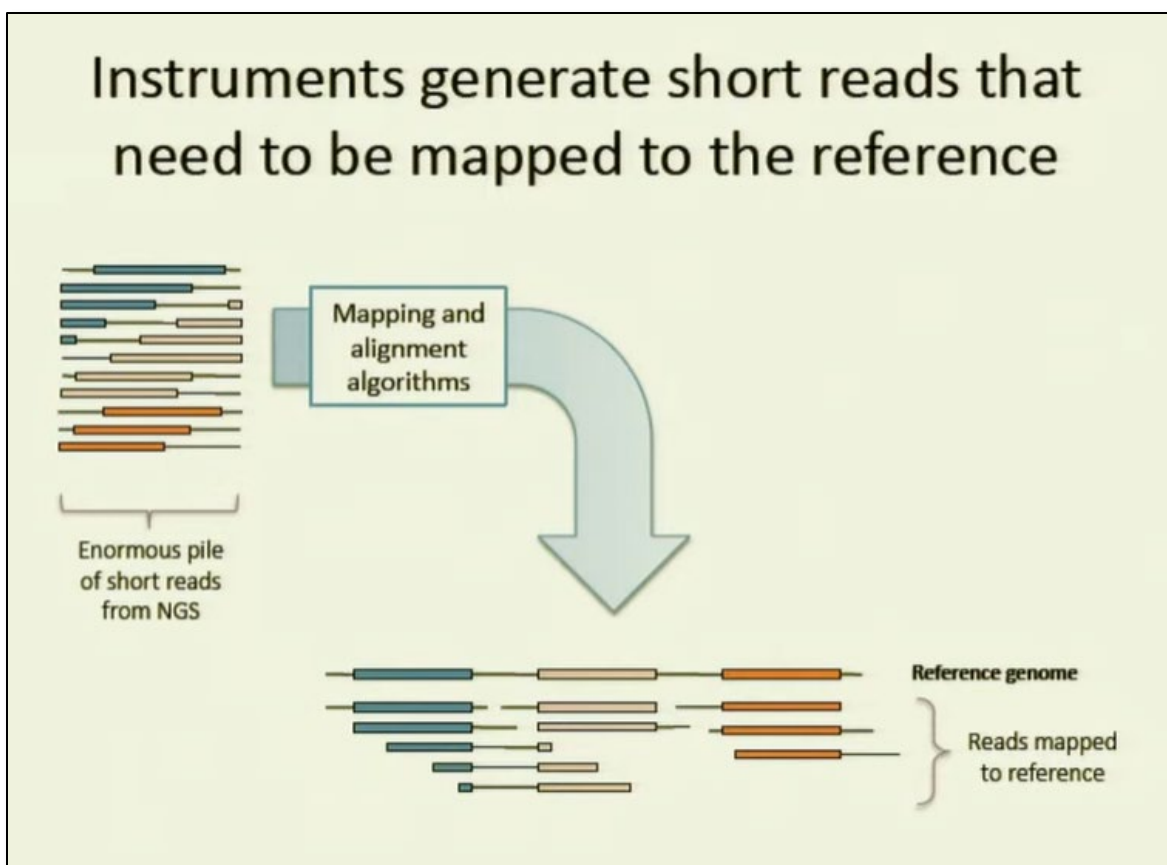
23. Processing steps to detect SNVs from sequence reads after obtaining the sequence reads could also be performed on the Illumina MiSeq instrument described in the ’596 patent, ’596 patent at 169:39, as shown below:



Ex. 4, Illumina Application Note: *Sequencing, Amplicon Sequencing from FFPE tissues on the MiSeq™ System* (2011) (“Illumina MiSeq Application Note”), Figure 1 (showing that variant calling is performed on the instrument, after base calling, which generates the sequence reads). See also Ex. 5, Daber et al., *Understanding the limitations of next*

generation sequencing informatics, an approach to clinical pipeline validation using artificial datasets, Cancer Genet. 206:441, 443 (2013) (“[m]apping reads to the amplicon target regions has been utilized by the onboard bioinformatics processing in the Illumina MiSeq as part of the amplicon workflow to reduce data processing times”). These data analysis steps after obtaining sequence reads are discussed in more detail below.

24. ***Alignment or mapping of sequence reads:*** Base calling results in many short sequence reads—typically millions or billions of short reads. Ex. 3, Broad Institute Presentation, *supra*, at 4:28-5:18. The sequence reads obtained by base calling are not organized by their locations in the genome—at this stage, the reads are like an enormous, disorganized “pile” of short strings of bases, as illustrated in the diagram below. The corresponding genomic locations of the sequence reads are not known without further processing. Determining whether a given base is an SNV, however, requires knowing its corresponding location in the genome: at one position, a “G” may match the normal sequence; at a different location, the normal sequence may be “T,” and “G” would represent a candidate SNV (or an error, as discussed in more detail below). To detect mutations, the corresponding genomic locations must therefore be identified for the sequence reads. See Ex. 3, Broad Institute Presentation, *supra*, at 5:22-5:45 (explaining that the location of sequence reads in the genome must be found in order to “assess whether there’s a mutation at that position”), 5:48-6:01 (“this is hugely important to understand that those pieces actually need to be mapped back to their original locations on the genome”).

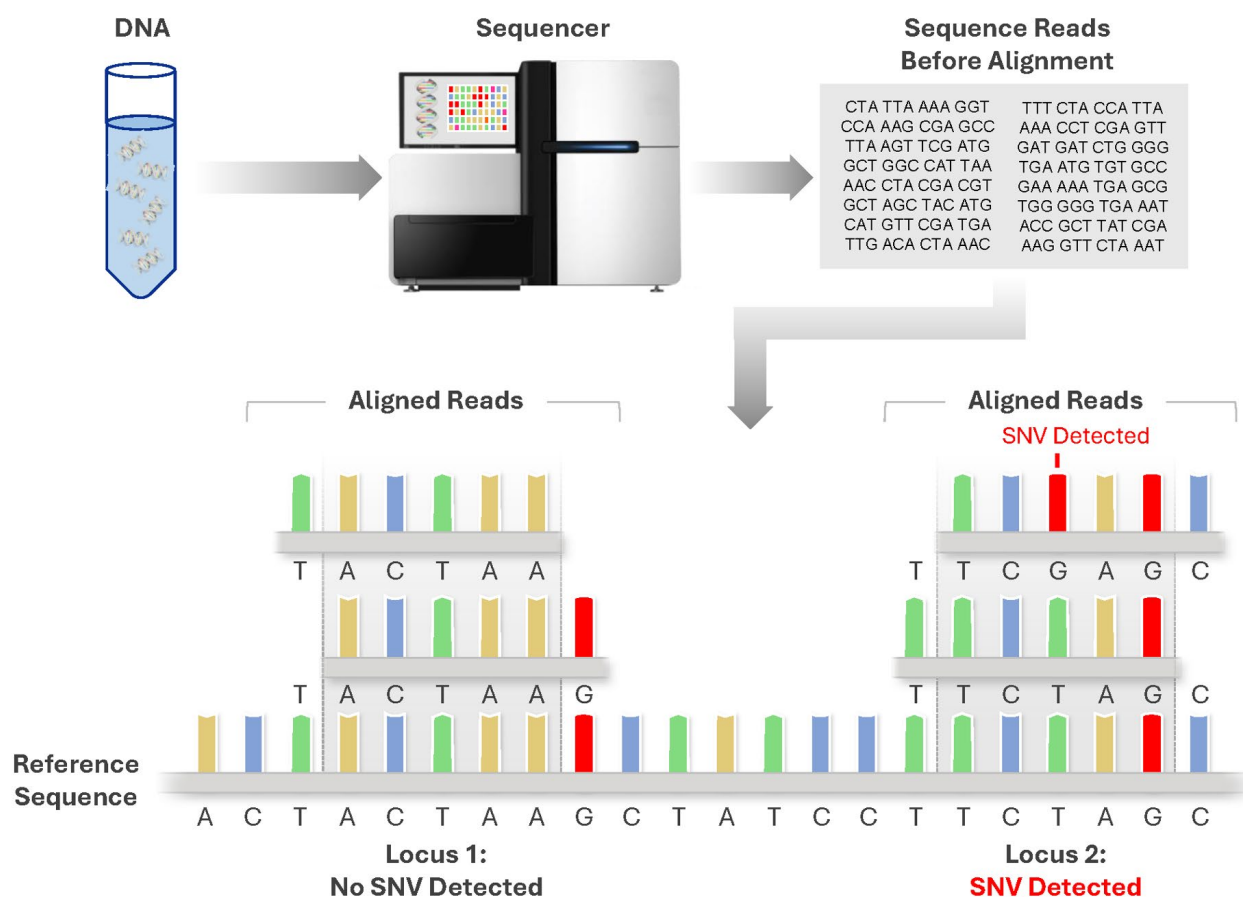


Ex. 3, Broad Institute Presentation, *supra*, at 5:33.

25. Read mapping typically involves aligning the reads to a reference sequence. The reference sequence could be the entire genome or a subset of it (*e.g.*, a reference sequence representing only the portion that was targeted). Ex. 5, Daber et al., *supra*, at 443. The location of origin of the sequence reads is determined “based on similarity of nucleotide sequence of the read and the genome sequence.” ’596 patent at 31:27-29. Read mapping could be performed automatically on the sequencing instrument as part of the sequencing step, or using a separate bioinformatic pipeline on the cloud or a separate computer cluster. *See, e.g.*, Ex. 5, Daber et al., *supra*, at 443 (“[m]apping reads to the

amplicon target regions has been utilized by the onboard bioinformatics processing in the Illumina MiSeq as part of the amplicon workflow to reduce data processing times”); *see also* Ex. 4, Illumina MiSeq Application Note, *supra*, at Figure 1.

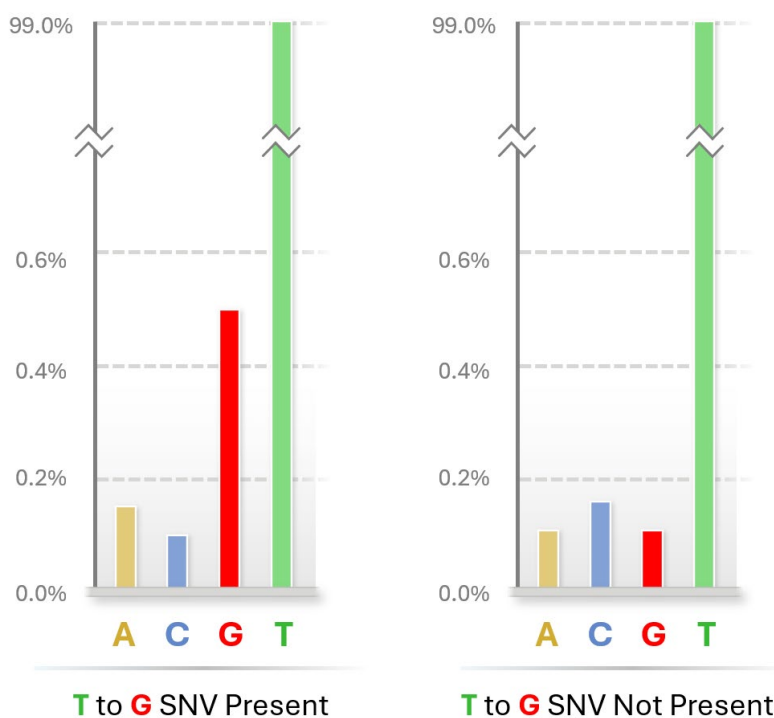
26. **SNV detection:** As explained above, an SNV mutation is a single-base change from the normal sequence at a particular locus. Detecting an SNV from a sequence read therefore requires mapping the corresponding locus for that read. *See* ’596 patent at 100:45-60 (describing allelic data for a SNV locus that is measured using high-throughput sequencing, including “the number of reads of each allele mapping to the locus of interest”). The figure below is a simplified explanation of how sequence reads are processed to detect an SNV from the sequence reads. The DNA used as input for the sequencer is short pieces of amplified DNA from multiple target locations in the genome. The sequencer “reads” the DNA to generate short sequence reads (strings of bases), as shown in the top right portion of the figure as “Sequence Reads Before Alignment.” The obtained reads are then mapped to their corresponding locations in the genome (e.g., “Locus 1” and “Locus 2” in the figure below) based on similarity to a reference sequence representing the genome or a portion thereof. Once the reads are mapped to the reference sequence, a base that differs from the normal sequence at that location (or that matches a known cancer SNV at that location) can be detected. In the example below, a “T” to “G” SNV is detected at Locus 2, but no mutation is detected at Locus 1.



27. The above figure provides a highly simplified illustration of read mapping and SNV detection. In reality, the process of mapping sequence reads is computationally intensive. Millions or billions of reads need to be aligned to the reference sequence, and, as I explain in the following paragraphs, amplification or sequencing errors may result in poorly mapping reads or reads that do not map to the reference sequence at all.

28. Once the corresponding locus of the sequence reads in the genome has been identified as shown above, a base that differs from the normal sequence or that matches a pre-defined SNV at that location can be identified. However, not all differences between a sequence read and the normal sequence represent actual SNVs. Both PCR

amplification and high-throughput sequencing can add the wrong bases during amplification and sequencing respectively. '596 patent, 65:34-37, 66:39-42. These amplification or sequencing errors can create lots of background 'noise' in the data that can make detecting actual SNV's difficult, particularly if an SNV is only present at low levels. This "noise" can for example create multiple differences between the bases in sequence reads and the bases in normal sample DNA. The figure below illustrates two examples of the percentage of mapped reads containing a particular base at one single location on the genome.



29. When sequencing a sample with an SNV present at low levels, the most prevalent base, in this example "T", is the normal base at that position. Each of the other three bases may be noise from amplification or sequencing, or may be an SNV. The left panel shows an SNV mutation of "T" to "G" that is determined to be present (detectable

above the level of background noise). In the right panel, the “T” to “G” mutation is not determined to be present. In the right panel, even if the “T” to “G” mutation were present in the sample, it would not be distinguishable from background noise. For this reason, an SNV is not necessarily detected each time a base in a sequence read is different from the normal base at the corresponding locus.

30. In summary, detecting a mutation involves determining that the SNV is present in the sample DNA. Variants are detected or “called” from sequence reads after obtaining the sequence reads. *See, e.g.,* Li et al., Ex. 6, *The Sequence Alignment/Map format and SAMtools*, Bioinformatics 25(16): 2078, 2078 (2009) (describing alignment of sequence reads and “analyses, including variant detection”), Ex. 7, Nielsen, *Genotype and SNP calling from next-generation sequencing data*, Nat. Rev. Genet. 12(6) Author Manuscript 1, 9 (2011) (available in PubMed Central 2013) (“[the] accuracy of the alignment has a crucial role in variant detection”); Ex. 3, Broad Institute Presentation, *supra*, at 4:28-5:18 (explaining that some types of sequencing errors can be removed during processing of sequence reads before “mutation variant detection”).

V. CLAIM CONSTRUCTION

31. It is my opinion that a skilled person would have understood the term “wherein an SNV mutation . . . is detected from the sequence reads” in claim 1 of the ’596 patent to mean “wherein an SNV mutation is determined to be present from the sequence reads.” The meaning of this term is clear from the language of the claim itself, which requires “performing high-throughput sequencing to obtain sequence reads” and

“wherein an SNV mutation . . . is detected from the sequence reads.” Because the claim requires detecting an SNV mutation “from” the sequence reads, it indicates to a skilled person that the information in the sequence reads is obtained first, and that this information is used to detect an SNV mutation. As I have explained in the technical background section above, this workflow is consistent with how high throughput sequencing worked at the time of the patent’s priority date. At a minimum, a scientist would understand that detecting an SNV requires finding or aligning the locations of sequence reads in a reference sequence. In other words, to ascertain whether a base such as “T” in a sequence read is the normal sequence or a mutation requires first knowing where that base is located and what ought to be in that position.

32. Second, as discussed below, the specification and prosecution history of the ’596 patent confirm that a scientist reading the term “wherein an SNV mutation . . . is detected from the sequence reads” would have understood it to mean “wherein an SNV mutation is determined to be present from the sequence reads.”

A. The Specification of the ’596 Patent

33. The ’596 patent’s specification supports this understanding, as it discloses several examples of data processing techniques for detecting SNVs from sequence reads, all of which disclose methods for making a determination about the presence of an SNV. *See, e.g.*, ’596 patent at 5:44-67 (describing using a computer to compare SNV positions with statistically significant depths of read compared to the background error at that position (or loci) and “thereby detecting one or more single nucleotide variants”); *id.* at

67:30-53 (same). These methods use normal (cancer free) samples as controls on the same sequencing run as the test sample “to account for run specific artifacts.” *Id.* 68:12-19 (explaining that noisy loci are removed from the analysis). The patent describes an alternative method that estimates background error using training data and using that to “differentiate the background error from real mutations” to detect an SNV. *Id.* at 165:48-55. The background error determined using the controls or training data can then be used when analyzing the data to help detect real SNV mutations as distinguished from “noise,” such as run-specific artifacts caused by amplification or sequencing. *Id.* at 68:12-19, 165:48-55.

34. Although the '596 patent does not expressly define “detected,” “detecting,” or “detection” of an SNV, a scientist in the field would have considered “detecting” an SNV to be synonymous with SNV calling that involves making a determination or “call” that an SNV is present. Scientists in this field understand that SNV calling is not the same thing as base calling, which is the process for obtaining sequence reads. Natera’s own expert, Dr. Metzker, has described SNV detection as a process requiring sequence alignment in a published paper describing sequencing technologies as of 2010. Ex. 8, Metzker, *Sequencing technologies — the next generation*, Nat. Rev. Genet. 11:31, 34 (2010) (“Sequence variants are called by aligning reads to a reference genome using bioinformatics tools . . .”); *id.* (describing “SNV detection using these alignment tools”). Natera’s expected argument that an SNV is detected “from” sequence reads as part of the

process of obtaining those sequence reads is contrary to the general understanding in the field.

35. Consistent with this general understanding, the specification equates “detecting” an SNV with determining whether a single nucleotide variant is present. For example, the specification states: “In certain aspects, provided herein are methods for detecting single nucleotide variants in a sample. Accordingly, provided herein is a method for determining whether a single nucleotide variant is present at a set of genomic positions in a sample from an individual . . .” *Id.* at 5:4-8. The specification then describes the method as including a step of “receiving observed nucleotide identity information for each genomic position in the sample.” *Id.* at 5:14-15. A skilled scientist would understand that this step uses the information from both base calling (i.e., “nucleotide identity information”) and alignment (i.e., “for each genomic position”). The next two steps of the method describe data processing steps to “determine the most-likely real variant percentage and confidence from the set of probabilities for each genomic position.” *Id.* at 5:16-26. A skilled scientist would understand these steps to describe determining whether an SNV mutation (i.e. “variant”) is present at each genomic position based on the most-likely percentage of real SNVs (as opposed to noise) at those positions.

36. The patent further ties “detecting” an SNV with “determining whether a single nucleotide variant is present” by using the terms interchangeably in the same paragraph:

In illustrative embodiments of the ***method for determining whether a single nucleotide variant is present***, the estimate of efficiency and the per cycle error rate is generated for a set of amplicons that span the genomic position. For example, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more amplicons can be included that span the genomic position. In certain embodiments of ***this method for detecting one or more SNVs*** the limit of detection is 0.015%, 0.017%, or 0.02%.

Id. at 5:27-34 (emphases added). In the first sentence of this paragraph, the patent refers to “embodiments of the method for determining whether a single nucleotide variant is present.” *Id.* at 5:27-28. Then, in the same paragraph, the patent refers to “embodiments of ***this*** method for detecting one or more SNVs.” *Id.* at 5:32-33 (emphasis added). The specification equates “detecting” an SNV with “determining whether a single nucleotide is present” in other places as well. *See, e.g.*, ’596 patent at 65:14-67 (section titled “Exemplary Embodiments for Detecting Single Nucleotide Variants” and describing methods for “determining whether a single nucleotide variant is present”). In my opinion, a skilled person reading these passages in the ’596 patent’s specification would understand that the purported inventors used the term “detecting” an SNV to mean determining that an SNV is present, consistent with the typical meaning of the term in this field.

37. The specification also describes “improved analytical methods” for detecting SNVs as forming part of the invention. *See* ’596 patent at 44:6-11. The analytical methods for detecting SNVs described in the ’596 patent relate to processing steps that happen after obtaining sequence reads and mapping the reads to a reference genome. *See, e.g.*, ’596 patent at 5:4-6:15, 65:14-67, 66:1-27, 67:11-24, 67:30-64, 67:65-

68:30, 165:19-167:26. For example, as discussed above, the '596 patent discloses several example data processing techniques for detecting SNVs from sequence reads. *See, e.g.,* '596 patent at 5:44-67 (describing using a computer to compare SNV positions with statistically significant depths of read compared to the background error at that position (or loci) and “thereby detecting one or more single nucleotide variants”); *id.* at 67:30-53 (same). These methods use normal, that is cancer free, samples as controls on the same sequencing run as the test sample “to account for run specific artifacts.” *Id.* 68:12-19 (explaining that noisy loci are removed from the analysis using normal plasma samples as part of the same sequencing run). In an alternative embodiment, the specification describes a method for detecting SNVs that estimates the background error by using training data to “differentiate the background error from real mutations.” *Id.* at 165:48-55. The sequence reads from these controls or training data can then be used when processing the data to help detect real SNV mutations as distinguished from noise, e.g., run-specific artifacts caused by amplification or sequencing. *Id.* at 165:48-55, 68:12-19. A skilled person reading these sections in the '596 patent's specification would understand that the “analytical methods” for detecting an SNV involved data processing for actually making a determination of whether an SNV is present from sequence reads.

B. Purpose of the '596 Patent

38. According to claim 1, the purpose of the purported invention claimed in the '596 patent is to “monitor[] the progression of cancer.” '596 patent at claim 1. But Natera proposes a construction that does not require that an SNV mutation—indicative of

cancer—is determined to be present from the sequence reads. Thus, it appears that Natera’s construction would only require obtaining sequence reads, which do not alone determine whether an SNV signifying cancer is present without additional data processing steps—including mapping the sequence reads to their locations in a reference sequence, and actually calling the SNV. Merely obtaining sequence reads provides no understanding as to what those sequence reads mean, and no information as to whether an SNV (and thus cancer) is present.

39. Claim 12 illustrates this point, as it requires “detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.” Detecting recurrence and/or metastasis of cancer from mutations detected in the cell-free DNA requires that the “mutations” have actually been determined to be present.

C. Prosecution History of the ’596 Patent

40. The prosecution history of the ’596 patent confirms that the claims were allowed based on an understanding that “wherein an SNV mutation . . . is detected from the sequence reads” in claim 1 of the ’596 patent meant “wherein an SNV mutation is determined to be present from the sequence reads.”

41. Natera added this term to the claims in the context of the limitation “wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.” Ex. 9, Office Action response dated June 1, 2021. Natera cited support from the specification including paragraphs [0052] and [0060] of the published application (Ex. 10, US2019/0194758).

Id. at 5. These paragraphs equate detecting an SNV mutation with determining that an SNV mutation is present.

42. Cited paragraph [0052] of Ex. 10, US2019/0194758 corresponds to Col. 5, lines 27-34 of the '596 patent. It refers to “the method for determining whether a single nucleotide variant is present,” and then, in the same paragraph, states that “[in] certain embodiments of *this* method for detecting one or more SNVs the limit of detection is 0.015%, 0.017%, or 0.02%.” '596 patent, 5:27-34 (emphasis added).

43. Cited paragraph [0060] of Ex. 10, US2019/0194758 corresponds to Col. 6, lines 16-21 of the '596 patent. Like paragraph [0052], paragraph [0060] refers to “*this* method for detecting one or more SNVs.” *Id.* at 6:19-20. A skilled scientist would understand that “this method” for detecting an SNV with a limit of detection of 0.015% described in paragraph [0060]—cited to support the claim amendment—requires aligning sequence reads to a reference and analyzing those sequence reads to determine that the SNV is present. The preceding paragraphs describe embodiments of SNV detection, all of which involve analyzing sequence reads. *See, e.g., id.* at 5:63-66 (describing detecting an SNV by “identifying using a computer, one or more single nucleotide variant positions with a statistically significant depth of read weighted mean compared to the background error for that position,” which requires knowing the positions of the sequence reads), 6:7-15 (describing embodiments for detecting SNVs involving calculating the observed “depth of read” for the SNV). Calculating “depth of read” in sequencing involves mapping the reads to a reference sequence and determining the number of reads that map

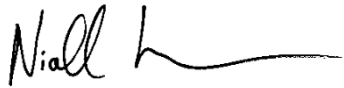
to a particular location—this number is referred to as “depth.” Therefore, the support cited for the claim amendment further indicates that the “wherein an SNV mutation . . . is detected from the sequence reads” term requires analysis of the sequence reads to detect the SNV, rather than merely obtaining the sequence reads.

44. Natera then argued that the references cited by the Examiner failed to teach the limitation “wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.” Ex. 9, Office Action response dated June 1, 2021 at 7. Specifically, Natera argued that “In contrast to the presently claimed invention, Diehn discloses “NSCLC DNA were accurately detected at fractional abundances between 0.025% and 10%” (paragraph 0792) and “fractions of tumor-derived DNA detected in plasma by SNV and/or indel reporters ranged from ~0.02% to 3.2%” (paragraph 0797).” *Id.* Diehn described mutation detection as involving “determining the presence of somatic mutations.” Ex. 11, U.S. Patent Pub. No. 2016/0032396 (“Diehn”) at [0019]. Natera did not dispute that Diehn taught a method wherein mutations including SNVs were detected, or Diehn’s description of mutation detection as requiring a determination that a mutation was present. Natera’s focus on the accuracy of the determination that an SNV was present in the prior art indicates that Natera considered the limitation to require actually making a determination regarding whether an SNV is present. The Examiner agreed and allowed the claims on that basis. Ex. 12, Mar. 14, 2022 Notice of Allowance (“Applicant’s claim amendments overcame all of the previously presented rejections. No new references were found

teaching or suggesting claim 1 which now requires detection of SNV mutation that is present in less than or equal to 0.015% of the cell free DNA having the SNV locus.”).

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

Executed on January 21, 2025

A handwritten signature in black ink, appearing to read "Niall" followed by a stylized surname.

Niall Lennon, Ph.D.