

**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 LENNON, PH.D., IN SUPPORT OF
DEFENDANT NEOGENOMICS LABORATORIES, INC.'S RESPONSIVE
CLAIM CONSTRUCTION BRIEF REGARDING U.S. PATENT NO. 11,319,596**

I, Niall Lennon, declare as follows:

1. I have been asked to provide my expert opinions in response to certain issues in Natera's Opening Claim Construction Brief and opinions by its expert, Dr. Michael Metzker. *See* Dkt. 372, Plaintiff Natera, Inc.'s Opening Claim Construction Brief Regarding U.S. Patent No. 11,319,596 (hereinafter "Natera's Op. CC Brief").

2. I base this declaration on information currently available to me. I reserve the right to supplement my opinions and to rebut any opinions provided by Natera's expert(s). I am able and willing to testify regarding the matters stated herein, if asked to do so by the Court.

3. I previously submitted a Declaration in the above-captioned litigation in connection with Defendant NeoGenomics, Inc.'s ("NeoGenomics") opening claim construction brief for U.S. Patent No. 11,319,596 (the "'596 patent"). *See* Dkt. 374, Declaration of Niall Lennon, Ph.D., on Claim Construction, dated January 21, 2025 (hereinafter, my "Opening Claim Construction Declaration"). I incorporate my Opening Claim Construction Declaration 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 '596 patent.

4. I understand that Natera asserts the '596 patent and U.S. Patent No. 11,530,454 ("the '454 patent") against NeoGenomics. The '596 and '454 patents are in the same family and share the same specification. NeoGenomics previously filed an IPR petition challenging the validity of the '454 patent based on the Forsheew prior art reference.

I provided a declaration in support of that IPR petition. In that IPR proceeding, Natera distinguished the challenged claims of the '454 patent from the Forshew prior art on the basis that its author “plainly did consider and reject use of multiplex amplification alone[.]” Dkt. 366-2 at 22. Specifically, Natera pointed to two amplification steps in the Forshew prior art that occurred after targeted multiplex amplification and before sequencing to support this statement: (1) a “single-plex PCR using a Fluidigm microfluidic system ‘to purify and select for intended targets’” and (2) “Then, additional sequencing adaptors and a barcode are added via additional PCR steps.” *Id.* at 13, 23. Polymerase chain reaction (PCR) is a method used to make copies of a specific pieces of DNA sample. Scientists use PCR amplification to amplify a small amount of DNA to get a sufficient amount to perform detailed studies of the DNA sample. Amplicons are copies of the DNA sample produced by the PCR amplification process. Accordingly, in the IPR petition, Natera took the position that a subsequent intermediate PCR amplification would lead to amplified DNA that is no longer the same as the amplified DNA obtained from the initial target amplification step.

5. I have reviewed Natera’s Opening Claim Construction Brief (Dkt. 372) and supporting documentation and NeoGenomics Opening Claim construction brief and supporting documentation (Dkt. 373-374). The parties dispute the meaning of the phrase “performing high-throughput sequencing of the amplified DNA” in claim 1 of the '596 patent. Specifically, the parties disagree on whether the proper construction of that term excludes intermediate PCR amplification steps between the targeted multiplex

amplification and high-throughput sequencing steps recited in claim 1 shown below:

(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.

6. I understand below are the parties' proposed constructions:

Natera's Proposed Construction	NeoGenomics' Proposed Construction
Plain and ordinary meaning, which permits intermediate steps after multiplex amplification but before sequencing.	Plain and ordinary meaning, which is performing high-throughput sequencing of the amplified DNA obtained from the targeted multiplex PCR amplification step.

7. Natera's Opening Claim Construction Brief (Dkt. 372) argues that the '596 patent's "performing high-throughput sequencing of the amplified DNA" limitation and the '454 patent's "sequencing the amplicons" limitation should be construed consistently. For the purpose of this declaration, I have applied this understanding to my analysis and opinions stated herein.

A. The claim language precludes PCR amplifications after the target multiplex amplification and before sequencing.

8. As an initial matter, the term “the amplified DNA” refers back to the amplified DNA generated by the preceding targeted multiplex amplification step.

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...

’596 patent at 172:1-15 (emphasis added). This claim language precludes additional PCR amplification steps between the targeted multiplex amplification step and the high-throughput sequencing step because intervening PCR amplifications would create new, different amplified DNA to be sequenced.

B. Amplified DNA from an intermediate PCR amplification step is not the same amplified DNA from the initial targeted multiplex amplification.

9. Natera argues the claim permits intermediate PCR amplification steps because amplified DNA obtained from these additional PCR steps still include the same DNA sequences as “the amplified DNA” from the initial targeted multiplex amplification step. Natera Op. CC Brief at 19 (citing Dkt. 145 (Metzker PI Reply Decl.), ¶ 270)). Natera further argues the claim permits intermediate PCR amplification steps because the claim does not require “direct” sequencing of the amplified DNA generated in the initial targeted multiplex amplification step. *Id.* (citing Dkt. 145 (Metzker PI Reply Decl.), ¶ 270). Specifically, Dr. Metzker states, “While the amplified products of the targeted PCR

reaction are further amplified to add sequencing-related sequences (adaptors and barcodes), *the same sequence* generated by targeted multiplex reaction (50-150 bp comprising the target loci) is subjected to sequencing, and sequence reads for that sequence are obtained.” *Id.* (emphasis added).

10. I disagree. The amplified DNA resulting from a subsequent intermediate PCR is not the same amplified DNA from the initial targeted multiplex amplification. That difference is important. This is because the amplified DNA generated by a further PCR amplification would not necessarily be faithful copies—they would not necessarily have the same sequence as “the amplified DNA” obtained from the initial targeted multiplex amplification step. An additional PCR amplification step risks introducing errors—which are not present in “the amplified DNA” obtained from the initial targeted multiplex amplification. Additional PCR amplification steps can also introduce other biases, which means that some of “the amplified DNA” representing target loci from the initial targeted multiplex amplification may not be present in the amplified DNA obtained from the further PCR amplification. When asked by Natera counsel during deposition, I also discussed how further PCR amplification could materially change the composition of the amplified DNA due to the introduced errors and biases. Ex. 13 (Niall Lennon 1/30/2025 Dep. Tr.) at 47:10-48:3. Notably, Natera’s expert, Dr. Michael Metzker, agrees with me. “Additional erroneous nucleotide changes could also be introduced during the PCR amplification steps. . . . Such accumulation of potential false positive errors . . . was known to limit the reliable analysis of true variants.” Dkt. 145 (Metzker Declaration ISO Natera Reply Motion for

Preliminary Injunction) ¶ 95. “[T]he sensitivity of preparing samples of and analyzing variants associated with a patient’s cancer, particularly when using cfDNA as source material, would have been hampered by errors introduced during the sample preparation process Such errors could be introduced at both the amplification and sequencing steps of preparing cfDNA preparations.” *Id.* ¶ 98. Because the amplified DNA produced from an additional PCR amplification step are not accurate copies of the amplified DNA from the initial targeted multiplex amplification, sequencing those inaccurate copies would be materially different from sequencing “the amplified DNA” from the initial targeted multiplex amplification.

C. An intermediate PCR amplification step is not needed to add barcodes and/or sequencing tags.

11. Natera cites Dr. Metzker’s testimony from the preliminary injunction proceedings earlier in the case for its contention that amplified DNA needs to be modified by adding sequence adaptors and may be modified by adding barcodes before sequencing. Natera’s Op. CC Brief at 21. Specifically, the following statements were made by Natera and Dr. Metzker:

POSAs understood that Illumina sequencers require modifying amplified DNA before sequencing can be performed.

Natera’s Op. CC Br. at 21 (citing Metzker PI Reply Decl. ¶ 274; PI Hr’g Tr. at 21:10-22:2).

[B]ased on how high-throughput sequencing works, a POSA would understand that additional steps are necessary before sequencing on Illumina systems . . . [a]nd where samples from multiple sources are run together on the Illumina system . . .

there is another permissible step: adding barcode sequences to amplified DNA before sequencing.

Natera's Op. CC Br. at 22 (citing Metzker PI Reply Decl. ¶ 272 & Dkt. 141-11 (Illumina protocol) at 1).

Claim 1 . . . encompasses a possible universal PCR step to add sequencing adaptors (or other sequencing-related sequences like barcodes) following targeted multiplex PCR in a single reaction volume, which one of ordinary skill in the art would have understood to be necessary for sequencing on certain instruments, including routinely and commonly-used Illumina sequencers . . .

Metzker PI Reply Decl. ¶ 274.

12. I have reviewed these statements and the Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing Protocol (Dkt. 141-11) cited by Natera and Dr. Metzker. Adding sequencing adaptors and barcodes does not need to be performed by additional PCR amplification steps between targeted multiplex amplification and sequencing, as explained below.

13. As an initial matter, barcodes and sequencing adaptors (sometimes called “tags” for sequencing) are DNA sequences that are not themselves part of the human genome. Multiplex PCR amplification involves multiple DNA targets at the same time, so barcodes are often added to DNA amplicons to help identify the amplicons produced from a particular DNA molecule, or to identify amplicons produced from a particular sample. Sequencing tags are sequences that are attached to DNA. For Illumina sequencers, sequencing tags allow the DNA to bind to the surface of the sequencing instrument, called the flow cell.

14. Barcodes are often used in sequencing experiments because it is useful to trace sequence reads back to their original samples during data analysis. Dr. Metzker correctly notes that the tags that allow DNA to bind to sequencing instruments are required for high-throughput sequencing. However, I disagree that these sequence tags need to be added by PCR amplification steps between the targeted multiplex amplification step and the sequencing step. Sequence tags, as well as barcodes, can be attached to amplified DNA during the multiplex amplification. Alternatively, such barcodes and/or tags could be attached directly to the amplified DNA by ligation. I explain both of these options in more detail below.

15. First, sequencing tags barcodes and could be added as part of the targeted multiplex amplification step, rather than in a subsequent and separate PCR amplification step. This is consistent with my review of the '596 patent's specification, which describes embodiments in which barcoding and tagging occur as part of targeted amplification—rather than after targeted amplification and before sequencing. For example, the specification states that the “selective enrichment and/or amplification” step (*i.e.*, the multiplex targeted amplification step) “may involve tagging each individual molecule with different tags, *molecular barcodes*, tags for amplification, and/or *tags for sequencing*.” '596 patent at 98:54-57 (emphases added). The specification thus supports my opinion that barcodes and/or tags for sequencing can be added as part of, rather than after, the multiplex amplification step.

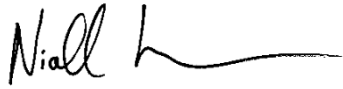
16. Second, barcodes and sequencing tags could also be attached directly to the

amplified DNA from the multiplex amplification using a process called ligation, without using a separate PCR amplification step. Direct attachment of sequencing tags was understood to be advantageous because it avoids errors and amplification biases that are introduced during PCR amplification, as I discussed in ¶ 10, *supra*. See Ex. 14, Kozarewa et al., *Amplification-free Illumina sequencing-library preparation facilitates improved mapping and assembly of GC-biased genomes*, Nat Methods. PMC Author manuscript (2009), 2. For example, full-length adapter tags could be “ligated”—that is, attached—directly to the ends of the amplified DNA generated in the targeted multiplex amplification step prior to sequencing, which would avoid introducing any amplification artifacts that might change the sequences of the amplified DNA. *Id.* at 2-3.

17. Natera also cites Dr. Metzker’s testimony at the preliminary injunction hearing that there is a “necessary amplification step that occurs *as part of sequencing* that makes additional copies of all amplified DNA before their sequences are determined.” Natera’s Op. CC Brief at 21 (emphasis added). I agree that some high-throughput sequencing technologies perform amplification as part of the sequencing process. For example, in certain Illumina sequencers, after DNA with sequencing tags is attached to the flow cell, the sequencer amplifies the attached DNA and makes more copies to facilitate reading the base sequences of the attached DNA. Because this amplification step occurs on the flow cell inside the sequencer as “part of sequencing,” it is irrelevant to whether the claims permit an additional amplification step after targeted multiplex amplification and before sequencing begins. *Id.*

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

Executed on February 3, 2025

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

Niall Lennon, Ph.D.