

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10X GENOMICS, INC,
Petitioner,

v.

BRUKER SPATIAL BIOLOGY, INC.,
Patent Owner.

Case IPR2023-01300
U.S. Patent No. 11,473,142

**DECLARATION OF DR. DAVID H. SHERMAN, PH.D., IN SUPPORT OF
PATENT OWNER'S RESPONSE**

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I. INTRODUCTION

1. I, David H. Sherman, Ph.D., make this Declaration in connection with *inter partes* review proceeding No. IPR2023-01300 concerning U.S. Patent No. 11,473,142 (“the ’142 Patent”). I am over the age of twenty-one and am competent to make this Declaration.

2. I have been retained by counsel for Bruker Spatial Biology, Inc. and Bruker Nano, Inc. (collectively, “Bruker”) in the above-captioned IPR as an independent technical expert to review and evaluate claims of the ’142 Patent. My opinions are based on my skills, knowledge, training, education, and experience in matters of this nature, and my examination of the materials used in preparing this Declaration.

3. I am being compensated on an hourly basis for my work performed in connection with this matter. My compensation does not depend upon the contents of this Declaration, any testimony I may provide, or the ultimate outcome of this matter.

II. BACKGROUND AND QUALIFICATIONS

4. I earned a Bachelor of Arts in Chemistry, with Honors, from the University of California, Santa Cruz in 1978. While an undergraduate student, I participated in research developing a novel gas chromatography mass spectrometry

method to identify and characterize polysaccharide natural products from marine alga under the guidance of Professor Phil Crews.

5. I then earned in my Ph.D. in Organic Chemistry from Columbia University in 1981. My graduate research focused on developing a general method for construction of trans-hydrindanone ring systems, and the total synthesis of 11-keto steroids using an intramolecular Diels-Alder approach.

6. I completed two postdoctoral research projects. At Yale University, I conducted studies on the immunosuppressive drug cyclosporin A to determine its effects on thymocyte populations in mice. At the Massachusetts Institute of Technology, I conducted studies on non-H-2 class I molecules of the mouse major histocompatibility complex. My work at MIT showed that specific subpopulations of T cells expressed structurally diverse molecules encoded in the Qa-2 locus.

7. After the completion of my postdoctoral work, I joined Biogen Research Corp. in Cambridge, Massachusetts as a Research Scientist. At Biogen, I expanded on my work from MIT by conducting molecular genetic studies on insulin-specific, class II MHC restricted T cell receptors in mice. I also conducted further biochemical and molecular studies on Qa-2 encoded MHC molecules.

8. After three years at Biogen, I joined the John Innes Institute in Norwich, U.K. as a Research Scientist. I conducted a molecular genetic analysis of type II

polyketide synthase systems to explore the identity and mechanism of construction of complex secondary metabolites in *Streptomyces*.

9. After three years at the John Innes Institute, I joined the University of Minnesota as an Assistant Professor in the Department of Microbiology and BioTechnology Institute. I held numerous positions during my time at the University of Minnesota, including earning the title of Professor, becoming Director of the Center for Microbial Physiology and Metabolic Engineering, and the Director of the Microbiology, Immunology, and Cancer Biology Graduate Program, and the Director of the UMN-NIGMS Biotechnology Training Program. It was also during my time at UMN when, on sabbatical, I was Senior Director of ChromaXome Corporation at San Diego, CA. I also founded and was the Chief Technical Consultant of Acera Biosciences, Inc.

10. In 2003, I begin my tenure at the University of Michigan, where I currently work. I started as a J. G. Searle Professor in the Department of Medicinal Chemistry, and a Professor in the Departments of Chemistry and Microbiology & Immunology. Over the last 20 years, I've held numerous appointments in the University of Michigan. I have been Director of the Center for Chemical Genomics in the Life Sciences Institute, the Associate Dean for Research and Graduate Education at the College of Pharmacy and am currently the Principal Investigator at the University of Michigan Natural Products Biosciences Initiative and the Faculty

Lead and co-Founder of the University of Michigan Natural Products Discovery Core. My research interests in the University of Michigan include synthetic chemistry, bioorganic and biochemical studies of natural product biosynthetic pathways from marine and terrestrial microbes. I also develop metabolic engineering and microbial genomic and proteomic technologies and use those technologies for analysis and production of novel biologically active molecules and identification of drug targets against infectious diseases and cancer. In my capacity as a researcher, I am familiar with 10x Genomics's Visium product line and have used it in my research to develop and understand the direct biomolecular target of HIV Nef inhibitors in human T cells.

11. In addition to my various academic posts at the University of Michigan, I also hold positions on various advisory and editorial boards. I am a member of the *Microbiology Spectrum* Advisory Board at the American Society for Microbiology Press, a member of the Editorial Board for the *Journal of Biological Chemistry* at the American Society for Biochemistry and Molecular Biology, and an Associate Editor for *Engineering Microbiology* at the Elsevier Press.

12. I have published over 300 peer-reviewed research articles, and currently have four papers that have been submitted, accepted for publication, or under revision. I have been listed as a co-inventor on 17 issued U.S. Patents, and

co-authored two books on biotechnology and organic synthesis. A complete list is available on my curriculum vitae.

13. A copy of my curriculum vitae, attached as Exhibit EX2017, contains further details on my education, experience, publications, and other qualifications to render an expert opinion in this matter.

III. MATERIALS CONSIDERED

14. In forming my opinions, I have reviewed the following documents and materials:

- The '142 Patent (EX1043)
- Dr. Paul Spellman's declaration submitted in support of the Petition for *inter partes* review (EX1044), as well as the exhibits Dr. Spellman cited
- The Board's institution decision in this matter (Paper 25)
- Materials cited in this Declaration

IV. LEGAL STANDARDS

15. I am not an attorney. Counsel has informed me of the legal standards applicable in this matter, and my understanding of the legal standards follows. I apply this understanding in rendering my opinions in this declaration.

A. Anticipation

16. I understand that anticipation is established only when a single prior art reference discloses each and every limitation of the claimed invention, either explicitly or inherently, as arranged in the claims. I also understand that a single

prior art reference may be deemed to inherently anticipate a claim if, and only if, a limitation that is not expressly stated in the reference is necessarily present in the product or process that the reference describes, not merely probably or possibly present in that product or process.

B. Nonobviousness

17. I understand that a patent claim is invalid as obvious if the differences between the subject matter of the patent and the prior art are such that the subject matter as a whole would have been obvious to a person of ordinary skill in the art at the time of invention. I understand that an obviousness analysis considers the following factors: (a) the scope and content of the prior art; (b) the differences between the prior art and the claims; (c) the level of ordinary skill in the art; and (d) any objective indicia of nonobviousness (such as unexpected results, skepticism in the field, industry praise, and commercial success). I understand that such objective indicia of nonobviousness must have a connection or “nexus” to the claims.

18. I also understand that when performing an obviousness analysis, it is insufficient that all the elements are found in the prior art. There must have been some motivation, or reason, for a person of ordinary skill to combine or modify the prior art to obtain the claimed invention. Furthermore, there must have been a

reasonable expectation that a person of ordinary skill would have succeeded in obtaining the claimed invention based on the prior art.

V. THE PERSON OF ORDINARY SKILL IN THE ART

19. I am knowledgeable about what the level of ordinary skill in the art (“POSA”) would have been at the time of the alleged invention based on my education and experience.

20. In determining the definition of a POSA, I have considered the following factors: (1) the level of education and experience of those in the field; (2) the complexity of the technology at issue; (3) the rate of innovation; (4) the kinds of problems faced by those in the art; and (5) solutions to such problems known in the prior art.

21. In my opinion, a POSA would have been someone with a Ph.D. in a relevant field, such as molecular biology, genetics, chemistry, biochemistry, organic chemistry, microbiology, or a related discipline, with at least two years of experience in the use and development of approaches for spatial detection of proteins and nucleic acids of interest in a biological sample, including multiplex detection of target analytes in a tissue sample for purposes including as an aid in the diagnosis and treatment of human diseases. Alternatively, a POSA would have been someone with greater relevant experience that has an equivalent understanding of approaches used in the field without the same amount of formal education. I

understand that Dr. Spellman has proposed a similar definition of a POSA. EX1044 ¶¶ 25-26. I agree with Dr. Spellman that the skill level of a POSA relevant to the '142 patent is high.

22. A POSA would have a strong understanding and/or experience with the design and application of various assays to manipulate polynucleotide sequences. Specifically, this includes the following techniques:

- Phosphoramidite chemistry. *See, e.g.,* EX2014 at 14268 (“The chemical synthesis of DNA and RNA is universally carried out using nucleoside phosphoramidites or H-phosphonates as synthons.”).
- Ligase chain amplification. *See, e.g.,* EX2007 at 1622-1623 (“LCR is an exponential amplification technology based on DNA ligase...Two pairs of complementary oligonucleotide probes are designed and hybridized with the perfectly matched target sequence.”).
- Rolling circle amplification. *See, e.g.,* EX2010 at 3324 (“Rolling circle amplification (RCA) is an isothermal enzymatic process where a short DNA or RNA primer is amplified to form a long single stranded DNA or RNA using a circular DNA template and special DNA or RNA polymerases.”).
- Stranded RNA sequencing. *See, e.g.,* EX2011 at 173 (“The information concerning strand origin is often lost during conventional

RNA-Seq; capturing this information would substantially increase the value of any RNA-Seq experiment. By manipulating the input cDNA during the template preparation stage it is possible to retain this vital information. This forms the basis of strand-specific RNA-Seq.”); EX2012 at 1 (“Without strand information it is difficult and sometimes impossible to accurately quantify gene expression levels for genes with overlapping genomic loci that are transcribed from opposite strands. It has recently become possible to retain the strand information by modifying the RNA-seq protocol, known as strand-specific or stranded RNA-seq.”).

These are at least some of the competencies that a POSA would have possessed. Other discussions regarding the knowledge of a POSA are incorporated into sections where such discussion is relevant.

VI. CLAIM CONSTRUCTION

23. I understand that claim terms are generally given their ordinary meaning to a person of ordinary skill in the art. I also understand that in a parallel district court litigation, the court has construed several terms from the ’142 Patent. I further understand that in its Decision Granting Institution of this *inter partes* review, the Board provided some preliminary constructions.

24. As to the terms that the District Court construed, I understand that the court gave the terms “under conditions that release” and “[first/second] location of the tissue sample” their plain and ordinary meaning. EX1052 at 38, 41. Independently of that court’s findings, I likewise apply the plain and ordinary meaning to these claim terms.

25. The parties agreed in the parallel litigation that “spatially detecting” means “identifying the presence of a specific target analyte within a specific region of interest in a sample.” EX1024 at 31. I apply this construction in my opinions.

A. “identifying a first plurality [and second plurality] of DNA products by sequencing”

26. The Board construed the term “identifying a first plurality [and second plurality] of DNA products by sequencing” to mean “locating the specific target analyte within the tissue sample by correlating the DNA product using the location-identifying nucleic acid sequences.” Paper 25 at 26-28. I disagree with this construction.

27. In my opinion, a POSA would have understood the claim term “identifying a first plurality [and second plurality] of DNA products by sequencing” by its plain and ordinary meaning. A POSA would have known each of the words in the claim term and understood their meaning.

28. As an example, a POSA would have understood “sequencing” to mean determining the sequence of a nucleic acid via a sequencing method, such as Sanger

sequencing or next-generation sequencing. Thus, a POSA would have understood that the DNA products, which incorporates the location-identifying sequence, undergo sequencing so that they are identified.

29. The Board's preliminary construction takes out the claimed requirement that the identity of the DNA products is determined "by sequencing." In my opinion, a POSA would not have understood "identifying a first plurality [and second plurality] of DNA products by sequencing" to refer to any method of determining the identity of DNA products that does not involve sequencing. To the extent the Board concludes that construction is necessary for this term, I find that "locating the specific target analyte within the tissue sample by correlating the DNA product using the location-identifying nucleic acid sequences, wherein the sequence of the DNA product and the sequence of the location-identifying nucleic acid sequences are determined via sequencing" is more aligned with how a POSA would understand the claim term "identifying a first plurality [and second plurality] of DNA products by sequencing." Both the plain and ordinary meaning and the alternative construction apply to my opinions throughout this declaration.

B. "synthesizing a first plurality [and second plurality] of DNA products by performing a synthesis reaction"

30. The Board construed the term "synthesizing a first plurality [and second plurality] of DNA products by performing a synthesis reaction" to mean "amplifying the collected plurality of oligonucleotides released after application of a force from

the location of the sample using primers that 1) have binding sites on the collected oligonucleotide and 2) contain location-identifying nucleic acid sequences that are incorporated into the DNA product upon synthesis.” Paper 25 at 19-22. I disagree with this construction.

31. First, the Board’s construction replaces the claim term “synthesizing” with “amplifying.” A POSA would have understood these terms differently. In the context of nucleic acids, synthesis refers to the assembly of a nucleic acid strand and amplification refers to creating many copies of a nucleic acid. I understand that Dr. Spellman likewise agrees that amplification is different from synthesis:

Q Would person of ordinary skill in the art understand what a DNA synthesis reaction is?

A I think a person of ordinary skill in the art would understand what DNA synthesis was, and that there are a large number of reactions capable of meeting the definition of a DNA synthesis reaction.

...

Q Is there a difference between a DNA synthesis reaction and a DNA amplification reaction?

A The first step of a DNA amplification reaction is a DNA synthesis reaction. So it would depend on context quite often.

...

Q Let's put it this way: Are there synthesis reactions that are not amplification reactions?

A Yes.

EX2009 at 28:3-29:22. In particular, I understand Dr. Spellman to have provided phosphoramidite synthesis as a method that is a synthesis reaction but not an amplification. I agree that phosphoramidite chemistry is often used as a method for de novo synthesis of nucleic acid strands of specific sequences and is not a method of amplification.

32. In addition, I understand that dependent claims such as claim 5 and claim 7 involves amplification while the independent claim 1 involves synthesis. Accordingly, I understand that the use of different terms in the claims is an indication that they refer to different concepts, which I have already provided above.

33. Second, the Board's preliminary construction requires "primers that have binding sites on the collected oligonucleotide." I understand from the use of multiple primer and multiple binding sites for each singular collected oligonucleotide that the Board's preliminary construction requires at least two primers for each DNA product that is synthesized. As discussed above, a POSA would not have found this requirement of at least two primers to be part of the plain and ordinary meaning of the "synthesizing a first plurality [and second plurality] of DNA products by performing a synthesis reaction," as a POSA would have understood that any DNA synthesis reaction, such as phosphoramidite synthesis which involves no primers, meets the claim requirement. A POSA would have likewise understood that amplification methods without two primers also meet the

claim requirements, such as ligase chain reaction and rolling circle amplification, methods that a POSA would have known and involve no primers and one primer, respectively. *See supra* § V.

34. Third, the Board’s preliminary construction requires “location-identifying nucleic acid sequences” to be incorporated into the DNA product. In my opinion, this is redundant, as claim 1(c) and (d) already includes this limitation:

synthesizing a first [and second] plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (a) [and (b)] as templates and incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the first [and second] plurality of DNA products;

EX1043 Claim 1(c), (d). Thus, the construction of “synthesizing a first plurality [and second plurality] of DNA products by performing a synthesis reaction” should not include this same limitation.

35. In my opinion, a POSA would have understood the plain and ordinary meaning of “synthesizing a first plurality [and second plurality] of DNA products by performing a synthesis reaction.” A POSA would have understood this claim limitation to require a synthesis reaction that generates DNA products. As part of the synthesis reaction that results in DNA products, the remainder of claim 1(c) and 1(d) require the collected oligonucleotides to be used as templates and require the

location-identifying sequences to be incorporated into the DNA products. A POSA would not have had difficulty interpreting this claim term.

VII. OVERVIEW OF THE '142 PATENT

36. The '142 Patent is owned by Bruker Spatial Biology, Inc. and is directed to a method of spatially detecting target analytes in a region of interest. The claimed methods are capable of detecting and quantifying proteins and/or nucleic acids from locations in a tissue sample. As the Abstract describes, the Patent relates to “probes, compositions, methods, and kits for simultaneous, multiplexed detection and quantification of protein and/or nucleic acid expression in a user-defined region of a tissue, user-defined cell, and/or user-defined subcellular structure within a cell that are adaptable for use with existing sequencing technologies.” EX1043 Abstract.

37. The '142 Patent was issued on October 18, 2022, from U.S. Patent Application No. 17/476,712 (“’712 Application”), filed on September 16, 2021. The '712 Application is a continuation of U.S. Patent Application No. 16/272,487 (“’487 Application”), filed February 11, 2019, which claims priority to U.S. Provision Applications Nos. 62/629,180, filed February 12, 2018, and 62/771,212, filed November 26, 2018.

38. Claim 1 of the '142 Patent recites:

1. A method comprising:

- a) collecting a plurality of oligonucleotides from a first location of a tissue sample under conditions that release the plurality of oligonucleotides from the first location of the tissue sample;
- b) collecting a plurality of oligonucleotides from a second location of the tissue sample under conditions that release the plurality of oligonucleotides from the second location of the tissue sample;
- c) synthesizing a first plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (a) as templates and incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the first plurality of DNA products;
- d) synthesizing a second plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step (b) as templates and incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the second plurality of DNA products; and
- e) identifying the first plurality of DNA products and the second plurality of DNA products synthesized in step (c) and step (d) by sequencing the first plurality of DNA products and the second plurality of DNA products, thereby spatially detecting the plurality of oligonucleotides collected from the first location of the tissue sample and the plurality of oligonucleotides collected from the second location of the tissue sample.

EX1043 Claim 1.

39. This claim is directed to the spatial detection of an oligonucleotide, *e.g.*, a nucleic acid, in a first and second location of a tissue sample. Claims 2-13 depend directly or indirectly from claim 1.

VIII. 10X GENOMICS HAS NOT PROVEN UNPATENTABILITY OF THE CLAIMS FROM THE '142 PATENT

A. GROUND 1: STAHL DOES NOT ANTICIPATE CLAIMS 1-4, 6-13

40. Petitioner argues that Stahl anticipates claims 1-4, 6-13 of the '142 patent. Based on my review, Petitioner's argument is deficient because it fails to show how Stahl meets claim elements involving (1) sequencing DNA products; (2) synthesizing DNA products that incorporate a nucleic acid sequence that identifies location; and (3) amplifying a library using DNA products as templates, as explained below. Accordingly, it is my opinion that Stahl does not anticipate claims 1-4, 6-13.

1. Stahl Does Not Disclose Sequencing DNA Products

41. Claim 1(e) requires "DNA products" to be sequenced:

e) identifying the first plurality of DNA products and the second plurality of DNA products synthesized in step (c) and step (d) by *sequencing the first plurality of DNA products and the second plurality of DNA products*, thereby spatially detecting the plurality of oligonucleotides collected from the first location of the tissue sample and the plurality of oligonucleotides collected from the second location of the tissue sample.

EX1043 Claim 1(e).¹ Thus, upon determining what Petitioner identified as the “DNA products” in Stahl, I conclude that Stahl does not sequence Petitioner’s identified “DNA product.”

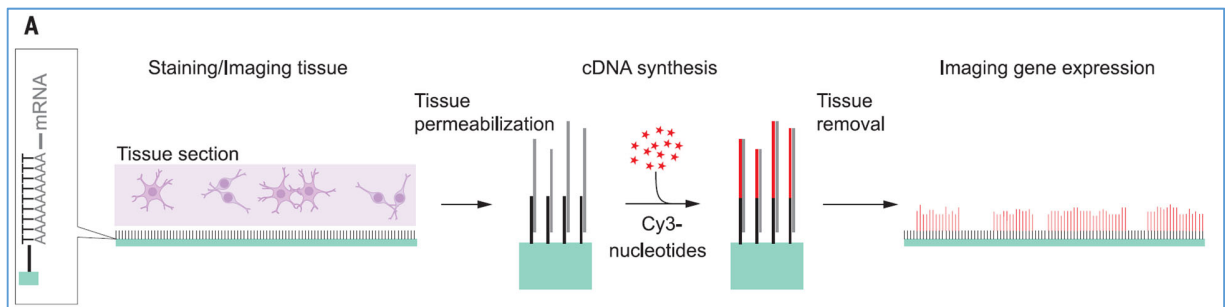
42. Stahl’s method involves capture probes that are immobilized on a microscope slide. The exposed ends of the capture probes are a series of poly-T sequences that are used to capture the poly-A tail of mRNA collected from tissue samples. After the sample mRNA hybridizes to the capture probe, a reverse transcription reaction is performed to synthesize cDNA by extending the capture probe.

43. In my opinion, Petitioner identifies the cDNA synthesized during reverse transcription of the captured mRNA as the claimed “DNA products.” This is because Petitioner states that “Stahl discloses the synthesis steps (c) and (d) because Stahl’s method uses a reverse transcription reaction to ‘synthesize’ cDNA products using the captured mRNAs as templates.” Paper 2 at 26. Petitioner is clear that it is the reverse-transcribed cDNA that meets the “DNA products” in the ’142 patent claims.

44. Petitioner further states that it is the cDNA synthesis in Stahl utilizing Cy3-nucleotides that meets the “DNA products” in the claims. This is evident

¹ Emphasis added throughout unless otherwise noted.

because Petitioner specifically states that “Stahl provides a figure depicting ‘spatially localized *cDNA synthesis*,’ which shows ‘*cDNA synthesis* with Cy3-labeled nucleotides,”” with added emphasis on “cDNA synthesis. *Id.* Petitioner further points to the red bar in Figure 1A: “Stahl’s Figure 1A depicts cDNA synthesis as red bars.” *Id.* Figure 1A is reproduced below:



EX1016 Fig. 1A. The caption states: “The tissue is sectioned, placed onto oligo(dT) primers, stained, and imaged. cDNA synthesis with Cy3-labeled nucleotides reveals fluorescent cDNA after tissue removal.” *Id.* Thus, it is clear that cDNA is synthesized using Cy3-nucleotides that are then imaged. No sequencing of the fluorescent cDNA occurs in Stahl. Stahl is clear that reverse transcription performed using Cy-3-nucleotides is “intended for generation of fluorescent cDNA footprints,” as opposed to reverse transcription performed with non-fluorescent nucleotides that is “intended for sequencing:”

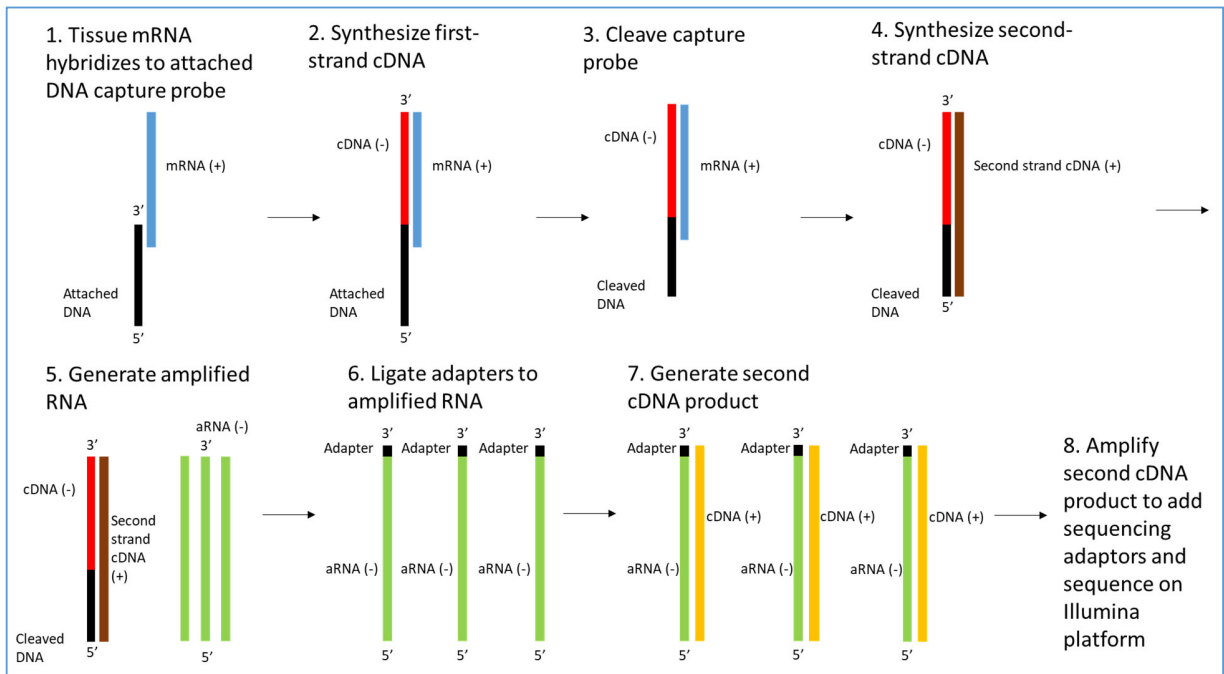
For spatially barcoded arrays belonging to samples *intended for sequencing*, the reverse transcription mix contained 1x First Strand Buffer (#18080-044, Invitrogen)...500μM of each dNTP (#R0192, Fisher Scientific)...For spatially barcoded arrays and surfaces with

poly-T reverse transcription primer *intended for generation of fluorescent cDNA footprints*, the reverse transcription mix contained the same reagents as above except for...*25μM of Cyanine 3-dCTP* (#NEL576001EA, PerkinElmer).

EX1016, Suppl. Materials at 5.

45. Even assuming that Petitioner had identified the reverse-transcribed cDNA “intended for sequencing” without the fluorescent nucleotides, it is my opinion that the first-strand cDNA in Stahl is not sequenced. Rather, as explained below, Stahl sequences a second cDNA product.

46. Stahl’s workflow is depicted in the diagram below:



As depicted, the sample mRNA is first released from the tissue sample and captured by the capture probe on an array (step 1). *See id.* (“70μl of 0.1 %

pepsin...dissolved in 0.1M HCl...was added to each well and incubated at 37°C for 10 minutes.”). Then, reverse transcription is used to synthesize a first-strand cDNA (depicted in red). *Id.* (“Each well was washed as previously described and 70µl of reverse transcription mix was added to each well and incubated overnight (ON).”) (step 2). Subsequently, the capture probe is cleaved (step 3) before synthesis of a second-strand cDNA (depicted in brown) that displaces the sample mRNA (step 4). *Id.* at 6 (“For each well, 70µl of release mix containing...0.1U/µl USER Enzyme...was added and incubated... 5µl of second strand mix containing...3.7U/µl DNA polymerase I...was added”). Next, a transcription reaction generates amplified RNA (step 5), followed by ligation of adapters to the 3’ end of the amplified RNA (step 6). *Id.* at 6-7 (“5.6µl of the samples were mixed with 10.4µl In Vitro Transcription mix with...1x T7 Enzyme Mix... Samples were heated at 70°C for 2 min. then placed on ice before 4.5µl ligation mix was added to a final content...20U/µl T4 RNA Ligase2...and 0.5µM Ligation Adapter.”). A reverse transcription reaction then generates second cDNA products (depicted in orange) from the amplified RNA (step 7). *Id.* at 7 (“Samples were... placed on ice, 8µl reverse transcription mix was added to a final content...500µM of each dNTP, 1mM RT-primer, 10U/µl Superscript III and 2U/µl RNaseOUT.”). This second cDNA product in Stahl is sequenced as Illumina sequencing adapters are appended to generate the sequencing library (step 8). *Id.* (“A total reaction volume of 10µl

containing...0.5µM PCR InPE1.0 (Eurofins), 0.01µM PCR InPE2.0 (Eurofins), 0.5µM PCR Index (Eurofins) and 2µl purified cDNA were amplified by qPCR...After determining the amount of cycles needed based on the qPCR, the samples were amplified in a total reaction volume of 25µl.”). The sequencing library was then sequenced using an Illumina sequencing platform. *Id.* (“Finished libraries were diluted to 4nM and sequenced on the Illumina NextSeq platform using paired-end sequencing according to the manufacturer's protocol.”). I understand Dr. Spellman to agree that it is the second cDNA that is sequenced in Stahl:

Q Let’s skip the next sentence and to the one after it, which starts with, “After determining the amount of cycles needed based on the qPCR, the samples were amplified in a total reaction volume of 25 microliter.” Do you see that?

A Yes.

Q Do you agree that the cDNA is being amplified in this step?

A Yes.

Q And if you read through the rest of this section, do you agree that the amplified cDNA is then sequenced on an Illumina NextSeq platform?

A Yes.

EX2009 at 75:16-76:8.

47. The second cDNA product (depicted in orange) is different from the first-strand cDNA (depicted in red). First, these are different molecules and only the second cDNA product (depicted in orange) is sequenced (step 8). Second, the

directionality of the second cDNA product and the first-strand cDNA is different. As shown in the diagram above, the second cDNA product has the same strand specificity as the sample mRNA, as shown by “(+).” This is critically important because Stahl’s workflow implements what is known in the art as stranded RNA sequencing or strand-specific RNA sequencing. *See generally* EX2011, EX2012; *see supra* § V. The objective of stranded RNA sequencing is to ensure that the strand specificity of the target mRNA is maintained as the sequence of the RNA is determined so that information on strand origin is not lost during sequencing. This was an area of active development around when Stahl was published, which highlighted the importance of determining strand origin:

The information concerning strand origin is often lost during conventional RNA-Seq; capturing this information would substantially increase the worth of any RNA-Seq experiment. By manipulating the input cDNA during the template preparation stage it is possible to retain this vital information. This forms the basis of strand-specific RNA-Seq.

EX2011 at 173;

While RNA-sequencing (RNA-seq) is becoming a powerful technology in transcriptome profiling, one significant shortcoming of the first-generation RNA-seq protocol is that it does not retain the strand specificity of origin for each transcript. Without strand information it is difficult and sometimes impossible to accurately quantify gene expression levels for genes with overlapping genomic loci that are transcribed from opposite strands. It has recently become possible to retain the strand information by modifying the RNA-seq protocol, known as strand-specific or stranded RNA-seq.

EX2012 at 1;

Among the over 57,800 annotated genes in Gencode release 19, there are an estimated 19 % (about 11,000) of overlapping genes transcribed from the opposite strands.

Id.;

Stranded RNA-seq provides a more accurate estimate of transcript expression compared with non-stranded RNA-seq, and is therefore the recommended RNA-seq approach for future mRNA-seq studies.

Id. Rather than sequencing the first cDNA product, Stahl performs single-stranded RNA amplification followed by appending an adapter on the 3' end. When amplified RNA is reverse-transcribed into a single-stranded cDNA, the directionality of this synthesized second cDNA product is the same as the captured mRNA. Thus, a POSA would not have understood the first-strand cDNA as being sequenced.

48. Petitioner includes only a single sentence to allegedly show how Petitioner's identified "DNA products" is being sequenced:

After synthesizing the cDNA products (first and second plurality of DNA products) on the array, Stahl discloses generating "sequencing libraries" by collecting the spatially barcoded cDNA synthesis products, amplifying them, and sequencing the amplified library "on the Illumina NextSeq platform using paired-end sequencing according to the manufacturer's protocol."

Paper 2 at 30. As is clear from the discussion above, however, Petitioner obfuscates Stahl's workflow in a manner contrary to a POSA's understanding. A POSA would have understood a "sequencing library" to be the set of molecules

ready for sequencing according to a manufacturer's protocol. Thus, the sequencing library in Stahl refers to the second cDNA product (depicted in orange). Additionally, a POSA would have understood sequencing a nucleic acid to refer to the nucleic acid being directly sequenced. Here, sequencing on the Illumina platform requires addition of Illumina adapters, which are appended to the second cDNA product (depicted in orange). Thus, Petitioner's incomplete description of Stahl's intentional stranded RNA sequencing workflow as "collecting the spatially barcoded cDNA synthesis products, amplifying them, and sequencing the amplified library" belies the second cDNA product (depicted in orange) that is specifically sequenced and not the first-strand cDNA (depicted in red).

49. Accordingly, for at least the reasons above, Stahl does not disclose sequencing the DNA products identified by Petitioner.

2. Stahl Does Not Disclose A Synthesis Reaction That Incorporates A Nucleic Acid Sequence That Identifies Location

50. In my opinion, Stahl also does not anticipate the '142 patent claims because Stahl does not incorporate a location-identifying sequence during the synthesis of Petitioner's identified "DNA products."

51. Claim 1(c) and (d) require a synthesis reaction that incorporates a location-identifying sequence:

synthesizing a first [and second] plurality of DNA products by *performing a synthesis reaction that* uses the plurality of

oligonucleotides collected in step (a) [and (b)] as templates and ***incorporates at least one nucleic acid sequence that identifies the first [and second] location of the tissue sample into each of the first [and second] plurality of DNA products;***

EX1043 Claim 1(c), 1(d). As discussed above, it is my opinion that a POSA would have understood “synthesizing a first [and second] plurality of DNA products by performing a synthesis reaction” to have a plain and ordinary meaning. *See supra* § VI.B. A POSA would have understood claim 1(c) and 1(d) to require a synthesis reaction that incorporates a location-identifying sequence into “DNA products” that are synthesized.

52. In my opinion, a POSA would have understood the use of the word “incorporates” in the claim language in a manner consistent with its usage in the art. In the context of DNA synthesis, which is relevant due to the synthesis reaction required by the claim step, a POSA would have understood “incorporate” to refer to addition of a sequence as each nucleotide is sequentially added during synthesis. For example, in the context of DNA synthesis, one sequence is generally incorporated by an enzyme that sequentially adds a nucleotide onto the end of a DNA strand. As a counterexample, a POSA would not have understood a primer tail to be incorporated as the primer is extended. In that instance, the primer tail sequence is considered incorporated when a complementary strand is synthesized.

53. The '142 patent usage of the term “incorporate” is consistent with a POSA’s understanding of the term. For example, the patent includes the following:

Thus, the nucleic acid sequencing reaction may comprise hybridizing a sequencing primer to a single-stranded region of a linearized amplification product, *sequentially incorporating one or more nucleotides into a polynucleotide strand* complementary to the region of amplified template strand to be sequenced, identifying the base present in one or more of the incorporated nucleotide(s) and thereby determining the sequence of a region of the template strand.

1043 at 44:60-67;

Once *the modified nucleotide has been incorporated into the growing polynucleotide chain* complementary to the region of the template being sequenced there is no free 3'-OH group available to direct further sequence extension and therefore the polymerase cannot add further nucleotides. Once *the nature of the base incorporated into the growing chain has been determined*, the 3' block may be removed to allow addition of the next successive nucleotide.

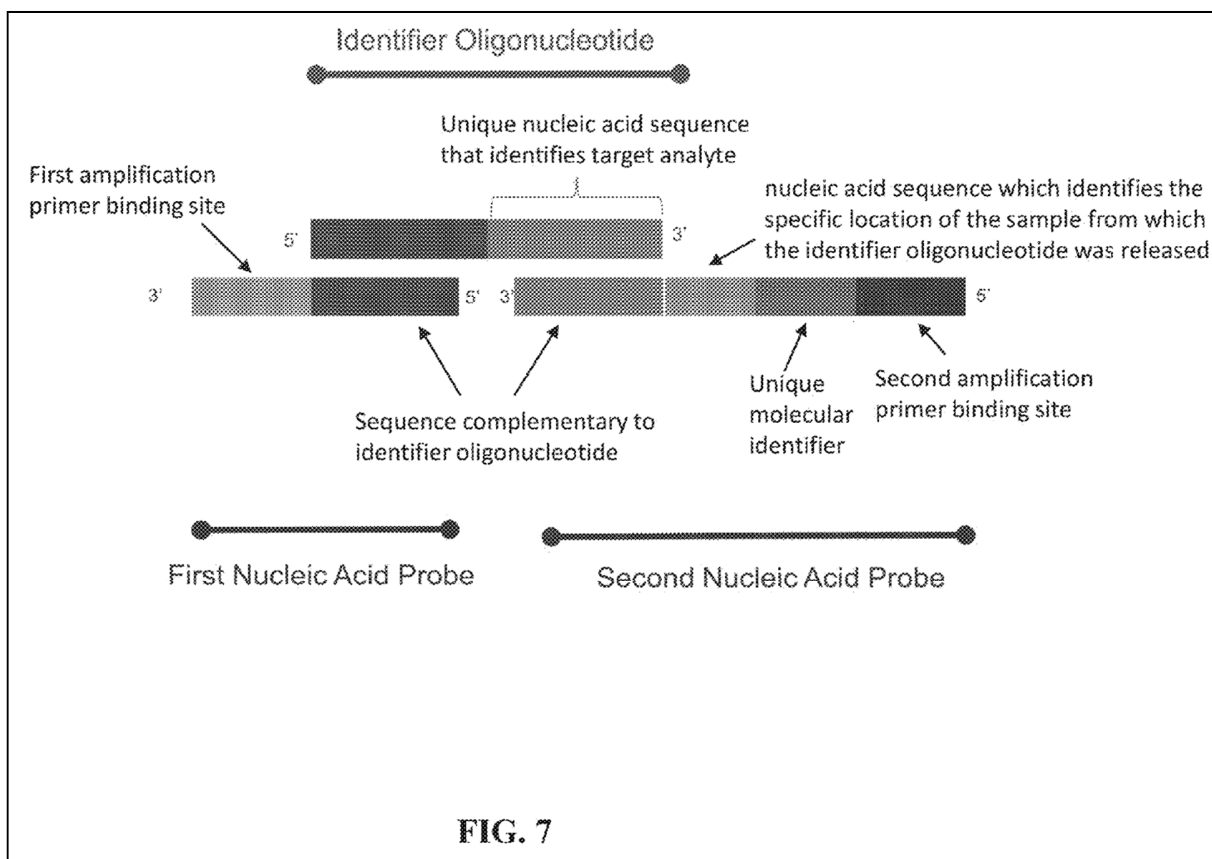
Id. at 45:3-11;

The invention is not intended to be limited to use of the sequencing method outlined above, as essentially any sequencing methodology which relies on *successive incorporation of nucleotides into a polynucleotide chain* can be used.

Id. at 45:33-37. Each of these usages of the term “incorporate” refers to the successive addition of nucleotides onto a polynucleotide strand.

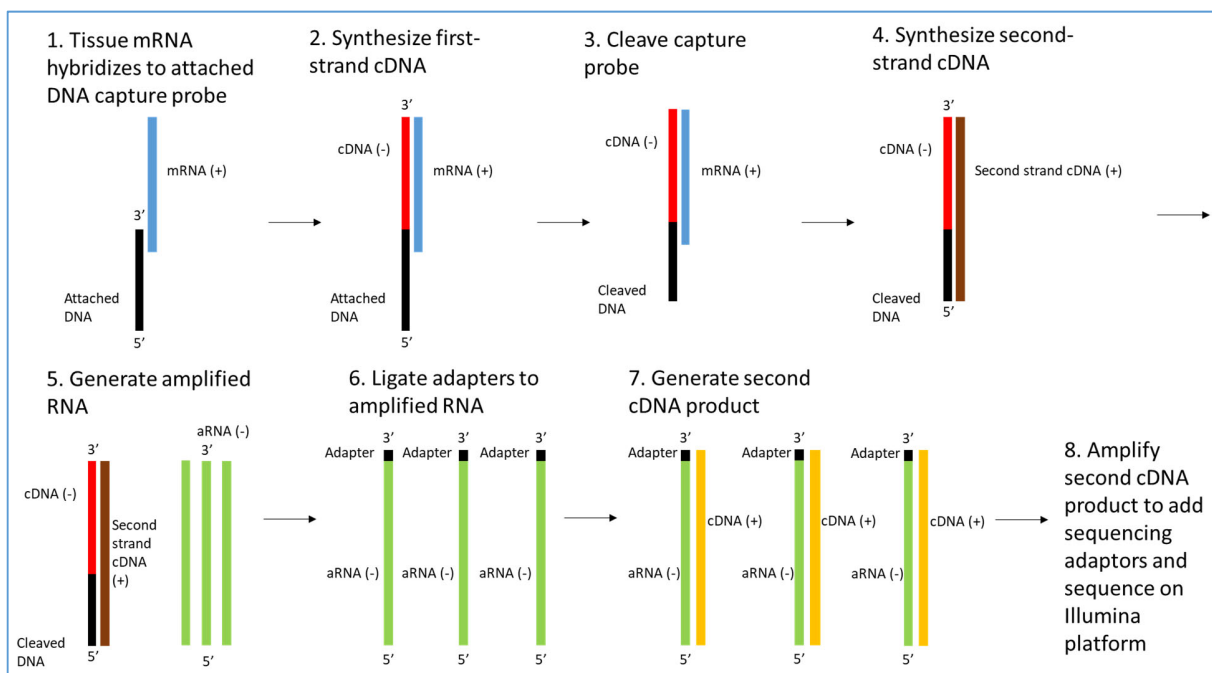
54. Additionally, the specification includes examples wherein the location-identifying sequence is synthesized. For example, the patent teaches that “a first nucleic acid probe and a second nucleic acid probe” can hybridize to an “identifier oligonucleotide” collected from a location of a tissue sample. EX1043 at 24:17-41. The “first or the second nucleic acid probe can also comprise a nucleic acid sequence which identifies the specific location of the sample from which the

identifier oligonucleotide was released.” *Id.* at 24:44-47. This is depicted in Figure 7:

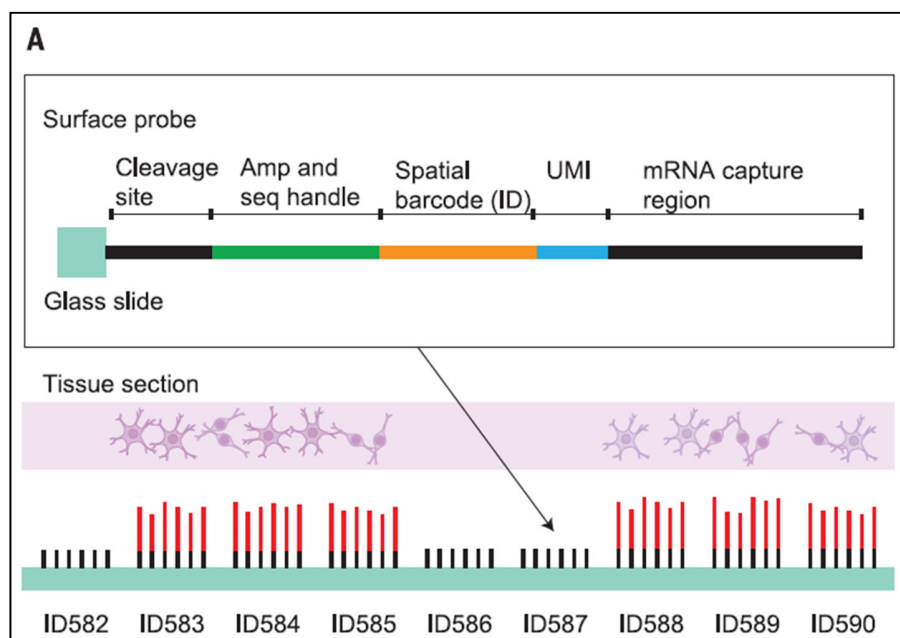


Id. Fig. 7. As the figure depicts, the location-identifying sequence is between the first and second amplification primer binding site. Thus, after the first and second nucleic acid probes are ligated together, when the ligated probe is amplified “using amplification primers that hybridize to the first and second amplification primer binding sites,” *id.* at 26:12-19, the location-identifying sequence is synthesized and incorporated into the complementary strand.

55. The first-strand cDNA in Stahl (with Cy3-nucleotides), which Petitioner identifies as the claimed “DNA products,” does not incorporate the location-identifying sequence during cDNA synthesis because the location-identifying sequence is not synthesized:



As Stahl shows in Figure 2A, the location-identifying sequence is already present as part of the capture probe that is extended during cDNA synthesis:



EX1016 Fig 2A (depicting “Spatial barcode” in orange). Petitioner does not dispute this. Instead, Petitioner contends that because the first-strand cDNA comprises the location-identifying sequence, the location-identifying sequence is incorporated. *See* Paper 2 at 29 (“Stahl’s synthesis reaction produces cDNA products comprising the spatial barcode of the capture probe, thus incorporating the spatial barcode (i.e., sequence that identifies a location on the array) into the cDNA products.”). As explained above, an extension of the capture probe does not incorporate the location-identifying sequence insofar as the location-identifying sequence is not synthesized. Thus, in my opinion, Petitioner fails to show that the location-identifying sequence is synthesized during synthesis of the first-strand cDNA with Cy3-nucleotides, Petitioner’s identified “DNA products.”

56. Accordingly, for at least the reasons above, Stahl does not disclose incorporating the location-identifying sequence into the DNA products identified by Petitioner during the DNA products' synthesis.

3. Stahl Does Not Disclose Amplifying A Library Using DNA Products As Templates

57. In my opinion, Stahl does not disclose generating a library using Petitioner's identified "DNA products" as templates, as required by claim 7:

7. The method of claim 1, further comprises amplifying a library using the first plurality of DNA products and the second plurality of DNA products as templates.

EX1043 Claim 7. This is because Petitioner's identified "DNA products" is not used as templates in an amplification reaction for a library.

58. A POSA would understand the term "template" to have a specific plain and ordinary meaning in the art. In an amplification reaction, a POSA would understand "template" to refer to the specific nucleic acid that is being amplified. A POSA would have understood this as a term in the art. For example, in a PCR, the template would be the target nucleic acid that is amplified and to which primers hybridize and the polymerase catalyzes amplification. *See generally* EX2015 at 12 ("The first group encompasses techniques where a single target sequence is amplified from single type tem-plate molecules using a single primer set. The template can be present in the test tube in multiple copies but all these copies have

the same sequence. This assay is referred to as single-template PCR (Fig. 1a) and is what is typically referred to as PCR.”). Thus, the product of the amplification reaction, such as the amplicons that result from PCR, would have the same sequence as the template. In this way, the template is amplified in an exponential fashion.

59. Dr. Spellman agrees with this understanding. During cross-examination, Dr. Spellman plainly stated that “the template is the thing being amplified” and “one of the strands [of the amplification product] hav[e] exactly the same sequence as the initial template.” EX2009 at 32:16-35:18. Additionally, Dr. Spellman agreed that in PCR primers bind to the template and the template is a substrate of the polymerase. *Id.* at 36:1-12.

60. With this understanding, a POSA would have understood claim 7 to require that the “DNA products” are used as templates to amplify a library. In my opinion, Petitioner fails to show that Petitioner’s identified “DNA products” in Stahl act as templates in an amplification reaction to generate a library.

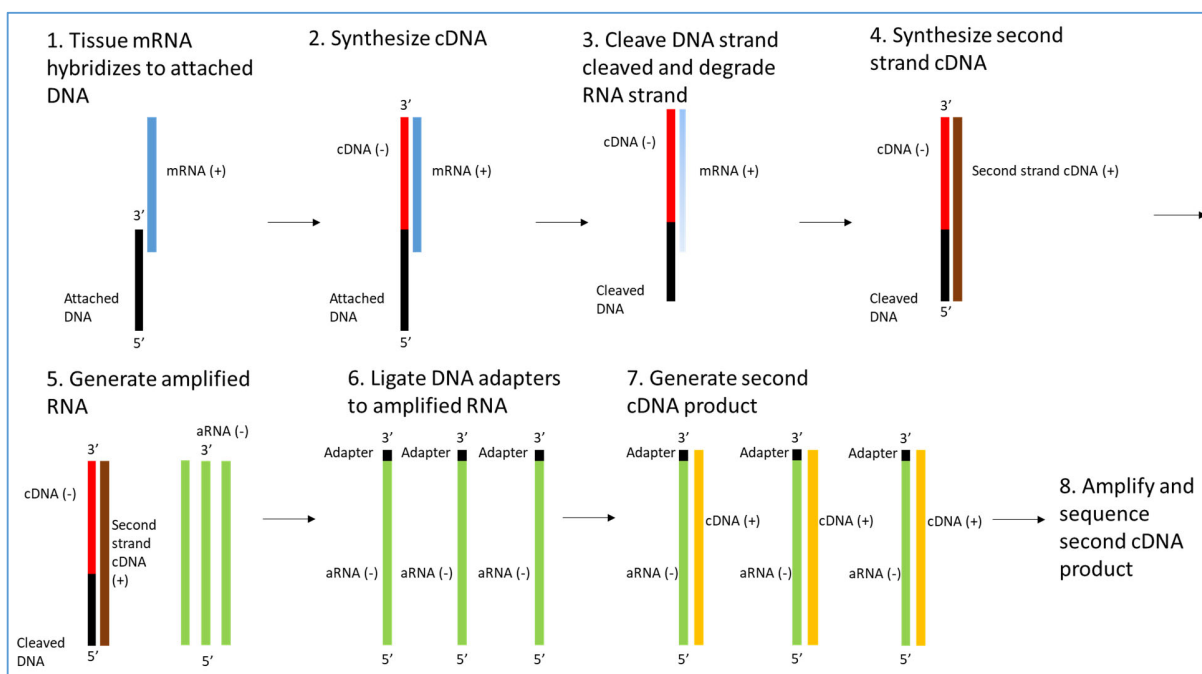
61. As discussed in Section VIII.A.1, Petitioner’s identified “DNA products” in Stahl is the first-strand cDNA synthesized with Cy3-nucleotides, as depicted in red in Stahl’s Figure 1A. In the section regarding claim 7, Petitioner points to **multiple** amplification reactions to allegedly meet the claim:

Stahl discloses amplifying “a library” when discussing the workflow for “cDNA library preparation for sequencing.” EX1016, Suppl. Materials, 6-7; EX1044, ¶142. After performing an initial *in vitro*

transcription/amplification to produce amplified RNA molecules, Stahl discloses adding DNA adapters to the ends of the amplified RNA, reverse-transcribing the amplified RNA into cDNA, and then performing PCR to *amplify the cDNA* to add Illumina paired-end (PE) sequencing flow-cell binding sites and index sequences before sequencing. EX1016, Suppl. Materials, 7 (“2µl purified cDNA were *amplified by qPCR* ... the samples were *amplified* in a total reaction volume of 25µl.”); EX1044, ¶142.

See Paper 2 at 37 (emphasis and modifications in original). Petitioner italicizes three separate reactions—“amplify the cDNA,” “amplified by qPCR,” and “amplified.” Petitioner’s identified “DNA products” are not templates in any of these amplification reactions.

62. In the first identified reaction—“performing PCR to *amplify the cDNA* to add Illumina paired-end (PE) sequencing flow-cell binding sites and index sequences before sequencing”—the template is the second cDNA product that was reverse-transcribed from amplified RNA (depicted in orange) (step 7). A POSA would not refer to a first-strand cDNA (depicted in red), Petitioner’s identified “DNA products,” as a template in a reaction multiple steps removed (step 2):



63. The second amplification reaction—“2µl purified cDNA were amplified by qPCR”—also involves the second cDNA product (depicted in orange) as the template. As Stahl explains, the qPCR is used to determine the amount of cycles needed to add Illumina sequencing adapters to the second cDNA product. *See EX1016 Suppl. Materials at 7* (“determining the amount of cycles needed based on the qPCR.”). The qPCR product is not subsequently sequenced. Thus, not only does this amplification reaction not involve Petitioner’s identified “DNA products” as template, but the reaction also does not generate a library that is sequenced.

64. The third amplification reaction—“the samples were amplified in a total reaction volume of 25µl”—involves amplifying the second DNA product to add Illumina sequencing adapters. This is the same reaction as Petitioner’s first

identified amplification reaction. For the same reason, this reaction does not involve Petitioner's identified "DNA products" as template.

65. Accordingly, for at least the reasons above, Stahl does not disclose the limitations in claim 7 because Petitioner's identified "DNA products" are not used as templates to amplify a library.

B. GROUND 2: CLAIMS 1-13 WOULD NOT HAVE BEEN OBVIOUS OVER STAHL

1. Claims 1-4, 6-13

66. Petitioner and Dr. Spellman provide conclusory arguments that, should Stahl not anticipate claims 1-4, 6-13, the claims would have been obvious.

67. I understand that to show obviousness, a Petitioner must show that there was a motivation to modify a prior art reference to arrive at the claimed invention, and that a Petitioner must show a reasonable expectation of success in modifying the reference. In my opinion, Dr. Spellman and Petitioner fail to provide any specific arguments as to motivation to modify the methods of Stahl or a reasonable expectation of success in modifying those methods. *See* Paper 2 at 44-45; EX1044 ¶¶ 161-164.

68. For example, Petitioner states that a "POSA reading Stahl would have been motivated to practice the claimed methods because Stahl teaches that its 'spatial transcriptomics' method 'provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections,' 'enabl[ing] novel

types of bioinformatics analyses.’” See Paper 2 at 45. This, however, shows only that Stahl is directed to a similar field of endeavor as the ’142 patent. Nothing suggests that a POSA would be motivated to modify Stahl to arrive at the claimed methods.

69. A POSA would further not have been motivated to modify Stahl to meet the missing disclosures discussed in Ground 1 in light of Petitioner’s arguments because Petitioner contends that “Stahl provides detailed descriptions and protocols, with demonstrated success in the form of extensive data and analyses.” *Id.* Moreover, Petitioner contends that “Stahl’s methods used commercially available materials and reagents, and that the protocol was straightforward.” *Id.* A POSA would not have had reason to modify Stahl’s “straightforward methods using routine materials” to arrive at the claimed methods if Stahl already “demonstrated success in the form of extensive data and analyses.” See *id.* Petitioner identifies no advantages that would motivate a POSA to deviate from Stahl, which Petitioner contends is already a successful method in the same field as the ’142 patent.

70. Accordingly, in my opinion, Petitioner has failed to provide any reason why a POSA would have modified Stahl to arrive at the claimed methods.

2. Claim 5

71. In my opinion, Stahl does not render obvious claim 5 of the ’142 patent. Claim 5 depends from claim 4 (which ultimately depends from claim 1), and further

requires that the recited amplification steps “comprises performing a solid-phase amplification reaction, wherein the solid phase amplification reaction is carried out on the microscope slide using the plurality of primers immobilized on the microscope slide.” EX1043 claim 5.

72. Petitioner agrees that Stahl does not perform solid-phase amplification, as Petitioner states that Stahl involves in solution amplification: “collecting the first-strand cDNAs and performing second-strand cDNA synthesis in solution prior to performing library amplification (also in solution).” Paper 2 at 46. Petitioner nonetheless argues that it would have been obvious for a POSA to modify Stahl to practice the limitation in claim 5 because a “POSA would have generally known that the second-strand cDNA synthesis and library amplification can be performed on Stahl’s array, i.e., on the microscope slide.” *Id.* at 47. I understand this to mean that Petitioner proposes modifying Stahl such that either the second-strand synthesis or the subsequent amplification steps can be performed in solid phase.

73. Neither of these modifications, even if successfully made, would meet the claim limitation of claim 5, which requires the steps of synthesizing DNA products to involve solid-phase amplification. Both of Petitioner’s suggested modifications—second-strand cDNA synthesis and library amplification—take place *after* the first-strand cDNA synthesis, Petitioner’s identified “DNA products.” Thus, steps after the first-strand cDNA synthesis in So-456 (claim 1(c) and 1(d))

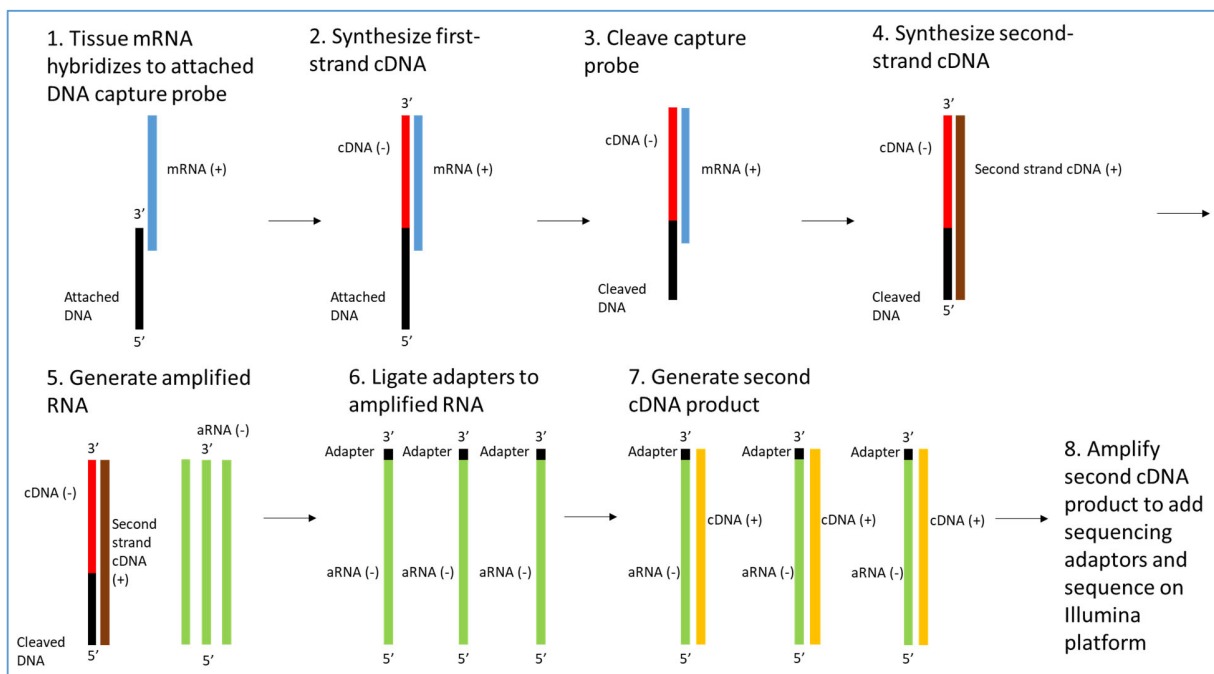
before sequencing of the DNA products (claim 1(e)) are unclaimed and cannot meet the recited claim limitation of claim 5.

74. In addition, in my opinion, neither Petitioner nor Dr. Spellman provide sufficient details on *how* a POSA could have modified Stahl to practice claim 5. In my opinion, Stahl's workflow involves multiple steps and it would not yield quantitative measurements of location-specific expression data if the workflow is modified without accounting for the implications of every modification. In my opinion, the closest Petitioner comes to providing details on the alleged modification to Stahl that would meet claim 5 is the following: "Stahl's first-strand synthesis is already 'solid-phase,' and a POSA would have viewed performing second strand synthesis on the substrate as a mere trivial modification." Paper 2 at 49. Besides calling the proposed modifications "trivial," Petitioner provides little detail. At a minimum, the polymerase used for solid-phase amplification are often different from those used for in solution amplification. This is because the polymerase needs to work efficiently in an environment where the movement of the DNA may be more restricted in solid phase compared to solution phase. Thus, the chosen polymerase should maintain activity under the cycling conditions that are optimized for surface-bound templates. Thus, key differences must be considered for just the polymerase choice that a POSA would not consider "trivial" in order to implement solid-phase amplification including:

- Performance on surfaces: Solid phase amplification may require polymerases that can operate effectively with the template tethered to a solid surface, which may limit enzyme accessibility or affect binding and elongation. This may require the use of specifically engineered polymerases for robust performance.
- Processivity and fidelity: Depending on the purpose, solid-phase amplification polymerases are typically optimized for high processivity (to rapidly synthesize long stretches of DNA), while solution-phase reactions may use polymerases chosen for their balance between processivity and high fidelity.
- Reaction Environment: Solid-phase environments can introduce additional constraints, such as steric hindrance or the need for surface compatibility, which are not concerns in solution phase amplification.

Petitioner provides no details regarding selection of polymerase for its proposed modifications.

75. I refer back to my discussion of Stahl's workflow:



See supra § VIII.A.1. In order to modify Stahl such that second-strand cDNA synthesis occurs in solid phase, the step involving the release mix where the capture probe is cleaved by a USER enzyme must be skipped. This is the only way for the second-strand cDNA to be synthesized while immobilized to the microscope slide.

I understand Dr. Spellman to agree with this assessment:

Q So if the second-strand cDNA is synthesized on the array, then the step in Stahl where the release mix with a user enzyme is added, that step needs to be skipped. Is that right?

A No, that's -- It doesn't need to be skipped. Oh, oh, sorry. To do it in - on the array itself, yes, you would -- you would either -- you would move the order of that potentially, or skip it.

EX2009 at 91:8-20 (objection omitted); *see also id.* 91:21-92:7. In my opinion, removing the cleavage step leads to a whole host of issues that cannot easily be resolved.

76. First, any reaction performed in solid-phase would be less efficient than its counterpart in Stahl where it is performed in solution. This is a characteristic of solid-phase reactions, where attached nucleic acids are limited to two-dimensional interactions with the other reactants in solution, in contrast to reactions in solution where nucleic acids have a higher probability of being close to other reactants. Due to the limitation in mobility of the attached nucleic acids, solid-phase reactants generally result in slower efficiency, yielding less products. Thus, by modifying Stahl to incorporate solid-phase amplification, a POSA would have to unnecessarily experiment with the concentrations and amounts of each reactant away from Stahl's existing workflow, which produces viable results as published.

77. In addition, if the cleavage step is skipped, such that the second-strand cDNA synthesis occurs in solid phase, the subsequent step of generating amplified RNA is unlikely to succeed. In Stahl, amplified RNA is synthesized from the first-strand/second-strand cDNA duplex by way of a T7 RNA polymerase. The T7 RNA polymerase's mechanism is such that it binds to a promoter region, opens up a DNA duplex, and transcribes DNA to synthesize RNA. This amplified RNA synthesis would be severely impacted because the promoter region for the T7 RNA polymerase is located very close to the surface on the microscope slide, such that T7 RNA polymerase (which is a large protein comprising almost 900 amino acids with a MW of 98,000 daltons) would have severe constraints binding to the substrate

cDNA duplex, particularly considering steric hindrance from neighboring attached strands. This is clear from Stahl. In Figure 2A, Stahl explains that the T7 promoter region is directly adjacent to the cleavage site, which in turn is attached to the glass slide:

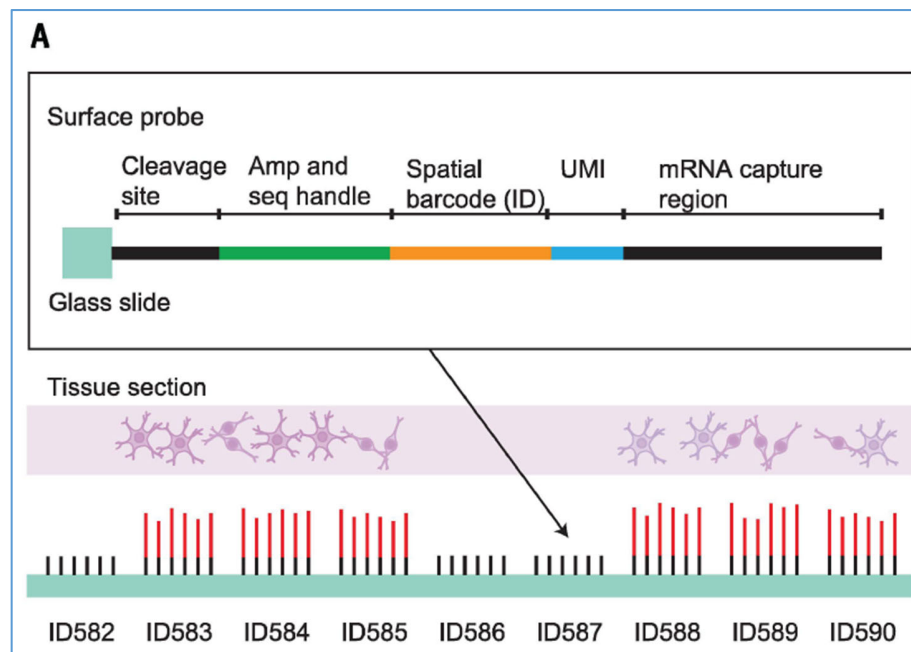


Fig. 2. Spatially resolved gene expression. (A) Each array feature contains unique DNA-barcoded probes containing a cleavage site, a T7 amplification and sequencing handle, a spatial barcode, a unique molecular identifier (UMI), and an oligo(dT) VN-capture region, where V is anything but T and where N is any nucleotide. cDNA (red) is generated from captured mRNA by reverse transcription. **(B)** Visualization of the expression of three genes by spatial tran-

EX1016 at Fig. 2A. Stahl also provides the exact sequence of the capture probe and I have identified the promoter region for the T7 RNA polymerase:

Surface spatially barcoded oligonucleotides:

[AmC6]UUUUUGACTCGTAATACGACTCACTATAGGGACACGACGCTCTCCGATCT[18
MER_SPATIALBARCODE]WSNNWSNNVTTTTTTTTTTTTTTTTTTTTTTVN

EX1016 Suppl. Materials at 3; *see also* EX2016 at 7. As the sequence above shows, the T7 RNA polymerase binds to a sequence that is very close to surface and would require significant optimization of Stahl if the amplified RNA is to be synthesized while the first-strand/second-strand cDNA duplex is immobilized on the surface.

78. Petitioner's other modification that "library amplification can be performed on Stahl's array," Paper 2 at 47, is also unlikely to succeed. As discussed previously, Stahl's workflow incorporates stranded RNA sequencing so as to maintain the orientation of the product that is eventually sequenced so that it matches the original mRNA. *See supra* § VIII.A.1. This is known as strand preservation. In order to modify Stahl such that amplification occurs completely on a solid surface to perform clonal amplification, significant modifications would have to be performed. This includes adding additional known sequences to the ends of the first-strand/second-strand cDNA duplex so that complementary sequences can be attached to the surface to enable clonal amplification. This is a drastic modification of Stahl's workflow and would require a significant amount of experimentation. In addition, clonal amplification functions primarily with DNA molecules, such that the amplification would entirely involve DNA and not RNA. As such, strand preservation would be lost with such a modification. Without strand preservation, the modified Stahl workflow would not succeed, as the data

collected would be compromised to that collected in Stahl, which does practice strand preservation. A POSA would have known that RNA sequencing without strand preservation leads to a loss of specificity and less accurate quantitative gene expression data. *See* EX2012 at 1 (“Without strand information it is difficult and sometimes impossible to accurately quantify gene expression levels for genes with overlapping genomic loci that are transcribed from opposite strands.”); *id.* (“Because stranded RNA-seq retains strand information of a read, we can resolve read ambiguity in overlapping genes transcribed from opposite strands, which provides a more accurate quantification of gene expression levels compared with traditional non-stranded RNA-seq.”); EX2011 at 174 (“While the use of RNA-seq is becoming more common throughout molecular biology, one significant short-coming of the standard RNA-seq protocol is that it loses the strand of origin information for each transcript. This is of particular concern due to the possible regulatory role carried out by antisense transcripts.”). In my opinion, a POSA would not forego the advantages of stranded RNA sequencing for modifications that yield less useful results.

79. In my opinion, a POSA would not been motivated to modify Stahl for reasons in addition to those above that they are unlikely to succeed. While Petitioner contends that a POSA would have modified Stahl because the authors knew about solid-phase amplification as exemplified by the Frisen patent

application, I note that Frisen was filed at least four years before Stahl's publication. Thus, there is no indication that the authors of Stahl, which allegedly disclosed the modification steps proposed by Petitioner in Frisen, purposefully published a workflow that was not optimized. Instead, a POSA would have understood that the authors of Stahl, while in possession of everything disclosed in Frisen, published the most optimized workflow for the purpose they sought at the time of publication. This is especially likely as Stahl was published in Science, a prestigious, well-regarded journal. It is contrary to common sense and practice by a POSA to argue that the authors of Stahl published an inferior workflow while in possession of a superior one. Dr. Spellman likewise agrees that Stahl's authors found something that worked, which was published in Stahl:

Q Do you have an explanation for why the authors of Stahl did not choose to perform solid-phase amplification?

A I'm not sure -- I'm not sure they didn't choose to. They published a method that doesn't. I'm not sure that it's -- they found something that worked.

Q Do you agree that the authors of Stahl knew about solid-phase amplification?

A Yes.

Q And you agree that the authors of Stahl knew that synthesis of the second-strand cDNA can occur on the array?

A They knew --

A I cite that directly in 171, Paragraph 171.

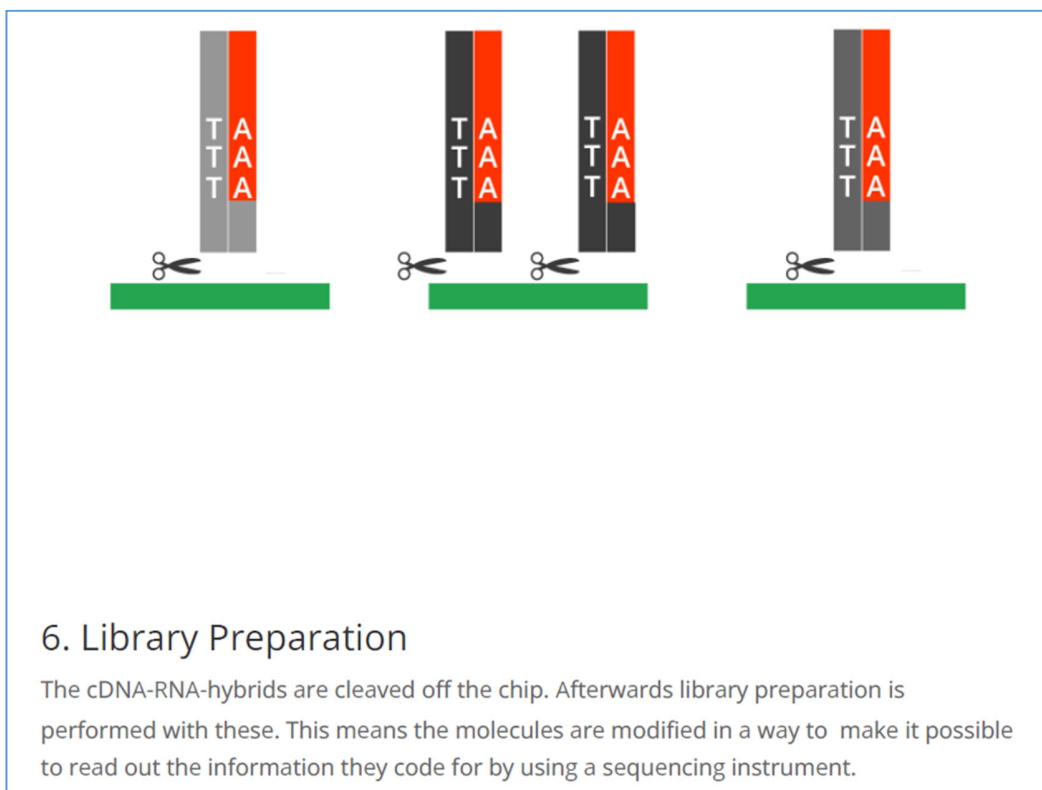
Q All right. So you agree that the authors of Stahl, who knew about

solid-phase amplification and knew that the synthesis of the second-strand cDNA can occur on the array, published a paper where they did not utilize either of these techniques?

A That is correct.

EX2009 at 100:3-101:10. Thus, even though allegedly in possession of Petitioner's proposed modifications, Stahl's authors did not publish them. The natural inference is that Petitioner's proposed modifications do not work.

80. Further, I understand that Petitioner credits Stahl's author as "the founders of a company called Spatial Transcriptomics. Stahl describes the fundamental Spatial Transcriptomics technology of collecting mRNAs onto spatially barcoded arrays, performing cDNA synthesis reactions to incorporate spatial barcodes into the cDNA products, collecting the cDNA products, processing the cDNA products, and analyzing them by sequencing." Paper 2 at 2. I note, however, that even in the workflow from Spatial Transcriptomics that Petitioner cite as an exhibit, dated 2023, solid-phase amplification is not performed. In particular, the second-strand cDNA is performed in solution. As the Spatial Transcriptomics workflow makes clear, after synthesis of the first-strand cDNA, the "cDNA-RNA-hybrids are cleaved off the chip":



EX1018 at 7.

81. Petitioner contends that solid-phase amplification can minimize undesirable primer interactions, such as the formation of primer dimers. Paper 2 at 48. This, however, is not a concern with Stahl. In Stahl's workflow, all mRNA hybridize to the capture probe via the same sequence, a poly-A tail. Subsequently, reverse transcription, second-strand cDNA synthesis, generation of amplified RNA, and second reverse transcription do not utilize primers. The next amplification step that utilizes primers is when sequencing adaptors are added to the second cDNA product. For this amplification, the same forward and reverse primers are used, as both ends of the molecules contain known sequences. Thus, with only one pair of

primers, there is already minimal undesirable primer interactions, such that the advantage cited by Petitioner is unlikely to motivate a POSA.

82. Another potential benefit of solid-phase amplification that Petitioner refer to is “sensitivity and selectivity.” These potential benefits, however, are highly dependent on the workflow and require significant experimentation to modify Stahl’s existing workflow. The reference that Petitioner relies on is in agreement, as it states that “the emergence of ‘active’ arrays *may* provide the sensitivity and selectivity for nucleic acid identification and genotyping.” EX1048 at 11. In addition, the reference states that the future of solid-phase amplification is in a microbead-platform, which is not what Petitioner is proposing. *See id.* Thus, any advantages that solid-phase amplification may offer over Stahl’s workflow is speculative.

83. A POSA would additionally have been concerned with the many drawbacks of solid-phase amplification. For example, these include electrostatic forces, the finite number of primers, polymerase and nucleotide availability, sterile molecules, and DNA rigidity, which can all affect a reaction. EX2013 at 40-41. These are issues that do not affect solution amplification. A POSA would also have been aware that solid-phase amplification is significantly restricted by steric hindrance—DNA strands that are attached to a surface will naturally inhibit the activity of neighboring strands. *See id.* at 32. Even a reference cited by Petitioner

acknowledges the many issues related to solid-phase amplification: “a molecule at the center of the colony (which is surrounded by others) will have a smaller duplication probability (its free end is less likely to find a matching primer on the surface,” “a sterile molecule still occupies space; and therefore it applies steric constraints to its neighbors and can prevent them from duplicating,” “a primer (or an attached molecule)...[can] break away from the surface,” and “it is very difficult for a molecule surrounded by others to bend so that its end can find a matching primer.” EX1050 at 2079, 2081, 2083-2084. As discussed above, the large size of the RNA polymerase in Stahl would be constrained to operate in a solid-phase environment in addition to the issues recited here. In my opinion, a POSA would have been well aware of all of these issues with solid-phase amplification and thus would not have been motivated to modify Stahl to implement solid-phase amplification.

84. I also note that Stahl’s first-strand cDNA is synthesized in solid phase as the capture probe is extended during reverse transcription of the hybridized sample mRNA. Thus, Stahl already benefits from employing a solid-phase reaction, such as being able to easily wash away unbound sample. A POSA would have recognized Stahl’s workflow to benefit from a solid-phase reaction and would not have further modified Stahl to implement solid-phase amplification for additional steps in light of the numerous issues discussed above.

85. As discussed above, a POSA would not have been motivated to modify Stahl to implement solid-phase amplification and would not have expected it to succeed. Accordingly, Stahl at least does not render obvious claim 5 of the '142 patent.

C. GROUND 3: CLAIM 5 WOULD NOT HAVE BEEN OBVIOUS OVER STAHL AND FRISEN

86. Ground 3 is similar to Ground 2 in that Petitioner also contends that Stahl in view of Frisen would have rendered claim 5 obvious. My opinions set forth for Ground 2 apply equally here. *See supra* § VIII.B.2.

87. Petitioner brings the same argument, essentially that Stahl can be modified to read on claim 5 because Frisen allegedly discloses “using solid-phase second-strand cDNA synthesis and solid-phase library amplification steps, both of which are ‘solid-phase amplification.’” Paper 2 at 51. As explained for Ground 2, a POSA would not have modified Stahl to incorporate either a solid-phase second strand cDNA or a solid-phase library amplification. *See supra* § VIII.B.2. In addition, similar to that discussed in Ground 2, neither second-strand cDNA nor library amplification are part of the synthesis of the first-strand cDNA, Petitioner’s identified “DNA products.”

88. Even if solid-phase second-strand cDNA synthesis or library amplification can read on the claimed limitation of claim 5, Frisen does not provide the motivation or the reasonable expectation of success that would lead a POSA to

modify Stahl. Frisen states that both second-strand cDNA synthesis and library amplification are optional steps:

The present invention relates to methods and products for the localised or spatial detection of nucleic acid in a tissue sample and in particular to a method for localised detection of nucleic acid in a tissue sample comprising: (a) providing an array comprising a substrate on which multiple species of capture probes are directly or indirectly immobilized such that each species occupies a distinct position on the array and is oriented to have a free 3' end to enable said probe to function as a primer for a primer extension or ligation reaction, wherein each species of said capture probe comprises a nucleic acid molecule with 5' to 3': (i) a positional domain that corresponds to the position of the capture probe on the array, and (ii) a capture domain; (b) contacting said array with a tissue sample such that the position of a capture probe on the array may be correlated with a position in the tissue sample and allowing nucleic acid of the tissue sample to hybridise to the capture domain in said capture probes; (c) generating DNA molecules from the captured nucleic acid molecules using said capture probes as extension or ligation primers, wherein said extended or ligated DNA molecules are tagged by virtue of the positional domain; (d) *optionally* generating a complementary strand of said tagged DNA and/or *optionally* amplifying said tagged DNA; (e) releasing at least part of the tagged DNA molecules and/or their complements or amplicons from the surface of the array, wherein said part includes the positional

domain or a complement thereof; and (f) directly or indirectly analysing the sequence of the released DNA molecules.

EX1017 Abstract. Thus, a POSA would have recognized that the two modifications proposed by Petitioner are optional and unnecessary to practice Frisen.

89. In my opinion, a POSA would not even attempt to modify Stahl in view of Frisen. Frisen does not provide any further information on why a POSA would want to practice these optional steps, much less modify an existing working method, Stahl. For example, Frisen teaches that “second strand cDNA synthesis may be achieved by any means known in the art” and is preferably in solution:

For instance, in tissue samples that comprise large amounts of RNA, the step of generating the double stranded cDNA may yield a sufficient amount of cDNA that it may be sequenced directly (following release from the array). In this case, second strand cDNA synthesis may be achieved by any means known in the art and as described below. The second strand synthesis reaction may be performed on the array directly, i.e. whilst the cDNA is immobilized on the array, or preferably after the cDNA has been released from the array substrate, as described below.

Id. at 39:1-8. In addition, Frisen is clear that this optional step of synthesizing the second-strand cDNA should be performed only “in tissue samples that comprise large amounts of RNA,” so that the double-stranded cDNA “may be sequenced directly.” *Id.* As discussed previously, Stahl purposefully practices stranded

RNA sequencing, which involves transcribing the double-stranded cDNA into amplified RNA so as to maintain strand preservation. This is completely contrary to Frisen's teaching that the second-strand RNA should be synthesized for direct sequencing.

90. Frisen also does not disclose solid-phase amplification. Frisen also discusses the second-strand cDNA acting as a template for further amplification:

In other embodiments it will be necessary to enhance, i.e. amplify, the amount of secured nucleic acid, e.g. synthesized cDNA to yield quantities that are sufficient for DNA sequencing. In this embodiment, the first strand of the secured nucleic acid, e.g. cDNA molecules, which comprise also the capture probe of the features of the array, acts as a template for the amplification reaction, e.g. a polymerase chain reaction. The first reaction product of the amplification will be a second strand of DNA, e.g. cDNA, which itself will act as a template for further cycles of the amplification reaction.

EX1017 at 39:9-16. Frisen, however, does not discuss solid-phase amplification, or any form of clonal amplification, in its discussion amplifying the synthesized cDNA.

91. In my opinion, a POSA would not have been motivated to modify Stahl in view of Frisen. I understand that Petitioner frames Frisen as "applications and modifications of Stahl's spatial barcoding array workflow" due to overlapping

authors and inventors between Stahl and Frisen. Paper 2 at 53. A POSA, however, would not understand it as such. This is because Stahl post-dates Frisen by at least four years. It is beyond common sense to expect that an earlier reference, Frisen, contains “applications and modifications” of a later publication, Stahl. In addition, it is immediately apparent that Frisen does not contain “applications and modifications of Stahl’s spatial barcoding array workflow” because nowhere in Frisen is there any mention of synthesizing amplified RNA, one of the key aspects of Stahl.

92. Petitioner also contends that “solid-phase second-strand cDNA synthesis and/or solid-phase library amplification offers certain advantages” that would motivate a POSA to modify Stahl. Paper 2 at 53. In my opinion, a POSA would not be motivated to modify Stahl because the advantages cited by Petitioner, “sensitivity,” “specificity,” and “scalability,” are not readily apparent in light of the many disadvantages of implementing solid-phase amplification. *See supra* § VII.B.2. Regardless, to the extent there are benefits to solid-phase reaction, Stahl already includes solid-phase synthesis of first-strand cDNA.

93. In addition, Frisen’s optional steps do not include clonal amplification, such that Petitioner’s claimed advantage of reduced “undesirable primer interactions” would not occur. This is because primers continue to be in solution unless they are attached to a solid surface to allow clonal amplification.

Regardless, primer dimer is not a major concern in Stahl because the same set of primers are used across the entire sample. In Stahl, primers are used only in the beginning of the workflow to capture the sample mRNA and at the end to add sequencing adapters to the ends of the amplified cDNA product. Thus, a POSA would have understood that undesirable primer interactions were not a concern in Stahl to begin with.

94. Likewise, Petitioner's claimed advantage of "parallelization of the DNA amplification process without any direct human intervention" is unlikely to motivate a POSA. Modifying Stahl's method so that there is additional parallelization of DNA amplification is unnecessary as each capture probe already contains a location-identifying sequence so that the sequenced result can be traced back to the location of the array where the mRNA was captured. Continuing to perform reactions on nucleic acids with the same location-identifying sequence in solid phase will only lead to lower efficiency along with issues that need to be tackled, as discussed previously. *See supra* § VIII.B.2. Similarly, as discussed, Petitioner provides no reason why a POSA would modify Stahl to replace the existing amplification steps with clonal amplification. *See id.* A POSA would not undergo a drastic modification of Stahl, which is shown to be a working method, to implement solid-phase amplification with dubious advantages.

95. A POSA would also not reasonably expect modifying Stahl as Petitioner proposes to succeed. In addition to the issues discussed above wherein Frisen does not readily teach either solid-phase second-strand cDNA or solid-phase library amplification, Petitioner provides no details on how Stahl can be modified. For example, amplified RNA cannot readily be generated if the first-strand/second-strand cDNA duplex is immobilized on a surface due to the access the T7 RNA polymerase would require to access the double strand. *See supra* § VIII.B.2. In addition, implementation of clonal amplification would require foregoing strand preservation, a major component of Stahl's workflow. *See id.*

D. GROUND 4: SO-456 DOES NOT ANTICIPATE CLAIMS 1-13

96. I understand Petitioner has argued that So-456 anticipates all claims of the '142 patent. In my opinion, So-456 fails to anticipate because it does not disclose key limitations in the '142 patent. Namely, So-456 does not teach (1) incorporating a nucleic acid sequence that identifies location, (2) does not teach a synthesis reaction that incorporates a location-identifying nucleic acid sequence, (3) does not teach conditions that release oligonucleotide probes, (4) does not disclose solid-phase amplification to synthesize DNA products, and (5) does not disclose amplifying a library using a synthesized DNA product.

1. So-456 Does Not Incorporate A Nucleic Acid Sequence That Identifies Location

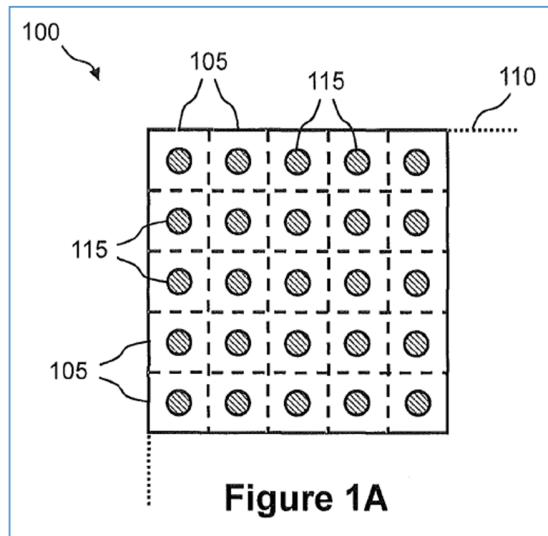
97. In my opinion, So-456 does not anticipate claim 1 of the '142 Patent at least because it does not teach incorporating a nucleic acid sequence that identifies location.

98. Claims 1(c) and 1(d) require synthesizing “DNA products” that incorporate a spatial barcode:

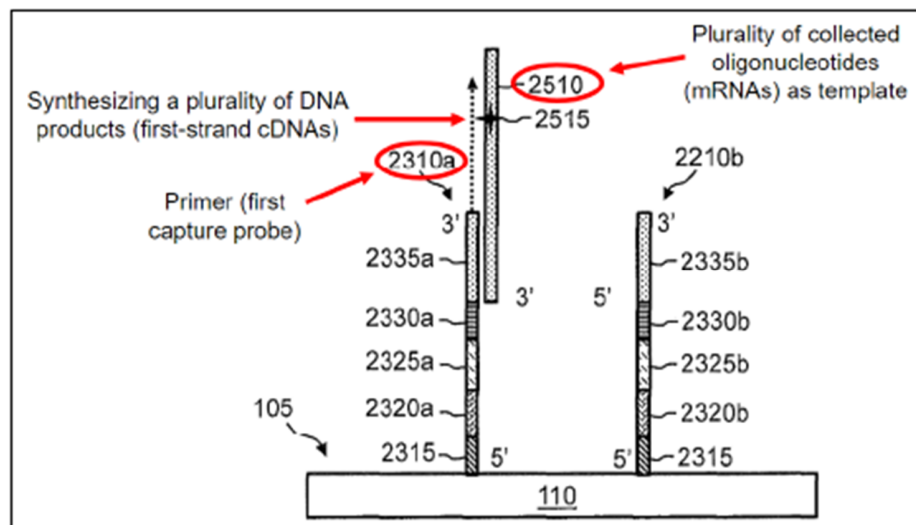
Synthesizing a [first]/[second] plurality of DNA products by performing a synthesis reaction that uses the plurality of oligonucleotides collected in step [(a)/(b)] as templates an incorporates at least one nucleic acid sequence that identifies the [first/second] location of the tissue sample into each of the [first/second] plurality of DNA products;

EX1043 Claims 1(c) and 1(d). Petitioner, and Dr. Spellman, identified a first-strand cDNA product as the claimed “DNA products. Petitioner argued that “So-456’s method uses the ‘gene-specific capture regions on the first capture probe’ (2310a) as ‘reverse transcriptase primers’ for ‘synthesis of first strand cDNA from captured mRNA molecules.’” Paper 2 at 61; *see also* EX1044 ¶¶ 209-210. Petitioner’s argument, however, is unsupported by the disclosures in So-456.

99. So-456 discloses an encoding scheme with an X-Y arrangement of capture sites, as shown in Figure 1A:



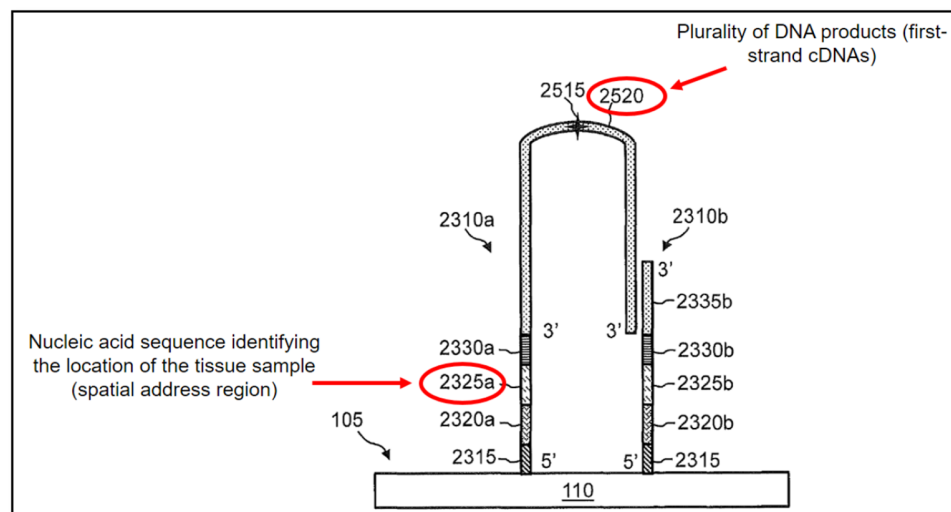
EX1005 Fig. 1A; *id.* ¶ 141. To encode the X-Y location of an analyte, two sequences are used in the capture probes that are immobilized to the array – in the figure below, as annotated by Dr. Spellman, these are 2325a and 2325b:



EX1005 Fig. 25A; EX1044 ¶ 209. As So-456 explains, “spatial address region 2325a” is a “unique spatial address for the X dimension” and “spatial address region 2325b” is a “unique spatial address for the Y dimension.” EX1005 ¶¶ 319-320; *see also* ¶ 302 (“capture probe 2310a comprises...spatial address region 2325a, which

comprises a unique spatial address sequence for the X dimension...Similarly, capture probe 2310b comprises...spatial address region 2325b, which comprises a unique spatial address region for the Y dimension...”).

100. As described by Petitioner and Dr. Spellman, what has been annotated as the “first capture probe” (2310a) is a “reverse transcriptase primer” to synthesize “first strand cDNA from captured mRNA molecules.” Paper 2 at 61. This cDNA product incorporates sequence 2325a:



Paper 2 at 64; EX1044 ¶ 214.

101. The very figure that Petitioner and Dr. Spellman rely upon, however, makes clear that only half the sequence necessary to encode location information is part of the first-strand cDNA, Petitioner’s identified “DNA products.” While sequence 2325a (encoding the X-coordinate) is part of the cDNA, sequence 2325b is not part of the of the DNA product.

102. Therefore, it is my opinion that the disclosures Petitioner relies upon do not disclose the recited “DNA products.” Petitioner contends that the first-strand cDNA products are the recited “DNA products,” which must “incorporate a nucleic acid sequence that identifies” the location in the tissue sample. The first-strand cDNA, however, only incorporates *half* the information necessary to identify the location. Therefore, the DNA products as identified by Petitioner do not incorporate a nucleic acid sequence identifying the location.

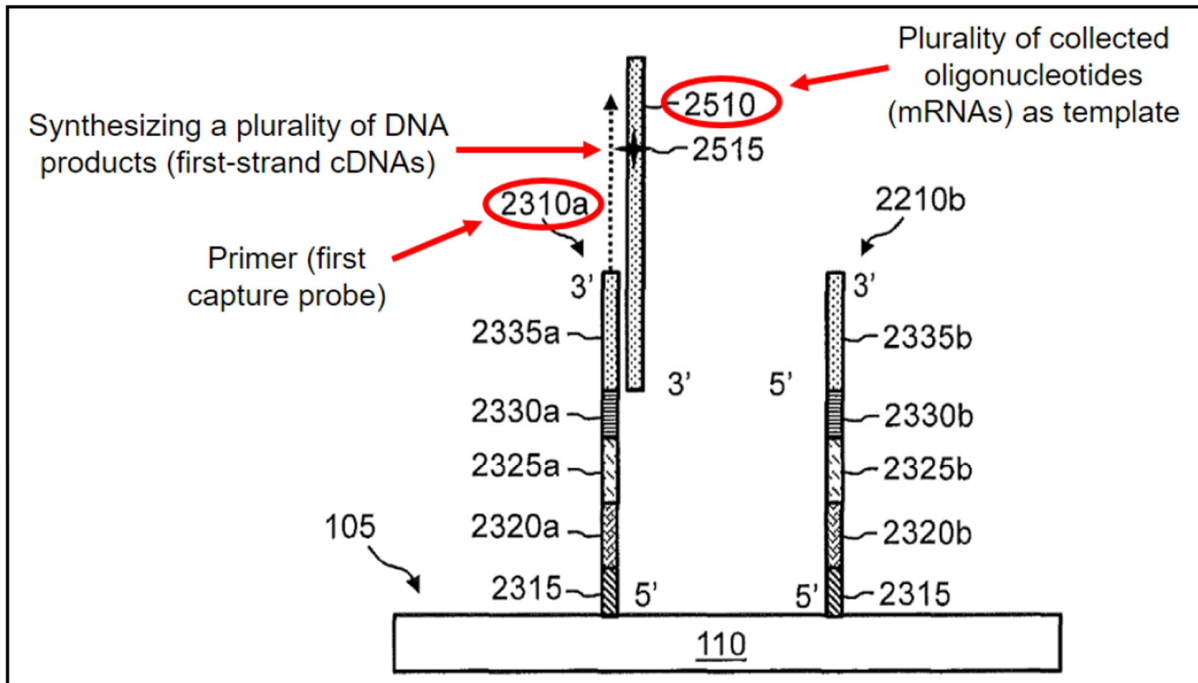
2. So-456 Does Not Disclose A Synthesis Reaction That Incorporates A Nucleic Acid Sequence That Identifies Location

103. In my opinion, So-456 does not anticipate claim 1 of the '142 patent because it does not disclose that the nucleic acid sequence used for location identification is incorporated during the synthesis reaction.

104. As I describe in connection with my opinions regarding Stahl's failure to disclose, claims 1(c) and 1(d) not only require the incorporation of a nucleic acid sequence that identifies the location of an analyte in the tissue sample, *i.e.*, a spatial barcode or location-identifying sequence, but also that the spatial barcode is incorporated during synthesis of the DNA product. *See supra* § VIII.A.2.

105. So-456 does not teach the incorporation of a spatial barcode via a synthesis reaction. As depicted in Dr. Spellman's own annotations and argued by

Petitioner, the spatial address region is already found in the capture probe that is extended:



Paper 2 at 61; EX1005, Fig. 25A; EX1044 ¶ 209. After an analyte hybridizes to the capture probe (2310a), extension occurs unidirectionally as shown by the arrow annotated “synthesizing a plurality of DNA products (first-strand cDNAs).” Importantly, the spatial address region (2325a) is not part of the synthesis reaction, as it is part of the primer from which extension occurs.

106. Therefore, it is my opinion that the spatial address region is not “incorporated” into Petitioner’s identified “DNA products,” as it is already present and not synthesized onto the first-strand cDNA, identified by Petitioner as the “DNA products.” So-456 therefore does not anticipate claim 1.

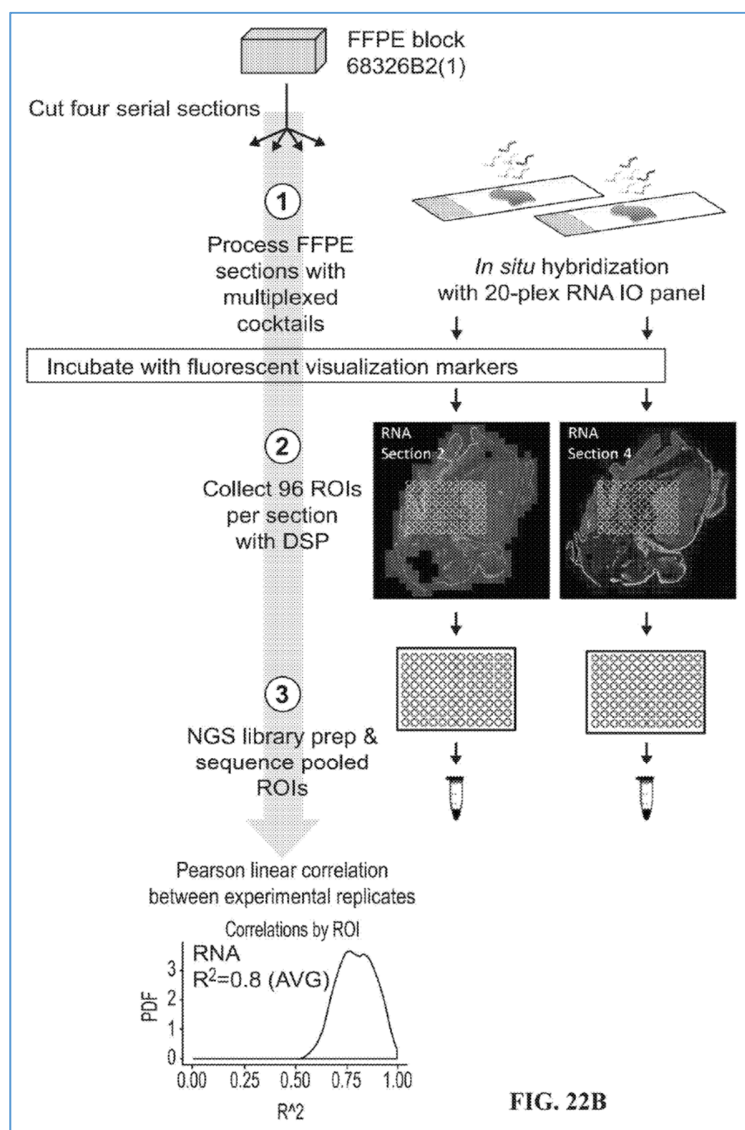
3. So-456 Does Not Disclose Conditions That Release Oligonucleotides

107. In my opinion, So-456 does not anticipate claim 1 of the '142 Patent because it does not disclose conditions that release oligonucleotides.

108. Claims 1(a) and 1(b) require “collecting a plurality of oligonucleotides from a [first]/[second] location of a tissue sample *under conditions that release the plurality of oligonucleotides* from the [first]/[second] location of the tissue.” EX1043 claim 1.

109. The claimed “conditions” would have been different from those in prior methods, such as in situ hybridization, which did not collect and identify nucleic acids from the tissue sample. For example, a POSA would have known that working with formalin-fixed paraffin-embedded (FFPE) tissue samples would have posed numerous challenges, including nucleic acid damage as a byproduct of formalin fixation, low input for the assay, poor signal to noise ratios, and difficulties with library preparation. In my opinion, based on my experience, any tissue sample must be treated in some manner so nucleic acids can be collected from the sample.

110. Demonstrating the importance of a treatment step, the '142 patent highlighted a “processing” step in the workflow:



EX1043 Fig. 22B. In this figure, not only is an FFPE block sectioned, but the sections are processed “with multiplexed cocktails” prior to incubating with fluorescent markers and subsequent analysis. *Id.* This “processing” step creates the claimed “conditions” for release. In fact, in my opinion it would be impossible to perform the analysis described in the workflow above on an FFPE sample without

first treating the sample in a manner to create the conditions for release. This holds similarly for other kinds of tissue samples.

111. I also note that Petitioner identified a permeabilization step in Stahl that involved treating the tissue with “0.01 pepsin dissolved in 0.1M HCl.” Paper 2 at 26. Although I do not provide an opinion on the sufficiency of that treatment, it is a recognition by Petitioner that a treatment is required to place a tissue under conditions such that nucleic acids are released.

112. The disclosures in the ’142 patent are different from what Petitioner and Dr. Spellman point to in So-456 as the claimed “conditions.” For instance, Petitioner cites to paragraph 152, which states that at step 210, “a tissue sample is prepared for analysis. In some embodiments, the tissue sample is a FFPE sample that is sectioned onto a slide.” A POSA would have recognized the tissue sample being “prepared for analysis” in this passage to refer to the sectioning, as the paragraph does not disclose any steps of treatment to facilitate nucleic acid release.

113. As yet another example, Petitioner and Dr. Spellman point to disclosures that the target nucleic acid in the tissue section is “transferred onto an array such that the position of a nucleic acid on the array can be correlated with a position on the tissue section,” that a “tissue sample that comprises a plurality of mRNA molecules is contacted with a capture array,” and that “the tissue sample containing mRNA molecule 2510 or a substrate comprising mRNA molecule 2510

derived from the tissue sample (not shown) is contacted with capture probes 2310.” EX1005 ¶¶ 154, 308, 315. These are all disclosures that are focused on the step of contacting a tissue sample with a probe, and not creating conditions that release the nucleic acids. In fact, a POSA would not be able to use these disclosures to create such conditions as recited.

114. Petitioner and Dr. Spellman provide only conclusory arguments that the So-456 discloses the claim element. According to Petitioner, because So-456 describes steps that involve the mRNA, it must be that “the tissue sample was treated to create a condition to ‘release’ the mRNA from the tissue onto the array.” Paper 2 at 60-61; *see also* EX1044 ¶¶ 207-208. In my opinion, this is conclusory and fails to provide any evidence underlying the argument. Merely stating that there are steps using mRNA is not disclosure of treatment, particularly in a case where the ’142 patent represents an advance over methods in the prior art.

115. It is my understanding that when Petitioner and Dr. Spellman contend that So-456 must have disclosed the claimed conditions while failing to identify any treatment or conditions, they are relying on inherency. It is my understanding that inherency is a doctrine in which a missing claim element can be considered disclosed by a prior art if the claim element is necessarily present or the natural result of the elements disclosed by the prior art. I note that neither Petitioner nor Dr. Spellman invoked inherency. I also note that Petitioner and Dr. Spellman explicitly invoked

inherency in a related IPR. *See* IPR2023-01298 Paper 2 at 23; *id.*, EX1002 ¶ 139. Accordingly, I take the lack of mention of inherency to mean that Petitioner is not relying on inherency for this claim element. I understand Dr. Spellman confirmed that he understood the doctrine of inherency and did not find it present in his declaration. EX2009 at 18:21-19:2, 19:22-26:7.

116. For the foregoing reasons, it is my opinion that So-456 does not disclose treating a tissue sample so that the oligonucleotides are released and does not anticipate claim 1 of the '142 Patent.

4. So-456 Does Not Disclose Solid-Phase Amplification To Synthesize DNA Products

117. In my opinion, So-456 also does not anticipate claim 5 of the '142 patent. Claim 5 depends from claim 4 (which ultimately depends from claim 1), and further requires that the recited amplification steps “comprise performing a solid-phase amplification reaction, wherein the solid phase amplification reaction is carried out on the microscope slide using the plurality of primers immobilized on the microscope slide.” EX1043 claim 5.

118. As I have detailed above, Petitioner identifies So-456's first-strand cDNA as the claimed “DNA products.” *See supra* § VIII.D.1. To show that So-456 discloses this claim limitation, therefore, Petitioner must show that synthesis of the first-strand cDNA is performed using a solid-phase amplification reaction.

119. Petitioner, and Dr. Spellman, instead identify So-456's *second-strand cDNA synthesis step* as being allegedly performed using a solid-phase amplification reaction. Paper 2 at 69-71; EX1044 ¶¶ 230-235. Petitioner does not argue, nor does Dr. Spellman identify, the second-strand cDNA as a "DNA product" as recited in claim 1. Synthesis of the second-strand cDNA takes place after synthesis of the first-strand cDNA, which Petitioner and Dr. Spellman identify as the recited "DNA products." Therefore, under Petitioner's own arguments, So-456 does not disclose that the "DNA products" are generated using solid-phase amplification.

5. So-456 Does Not Disclose Amplifying A Library Using DNA Products

120. In my opinion, So-456 does not anticipate claim 7 of the '142 Patent because it does not disclose amplifying a library using DNA products as templates. Claim 7 depends from claim 1, and further requires "amplifying a library using the first plurality of DNA products and the second plurality of DNA products as templates." EX1043 claim 7.

121. As I describe in connection with Section VIII.D.4 above, Petitioner and Dr. Spellman identify the first-strand cDNA in So-456 as the recited "DNA products." Therefore, the first-strand cDNA must be used as the template for a library to read onto this claim. Petitioner, however, states that the "second strand cDNAs (2525) are released from the array and 'amplified to generate a sequencing library.'" Paper 2 at 72; *see also* EX1044 ¶ 239 ("So-456 elaborates that 'cDNA

molecule 2525 is *amplified* using an SBS primer 2530 and a second SBS primer 2535' to generate a 'library *amplicon* 2550.'). As is plain from Petitioner's own argument, the actual molecule being amplified is not the first-strand cDNA, but rather the second-strand cDNA. This is consistent with a POSA's understanding of the method described in So-456.

122. Indeed, So-456 explicitly describes the difference between the first and second strand cDNA molecule. So-456 discloses that a "second strand cDNA is synthesized in an extension reaction using capture region 2335b as a primer." EX1005 ¶ 318. This molecules is "released from capture site 105 by cleavage of a cleavable region 2315." *Id.* ¶ 319. Finally, the second strand "cDNA molecule 2525 is amplified using an SBS primer 2530 and a second SBS primer 2535." *Id.* ¶ 320.

123. As I describe above, the first-strand cDNA, which is what Petitioner identifies as the recited "DNA products," is different from the second-strand cDNA that Petitioner and Dr. Spellman attempt to rely upon as disclosure to anticipate this claim. To permit amplification, the second-strand cDNA includes additional sequences that are not part of the first-strand cDNA, such as "SBS primer 2535 comprises an SBS region 2320b' that is complementary to SBS primer region 2320b." *Id.* In the cited passage, primer region 2320b is part of the second-strand

cDNA but not part of the first-strand cDNA. Therefore, the amplification primers that hybridize to that region would not be able to amplify the first-strand cDNA.

E. GROUND 5: CLAIMS 1-13 WOULD NOT HAVE BEEN OBVIOUS OVER SO-456

124. Petitioner and Dr. Spellman also provide conclusory arguments that, should So-456 not anticipate claims 1-13, the claims would have been obvious over So-456.

125. I understand that to show obviousness, a Petitioner must show that there was a motivation to modify a prior art reference to arrive at the claimed invention, and that a Petitioner must show a reasonable expectation of success in modifying the reference. In my opinion, Dr. Spellman and Petitioner fail to provide any specific arguments as to motivation to modify the methods of So-456 or a reasonable expectation of success in modifying those methods.

126. For instance, Petitioner argues that a “POSA reading So-456 would have been motivated to practice the claimed methods because, e.g., So-456 teaches a ‘method ... of spatial detection and analysis of a nucleic acid (i.e., at least one target analyte) in a tissue sample’ using spatially barcoded arrays.” Paper 2 at 78; *see also* EX1044 ¶ 254 (stating the same). This argument merely shows that So-456 and the ’142 patent are directed to similar fields. In my opinion, this does not show that a POSA would have been motivated to modify the methods of So-456, as

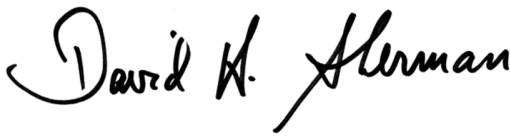
Petitioner and Dr. Spellman have failed to provide any evidence as to why So-456 would be modified to arrive at the claimed invention.

DECLARATION

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

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

November 20, 2024
Dated: _____


By: _____