

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

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

**DECLARATION OF DR. MICHAEL L. METZKER IN SUPPORT OF
PLAINTIFF NATERA, INC.'S RESPONSIVE CLAIM CONSTRUCTION BRIEF**

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I, Michael Metzker, declare as follows:

1. I previously submitted two Declarations in the above-captioned litigation in connection with Plaintiff Natera, Inc.'s ("Natera") motion for a preliminary injunction regarding NeoGenomics Laboratories, Inc.'s ("NeoGenomics") RaDaR™ test.¹ I also submitted two Declarations² in connection with Natera's opening³ and responsive claim construction briefs, respectively. I incorporate those Declarations herein by reference, including information regarding my qualifications and expertise, my curriculum vitae, as well as my understanding of relevant legal principles, the technology background, and the '454 Patent (Babiarz)⁴ and the '596 Patent (Babiarz)⁵ (collectively, the "Asserted Natera Patents").

¹ See Doc. 17, *Declaration of Dr. Michael L. Metzker in Support of Plaintiff Natera, Inc.'s Motion for a Preliminary Injunction*, dated July 29, 2023 [hereinafter "Metzker Op. PI Decl." or my "Opening PI Declaration"; see also Doc. 145, *Reply Declaration of Dr. Michael Metzker*, dated Nov. 6, 2023 [hereinafter "Metzker Reply PI Decl." or my "Reply PI Declaration"]].

² See Doc. 263, *Declaration of Dr. Michael L. Metzker in Support of Plaintiff Natera, Inc.'s Opening Claim Construction Brief*, dated April 8, 2024 [hereinafter "Metzker Op. CC Decl." or my "Opening CC. Declaration"]; Doc. 273, *Declaration of Dr. Michael L. Metzker in Support of Plaintiff Natera, Inc.'s Responsive Claim Construction Brief*, dated April 22, 2024 [hereinafter "Metzker Resp. CC Decl." or my "Responsive CC. Declaration"];

³ See Doc. 262, *Plaintiff Natera, Inc.'s Opening Claim Construction Brief*, dated April 8, 2024 [hereinafter "Natera Op. CC Brief"]; Doc. 272, *Plaintiff Natera, Inc.'s Responsive Claim Construction Brief*, dated April 22, 2024 [hereinafter "Natera Resp. CC Brief"];

⁴ See Doc. 1-1, NAT-NEO-00000001–00000222, Babiarz *et al.*, *Detecting mutations and ploidy in chromosomal segments*, U.S. Patent No. 11,530,454 (2022) (the "'454 Patent (Babiarz)").

⁵ See Doc. 352-10, Babiarz *et al.*, *Detecting mutations and ploidy in chromosomal segments*, U.S. Patent No. 11,319,596 (2022) (the "'596 Patent (Babiarz)").

2. I have been asked to provide my expert opinions as to the technical background and meaning of certain terms of the '596 Patent (Babiarz) in response to the NeoGenomics '596 Patent Op. CC Br.⁶ and the Lennon '596 Patent Op. CC Decl.⁷ I have been informed and understand that Natera has asserted the following claims against NeoGenomics: Claims 1, 8, 10, 12, 14, 15, 22, 24, 26, and 28 of the '454 Patent (Babiarz) and Claims 1, 11, 12, and 18 of the '596 Patent (Babiarz) (collectively, the "Asserted Natera Claims").

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

I. SUMMARY OF OPINIONS

4. Regarding Claim 1 of the '596 Patent (Babiarz), it is my opinion that one of ordinary skill in the art would have understood the term "performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence

⁶ See Doc. 373, *Defendant NeoGenomics, Inc. 's '596 Patent Opening Claim Construction Brief* [hereinafter "NeoGenomics '596 Patent Op. CC Br."].

⁷ See Doc. 374, *Declaration of Niall Lennon,, Ph.D. on Claim Construction* [hereinafter "Lennon '596 Patent Op. CC Decl."].

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” to mean (i) the terms are all part of the “performing high-throughput sequencing . . . to obtain sequence reads” step, (ii) the “wherein” clause informs the mechanics of how the high-throughput sequencing is performed, and (iii) “the amplified DNA” may be modified before being sequenced. Nothing in the NeoGenomics ’596 Patent Op. CC Br. or the Lennon ’596 Patent Op. CC Decl. change my opinions.

II. BACKGROUND AND QUALIFICATIONS

5. I previously identified my background and qualifications in my Opening PI Declaration,⁸ and incorporate that discussion here.

6. As an expert in molecular biology, I am qualified to provide an opinion as to what one of ordinary skill in the art would have understood, known, or concluded as of the relevant timeframe, which is as of 2015, or several years prior to that.

7. In formulating my opinions, I have relied upon my 30+ years of education, training, knowledge, and experience in the relevant art. But at all times, I have understood and interpreted the Asserted Natera Claims from the point of view of one of ordinary skill in the art, as discussed in Section IV *infra*.

⁸ See Doc. 17 (Metzker Op. PI Decl.) at ¶¶ 4–19, with one exception: I am no longer Chief Technology Officer at 454 Bio.

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

III. UNDERSTANDING OF THE LAW

9. I am not an attorney, nor have I independently researched the law. In this Responsive Declaration, I have applied certain legal principles, including with relation to claim construction, of which I have been informed by Natera's counsel as described in my Opening PI Declaration.⁹

10. I have been informed and understand from counsel that patent claims should be generally given their plain and ordinary meaning from the perspective of one of ordinary skill in the art at the time that the claimed subject matter was applied for. I also understand that patent claims must also be read in light of the specification of the patent and associated prosecution histories, and that the specification is viewed as the best source for understanding a technical claim term. I further understand that the only exceptions to giving the words in a claim their plain and ordinary meaning in the art are (i) when the patentee acts as their own lexicographer; and (ii) when the patentee disavows or disclaims the full scope of a claim term in the specification. With respect to the former, I have been informed and understand that to act as their own lexicographer, the applicant must clearly set forth a special definition of a claim term in the specification that differs from the plain

⁹ See Doc. 17 (Metzker Op. PI Decl.) at ¶ 24.

and ordinary meaning it would otherwise possess. I also understand that the specification may also include an intentional disclaimer, or disavowal, of claim scope, which must be clear and unmistakable.

11. I have been informed and understand from counsel that it is improper to import claim limitations from the specification. I also understand that a claim construction that reads disclosed embodiments out of the claim is rarely correct absent evidence of a clear disclaimer. I further understand that extrinsic evidence cannot “alter the meaning” of a claim term where the intrinsic evidence is otherwise clear.

IV. LEVEL OF ORDINARY SKILL IN THE ART

12. I set out my understanding of one of ordinary skill in the art in my Opening PI Declaration,¹⁰ and I incorporate that discussion here. Additionally, one of ordinary skill in the art would have had an understanding of cancer biology as it relates to mutations associated with cancer, including as related to tumor monitoring, progression, and treatment.

13. In or around 2015, I would have been at least one of ordinary skill in the art with respect to the subject matter of the '596 Patent (Babiarz).

V. MATERIALS CONSIDERED

14. In formulating my opinions, I considered all of the references cited in this Responsive Declaration, the claims and specification of the '596 Patent (Babiarz), the prosecution history of the '596 Patent (Babiarz), the prosecution histories of the related

¹⁰ See Doc. 17 (Metzker Op. PI Decl.) at ¶ 32.

patents cited on the face of the '596 Patent (Babiarz), the NeoGenomics '596 Patent Op. CC Br.,¹¹ the Lennon '596 Patent Op. CC Decl.¹² and exhibits thereto, and Dr. Lennon's deposition testimony.¹³

VI. TECHNOLOGY BACKGROUND

15. I incorporate by reference the technology background sections from each of my prior declarations in this case.

A. DNA

16. DNA is made of four different nucleotides or bases, each represented by a different letter: adenine (A), thymine (T), cytosine (C) and guanine (G). The order of these letters (*i.e.*, the DNA sequence) encodes the information that instructs cells when and how to perform certain functions.

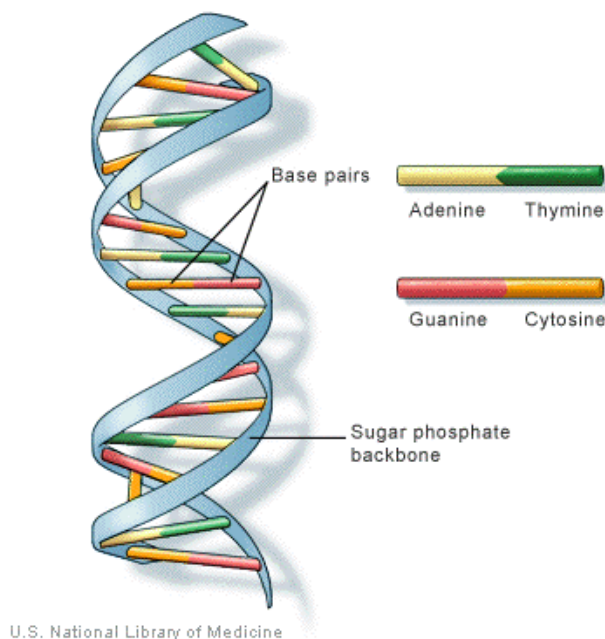
17. The four DNA different bases form stable hydrogen bonds according to specific base-pairing rules. "A" and "T" can form stable hydrogen bonds, and "C" and "G" can form stable hydrogen bonds. Said in another way, "A" only pairs with "T" and does not pair with "G" or "C". Likewise, "C" only pairs with "G" and does not pair with "A" or "T."

¹¹ See Doc. 373, NeoGenomics '596 Patent Op. CC Br.

¹² See Doc. 374, Lennon '596 Patent Op. CC Decl.

¹³ See Doc. 378-4, January 30, 2025 Deposition Tr. of Dr. Niall Lennon [hereinafter "Lennon Jan 30, '25 Tr."].

18. The formation of base-pairs between nucleotides on the two opposite strands causes the two strands to coil around each other to form a double helix structure as shown *infra*.



19. According to the base-pairing rules, stretches of nucleotides in one strand can form hydrogen bonds only with a complementary stretch of nucleotides in the other strand. Two single strands of DNA that come together by way of hydrogen bonding between stretches of complementary (or substantially complementary) nucleotides are said to be hybridized to one another. Because of the specificity of base-pairing, under most environmental conditions, a strand of DNA will have a specific complementary sequence.

B. PCR

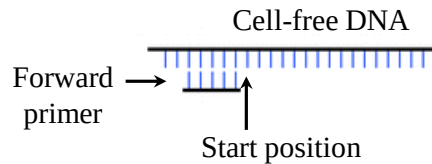
20. Polymerase Chain Reaction or “PCR” is a technique for replicating or amplifying nucleic acids in a laboratory setting. I previously explained the fundamentals

of PCR in a book chapter I authored, which was published in 2006 and discussed during my deposition.¹⁴

21. Generally speaking, PCR involves repeated denaturation and replication of DNA sequences *in vitro* (i.e., outside of an organism). An enzyme called *DNA polymerase* copies or synthesizes the complementary strand from a single-stranded template. For this enzymatic reaction to occur, an *oligonucleotide* or *primer* (that is, a short DNA molecule typically between 15 and 30 bases) will first hybridize to a single-stranded template molecule. Because of the base-pairing rules, primer sequences can be designed to target specific nucleic acid sequences. DNA polymerase initiates synthesis of the nascent strand from the hybridized primers in a 5'-to-3' direction to create double-stranded DNA. Two primers are used for each template strand in PCR that define the targeted sequence of interest: a *forward primer* will hybridize to the 5'-end of one single-stranded template DNA, such as cell-free DNA. This is important because the specific hybridization of a forward target-specific primer to the cell-free DNA target of interest defines the *start*

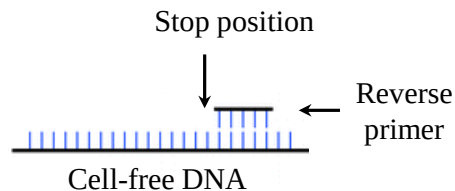
¹⁴ See Doc. 141-6, Metzker & Caskey, *Polymerase chain reaction*, In ENCYCLOPEDIA OF MEDICAL DEVICES AND INSTRUMENTATION, Second Edition, Volume 5, pp. 380–387 (2006) (“Metzker (2006)”) (cited as Ex. 7 in the September 26, 2023 Deposition of Michael Metzker Transcript (Doc. 116-6)).

position of the amplified target (*i.e.*, the first base of the amplicon). The start position of the amplicon is illustrated *infra*.



All amplicons of a given target will have the same start position. For multiplex targeted PCR, each amplicon of a given target loci would have the same start positions. That is, the amplicon of loci 1 would have the known start position and the amplicon of loci 2 would have the different, but known start position.

22. DNA polymerase binds to this partially double-stranded complex and extends the primer by copying of sequence of the single-stranded template in the 5'-to-3' direction. Primer extension will build a complementary strand of DNA.¹⁵ Also in the same cycle, a *reverse primer* will hybridize to the 3'-end of the other single-stranded template DNA, again such as cell-free DNA. This is also important because the specific hybridization of a reverse target-specific primer to the cell-free DNA target of interest defines the *stop position* of the amplified target (*i.e.*, the last base of the amplicon). The stop position of the amplicon is illustrated *infra*.

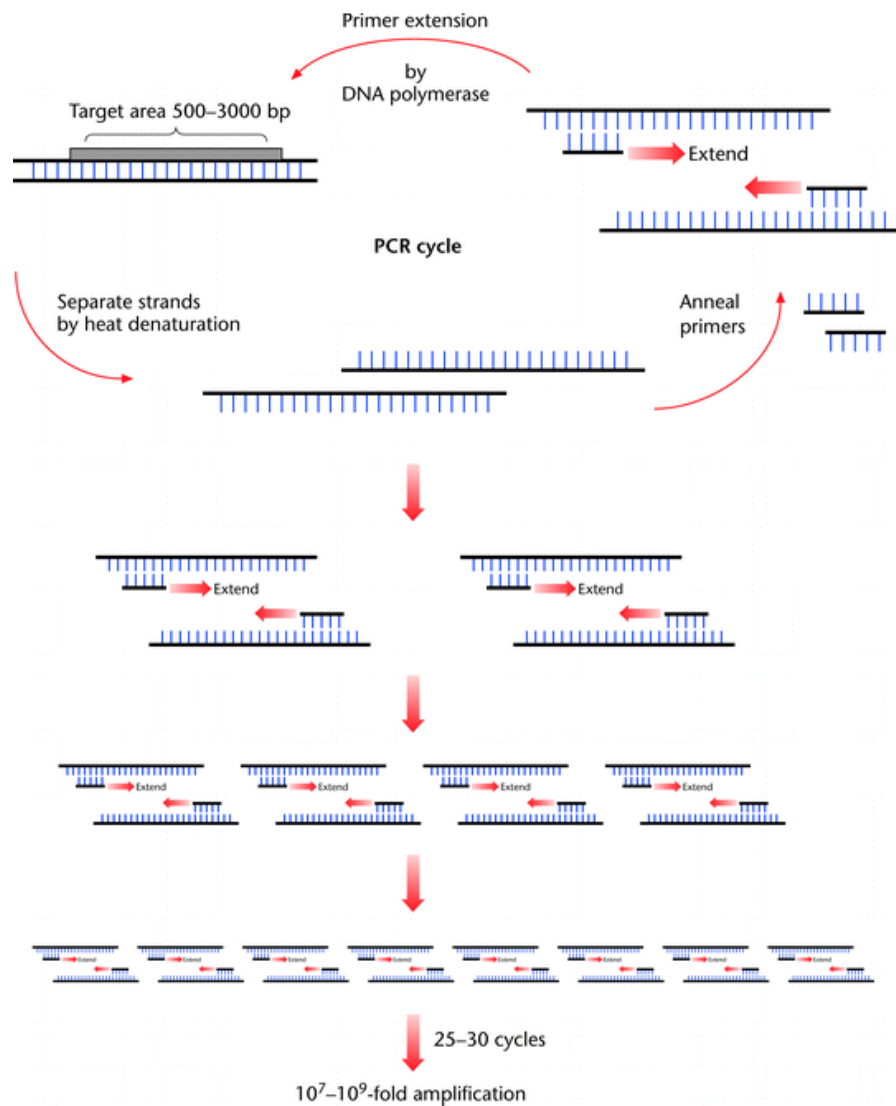


¹⁵ *Id.* at 380.

All amplicons of a given target will have the same stop position. For multiplex targeted PCR, each amplicon of a given target loci would have the same stop positions. That is, the amplicon of loci 1 would have the known stop position and the amplicon of loci 2 would have the different, but known stop position. Knowing the start and stop positions of each amplicon is important because the position of a previously identified SNV mutation will also be known, as described in more detail *infra*.

23. Similarly, DNA polymerase binds to this partially double-stranded complex and extends the primer by copying of sequence of the single-stranded template in the 5'-to-3' direction. Primer extension will build another complementary strand of DNA. The general concept of PCR is illustrated by the figure *infra*.¹⁶

¹⁶ *Id.* at Figure 1.



24. Going counterclockwise in the figure *supra*, the first step of a PCR reaction is to *denature* double-stranded DNA to yield single-stranded DNA by raising the reaction temperature to melt or by chemical means to separate double-stranded DNA or fragments into single strands.¹⁷ The second step of PCR, called *annealing*, involves lowering the temperature of the reaction mixture to allow a primer to bind to a complementary sequence

¹⁷ *Id.* at 380–381.

on the single-stranded DNA.¹⁸ In a last step called *extension*, the reaction mixture is heated to allow DNA polymerase to extend the annealed primer sequence and synthesize a complementary strand from a single-stranded template.¹⁹ Generally, the quantity of the original target sequence of interest, which is flanked by the two primers and as described *supra*, define the start and stop positions of the amplicon, will have been essentially doubled in one PCR cycle.²⁰

25. Next, the PCR cycle is repeated multiple times. Specifically, the products of a prior cycle will serve as the templates to which primers will anneal for the next cycle of PCR. This is accomplished by denaturing the double-stranded template, annealing the primers, and extending the primers with DNA polymerase to synthesize new double-stranded templates. At each PCR cycle, the target sequence that lies between the primers (including the primer sequences themselves) will be replicated. This process is repeated iteratively for, on average, 25-35 cycles whereupon the result is about a 10^7 -to- 10^9 fold amplification of the starting template.²¹ Theoretically, a target sequence will be amplified exponentially as described by 2^n , where n is the number of cycles.

¹⁸ *Id.* at 381.

¹⁹ *Id.*

²⁰ *Id.* at 380. (“**Each cycle doubles** the region marked by the primer sequences.”) (emphasis added).

²¹ *Id.* at 380. (“The optimal number of cycles is dependent on the starting concentration or copy number of the target DNA and **typically ranges from 25 to 35 cycles**.”) (emphasis added); see also *id.* at Figure 1 (“ **10^7 to 10^9 -fold amplification**”) (emphasis added).

C. High-Throughput Sequencing

26. Next generation sequencing (“NGS”), also called “high-throughput sequencing,” technologies enable exponential sequencing capacity.²² NGS platforms enable high-throughput sequencing of nucleic acids both on a genome-wide scale and in a targeted, highly multiplexed manner.²³

27. In 2010, I published a review article about the specific methodologies used in various commercial NGS platforms, including Illumina sequencers, which Dr. Lennon cites and attaches as Exhibit 8 to his Declaration.²⁴ For example, the Genome Analyzer II sequencer was developed after the acquisition of Solexa by Illumina in 2007. Since 2009, Illumina has released various instruments including multiple versions of HiSeq®, MiSeq®, NextSeq® and NovaSeq® systems.

28. I am familiar with and have used Illumina’s technical product brochures for its NGS platforms, including before 2015.²⁵

²² See Doc. 374-8, NEOGEN00289233–00289248, Metzker, *Sequencing technologies - the next generation*, NATURE REVIEW GENETICS 11:31–46 (2010) (“Metzker (2010)”) at 31.

²³ *Id.* at 31.

²⁴ See generally *id.*, Metzker (2010).

²⁵ See Doc. 372-2, Illumina System Specification Sheet: Sequencing, *HiSeq® 2500 Sequencing System* (2015) (hereafter “Illumina HiSeq 2500 Sequencing System”); see also Doc. 374-2, *Illumina Quality Scores for Next-Generation Sequencing* (2011); Ex. 1, *Illumina Technology Spotlight: Illumina Sequencing* (2010); Ex. 2, *Illumina TruSeq RNA and DNA Sample Preparation Kits* (2010); Ex. 3, *Illumina TruSeq RNA and DNA Sample Preparation Kits*, (2010); Ex. 4, NAT-NEO-01061417–01061418, *Illumina Understanding Illumina Quality Scores* (2012-2014). I am familiar with and have reviewed each of these Illumina technical product sheets before 2015.

29. To use an NGS platform for whole genome sequencing, DNA template fragments must be made *sequence-ready* through what is called “library preparation.” As described *infra*, PCR can also be used to target regions of interest to create a library of fragments. In both case, short adaptors are attached or ligated to both ends of the DNA template fragments to make those libraries sequence-ready.²⁶ As one example, Bentley (2008)²⁷ described that ligated products, which contain the adaptor sequences, could be PCR amplified to include additional sequences used in subsequent amplification and sequencing steps.

30. In NGS platforms, template DNA is typically attached or immobilized to a solid surface or support. The immobilization of spatially separated template sites allows thousands to billions of sequencing reactions to be performed simultaneously.²⁸ Most imaging systems have not been designed to detect single molecule fluorescent events, so amplified templates are required. Solid-phase amplification, also called *bridge amplification*, is commonly used in NGS sequencers to produce randomly distributed,

²⁶ See 374-8, Metzker (2010) at 32.

²⁷ See Ex. 5, NEOGEN00289044–00289050, Bentley *et al.*, *Accurate whole human genome sequencing using reversible terminator chemistry*, NATURE 456:53–59 (2008) (“Bentley (2008)”) at Figure 1 legend.

²⁸ See 374-8, Metzker (2010) at 32.

clonally amplified clusters from DNA template fragments.²⁹ In 2015, Illumina introduced a different solid-phase amplification method, called *exclusion amplification* or *ExAmp*.³⁰

31. Bridge amplification or ExAmp is performed on Illumina’s NGS platforms and requires library fragments to be appended at each end with Illumina-specific “P5” and “P7” adaptor sequences so that the fragments can adhere to the flow cell within the Illumina instrument.³¹ As part of bridge amplification or ExAmp, the P5 and P7 regions of single-stranded library fragments hybridize to their complementary oligonucleotides that are immobilized on the flow cell surface.³² Bridge amplification or ExAmp is then used to make numerous copies of the DNA template. Following amplification, clusters are formed on the flow cell that contain copies of the single original DNA template fragment. The result is that millions of template clusters are formed.³³

²⁹ *Id.*

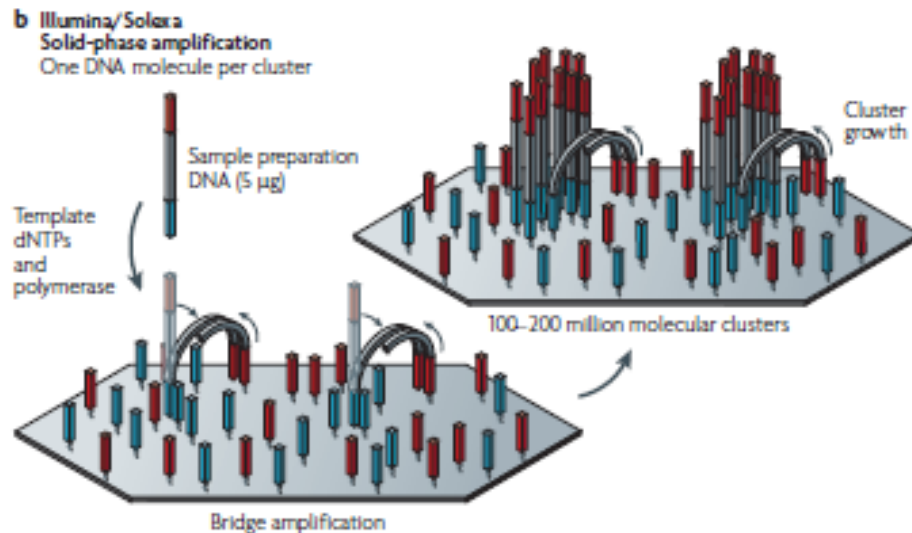
³⁰ See Ex. 6, Sinha *et al.*, *Index switching causes “spreading-of-signal” among multiplexed samples in Illumina HiSeq 4000 DNA sequencing*, BIORXIV preprint doi: <https://doi.org/10.1101/125724> (2017) (“Sinha (2017)”) at Abstract. (“**In 2015, a new chemistry of cluster generation was introduced in the newer Illumina machines (HiSeq 3000/4000/X Ten) called exclusion amplification (ExAmp)**, which was a fundamental shift from the earlier method of random cluster generation by bridge amplification on a non-patterned flow cell.”) (emphasis added).

³¹ See Ex. 1, Illumina *Technology Spotlight: Illumina Sequencing* (2010); Ex. 2, Illumina *TruSeq RNA and DNA Sample Preparation Kits*; Ex. 3, Illumina *TruSeq RNA and DNA Sample Preparation Kits* (2010).

³² *Id.*

³³ See 374-8, Metzker (2010) at 34.

128. The following is an illustration of bridge amplification from my 2010 review article.³⁴



32. Illumina NGS instruments utilize a strategy called *sequencing by synthesis* (“SBS”), which is otherwise known as cyclic reversible termination (“CRT”) that enable the identification of single bases as they are introduced into DNA strands.³⁵ For this declaration, I will refer to the Illumina sequencing method as sequencing by synthesis or SBS.

33. After bridge amplification or ExAmp, a universal sequencing primer is added to the flow cell to hybridize to all templates amplified by bridge amplification or ExAmp. Generally, all four nucleotides contain a unique fluorescent label. Each nucleotide, however, is modified to act as a reversible terminator, which is an important feature of the

³⁴ *Id.* at Fig. 1b.

³⁵ *Id.* at 32; see also Doc. 372-2, *HiSeq 2500 Sequencing System*, Illumina Inc. (2015) at 1.

technique. In the first step, all four reversible terminators are added simultaneously to the flow cells along with DNA polymerase.³⁶ Here, a DNA polymerase, bound to the primed templates within each cluster, adds or incorporates just one fluorescently-labeled reversibly terminating nucleotide, which represents the complement of the template base.³⁷ The terminating group on the incorporated nucleotide blocks any other nucleotides from subsequently being incorporated. Following incorporation, the remaining unincorporated reversible terminators are washed away. Imaging is then performed to detect the identity of the incorporated nucleotide based on the fluorescent signal emitted upon laser excitation.

34. Imaging is followed by a cleavage step, which removes the terminating group and the fluorescent label on the incorporated nucleotide. Additional washing is performed before starting the next incorporation step, and the fluorescent identity for each cluster is read using sequential images.³⁸ As shown *infra*,³⁹ the cycle of nucleotide incorporation, fluorescence imaging, and cleavage is repeated multiple times such that the number of cycles is the sequence read length.

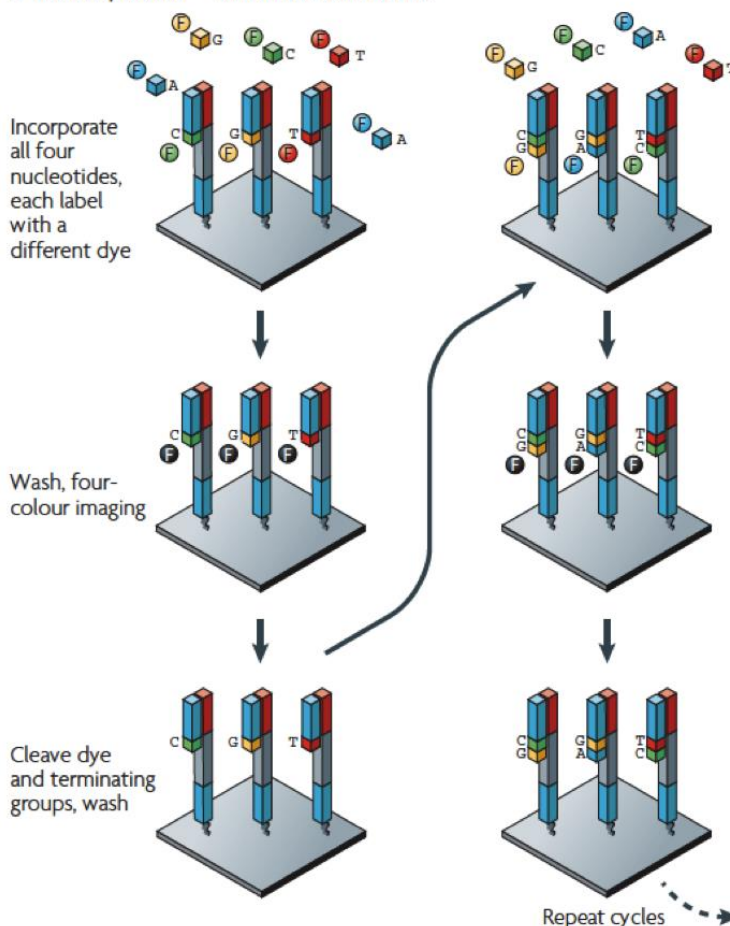
³⁶ See 374-8, Metzker (2010) at 34.

³⁷ *Id.*

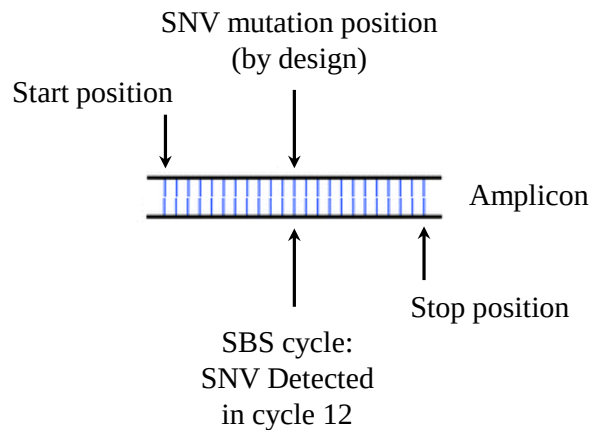
³⁸ *Id.* at Figure 2a (depicting the four-label SBS method).

³⁹ *Id.*

a Illumina/Solexa — Reversible terminators



35. For example, a SBS run with 20 cycles yields a sequence read length of 20 bases. In this 20 cycle example, the first cycle detects the start position of the amplicon (*i.e.*, the first base of the target of interest). The second cycle detects the second base of the amplicon and so on. If the SNV mutation is positioned at base 12 of the amplicon, the 12th cycle will detect the base on that SNV mutation, as illustrated *infra*.

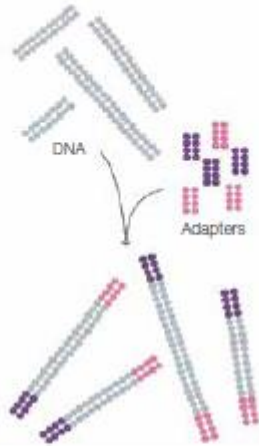


Illumina instruments including multiple versions of HiSeq®, MiSeq®, NextSeq® and NovaSeq® systems, use the same SBS method described *supra*.

36. As shown *infra*, Illumina’s NGS product brochure depicts its overall sequencing methodology, including attachment of adaptors to the library fragments and subsequent annealing of single-stranded fragments with adaptor ends to the complementary adaptor sequences on the surface of the flow cell for bridge amplification.⁴⁰

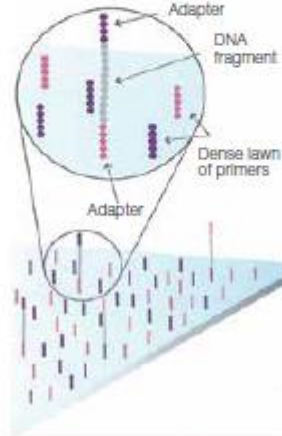
⁴⁰ See Ex. 1, Illumina *Technology Spotlight: Illumina Sequencing* (2010) at Figs. 2–3.

Figure 2: Prepare Genomic DNA Sample



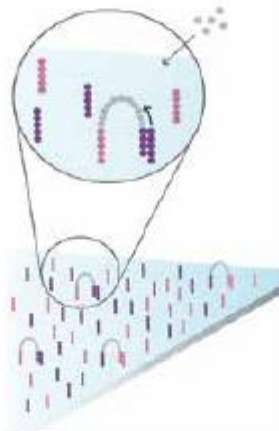
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

Figure 3: Attach DNA to Surface



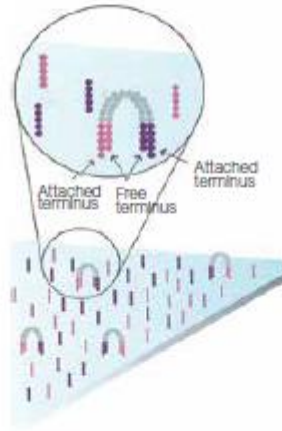
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

Figure 4: Bridge Amplification



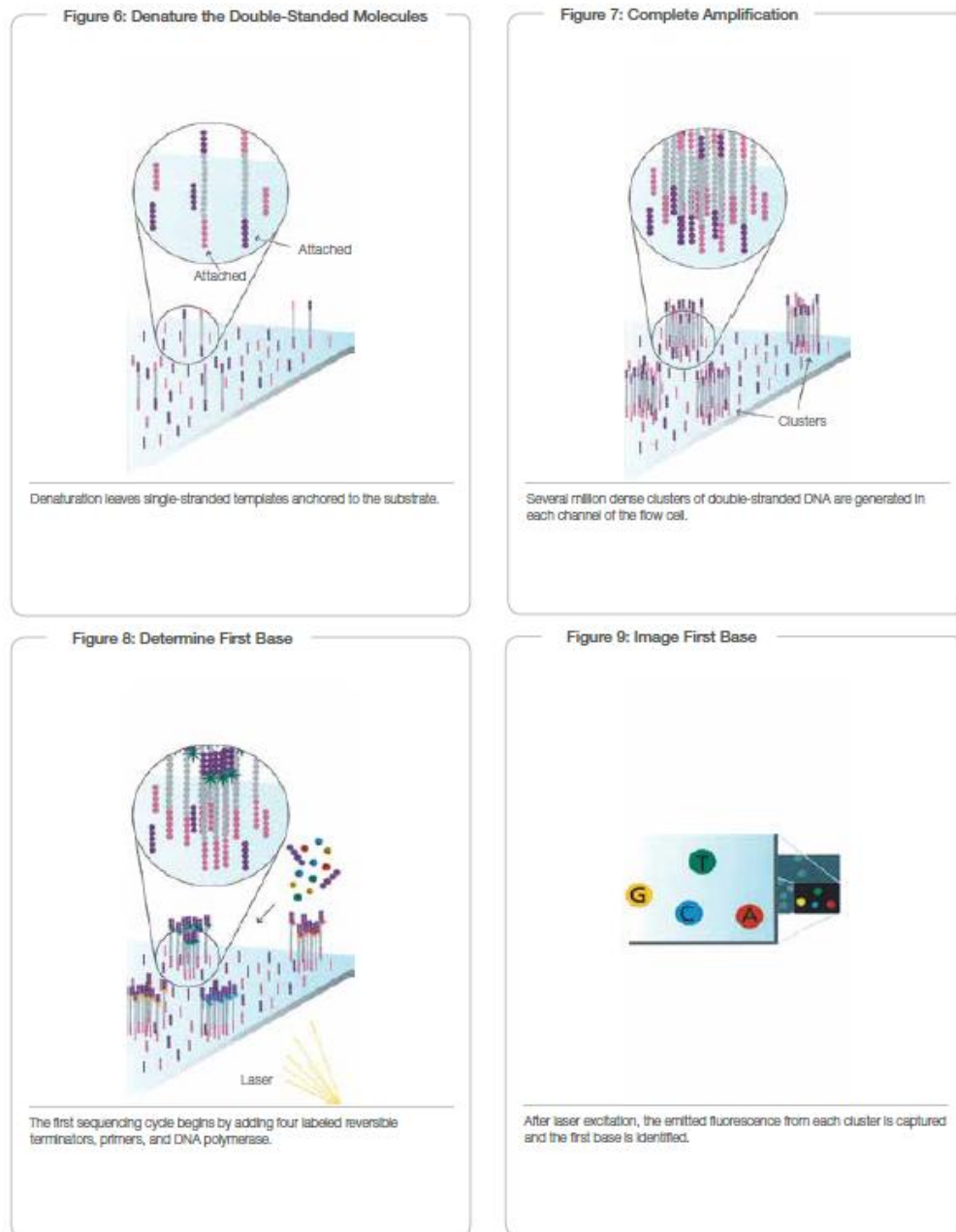
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

Figure 5: Fragments Become Double Stranded



The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

37. The product brochure also shows the steps of bridge amplification, addition of nucleotides using reversible terminators, and image capture occurs in the flow cell to generate a sequence read.⁴¹



⁴¹ *Id.* at Figs. 4–9.

38. According to Illumina, base calls are made in the next generation sequencer during each sequencing cycle and BCL files generated in the sequencer contain quality scores (called “Q scores”) that measure the base calling accuracy.

During a sequencing run, a quality score is assigned to each base call for every cluster, on every tile, for every sequencing cycle. Illumina quality scores are calculated for each base call in a two-step process:

1. For each base call, a number of quality predictor values are computed. *Quality predictor values* are observable properties of clusters from which base calls are extracted. These include properties, such as intensity profiles and signal-to-noise ratios, measure various aspects of base call reliability. They have been empirically determined to correlate with the quality of the base call.
2. A *quality model*, also known as a *quality table* or *Q-table*, lists combinations of quality predictor values and relates them to corresponding quality scores; this relationship is determined by a calibration process using empirical data. To estimate a new quality score, the quality predictor values are computed for a new base call and compared to values in the pre-calibrated quality table.

Quality scores are recorded in base call files (*.bcl) that contain the base call and quality score per cycle.⁴²

During each sequencing cycle, a single labeled deoxynucleoside triphosphate (dNTP) is added to the nucleic acid chain. The dNTPs contain a reversible blocking group that serves as a terminator for polymerization, so after each dNTP incorporation, the fluorescent dye is imaged to identify the base and then enzymatically cleaved to allow incorporation of the next nucleotide. Since all four reversible terminator-bound dNTPs (A, C, T, G) are present as single, separate molecules, natural competition minimizes incorporation bias, which can be problematic with serial nucleotide incorporation

⁴² See Ex. 4, NAT-NEO-01061417–01061418, *Understanding Illumina Quality Scores*, Illumina, Inc. (2012-2014) at NAT-NEO-01061417 (emphases added; original emphasis for italicized phrases only).

chemistry used in Sanger sequencing. **Base calls are made directly from signal intensity measurements during each cycle, greatly reducing raw error rates compared to other technologies. The result is highly accurate base-by-base sequencing that eliminates sequence context specific errors, enabling robust base calling** across the genome, including repetitive sequence regions and homo polymers.

Q scores are used to measure base calling accuracy, one of the most common metrics for assessing sequencing data quality. Low Q scores can lead to increased false-positive variant calls, resulting in inaccurate conclusions and higher costs for validation experiments. **Illumina's sequencing chemistry delivers unparalleled accuracy, with a vast majority of bases scoring Q30 and above. This level of accuracy is ideal for a range of sequencing applications, including clinical research.**"⁴³

39. Generally, a high quality score means that a base call is more reliable and less likely to be incorrect. Illumina uses terms such as Q10, Q20, Q30, Q40, *et. cetera* as a way to communicate probabilities for base calls with a quality score. Q scores are defined as a property that is logarithmically related to the base calling error probabilities⁴⁴. Table 1 *infra* shows the relationship between base quality scores and error probabilities.⁴⁵

⁴³ See Doc. 374-2, NAT-NEO-01061415–01061416, Illumina: *Quality Scores for Next-Generation Sequencing* at NAT-NEO-01061416 (emphases added).

⁴⁴ *Id.* at NAT-NEO-01061415.

⁴⁵ *Id.*; see also Ex. 4, NAT-NEO-01061417–01061418, *Understanding Illumina Quality Scores*, Illumina, Inc. (2012-2014).

Table 1: Q-Scores and Error Probabilities

Quality Score	Error Probability
Q40	0.0001 (1 in 10,000)
Q30	0.001 (1 in 1,000)
Q20	0.01 (1 in 100)
Q10	0.1 (1 in 10)

40. Here, Q40 means that the base call has an accuracy of 99.99% (*i.e.*, an error probability of 0.0001 or one in 10,000 chance that the base call is incorrect. A base call of Q30 means that the base call has an accuracy of 99.9% (*i.e.*, an error probability of 0.001 or one in 1,000 chance that the base call is incorrect. A base call of Q20 means that the base call has an accuracy of 99.0% (*i.e.*, an error probability of 0.01 or one in 100 chance that the base call is incorrect.

VII. THE '596 PATENT (BABIARZ)

A. Claims

41. The '596 Patent (Babiarz) is titled, *Detecting mutations and ploidy in chromosomal segments*, and issued on May 3, 2022.⁴⁶

42. Claims 1, 11, 12, and 18 of the '596 Patent (Babiarz) are reproduced *infra*.

Claim 1. 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;

⁴⁶ See Doc. 352-10, '596 Patent (Babiarz) at cover page.

(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.⁴⁷

Claim 11. The method of claim 1, wherein amplifying comprises targeted multiplex PCR amplification of 10 to 50 of the target loci.⁴⁸

Claim 12. The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.⁴⁹

Claim 18. The method of claim 1, wherein step (a) comprises performing whole exome sequencing on a tumor biopsy sample of the subject to identify the plurality of tumor-specific mutations.⁵⁰

⁴⁷ *Id.* at Col. 171:51–172:15 (emphases added).

⁴⁸ *Id.* at Col. 172:41–43.

⁴⁹ *Id.* at Col. 172:44–48.

⁵⁰ *Id.* at Col. 172:58–61.

B. '596 Patent (Babiarz) Specification

43. The '596 Patent (Babiarz) discloses high throughput sequencing following targeted multiplex PCR amplification. For example, there are several passages that describe high throughput sequencing following targeted multiplex PCR amplification.

In certain illustrative embodiments, the nucleic acid sequence data is generated by ***performing high throughput DNA sequencing of a plurality of copies of a series of amplicons generated using a multiplex amplification reaction***, wherein each amplicon of the series of amplicons spans at least one polymorphic loci of the set of polymorphic loci and wherein each of the polymeric loci of the set is amplified.⁵¹

In certain exemplary system embodiments provided herein the nucleic acid sequence data is generated by ***performing high throughput DNA sequencing of a plurality of copies of a series of amplicons generated using a multiplex amplification reaction***, wherein each amplicon of the series of amplicons spans at least one polymorphic loci of the set of polymorphic loci and wherein each of the polymeric loci of the set is amplified. In certain exemplary system embodiments provided herein, wherein the multiplex amplification reaction is performed under limiting primer conditions for at least ½ of the reactions. In certain exemplary system embodiments provided herein, wherein the sample has an average allelic imbalance of between 0.4% and 5%.⁵²

44. The '596 Patent (Babiarz) also discloses the use of Illumina sequencers in multiple embodiments, including attachment of sequencing tags and barcodes to amplification products prior to sequencing.

In some embodiments, universal tagged adaptors are added to make a library. Prior to ligation, sample DNA may be blunt ended, and then a single adenosine base is added to the 3-prime end. Prior to ligation the DNA may be cleaved using a restriction enzyme or some other cleavage method. During ligation the 3-prime adenosine of the sample fragments and the

⁵¹ *Id.* at Col. 58:32–39 (emphasis added).

⁵² *Id.* at Col. 62:1–13 (emphasis added).

complementary 3-prime tyrosine [*sic*] overhang of adaptor can enhance ligation efficiency. In some embodiments, adaptor ligation is performed using the ligation kit found in the AGILENT SURESELECT kit. In some embodiments, the library is amplified using universal primers. In an embodiment, the amplified library is fractionated by size separation or by using products such as AGENCOURT AMPURE beads or other similar methods. ***In some embodiments, PCR amplification is used to amplify target loci. In some embodiments, the amplified DNA is sequenced (such as sequencing using an ILLUMINA IIGAX or HiSeq sequencer).***⁵³

An aliquot of the STAR 2 products was then amplified by standard PCR for 12 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries. An aliquot of each library was mixed with libraries of different barcodes and purified using a spin column.

In this way, 19,488 primers were used in the single-well reactions; the primers were designed to target SNPs found on chromosomes 1, 2, 13, 18, 21, X and Y. ***The amplicons were then sequenced using an ILLUMINA GAIIX sequencer.*** If desired, the number of sequencing reads can be increased to increase the number of targeted SNPs that are amplified and sequenced.⁵⁴

Full sequence tags and barcodes were attached to the amplification products and amplified for 9 cycles using adaptor specific primers. Prior to sequencing, the barcoded library product were pooled, purified with the QIAquick PCR Purification Kit (Qiagen), and quantified using the Qubit® dsDNA BR Assay Kit (Life Technologies). ***Amplicons were sequenced using an Illumina HiSeq 2500 sequencer.***⁵⁵

45. One of the references cited in the '596 Patent (Babiarz) refers to a product specification sheet for the Illumina HiSeq 2500 sequencing system.⁵⁶ According to the

⁵³ *Id.* at Col. 96:23–40 (emphasis added).

⁵⁴ *Id.* at Col. 152:55–67 (emphases added).

⁵⁵ *Id.* at Col. 164:7–14 (emphases added).

⁵⁶ *Id.* at p. 24 citing Doc. 372-2, Illumina *HiSeq 2500 Sequencing System* (2015).

specification sheet, the HiSeq 2500 system detects single bases as they are incorporated into DNA template strands, such as amplicons.

With the HiSeq 2500 System, high data quality is ensured by leveraging the most widely adopted chemistry in the industry: sequencing by synthesis (SBS). SBS technology on the HiSeq 2500 utilizes a proprietary reversible terminator-based method that ***detects single bases as they are incorporated into DNA template strands***. As all 4 reversible, terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias and greatly reduces raw error rates compared to other technologies. The result is highly accurate base-by-base sequencing that virtually eliminates sequence-context-specific errors, even within repetitive sequence regions and homopolymers. Illumina sequencing delivers the most accurate human genome, the highest yield of error-free reads, and the highest percentage of base calls above Q30 in the industry.⁵⁷

46. Under the “Definitions” section, the ’596 Patent (Babiarz) provides a description of a sequence read:

Sequence Read refers to data representing a sequence of nucleotide bases that were measured, *e.g.*, using a clonal sequencing method.... A sequence read may also have associated ***quality score at each base position of the sequence indicating the probability that nucleotide has been called correctly***.⁵⁸

47. The 596 Patent (Babiarz)’s definition for “sequence read” makes specific reference to a quality score at every base position just as Illumina describes Q scores during base calling.⁵⁹

⁵⁷ See Doc. 372-2 at 1 (emphasis added, citations omitted).

⁵⁸ See Doc. 352-10, ’596 Patent (Babiarz) at Col. 31:16–24 (emphasis added).

⁵⁹ See Section VI.C *supra*; see also Doc. 374-2, NAT-NEO-01061415–01061416, Illumina: *Quality Scores for Next-Generation Sequencing* at NAT-NEO-01061416.

VIII. OPINIONS RESPONDING TO NEOGENOMICS AND DR. LENNON

A. The Term “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” (’596 Patent (Babiarz), Claim 1) Should Be Given Its Plain and Ordinary Meaning

48. I understand that the parties dispute the meaning of the term “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,” which appears in Claim 1 of the ’596 Patent (Babiarz).

49. Specifically, I understand Natera’s position is that this phrase should be given its plain and ordinary meaning.⁶⁰ As described *supra*,⁶¹ it is my opinion that one of ordinary skill in the art would have understood that (i) the terms in the phrase are all part of the “performing high-throughput sequencing . . . to obtain sequence reads” step, (ii) the “wherein” clause informs the mechanics of how the high-throughput sequencing is performed, and (iii) “the amplified DNA” may be modified before being sequenced.

50. I understand that NeoGenomics proposes a plain and ordinary construction for the full “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

⁶⁰ See Doc. 372, *Plaintiff Natera, Inc.’s Opening Claim Construction Brief Regarding U.S. Patent No. 11,319,596* [hereinafter “Natera ’596 Patent Op. CC Br.”] at 10.

⁶¹ See *supra* at ¶ 4.

in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads” term, but also states that no construction is necessary for the full term beyond the two sub-terms “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” and “performing high-throughput sequencing of the amplified DNA.”⁶²

51. I understand that NeoGenomics proposes the following construction for the term “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” as “[p]lain and ordinary meaning, which is wherein an SNV mutation is determined to be present from the sequence reads.”⁶³

52. I understand that NeoGenomics proposes the following construction for the term “performing high-throughput sequencing of the amplified DNA” as “[p]lain and ordinary meaning, which is performing high-throughput sequencing of the amplified DNA obtained from the targeted multiplex PCR amplification step.”⁶⁴ I also understand that NeoGenomics contends this term requires sequencing to be “performed on the amplified DNA from the earlier step (rather than other DNA that has been altered by intervening amplification), . . .”⁶⁵

⁶² See Doc. 373, NeoGenomics ’596 Patent Op. CC Br. at 13-14, 20.

⁶³ *Id.* at 14.

⁶⁴ *Id.* at 20.

⁶⁵ *Id.* at 2.

53. As described *infra*,⁶⁶ I disagree with NeoGenomics' constructions.

1. The terms in the phrase are all part of the “performing high-throughput sequencing . . . to obtain sequence reads” step

54. I understand that Dr Lennon opines that “wherein an SNV mutation . . . is detected from the sequence reads” in the claim means “wherein an SNV mutation . . . is determined to be present from the sequence reads.”⁶⁷ I disagree. Dr. Lennon appears to be suggesting that SNV mutations are not detected during sequencing, but rather in a subsequent computational step involving data processing. Dr. Lennon's reading is not supported by the plain claim language, which does not mention a separate computational, noise-reducing, or data processing step after sequencing. Dr. Lennon's interpretation imposes additional requirements that are inconsistent with one of ordinary skill in art's understanding as the claim is drafted.

55. In forming my opinion of the term “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,” I considered, *inter alia*,⁶⁸ the claim as a whole, including its language, structure and formatting. It is my opinion that the claim is divided into three main steps designated as “(a)”, “(b)”, “(c),” respectively. Each of these steps is separated by a semicolon. Under step “(c),” the claim is further

⁶⁶ See *infra* Section VIII.A and cited references therein.

⁶⁷ See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 31.

⁶⁸ See Section V *supra*.

divided into two sub-steps each presented as a separate indented paragraph and separated by a semicolon. Regarding the latter sub-step, there exists a “wherein” clause, which is preceded by a comma and not introduced by separators used earlier in the claim (*e.g.*, a semicolon, independent paragraph).

56. One of ordinary skill in the art would have understood that in the context of the structure, formatting, and language of Claim 1 of the '596 Patent (Babiarz) as a whole, that the “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” term all pertains to a single step, not two separate steps as NeoGenomics and Dr. Lennon assert. One of ordinary skill in the art would have understood that the “wherein” clause modifies the step of “performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads.”

57. I note that Dr. Lennon testified⁶⁹ that he did not take into consideration the structure and formatting of the claim (*i.e.*, presence of the “wherein” clause in Claim 1(c) of the '596 Patent would not make a difference), which is inconsistent with how one of ordinary in the art would have approached understanding the claim term.

⁶⁹ See Doc. 378-4, Lennon Jan 30, '25 Tr. at 19:11–20:20, citing testimony at 20:17–20 (“No, again, because I would – I;m reading ***it*** with a -- with a scientific lens, I guess, a technical lens. So, to me, ***it*** wouldn’t make a difference.”) (where the bold and italicized “it” refers to the “wherein” clause at 20:9.

2. The “wherein” clause informs the mechanics of how the high-throughput sequencing is performed

58. Dr. Lennon asserts that “a skilled person in the field of the ’596 patent would have a deep understanding of genomics, including nucleic acid detection,”⁷⁰ and further that “a skilled person would stay apprised of recent literature on genomic assays, such as multiplex PCR, next-generation nucleic acid sequencing techniques, [and] methods for detecting SNVs.”⁷¹ Dr. Lennon, however, does not appear to apply these skill sets in forming his opinions about what detection means and when it occurs in the context of high-throughput sequencing, particularly following targeted multiplex amplification, as described *infra*. For this reason, Dr. Lennon’s interpretation of the plain and ordinary meaning of the “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” term is incorrect.

(a) *Detection Occurs During Sequencing*

59. Dr. Lennon devotes much of the Lennon ’596 Patent Op. CC Decl. to the proposition that “[t]he process of obtaining [] 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.”⁷² Dr. Lennon further opines that “a series of steps after obtaining

⁷⁰ See Doc. 374, Lennon ’596 Patent Op. CC Decl. at ¶ 14.

⁷¹ *Id.* at ¶ 15.

⁷² *Id.* at ¶ 20; see also *id.* at ¶¶ 21, 24

sequence reads are required to detect sequence variants from the sequence reads,”⁷³ including alignment and mapping to a reference sequence and reducing background noise.⁷⁴ Dr. Lennon’s opinions are flawed because (i) he explains how sequence reads from whole genome sequencing are analyzed,⁷⁵ which is not relevant for targeted multiplexed PCR methods and (ii) he does not take into account the full context of the claim, which is about identifying and targeting specific SNV loci from a plurality of target loci specific to the subject have already been identified [*i.e.*, see Claim 1(b) of the ’596 Patent (Babiarz)].

60. As shown *infra* in bold and underline, the claim recites multiple steps that are directed to a specific workflow of identifying SNV from a tumor biopsy sample and targeting those identified SNV loci in cell-free DNA. Those steps are highlighted in Claim 1 of the ’596 Patent (Babiarz) *infra*.

(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:

⁷³ *Id.* at ¶ 22.

⁷⁴ *Id.* at ¶¶ 24–30.

⁷⁵ *Id.*

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.**⁷⁶

61. One of ordinary skill in the art would have understood that the claim language requires identifying a plurality of tumor-specific SNVs from a tumor biopsy sample [step 1(a)], determining a plurality of target loci each having known locations in the genome spanning an SNV from that sample [step 1(b)], and amplifying those targeted SNV loci using target-specific primers in the same multiplex PCR reaction volume [step 1(c)]. After identifying the specific SNV loci having known locations in the genome in steps 1(a) and 1(b) of the '596 Patent (Babiarz), I discuss *infra* how and why step 1(c) contributes to providing orientation when a sequence read is being generated to know what SNV locus is being read without subsequent data processing.

62. For example, steps 1(a) and 1(b) of the '596 Patent (Babiarz) requires identifying tumor-specific mutations comprising SNVs and determining the locations in the genome of the SNVs, wherein the target locus spans a tumor-specific mutation. In targeted multiplex PCR, target-specific primers operate in the same manner as in described

⁷⁶ See Doc. 352-10, '596 Patent (Babiarz) at 171:54–172:14.

supra,⁷⁷ whereby each primer-pair can be designed to flank the already determined SNV locus on either side by specifically hybridizing to known genomic sequences adjacent to the SNV locus of interest.⁷⁸ One of ordinary skill in the art would have understood that, generally speaking, primers are about 18-25 bases in length⁷⁹ and cell-free DNA fragments are about 140-170 bases in length.⁸⁰

63. Multiplex PCR requires at least two or more sets of target-specific primers, and can include dozens of different primer sets in the same reaction. The mixture of many target-specific primers with different sequences allows targeting of many sequences with known locations within the template DNA, and results in amplification of a plurality of specific target loci. Notably, in designing primer sequences, in addition to knowing the identity and length of the amplicon on the target sequence, one of ordinary skill in the art would have also known the precise location of the SNV position relative to the start and stop positions in the amplicon. In other words, one of ordinary skill in the art would have understood what each target amplicon sequence will be, the start and stop positions in those

⁷⁷ See Section VI.B *supra*.

⁷⁸ See Sections VI.A and VI.B. *supra*, whereby specific hybridization of primer sequences are based on Watson-Crick base-pairing rules and the designed positioning of the primer sequences for any given loci determine the start and stop positions of the amplicon.

⁷⁹ See Doc. 141-6, Metzker (2006) at 380.

⁸⁰ See Doc. No. 17-7, NEOGEN00017097–00017109, 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)”) at p. 1.

amplicons, and the exact position within each amplicon of where the SNV mutation is located.

64. Following performing multiplex PCR amplification in a single reaction volume on the plurality of SNV target loci using the specifically designed target-specific primers, one of ordinary skill in the art would have understood that they need to create a library prior to high-throughput sequencing, as described *supra*.⁸¹ Library generation involves the attachment of sequencing adaptors specific to the NGS platform on either end of the amplification products (*e.g.*, P5 and P7 are the sequencing adaptors for Illumina sequencers).⁸² Sequencing adaptors have a known common sequence and length and are incorporated at the ends of the amplification products, enabling the sequencer to read to the exact start and stop positions in amplicons derived from the same target loci. The ability to generate sequence reads that start and stop at the same position coupled with the targeted multiplex PCR to amplify specific sequences containing SNV loci having the same start and stop positions would have enabled one of ordinary skill in the art to know the identity of the sequence read and the SNV mutation as it is being generated in the sequencer.

65. The notion that alignment and mapping is not always required to know the location of the sequence read in the genome is further supported by one of the references

⁸¹ See Section VI.C. *supra*.

⁸² *Id.*

on which Dr. Lennon relies describing that sequence reads from amplicons have the same start and stop positions.

Since the data utilized in this approach was generated from amplicons with the **same starts and stops to every read**, indels affecting only one mate, and therefore demonstrating discordant read counts over the amplicon, could be visually detected by reviewing the mapped data in the Integrative Genomics Viewer...⁸³

To help **identify amplicons with a large fraction of unmapped reads**, we developed a tool called GarbagePicker. Since every **amplicon contains reads with the same start and stop primer sequence, we were able to capture flag-value statistics (i.e., both mates mapped, one mate unmapped, both mates unmapped) for every amplicon read based upon searching for its primer sequences** in the post-alignment data..⁸⁴

66. For additional specificity, barcodes and unique index tags can be incorporated during library preparation to help distinguish the unique SNV loci target sequences as sequence reads are being generated. Assigning barcodes that are specific to a target loci containing a SNV mutation is well within the skill set of the ordinary artisan. For example, Akhras (2006) explains that a unique barcode can contain both the patient ID and the genotype (i.e., a SNV mutation) that can be used to simplify the downstream analysis in sequencing applications.

The unique barcode now carries two dimensions of information, i.e. genotype and patient ID. Post-reaction CIPers can be combined into multiple patient pools and all screened simultaneously, **essentially**

⁸³ Doc. 374-5, Daber *et. al.*, *Understanding the limitations of next generation sequencing informatics, an approach to clinical pipeline validation using artificial data sets*, CANCER GENETICS, 441–448 (2014) (“Daber (2014)”) at 444 (emphasis added).

⁸⁴ *Id.* (emphases added).

lowering costs involved with downstream validations, both for hybridization-based techniques and sequencing procedures.⁸⁵

67. As described *supra*,⁸⁶ in Illumina sequencing instruments, a sequence read is generated one base at a time through iterative cycles in the sequencer. The Illumina SBS sequencing system works this way because only one complementary base is added at each cycle to the a template strand corresponding to the amplified SNV target loci. For each position in the amplicon sequence, the sequencer captures images and detects fluorescence corresponding to each incorporated nucleotide and a base call is made at that position in the amplicon sequence. The sequencer also assigns a quality score indicate the likelihood the call is correct.⁸⁷ The quality score will indicate the probability that the base corresponding to the SNV mutation is called correctly when the sequencer reaches this position in the growing sequence read on the amplicon sequence. This is consistent with the '596 Patent (Babiarz)'s definition of sequence read, which specifically explains that “a quality score at each base position of the sequence indicating the probability that nucleotide has been called correctly.”⁸⁸ One of ordinary skill in the art would have understood that, as soon as the base call is made for a sequence read at the SNV mutation position, the sequencer detects whether the tumor-specific SNV is present or not.

⁸⁵ See Ex. 7, Akhras *et al.*, *Connector inversion probe technology: A powerful one-primer multiplex DNA amplification system for numerous scientific applications*, PLOS ONE 2:e915pp. 1–6 (2007) (“Akhras (2007)”) at 5 (Figure 6S legend) (emphases added).

⁸⁶ See Section VI.C *supra* for more details.

⁸⁷ *Id.*

⁸⁸ See Doc. 352-10, '596 Patent (Babiarz) at Col. 31:16–24.

68. In summary, target SNVs and their precise positions are known and selected in advance, and target-specific primers are designed so that SNV mutations occur at specific locations (*e.g.*, see the example *supra* that the SNV mutation occurs at cycle 12 of the SBS method). Through targeted multiplex PCR in the same reaction volume, these targeting primers selectively amplify the plurality of target loci and create amplicons containing SNV mutations at known locations, which are then detected by sequencing. One of ordinary skill in the art would have already had an expectation of the location of the tumor-specific mutation at each target SNV locus prior to sequencing. Thus, one of ordinary skill would have understood that, in the context of the claimed methods, tumor-specific SNVs contained in the cell-free DNA are detected as soon as the SBS cycle reaches the position where the SNV mutation is located in the growing sequence reads that the sequencer generates.

69. Neither NeoGenomics or Dr. Lennon acknowledge that the claimed method involves identifying and targeting specific loci that contain SNV mutations (as described *supra*) and how this would have necessarily informed one of ordinary skill in the art what to look for in the sequence reads. One of ordinary skill in the art would have understood that the target loci spanning each SNV are unique and had been previously identified and, therefore, their location in the genome is known from steps 1(a) and 1(b) of the Claim of

the '596 Patent (Babiarz), which provides orientation as sequence reads are being generated.⁸⁹

(b) *Dr. Lennon's Previous Testimony*

70. One of ordinary skill in the art would have understood that base calling as described *supra*⁹⁰ is detection—put simply, detection happens in the sequencer from the growing sequence read. Several examples of Dr. Lennon's testimony support this understanding of what one of ordinary skill in the art would have had.

71. For example, the Lennon '596 Patent Op. CC Decl.⁹¹ and recent Dr. Lennon's deposition testimony shown *infra* supports the understanding that detection occurs in the sequencer from a growing sequence read.

Q Could you explain to me what occurs as a process of sequencing in the high-throughput sequencer?

A So there's many types of sequencing, but as a -- as a general application, a sample that has been prepared for sequencing is first loaded onto the substrate that the Illumina sequencer uses, which we call a flow cell. The flow cell is loaded into the instrument itself.

The Illumina sequencer uses a process called sequencing by synthesis. . . .

⁸⁹ See Ex. 2, *TruSeq RNA and DNA Sample Preparation Kits*, Illumina, Inc. (2010); Ex. 3, *TruSeq RNA and DNA Sample Preparation Kits*, Illumina, Inc. (2010).

⁹⁰ See Section VI.C *supra*.

⁹¹ See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 19 (“***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.”) (emphases added).

Your template molecule, the molecule you're trying to sequence, which has gone through sort of preparation on the surface of the flow cell is ready -- is ready and, essentially, has a primary attached to it. And a polymerase is present in the mix.

If -- you know, whichever of the four labeled nucleotides is complementary to the first single-stranded template piece of DNA, ***whichever base is complementary to that will get incorporated.*** So this is the synthesis part, just polymerase doing what it does, their nucleotides are chemically modified ***so that just a single -- you know, a single base extension happens at each step,*** another nucleotide can't attach on and continue the growing strand of DNA as it would in a normal PCR.

And after that incorporation event, a -- there's a laser excitation of the surface of the flow cell which is essentially an optically clear glass slide, and a -- ***the camera, essentially, takes a photograph or, essentially, you know, detects any emission spectra or any, you know, signal that comes off of those incorporated bases.***

The computer then essentially looks at the emission spectra, determines which of the four bases had been incorporated at that site. It digitizes this information into a base call, and then it associates some other information with that base call based on the -- sort of the macro-environment, you know, how confident it is in that base based on the signal to noise of the ratio at that incorporation event.

Then a -- after that -- you know, that first base incorporation has been recorded across -- it does it in tiles across the surface of the flow cell because it's actually quite a large area to cover, then they flow across a -- you know, they wash out the unbound nucleotides and they flow in, you know, chemical treatment to remove the -- you know, the terminator molecule. ***And then that allows another base to be incorporated, and so then they flow in more fluorescent bases.***

The next base gets incorporated. They detect that, record it as a base call and so on and so forth for many cycles until you reach the, you know, predetermined cycle number that you've set on the particular sequencer. In Illumina, that's somewhere between 150 to 300 incorporation events.

And it's then producing data as it goes along which are digital files which incorporate what the bases are in this contiguous stretch that

we call a sequencing read and then the associated sort of quality information with those base calls in the sequencing read.

And then -- so that's -- at the high level, that's the sequencing on Illumina.⁹²

72. Additionally in this proceeding, Dr Lennon opines that “wherein an SNV mutation . . . is detected from the sequence reads” has a plain and ordinary meaning that is “wherein an SNV mutation is determined to be present from the sequence reads,”⁹³ Dr. Lennon does *not* use this same interpretation in the co-pending pre-institution IPR proceeding of the '596 Patent (Babiarz). In that IPR proceeding, Dr. Lennon explains that that he understands “that the words of the claims of a patent are generally given their plain and ordinary meaning,”⁹⁴ and in applying that understanding, Dr. Lennon never equates “detected” with “determined to be present.”⁹⁵ Instead, Dr. Lennon acknowledges that high-throughput sequencing platforms use “sequencing and detection biochemistry . . . to produce sequence reads,”⁹⁶ consistent with one of ordinary skill in the art’s interpretation that sequencing and detection would have been part of one high-throughput sequencing step.

⁹² See Doc. 378-4, Lennon Jan. 30, '25 Tr. at 22:1–25:3 (emphases added, objection omitted).

⁹³ See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 31.

⁹⁴ See Doc. 378-3, IPR2025-00455, Exhibit 1004, *Expert Declaration of Niall Lennon, Ph.D. in Support of Petition for Inter Partes Review of U.S. Patent No. 11,319,596, NeoGenomics Laboratories, Inc. v. Natera, Inc.*, (P.T.A.B. Jan. 15, 2025) [hereinafter “Lennon '596 Patent IPR Decl.”] at ¶ 15.

⁹⁵ *Id.* at ¶¶ 16–19.

⁹⁶ *Id.* at ¶ 81.

73. Finally, in a prior case, *ArcherDx v. Qiagen LLC*, Dr. Lennon served as Archer Dx's technical expert and I served as Qiagen's technical expert. In that case, Dr. Lennon also testified that detection occurred during sequencing:

Q. And a sequencing primer is a primer, another piece of DNA, that on the Illumina sequencer comes in and does something; right?

A. Correct.

Q. And on that universal oligo tail adaptor, there has to be sequences of two different sequencing primers; right?

A. I believe that's correct.

Q. And one of the sequencing primers that you have identified is something called a P7 sequence; right?

A. Identify a sequence that is identical to the P7.

Q. P7 is just a name. It doesn't have a scientific significance; right? It's just a name?

A. That's the name of it, correct.

Q. So there is the P7 and then there is another sequence that you have identified called the read -- I believe it's the Read 2 sequence; correct?

A. I believe that's correct.

Q. And you're saying those are both sequencing primers; right?

A. Yes.

Q. They have totally different functions; right?

A. Can you elaborate.

Q. You don't understand that question?

A. Could you make it more specific.

Q. Sure, I'll try to help you out. The Read 2 sequencing primer, that primer gets used during the -- when it's in the machine, and when it

lands, it helps -- it actually is part of the process that spits out hey, next one is an A, next one is a G, next one is a T, next one is a C. So it actually -- it's actually a participant in the actual thing where a sequence is getting spit out of the machine; right?

A. ***Right. We call that the detection step.***

Q. So the sequencing step; right?

A. ***It's where we're doing the interrogation of the colors to understand what bases are there.***

Q. Again, an easy concept. That's where the actual sequencing is taking place, where the letters are read out; right?

A. Again, "sequencing" is a very broad term.

Q. Dr. Lennon, simple question. That's the step where the letters actually get read out; right?

A. ***That is correct.***⁹⁷

(c) *The Specification Does Not Support Substituting the Term "Determined to be Present" For the Term "Detected"*

74. Both NeoGenomics and Dr. Lennon draw a false equivalency between "detected" and "determined to be present."⁹⁸ Dr. Lennon and NeoGenomics cite to disclosures in the specification that are directed to various predictive models to determine a set of probabilities that SNVs result from real mutations or background error models for

⁹⁷ See Doc. 378-1 (trial testimony of Dr. Niall Lennon from *ArcherDX, LLC v. QIAGEN Sciences, LLC*, 1:18-cv-01019, D.I. 487 (D. Del. Aug. 25, 2021)) at 593:23–595:15 (emphases added).

⁹⁸ See Doc. 373, NeoGenomics '596 Patent Op. CC Br. at 15–16; see also Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 35.

noise,⁹⁹ despite Dr. Lennon acknowledging that the claims do not recite noise.¹⁰⁰ None of these disclosures recite sequencing or are related to the claimed steps of identifying and targeting SNVs beforehand. Dr. Lennon does not cite to a single embodiment from the '596 Patent (Babiarz) specification that is covered by the claims where “detect” is used as a synonym for “determined be present.” For this reason, it is my opinion that Dr. Lennon’s interchangeable use of “determined to be present” with “detected” clause lacks intrinsic support.

(d) *Claim 12*

75. Dr. Lennon contends that Claim 12 of the '596 Patent (Babiarz) supports his opinion that detecting SNVs means “actually calling the SNV.”¹⁰¹ I disagree. First, the full language of Claim 12 of the '596 Patent (Babiarz) recites “[t]he method of Claim 1, wherein the method **further comprises** detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.”¹⁰² One of ordinary skill in the art would have understood that “further comprises” means that “detecting recurrence and/or metastases” is *not* part of the actions recited in Claim 1 of the '596 Patent (Babiarz). Dr. Lennon testified at his deposition that “further comprises” refers to “in addition it also

⁹⁹ *Id.*

¹⁰⁰ See Doc. 378-4, Lennon Jan. 30, '25 Tr. at 34:15-16.

¹⁰¹ See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 38; *see also id.* at ¶ 39.

¹⁰² See Doc. 352-10, '596 Patent (Babiarz) at 172:44–47 (Claim 12).

includes,”¹⁰³ which undermines the opinion the Lennon ’596 Patent CC Decl. about how “further comprises” informs the “detected” language in Claim 1 of the ’596 Patent (Babiarz). Second, neither Claim 1 nor Claim 12 of the ’596 Patent (Babiarz) require a specific number or proportion of the plurality of SNVs to be called correctly—Dr. Lennon agreed there is no claim requirement for correctness at his deposition.¹⁰⁴

(e) *Prosecution History*

76. I understand that Dr. Lennon refers to the “prosecution history” of the ’596 Patent (Babiarz) in forming his opinions that “wherein an SNV mutation . . . is detected from the sequence reads” in the claim means “wherein an SNV mutation . . . is determined to be present from the sequence reads.”¹⁰⁵ However, Dr. Lennon testified that he has not reviewed the entirety of the prosecution history of the ’596 Patent (Babiarz), but only the two documents attached as Exhibits 9 and 10 to his Declaration, which refer to a Response to the June 1, 2021 Office Action and the published application of the ’596 Patent (Babiarz) (i.e., the ’758 Patent Pub. (Babiarz), respectively.¹⁰⁶ Dr. Lennon further testified that he is

¹⁰³ See Doc. 378-4, Lennon Jan. 30, ’25 Tr. at 37:9–38:4. (“So, again, it -- ***it informs my opinion*** to the extent of understanding the plain English meaning of ‘further comprises.’ ***And, to me, that means sort of ‘in addition it also includes.’***”) (emphases added).

¹⁰⁴ *Id.* at 32:12–15 (“I don’t think Claim 1 has any wording around correctness.”).

¹⁰⁵ See Doc. 374, Lennon ’596 Patent Op. CC Decl. at ¶¶ 40–44.

¹⁰⁶ See Doc. 378-4, Lennon Jan. 30, ’25 Tr. at 58:23–60:21; *see also* Doc. 374-9, Response to June 1, 2021 Office Action; Doc. 374-10, Babiarz *et al.*, *Detecting mutations and ploidy in chromosome segments*, U.S. Patent Application Publication No. US 2019/0194758 (2019) (the “’758 Patent Pub. (Babiarz)”).

“not familiar with an Office Action” nor an Office Action Response¹⁰⁷. I disagree that the cited portions of the prosecution history support Dr. Lennon’s opinion that “detect” is synonymous with “determined to be present.”

77. Regarding the Response to the June 1, 2021 Office Action,¹⁰⁸ I disagree with Dr. Lennon’s characterization and opinion. In amending the claims to add multiple limitations, Natera cited multiple paragraphs from the specification (*i.e.* ¶¶ [0052], [0060], [0412], and [0538] from the ’758 Patent Pub. (Babiarz).

78. Dr. Lennon first focuses on ¶ [0052] to draw a false equivalency between detect and determine, citing a portion of the sentence “[i]n 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.”¹⁰⁹ The portion of the sentence omitted by Dr. Lennon is telling, and confirms that this disclosure refers to estimating efficiency and the error rate per amplification cycle, which is not recited or required by Claim 1 of the ’596 Patent (Babiarz). The disclosure relevant to the claim amendment in ¶ [0052] is the last sentence, which explains “[i]n certain embodiments of this method for detecting one or more SNVs the limit of detection is 0.015%, 0.017%, or 0.02%.”¹¹⁰ This passage refers to the method

¹⁰⁷ See Doc. 378-4, Lennon Jan. 30, ’25 Tr. at 60:5–10.

¹⁰⁸ See Doc. 374-9, Response to June 1, 2021 Office Action.

¹⁰⁹ See Doc. 374, Lennon ’596 Patent Op. CC Decl. at ¶ 42 (underlined portion cited).

¹¹⁰ See Doc. 374-10, ’758 Patent Pub. (Babiarz) at ¶ [0052].

of Claim 1 of the '596 Patent (Babiarz) overall and directly supports the “SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA” limitation that was added during the amendment.¹¹¹ One of ordinary skill in the art reading the specification, claim amendments, and Response to the Office Action would have understood that the Applicants of the '758 Patent Pub. (Babiarz) did not equate the terms detect and determine, as suggested by Dr. Lennon.

79. Dr. Lennon next focuses on ¶ [0060] of the '758 Patent Pub (Babiarz) to draw a false correlation between detect and aligning sequence reads to a reference.¹¹² Paragraph [0060] explains “[i]n certain embodiments of this method for detecting one or more SNVs the sequencing run comprises a multiplex amplification reaction performed under limited primer reaction conditions. In certain embodiments of this method for detecting one or more SNVs the limit of detection is 0.015%, 0.017%, or 0.02%.”¹¹³ Nothing in ¶ [0060] mentions alignment of sequence reads to a reference sequence.

80. Dr. Lennon asserts that the “preceding paragraphs describe embodiments of SNV detection, all of which involve analyzing sequence read.”¹¹⁴ However, the Applicants of the '758 Patent Pub. (Babiarz) did not rely on the “preceding paragraphs” in support of its amendment. Moreover, Dr. Lennon misapprehends how one of ordinary skill in the art

¹¹¹ See Doc. 374-9, Response to June 1, 2021 Office Action at p. 2.

¹¹² See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 43.

¹¹³ See Doc. 374-10, '758 Patent Pub. (Babiarz) at ¶ [0060].

¹¹⁴ See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 43.

would have understood the '596 Patent (Babiarz) specification. In my opinion, one of ordinary skill in the art would have interpreted ¶ [0060] in the context of the '758 Patent Pub. (Babiarz) as a whole and the claimed method, not simply limited to its proximity to preceding paragraphs.

81. Finally, Dr. Lennon focuses on arguments Natera made in response to the Patent Examiner's rejection of the pending claims based on the '396 Patent Pub. (Diehn).¹¹⁵ Natera explained the following.

In addition, Diehn also fails to teach or suggest “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.” 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). Further, Murtaza discloses detection of mutant allele frequency (AF) between 3% to 34% in patient plasma samples (Table 1).¹¹⁶

82. Dr. Lennon opines that “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.”¹¹⁷ I disagree. While Natera quoted disclosure from the '396 Patent Pub. (Diehn), Natera did

¹¹⁵ *Id.* at ¶ 44; see also Doc. No. 374-11, Diehn *et al.*, *Identification and use of circulating nucleic acid tumor markers*, U.S. Patent Application Publication No. US 2016/0032396 (2016) (the “'396 Patent Pub. (Diehn)”).

¹¹⁶ See Doc. 374-9, Response to June 1, 2021 Office Action at p. 7.

¹¹⁷ See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 44.

not distinguish Claim 1 of the '596 Patent (Babiarz) based on accuracy, as evidenced by the fact that the allowed claim does not recite anything about a threshold or number of SNVs that must be called correctly. Notably, Dr. Lennon agrees that Claim 1 of the '596 Patent (Babiarz) does not require correctness:

Q Does Claim 1 require that a certain proportion of SNVs be called correctly?

A ***I don't think Claim 1 has any wording around correctness.***¹¹⁸

83. Overall, Dr. Lennon's analysis of the prosecution history does not support his contention that "detected" is synonymous with "determined to be present." Nothing in the disclosures Dr. Lennon's cites are inconsistent with one of ordinary skill in the art's understanding that the "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" informs how the high-throughput sequencing would be performed.

(f) *Purpose of Claimed Invention*

84. Dr. Lennon contends that Natera's construction "does not require that an SNV mutation—indicative of cancer—is determined to be present from the sequence reads"¹¹⁹ and is thus incompatible with the intended purpose of Asserted Natera Claims. I disagree for the reasons explained *infra*.

85. First, Dr. Lennon does not correctly opine on the scope of the intended purpose. Dr. Lennon contends that "the purpose of the purported invention claimed in the

¹¹⁸ See Doc. 378-4, Lennon Jan. 30, '25 Deposition Tr. at 32:12–15 (emphasis added).

¹¹⁹ See Doc. 374, Lennon '596 Patent Op. CC Decl. at ¶ 38.

'596 patent is to 'monitor[] the progression of cancer.'"¹²⁰ I disagree. The purpose of the claimed invention is stated in the preamble, which in full context, recites "[a] method for preparing biological samples useful for monitoring the progression of cancer in a subject."¹²¹

86. Second, base calls made in the sequencer already contain a quality score as a measure of their correspondence to an SNV, as described *supra*.¹²²

87. Third, Claim 1 of the '596 Patent (Babiarz) does not recite a specific threshold amount or proportion of tumor-specific SNV loci that must be called correctly among the plurality of SNV mutations. Claim 1 of the '596 Patent (Babiarz) only requires that "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." One of ordinary skill in the art would have understood that a sequencer detecting one SNV from a single sequence read among 7,000 other reads of that same position—which amounts to 1/7000 or about 0.014%— is sufficient to meet the Claim 1. Dr. Lennon's opinion in the Lennon '596 Patent IPR Decl. is consistent with this understanding:

A POSA would understand that detecting one mutant template among 8,000–80,000 genome equivalents (1/8,000–1/80,000) in a single reaction would be equivalent to detecting an SNV that is present in 0.0125%–0.00125% of the cfDNA having the SNV locus, which is less than 0.015%. To illustrate this concept: ***if a reaction contains at least 7,000 copies of a particular cfDNA template locus*** (e.g., 7,000 genome equivalents) and ***only one of those copies is a mutant copy, then a POSA would understand that the single mutant***

¹²⁰ *Id.*

¹²¹ See Doc. 352-10, '596 Patent (Babiarz) at 171:51–53 (Claim 1 preamble).

¹²² See *supra* Section VI.C and VIII.A.2.(a).

copy of that template would be present in less than 0.015% of the cfDNA containing the locus. This is because 1/7,000 is about 0.014%, which is less than 0.015%.¹²³

88. Dr. Lennon contends that “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.”¹²⁴ Dr. Lennon does not cite any evidence in support of his contention. To the extent NeoGenomics and Dr. Lennon assert that scientists would obtain and combine ctDNA samples from a patient at different time points, it is my opinion that this too is unsupported. This assertion would also be inconsistent with the intended purpose of Claim 1 of the ’596 Patent (Babiarz) claims because it would not enable monitoring cancer progression over time, which necessarily requires that samples from different timepoints are analyzed separately.

3. “the amplified DNA” may be modified before being sequenced.

89. I understand that NeoGenomics proposes the following construction for the term “performing high-throughput sequencing of the amplified DNA” as “[p]lain and ordinary meaning, which is performing high-throughput sequencing of the amplified DNA obtained from the targeted multiplex PCR amplification step.”¹²⁵ I understand further that NeoGenomics contends this term requires sequencing to be “performed on the amplified

¹²³ See Doc. 378-3, Lennon ’596 Patent IPR Decl. at ¶ 240 (emphases added).

¹²⁴ See Doc. 374, Lennon ’596 Patent Op. CC Decl. at ¶ 18.

¹²⁵ See Doc. 373, NeoGenomics ’596 Patent Op. CC Br. at 20.

DNA from the earlier step (rather than other DNA that has been altered by intervening amplification), ...”¹²⁶

90. I understand that Dr. Lennon will be submitting a second declaration in which he opines on the meaning of “performing high-throughput sequencing of the amplified DNA.”¹²⁷ Dr. Lennon testified that “depending on what the intervening steps are, [] the thing you perform high-throughput sequencing on may be changed from the amplified DNA that’s described in the first sub-clause.”¹²⁸ According to Dr. Lennon, “if you were to perform another PCR after the multiplex PCR to add those adapter sequences, that -- for the reasons I’ve spoken about, that you may not be then sequencing the same amplified DNA that’s described in the first step because you will have ***changed the composition of the mix of what’s in there***”¹²⁹. Dr. Lennon stated that “some amplicons could drop out,” or there may be errors due to a subsequent amplification.¹³⁰

91. I disagree with NeoGenomics’ position and Dr. Lennon’s anticipated opinion. One of ordinary skill in art would have understood that the term “performing high-throughput sequencing of the amplified DNA” permits intervening steps between multiplex PCR and high-throughput sequencing, particularly where those steps are modifications required for sequencing.

¹²⁶ *Id.* at 2.

¹²⁷ *See* Doc. 378-4, Lennon Jan. 30, ’25 Tr. at 54:17–57:23.

¹²⁸ *Id.* at 55:23–55:6, citing testimony at 56:2–.

¹²⁹ *Id.* at 56:7–17, citing testimony at 56:11–17 (emphasis added).

¹³⁰ *Id.* at 46:6–48:3, citing testimony at 47:12–48:3.

92. I incorporate by reference my opinions in my Reply PI Declaration at ¶¶ 270-274 with respect to the '454 Patent (Babiarz), which are applicable to this term due to the similar claim language and the fact that the '454 Patent (Babiarz) and '596 Patent (Babiarz) share the same specification.

93. One of ordinary skill in the art would have understood that sequencing amplified DNA could require subsequent ligation or universal amplification to add sequencing adaptors (*e.g.*, sequencing adaptors are required for sequencing on an Illumina sequencer, which the '596 Patent (Babiarz) teaches can be used for the sequencing step).¹³¹ I disagree with Dr. Lennon's contention that an intervening step between targeted multiplex PCR amplification and sequencing (such as the aforementioned ligation or universal PCR step) would change the composition of what is sequenced such that the sequencer would not detect SNV mutations from amplicons created by the targeted multiplex PCR step.

94. 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 SNV

¹³¹ See Section VII.B *supra*; see also Section VI.C.

mutations will not be diluted. Therefore, universal PCR will amplify the final products from the targeted multiplex PCR step.

95. Dr. Lennon testified that the composition will not change under certain conditions, which undermines his opinion that intervening steps, such as amplification or ligation would be excluded from the scope of the claim:

Q Your testimony is that “It’s a possibility that for some target loci, they do not receive a sequencing adapter in a subsequent amplification.”

And my question is: What happens to the other target loci?

A So if there are target loci from the first reaction that are well-represented that you then put into a subsequent PCR amplification to add adapter sequences, for instance, they will get copied, amplified, and ***the amplicons that are created will have, you know, presumably the correct adapters appended to the tails.***¹³²

Q In the case of ligation, the target loci sequences are attached to sequencing adapters, correct?

A So, again, you know, it is typical to take a product and make sure you’re adding the right amount to a ligation reaction. So the same possibility exists that even in a ligation-based addition, you may not - you may lose some molecules from the first reaction.

The molecules that are there, in theory, although ligation is not a 100 percent efficient process either, ***you would expect the majority of the molecules that are present to have adapters ligated.***¹³³

Thus, Dr. Lennon’s testimony supports my opinion that one or more intervening steps between targeted multiplex PCR amplification and sequencing would *not* change the

¹³² See Doc. 378-4, Lennon Jan. 30, ’25 Tr. at 52:20–53:8 (emphasis added).

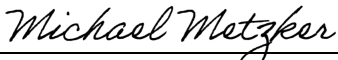
¹³³ *Id.* at 53:21–54:8 (emphasis added).

amplicons such that that the sequencer would not detect SNV mutations from amplicons created by the targeted multiplex PCR step.

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: February 3, 2025



Michael Metzker, Ph.D.