

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 IPR2023-01298
U.S. Patent No. 11,377,689

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

I. INTRODUCTION

1. I, David H. Sherman, Ph.D., make this Declaration in connection with *inter partes* review proceeding No. IPR2023-01298 concerning U.S. Patent No. 11,377,689 (“the ’689 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 ’689 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 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 inhibitors of HIV Nef 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 EX2014, 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 '689 Patent (EX1001)
- Dr. Paul Spellman's declaration submitted in support of the Petition for *inter partes* review (EX1002), as well as the exhibits Dr. Spellman cited
- The Board's institution decision in this matter (Paper 14)
- 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.

C. Written Description

19. I understand that written description of a subject matter is assessed from the viewpoint of a person of ordinary skill in the art. I understand that assessing written description requires determining that the disclosures in the application convey that inventors had “possession” of the claimed invention. Possession, I understand, does not require physical possession in the context of the written description analysis. I understand that sufficient description is determined by the sufficiency of the disclosures in the application, including any patents or patent applications incorporated by reference within the disclosures, and the state of the art at the time of filing. The state of the art is determined by the general knowledge of a POSA, and can be demonstrated by the published literature at the time.

20. Furthermore, I understand that a patent application does not need to disclose a working example to satisfy the written description requirement. As long as there is a sufficient disclosure in the application, separate parts of the disclosure can be combined.

V. THE PERSON OF ORDINARY SKILL IN THE ART

21. 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.

22. 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.

23. 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.

EX1002 ¶¶ 24-25. I agree with Dr. Spellman that the skill level of a POSA relevant to the '689 patent is high.

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

- Amplification and sequencing of nucleic acids, including PCR and next-generation sequencing. *See, e.g.*, EX1009 ¶ 177 (“the ligation product is amplified via PCR using amplification primers that hybridize to the first and second amplification primer binding sites”); *id.* ¶ 230 (“the identifier oligonucleotides are sequenced using next generation sequencing methods to spatially detect at least one target analyte in the sample”).
- Design of oligonucleotide strands with specific sequences, including attachment of different labels or molecules. *See, e.g.*, EX2013 (describing oligonucleotide synthesis, addition of molecules such as labels, and their applications).
- Ligating nucleic acids, including using ligases. *See, e.g.*, EX2015 at 17, 23-25 (describing DNA ligases); EX2001 at 1 (teaching “binding of pairs of specific protein-binding reagents to the same target-protein

molecule” and ligating them together when “a mixture is added that contains all components required for ligation”).

- Immunohistochemical and in situ hybridization methods. *See, e.g.,* EX1009 ¶ 3 (“Standard immnohistochemical and *in situ* hybridization methods allow for simultaneous detection of, at most, six to ten protein or nucleic acid targets, with three to four targets being typical.”).

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

VI. CLAIM CONSTRUCTION

25. 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 '689 Patent. I further understand that in its Decision Granting Institution of this *inter partes* review, the Board provided some preliminary constructions.

26. As to the terms that the District Court construed, I understand that the court gave those terms their plain and ordinary meaning. EX1052. Therefore, the District Court’s claim construction order has no bearing on my opinions in this declaration.

27. The Board construed the terms “nucleic acid probe” and “collecting a nucleic acid probe.” The Board construed the term “nucleic acid probe” to refer to an oligonucleotide comprising a target binding domain that binds to one or more target analytes, and is modified after collection to comprise a location-identifying nucleic acid sequence for use in identifying the location of the target analyte. Paper 14 at 23. To the extent that the Board’s construction refers to the use of an extension reaction to encode spatial information, I agree with the Board’s construction.

28. The Board construed the “collecting the nucleic acid probe” to mean releasing all or a portion of the nucleic acid probe bound to the at least one target analyte through application of a force followed by retrieval of the released probe or portion of the probe. Paper 14 at 24. I agree with this construction, and apply it in my opinions below.

VII. OVERVIEW OF THE '689 PATENT

29. The '689 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.” EX1001 at Abstract.

30. The '689 Patent was issued on July 5, 2022, from U.S. Patent Application No. 17/476,707 (“’707 Application”), filed on September 16, 2021. The '707 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 18, 2018, and 62/771,212, filed November 26, 2018. The specification of the '707 Application and the '487 Application are identical.

31. Claim 1 of the '689 Patent recites:

1. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:
 - a) contacting the tissue sample with a plurality of nucleic acid probes, wherein each of the nucleic acid probes comprise a target binding domain that binds to the at least one target analyte, wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte;
 - b) collecting the nucleic acid probes, or portions thereof, bound to the at least one target analyte in a first location of the tissue sample under conditions that release the nucleic acid probes, or portions thereof, from the first location of the tissue sample;
 - c) collecting the nucleic acid probes, or portions thereof, bound to the at least one target analyte in a second location of the tissue sample under conditions that release the nucleic acid probes, or portions thereof, from the second location of the tissue sample;

d) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the nucleic acid probes, or portions thereof, collected in step (b), thereby forming a first plurality of extension products that comprise the nucleic acid probes, or portions thereof, collected in step (b) and the at least one nucleic acid sequence that identifies the first location of the tissue sample;

e) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the nucleic acid probes, or portions thereof, collected in step (c), thereby forming a second plurality extension products that comprise the nucleic acid probes, or portions thereof, collected in step (c) and the at least one nucleic acid sequence that identifies the second location of the tissue sample; and

f) identifying the first plurality of extension products and the second plurality of extension products by sequencing the first plurality of extension products and the second plurality of extension products, thereby spatially detecting the at least one target analyte in the first location of the tissue sample and the second location of the tissue sample.

EX1001 Claim 1.

32. This claim is directed to the spatial detection of a target analyte, *e.g.*, a protein or a nucleic acid, in a first and second location of a tissue sample. Furthermore, as the prosecution history makes clear, this claim encompasses “methods wherein steps (b), (e) and (f) are repeated at one or more additional locations of the tissue sample (*e.g.* a third location, a fourth location, a fifth location, etc.,” and so this claim is directed to the spatial detection of a target analyte across a plurality of locations. EX2011.269. Claims 2-15 depend from claim 1.

33. The '689 Patent has a second independent claim, claim 16:

16. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:

a) contacting the tissue sample with a first plurality of nucleic acid probes and a second plurality of nucleic acid probes,

wherein each of the nucleic acid probes in the first plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte,

wherein each of the nucleic acid probes in the second plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte,

wherein the tissue sample is treated to facilitate binding of the first plurality of nucleic acid probes and the second plurality of nucleic acid probes to the at least one target analyte;

b) forming ligated probes by ligating each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are bound to each of identical molecules from the at least one target analyte;

c) collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte in a first location of the tissue sample under conditions that release the ligated probes, or portions thereof, from the first location of the tissue sample;

d) collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte in a second location of the tissue sample under conditions that release the probes, or portions thereof, from the second location of the tissue sample;

e) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (c), thereby forming a first plurality of extension products that comprise the ligated probes, or portions thereof, collected in step (c) and the at least one nucleic acid sequence that identifies the first location of the tissue sample;

f) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (d), thereby forming a second plurality extension products that comprise the ligated probes, or portions thereof, collected in step (d) and the at least one nucleic acid sequence that identifies the at least second location of the tissue sample; and

g) identifying the first plurality of extension products and the second plurality of extension products by sequencing the first plurality of extension products and the second plurality of extension products, thereby spatially detecting the at least one target analyte in the first location of the tissue sample and the second location of the tissue sample.

EX1001 Claim 16.

34. The method recited in claim 16 is different from the method recited in claim 1 in that it requires a first and second plurality of probes. These probes are ligated to form ligated probes that are subsequently collected and used for analysis.

Claims 17-30 depend from claim 16.

VIII. 10X GENOMICS HAS NOT PROVEN UNPATENTABILITY OF THE CLAIMS FROM THE '689 PATENT

A. GROUND 1: SO-456 DOES NOT ANTICIPATE CLAIMS 1-8, 11-23, AND 26-30

35. I understand that Petitioner has argued in Ground 1 that So-456 anticipates the above-listed claims of the '689 Patent, *i.e.*, that all elements of the claims are found in So-456, as they are arranged in the claims. In my opinion, however, So-456 does not disclose several key elements of the two independent claims: So-456 does not disclose collecting the nucleic acid probes, as required by

claim steps 1(b)-(c) and 16(c)-(d); So-456 does not disclose a treated tissue sample, as required by claim steps 1(a) and 16(a); So-456 does not disclose an extension reaction to incorporate a nucleic acid sequence that identifies a location, as required by claim steps 1(d)-(e) and 16(e)-(f); and So-456 does not disclose forming ligated probes, as required by claim step 16(b).

36. Claims 1 and 16 are the only independent claims that Petitioner challenges, and all other challenged claims depend from either claim 1 or 16. Because So-456 does not disclose all the limitations of the independent claims, it is my opinion that it cannot anticipate any of the claims that depend from the independent claims.

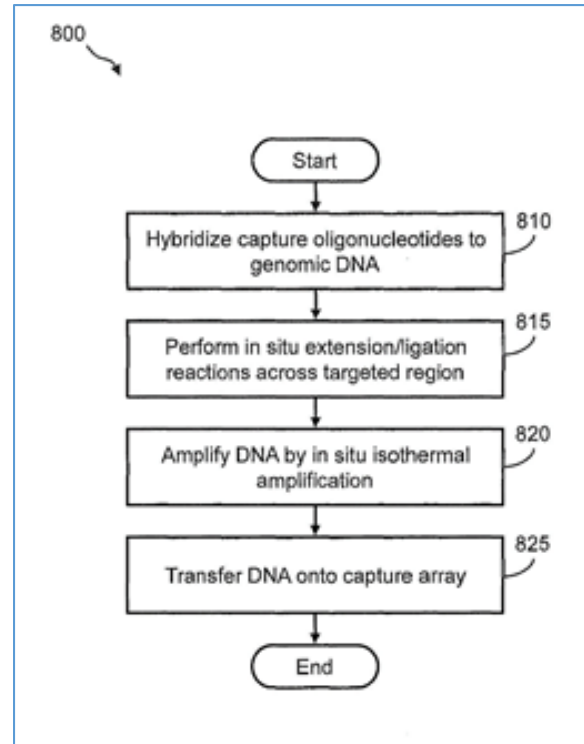
1. So-456 Does Not Disclose Collecting the Nucleic Acid Probes

37. In my opinion, So-456 does not disclose the “collecting” steps required by claims 1(b)-(c) and 16(c)-(d). I understand that in its decision granting institution of this IPR, the Board construed “collecting the nucleic acid probes” to mean “releasing all or a portion of the nucleic acid probe bound to the at least one target analyte through application of a force followed by retrieval of the released probe or portion of the probe.” Paper 14 at 24. As I describe above, I agree with this construction, and apply it to my analysis.

38. Claim 1 requires “collecting nucleic acid probes, or portions thereof, bound to the at least one target analyte” at a first and second location in a tissue

sample, while Claim 16 similarly requires “collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte” at a first and second location in the tissue sample. In both claims, an extension, or amplification step, is performed on the collected probes.

39. So-456, however, does not disclose “collecting” the probes, but rather collecting amplified products. Figure 8, for example, demonstrates So-456’s method:



EX1005 Fig. 8. In the method described by this figure, the capture oligonucleotides are hybridized to genomic target DNA at step 810. This is followed by in situ reactions across targeted regions at step 815. Importantly, after

capture, the *probes are not yet collected*. These reactions, occurring *in situ*, are occurring in the tissue sample.

40. At step 820, “DNA flanked by capture oligonucleotides is amplified by *in situ* isothermal amplification to generate multiple copies of the region of interest, i.e., multiple genomic amplicons.” EX1005 ¶ 180. This amplification step again occurs *in situ*, before the probes are collected. Subsequently, at step 825, the “genomic amplicons are transferred onto an array and captured by hybridization to universal capture regions on the array.” *Id.* ¶ 181. Therefore, So-456 does not teach collecting the probes, but rather teaches collecting copies of the probes. Such a distinction is a meaningful one, as the claim not only requires collecting the probes themselves, but explicitly requires extension and amplification of the collected probes.

41. The Petition does not address this distinction, but rather confirms that So-456’s method does not capture the probes. Petitioner, challenging claim 16, explains that after “ligating the primary probe pairs, So-456’s method amplifies the ligated probes (950) ‘to generate multiple copies...of the targeted region of interest’ by ‘isothermal amplification.’” Paper 2 at 20. It is the “amplified ligated probes (950)” that are captured. *Id.* Thus, Petitioner acknowledges that the probes themselves are not collected, but rather the amplicons.

42. Dr. Spellman, supporting the Petition, likewise notes that the ligated probes are “amplified by in situ isothermal amplification” and that the “amplicons generated by isothermal amplification are free from (i.e., not bound to) the target nucleic acid molecules.” EX1002 ¶¶ 134-135. Dr. Spellman’s testimony confirms that So-456 discloses capturing amplicons that have never been bound to a target, and not the probes that were bound.

43. My opinions are wholly consistent with the Board’s determination that “Petitioner has not demonstrated that So-456 teaches this claim limitation [collecting the nucleic probes] or otherwise demonstrates that this limitations would have been inherent in the disclosure of So-456 for claims 1 and 16.” Paper 14 at 24.

2. So-456 Does Not Disclose Treated Tissue Sample

44. Claim elements 1(a) and 16(a), the “contacting” steps of the claimed methods, require that the tissue “is treated to facilitate binding of the nucleic probes to the target analyte.” In my opinion, So-456 does not disclose treating a tissue sample to facilitate binding of nucleic acid probes to a target analyte.

45. The ’689 Patent’s disclosures lead a person of ordinary skill to recognize the importance of treating the tissue sample to facilitate the binding of nucleic acid probes. For example, Figure 21B depicts an exemplary workflow on the claimed methods:

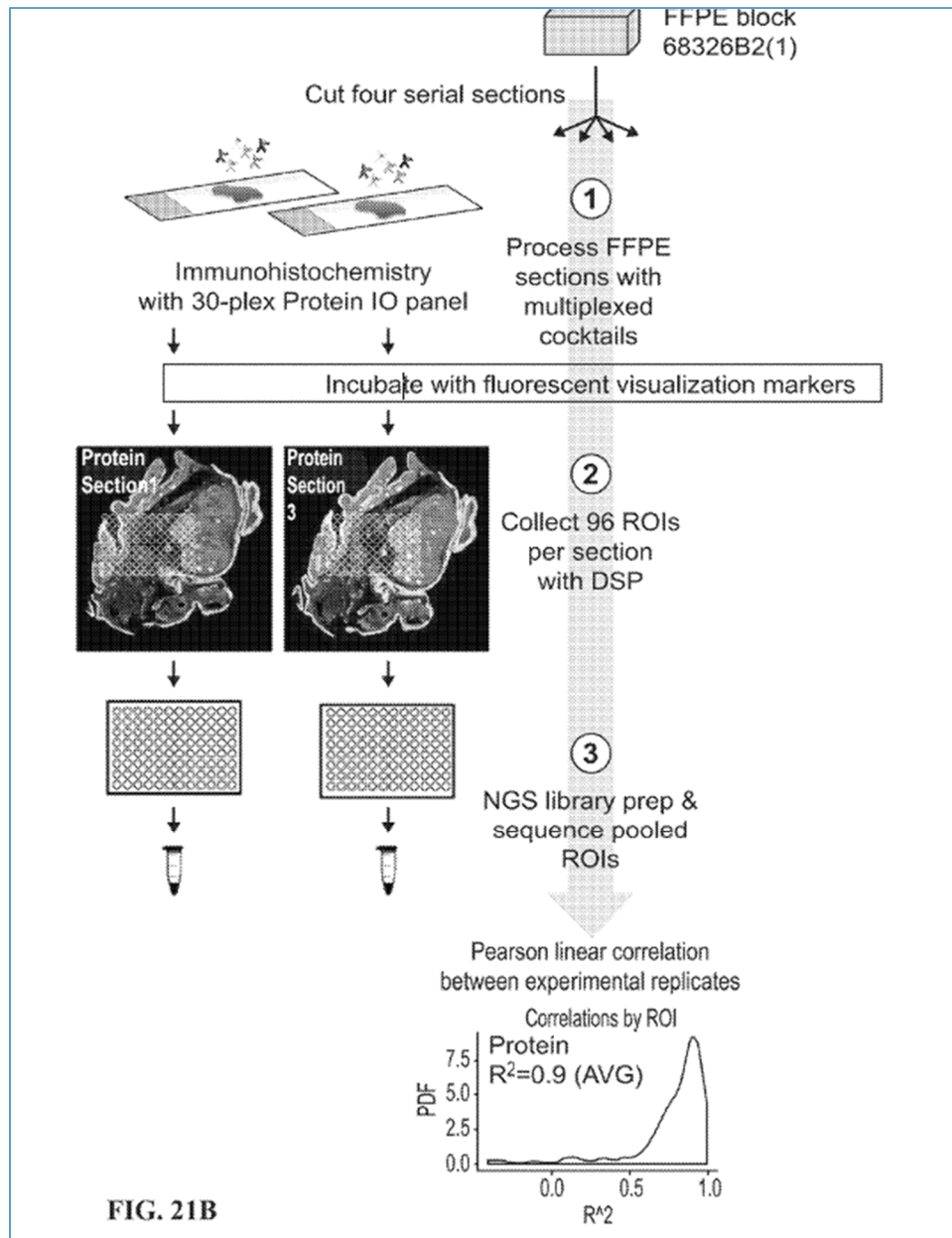


FIG. 21B

EX1001 Fig. 21B. In this figure, the method begins with the use of a formalin-fixed paraffin-embedded (FFPE) block. This tissue sample has been subject to preservation processes that would make working with the tissue directly from the block difficult. The first step of this figure shows that cut serial sections of the tissue must be “process[ed]” with “multiplexed cocktails” to permit further analysis.

Figure 22B, which showcases yet another exemplary workflow, also features such a step. *Id.* Fig. 22B.

46. Examples 7-9 further confirm the importance of treating the tissue sample to facilitate probe binding. All three examples analyze an FFPE sample, which could not be contacted with probes and analyzed for meaningful data without treatment. EX1001 at 75:50-78:3.

47. Further, other disclosures in the '689 Patent would lead a person of ordinary skill to understand the importance of treating the tissue sample to facilitate the binding of probes. For example, the Patent states that in some aspects, “probes are provided to a sample at concentrations typically less than that used for immunohistochemistry (IHC) or for in situ hybridization (ISH)” including at concentrations “significantly less than used for IHC or ISH.” *Id.* at 40:4-8. In my opinion, a person of ordinary skill would have recognized that to provide probes at lower concentrations than were used in prior art techniques (such as IHC and ISH), the sample must be treated in a manner that facilitates binding.

48. My opinions as to the importance of the tissue treating step are supported by the prosecution history of the '689 Patent and its parent, the '487 Application. When the '487 Application was rejected, in part, over a failure to enable the claims because the Examiner noted that without some form of permeabilization to permit probes to enter the tissue, the Applicant added an

identical limitation to the one found in the '689 Patent for treating a tissue sample. At the time this was added, the Applicant pointed the Examiner to paragraph 266 of the Application, explaining that “there are reagents, for example buffers and other reagents, that are known in the art to be useful in facilitating binding of a protein or a nucleic acid in a sample,” and so it would be “well within the purview of a person of ordinary skill in the art” to treat a tissue sample as recited by the claim. EX1012 at 1560. In my opinion, this is consistent with the state of the art as of the priority date of the '689 Patent.

49. During the prosecution of the '689 Patent, when Patent Owner amended claims 1 and 16 to include the limitation “wherein the tissue sample has been treated to facilitate binding of the probes to the target analyte,” Patent Owner highlighted Example 7, Figure 21, and Figure 22 as enabling a person of ordinary skill to practice the claimed invention. EX2011.270.

50. Importantly, these disclosures contrast with the disclosures from So-456 that Petitioner relies upon. Petitioner cites So-456 to state that the “tissue sample is prepared for analysis” and that “in situ biochemistry is performed on the tissue section to facilitate subsequent manipulation of a nucleic acid sample.” EX1005 ¶¶ 152-153. These disclosures, however, do not describe treating a tissue sample to facilitate the binding of nucleic acid probes.

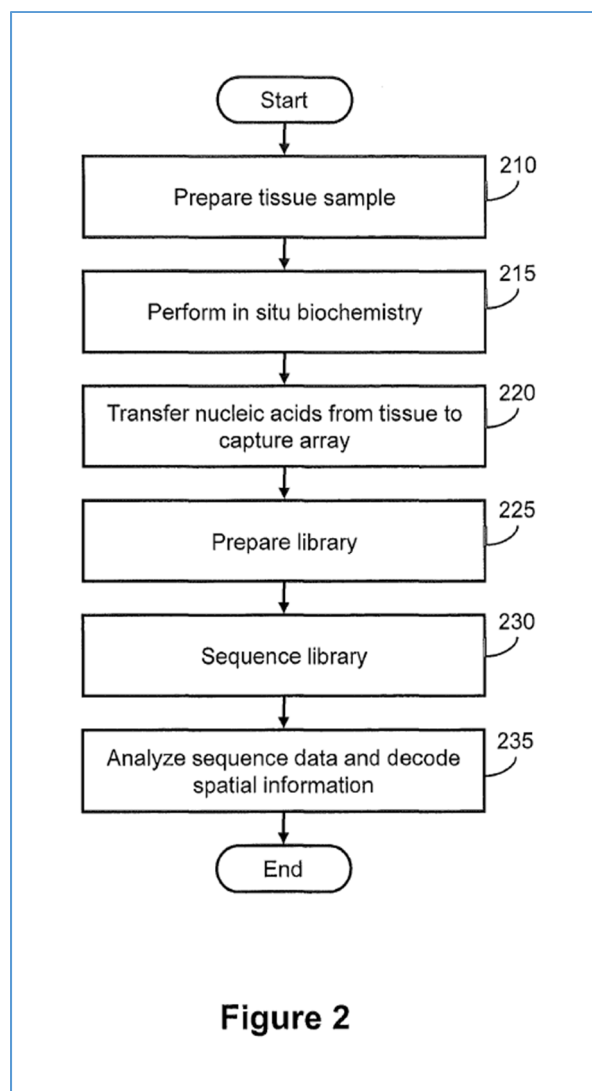
51. Paragraphs 152 and 153, in full, state:

[152] At a step 210, a tissue sample is prepared for analysis. In some embodiments, the tissue sample is a FFPE tissue sample that is sectioned onto a slide. Other examples include fresh tissue, frozen tissue, etc.

[153] At a step 215, in situ biochemistry is performed on the tissue section to facilitate subsequent manipulation of a nucleic acid in the sample. In some embodiments, an in situ reverse transcription reaction is used to synthesize cDNA from targeted mRNA in the tissue sample. In some embodiments, an in situ amplification reaction can be used to produce multiple genomic DNA amplicons from targeted genes in the tissue sample. In some embodiments, there is no in situ biochemistry step, and synthesis of cDNA is performed after capture or extraction of the RNA from the tissue.

EX1005 ¶¶ 152-153. These paragraphs, unlike disclosures in the '689 Patent, do not describe a step of treating a tissue sample prior to applying probes for contacting, for the purpose of facilitating binding. Rather, they describe a “sectioning” step (210) and *then* in situ biochemistry for subsequent manipulation. In my opinion, a person of ordinary skill would recognize that neither of these disclosures facilitate probe binding.

52. My opinion is further supported by Figure 2 of So-456, which depicts the aforementioned steps:



EX1005 Fig. 2. While steps 210 and 215 occur before the step of “transfer[ring] nucleic acids from tissue to capture array,” nothing in those steps *facilitates the binding* of probes from, *e.g.*, the capture array to the tissue sample. The 210 step is merely sectioning, which is not a treatment that facilitates binding. And at 215, the *in situ* biochemistry is not treating the sample to facilitate binding, but rather performing reverse transcription or amplification prior to capture. Neither step is,

in my opinion, analogous to the step in the '689 Patent of treating a sample with a “multiplexed cocktail.” EX1001 Fig. 22B.

53. Because So-456 does not disclose any method of treating a tissue sample, nor are there any disclosures in So-456 that would lead a person of ordinary skill to recognize the importance of treating a tissue sample to facilitate probe binding, it is my opinion that So-456 does not disclose this limitation in claims 1(a) and 16(a). Indeed, the lack of any discernible step to prepare an FFPE tissue sample for analysis suggests that the approach outlined in So-456 is speculative and lacks any consideration of the practical issues that would need to be overcome to have a working method.

3. So-456 Does Not Teach Required Extension Reaction Steps

54. In my opinion, So-456 does not disclose the “extension reaction” steps that are recited in Claims 1(d)-1(e) and 16(e)-16(f). Both independent claims require performing an “extension reaction” incorporating a nucleic acid sequence that identifies the location of the target analyte:

d) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the nucleic acid probes, or portions thereof, collected in step (b), thereby forming a first plurality of extension products that comprise the nucleic acid probes, or portions thereof,

collected in step (b) and the at least one nucleic acid sequence that identifies the first location of the tissue sample;

EX1001 Claim 1(d); *see also id.* Claims 1(e), 16(e), 16(f).

55. Both Petitioner and Dr. Spellman acknowledge that So-456 does not explicitly disclose an extension step. Rather, Petitioner argues, and Dr. Spellman opines, that So-456 inherently discloses an extension step incorporating a spatial barcode. Paper 2 at 23; EX1002 ¶ 139. I understand that inherent disclosure requires that the limitation at issue must either 1) be necessarily present in the prior art, if not explicitly stated, or 2) must be the natural result of the combination of elements disclosed in the prior art. I further understand that merely because something may result from a given set of circumstances is not sufficient to show inherency, and Petitioner must meet a high standard to show inherency.

56. In my opinion, none of the disclosures that Petitioner and Dr. Spellman point to meet this high standard. As an initial point, I note that it appears that Petitioner attempts to draw from different parts of the specification and stitch them together to support Petitioner's inherency argument. As is detailed below, Petitioner appears to rely chiefly on Figure 9 to frame the argument that So-456 anticipates this claim. Petitioner, however, improperly relies on labeling and disclosures from other figures and embodiments to supplement the lack of disclosure in Figure 9, including contradictory disclosures. Therefore, even if these elements

were expressly disclosed, it is my understanding that anticipation requires all elements to be arranged as they are in the claim. For at least that reason, So-456 fails to anticipate claims 1 and 16.

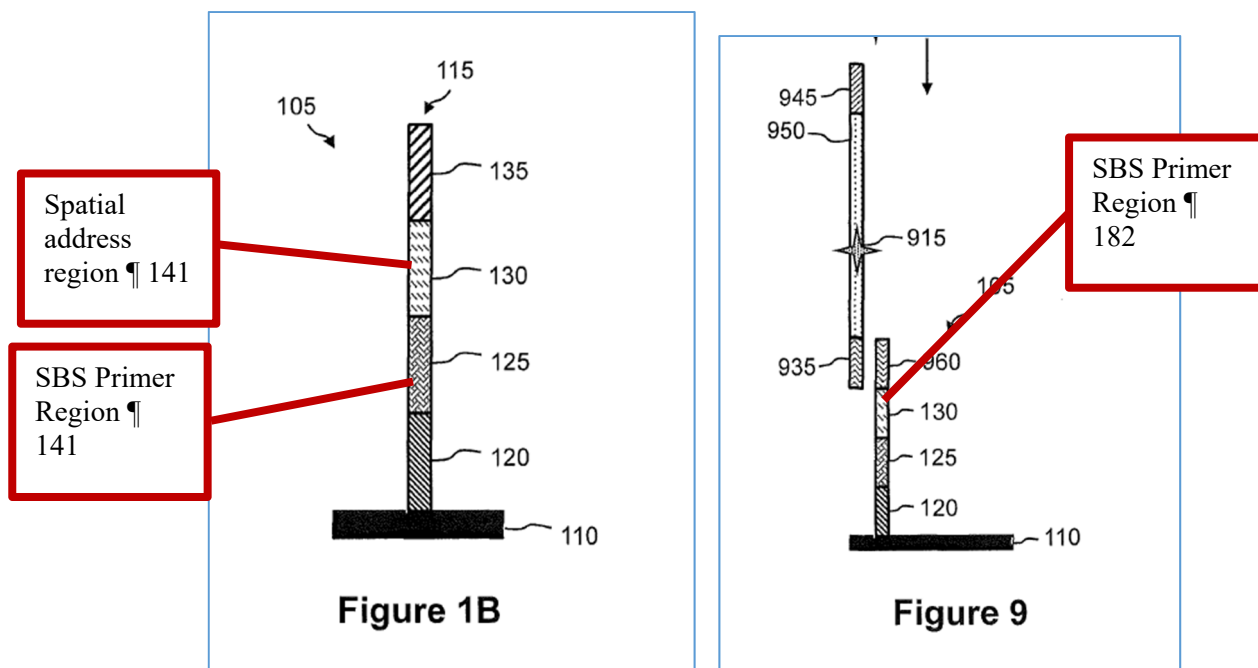
57. Furthermore, it is my opinion that So-456 does not inherently disclose an extension reaction incorporating a spatial barcode. Petitioner and Dr. Spellman rely on scant disclosure to conclude inherent disclosure, where So-456's process does not require that a spatial barcode is necessarily added by an extension reaction after collecting bound probes.

58. First, as I detail above, So-456 does not disclose collecting bound probes. Therefore, So-456 cannot, as a matter of logic, disclose claim limitations 1(d)-(e) nor 16(e)-(f). These claim limitations require that an extension reaction is performed on "each of the nucleic acid probes, or portions thereof, collected in" the relevant "collecting step," *e.g.* claim 1(b), 1(c), 16(c), or 16(d). Because So-456 does not collect the nucleic acid probes, the method cannot teach performing an extension reaction on those probes.

59. Second, the disclosures that Petitioner relies upon do not inherently disclose these claim limitations. Petitioner, and Dr. Spellman, rely primarily on Figure 9 in So-456. According to Petitioner, "So-456's ligated probes (950) captured on the array in the previous step are not yet tagged with a spatial barcode and do not include functional sequences...necessary for performing by sequencing-

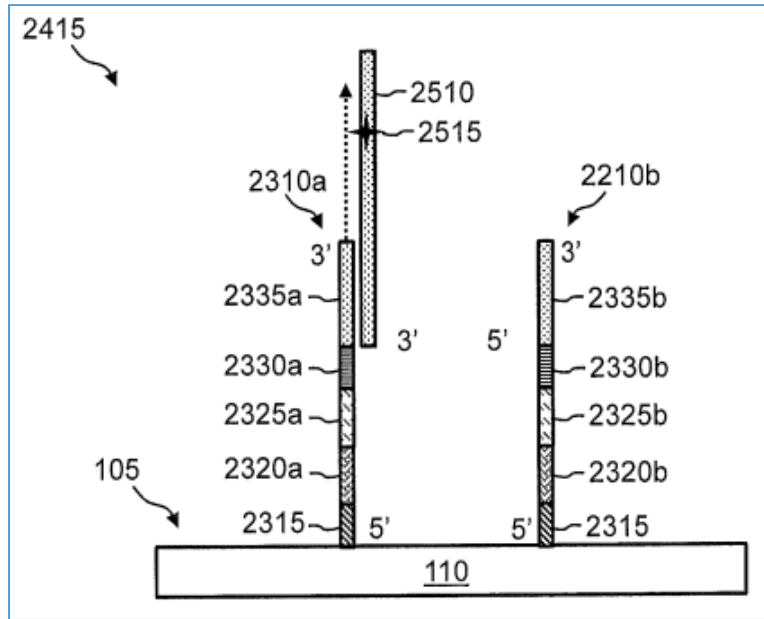
by-synthesis (SBS) in subsequent steps.” Paper 2 at 24; EX1002 ¶ 142. Figure 9, however, does not include a spatial barcode at all. Dr. Spellman attempts to map a unique “spatial address region 130” to the spatial barcode. EX1002 ¶ 143. Dr. Spellman, however, is mistaken—as the description of Figure 9 makes clear, region 130 is a sequencing by synthesis primer region. EX1005 ¶ 182 (“SBS primer region 130 is SBS3”).

60. As I noted earlier, Petitioner and Dr. Spellman attempt to string together disparate disclosures to put together an argument for inherent anticipation. In this case, they cite to paragraph 141, which describes the generation of arrays. In this paragraph, the “SBS primer region” is marked as region 125, while a spatial address region is marked region 130. As I have annotated below, the two cited figures demonstrate that So-456 is referring to different molecular biological elements in different embodiments:



EX1005 Figs. 1B, 9. Given that So-456 labels different regions differently across its embodiments, it is my opinion that a person of ordinary skill would not have recognized the spatial address region, as labeled in Figure 1B, is the same as the SBS primer region as labeled in Figure 9.

61. Furthermore, even if Figure 9 contained some form of spatial barcode (which So-456 does not disclose), I disagree with Dr. Spellman's annotations and conclusions regarding bi-directional extension. So-456 includes numerous embodiments that feature uni-directional extension of the capture probe to incorporate the target analyte's sequence. One example of such a reaction is demonstrated by Figure 25A:

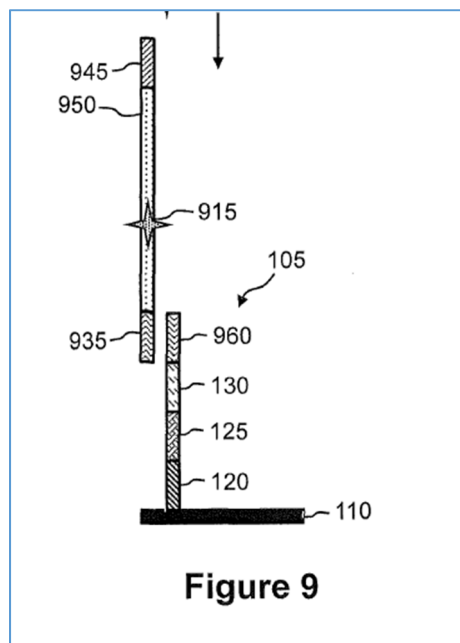


EX1005 Fig. 25A. This Figure depicts a “first strand cDNA” that is “synthesized using capture 2335a as a primer in a reverse transcription reaction.” *Id.* ¶ 316. So-456 is replete with examples of uni-directional extension, depicted across several figures and numerous embodiments. *Id.* at Figs. 22, 23A, 24, 25A, 25B, 29A, 39, 40; *see also id.* ¶¶ 297-301, 307-320, 332-334, 391-395, 410-412 (“Capture probe [] hybridizes to nucleic acid molecule [] from a tissue sample and is extended to form a complementary nucleic acid molecule”).

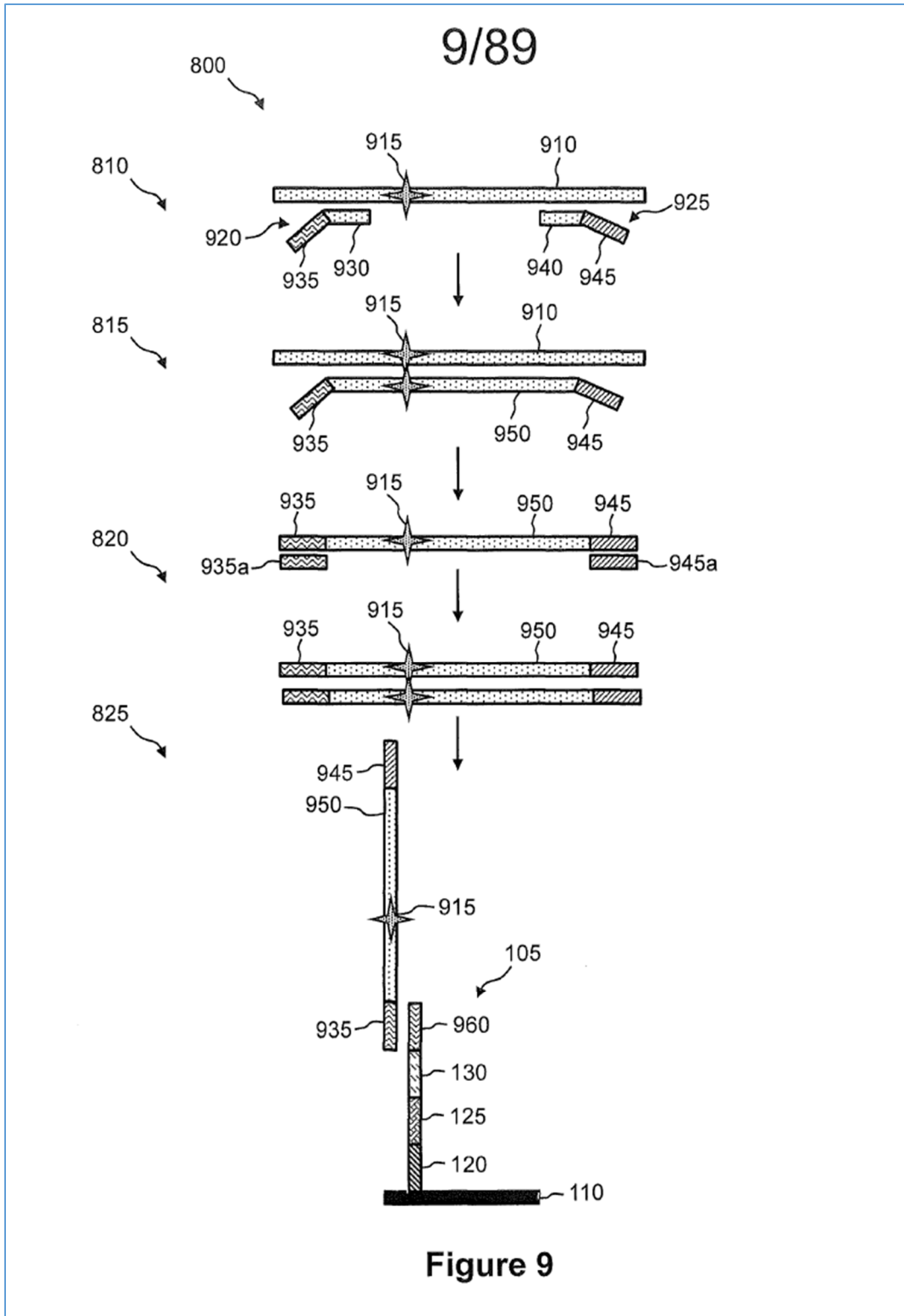
62. None of the aforementioned extension reactions, and none of the extension reactions disclosed in So-456 at all, incorporate a spatial barcode. I disagree with Petitioner and Dr. Spellman that the incorporation of a spatial barcode would be inherent to any of the disclosed methods. Even if the capture probe contains a spatial address region, that sequence would not be incorporated by any of the uni-directional embodiments. To use Dr. Spellman and Petitioner's own

example, in Figure 9, a single extension reaction takes place. This extension incorporates the target analyte sequence to the capture probe, *but does not necessarily incorporate the capture probe sequence back onto the target analyte strand.*

63. Without Dr. Spellman's annotations to Figure 9, it is clear that bi-directional extension is not necessarily occurring:



EX1005 Fig. 9. In fact, the amplification described in the specification only makes sense in the context of the entirety of Figure 9:



Id. In this Figure, step 815 features an “extension/ligation reaction” wherein the amplicon, containing a mutation (915) is extended using a gene-specific primer with

a universal capture region (935). This amplified molecule is then subjected to capture on an array, where the universal capture region hybridizes to a capture sequence (960). Despite Dr. Spellman's annotations, there is no evidence of any amplification occurring at the final step. On the contrary, the specification would appear to state the opposite:

At step 825, genomic amplicons 950 are captured onto the capture site 105. For example, the tissue sample containing genomic amplicons 950 or a substrate comprising genomic amplicons 950 derived from the tissue sample (not shown) is contacted with the capture site 105. In this example. Capture probe 115 on the capture site 105 by hybridization of universal capture region 935 to capture region 960.

Id. ¶ 182. Aside from Dr. Spellman's presumptive annotations, neither Dr. Spellman nor Petitioner point to a single example or embodiment where bi-directional extension occurs.

64. It is further my opinion that Dr. Spellman's annotations do not show such bi-directional extension necessarily occurs as a consequence of any reactions in So-456. A person of ordinary skill would have recognized challenges with bi-directional extension, and the requirements for unique chemistry and application of enzymes to permit such extension. Merely adding enzymes would not necessarily cause a bi-directional extension reaction to occur. Thus, contrary to Petitioner's assertion that extension is necessarily bi-directional because "So-456 did not teach

methods for preventing the extension from occurring,” the prior art would actually need to teach a method to permit bi-directional extension as drawn by Dr. Spellman. Paper 2 at 25.

65. My opinions in this declaration are wholly consistent with the Board’s conclusion that “Petitioner does not show sufficient evidence of inherently bi-directional extension,” and that in particular, the Board did “not find that one of ordinary skill in the art would have read So-456 to disclose an extension reaction replicating the spatial barcode due to the lack of disclosure on this issue.” Paper 14 at 28.

4. So-456 Does Not Disclose Forming Ligated Probes

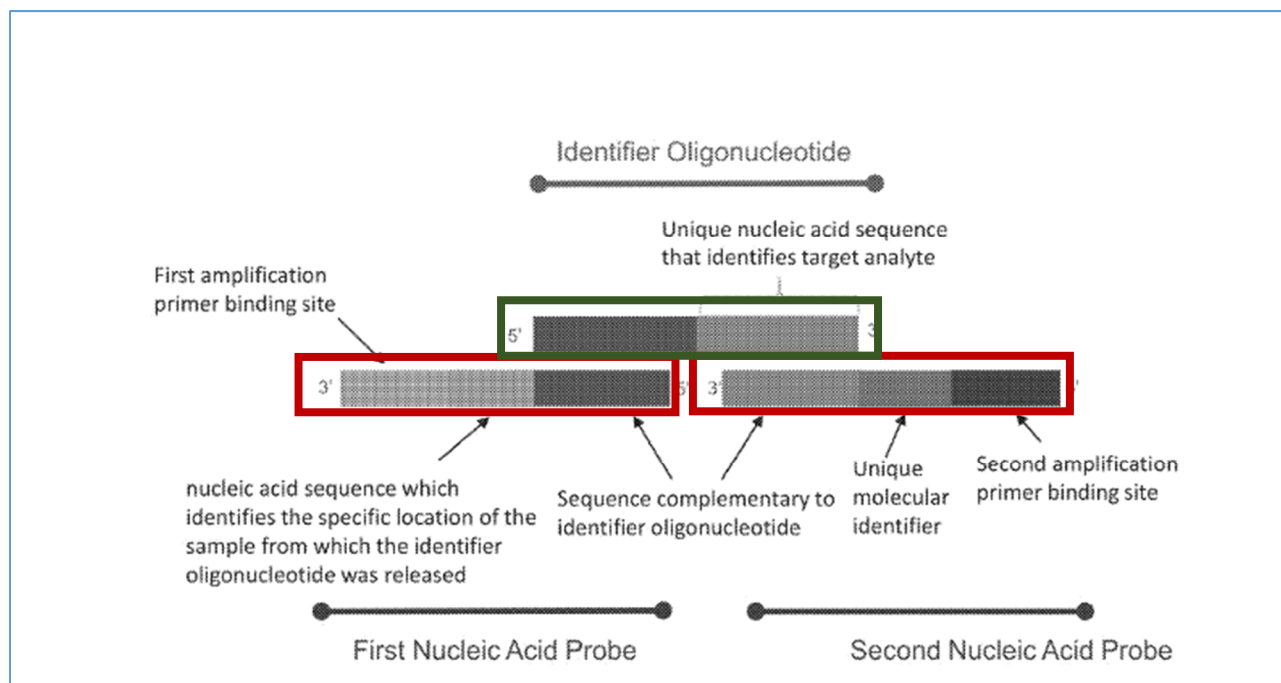
66. In my opinion, So-456 does not anticipate the claims of the ’689 Patent because it does not disclose forming ligated probes, as required by claim 16(b). Claim 16(b) recites:

b) forming ligated probes by ligating each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are bound to each of identical molecules from the at least one target analyte;

EX1001 Claim 16. In my analysis of this claim, I first address the ’689 Patent’s disclosures to clarify the meaning of “ligating the probes,” and then address

Petitioner's and Dr. Spellman's faulty contentions that So-456 discloses ligating the probes.

67. The '689 Patent discloses that adjacent first and second nucleic acid probes are ligated. In other words, a first nucleic acid probe hybridizes to a target sequence, a second analyte probe hybridizes nearby, and the two individual probes can be ligated together. To help clarify this, Figure 5 of the '689 Patent is reproduced below:



EX1001 Fig. 5. In red, I have highlighted the two nucleic acid probes, which includes two sequences that are complementary to adjacent portions of an identifier oligonucleotide. In green, I have highlighted the identifier oligonucleotide. Importantly, the '689 Patent specifies that the "first nucleic acid probe and the second nucleic acid probe can hybridize to the identifier oligonucleotide such that

the first and the second nucleic acid probes are adjacent and are not overlapping.” *Id.* at 25:51-54. The ligation can occur “by performing a nick repair reaction.” *Id.* at 25:64-65.

68. Therefore, “ligating probes” in the ’689 Patent is directed specifically to ligating adjacent, nonoverlapping probes with, for example, a nick repair reaction. *See, e.g., id.* at 6:33-38 (“wherein the first and the second nucleic acid probes hybridize to the identifier oligonucleotide such that the first and the second nucleic acid probes are adjacent but not overlapping; (5) performing nick repair such that the hybridized first and second nucleic acid probes are ligated together;”) (emphasis added); *id.* at 5:66-6:3, 8:42-47, 9:13-18, 12:48-52, 27:42-47, 28:35-40, 30:53-58, 69:22-26, 70:63-67. This is consistent with the state of the art as of the priority date of the ’689 Patent, as performing a nick repair ligation reaction would have been well within the province of a person of ordinary skill.

69. I now turn to Petitioner’s and Dr. Spellman’s mistaken contentions that So-456 discloses this claim limitation. Petitioner relies on So-456’s disclosure that “an extension/ligation reaction is performed in situ between the flanking capture oligonucleotides 920 and 925 across the region of interest.” EX1005 ¶ 182. According to Petitioner, this forms ligated probes as claimed. Paper 2 at 19. Dr. Spellman’s testimony adds little further analysis to this issue.

70. Both Petitioner and Dr. Spellman ignore a vital step to So-456's method – that the disclosed reaction is not exclusively a ligation reaction, but rather an extension/ligation reaction. Dr. Spellman's annotation of Figure 9, calling a mid-workflow step a "ligated probe," is misleading as "ligated probe" is used in the '689 Patent. I have reproduced Figure 9 in its entirety below, and annotated it to more accurately describe what is occurring:

9/89

810: hybridization of probes with target

815: extension capturing mutation and ligation of extension product with probes

820: isothermal amplification

825: capture

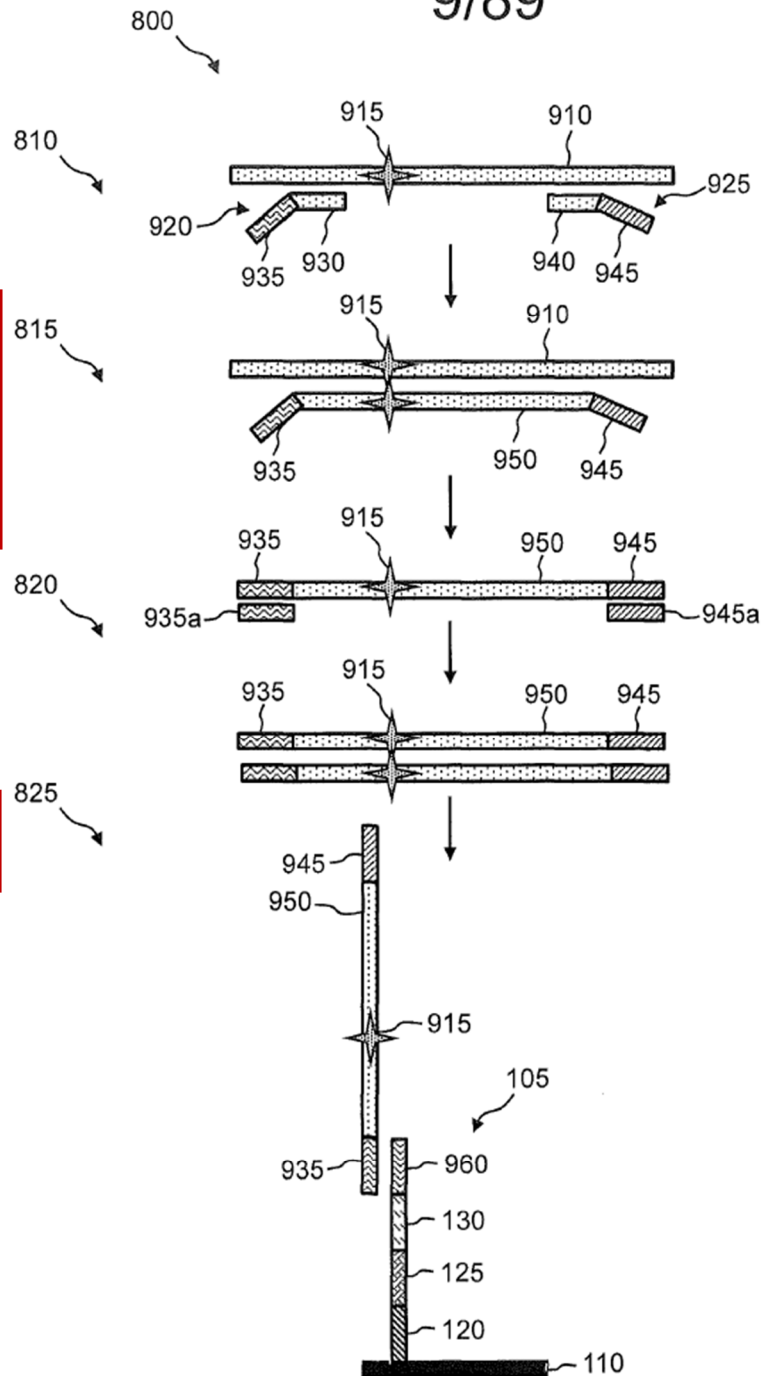
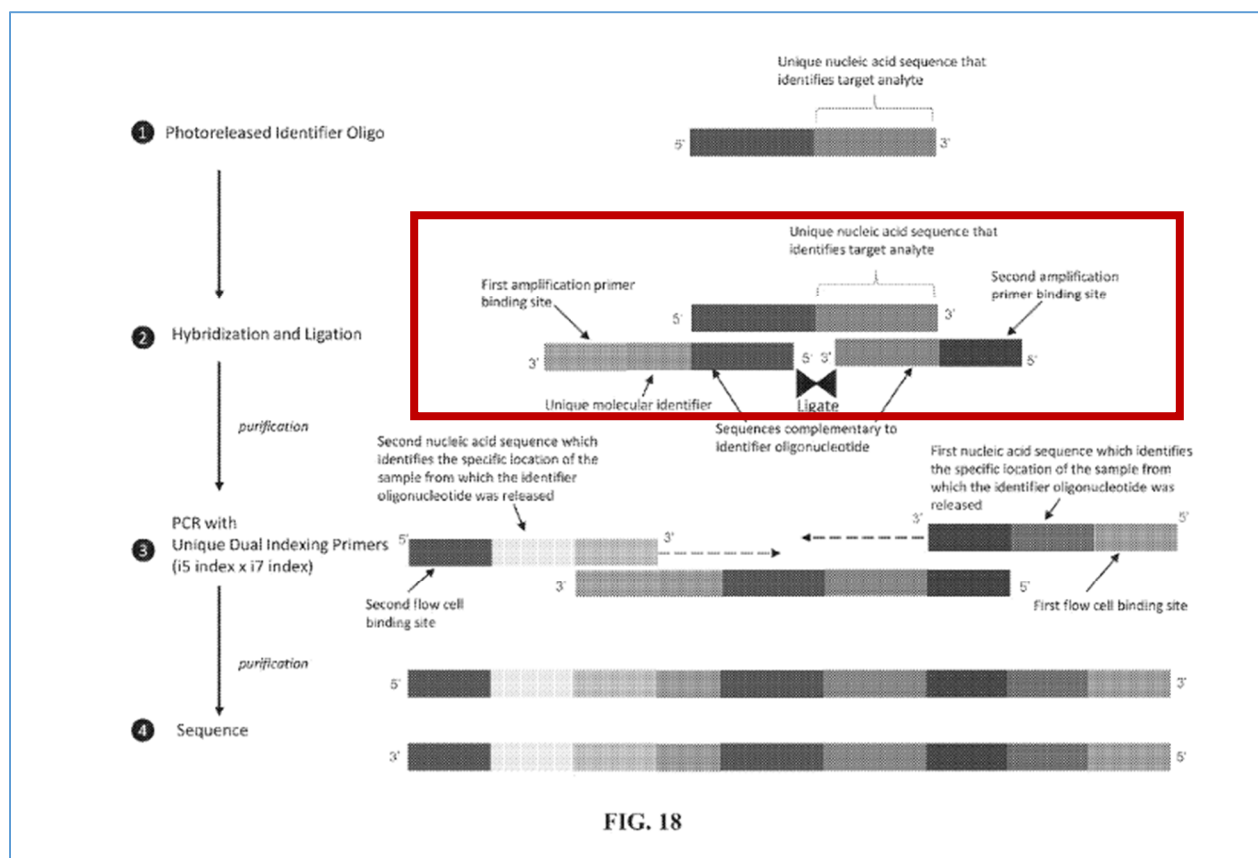


Figure 9

EX1005 Fig. 9. As I have annotated above, step 815 includes an extension reaction that captures the oligo of interest, and does not ligate the probes together. Rather, the referenced ligation is the extension product with the probe. This ligated molecule is then used as the basis for isothermal amplification, generating many copies prior to capture. *Id.* ¶ 182. To contrast, I have reproduced Figure 18 of the '689 Patent, which depicts the ligation step:



EX1001 Fig. 18. As depicted in highlighted step 2 of the depicted workflow, ligation is acting directly on the probes, and the subsequent PCR occurs on the ligated probe product.

71. To the extent that Petitioner or Dr. Spellman rely on the '689 Patent's disclosure that ligation occurs by "performing a gap extension reaction and a nick repair reaction" and equate a gap extension reaction to So-456's extension, it is a false equivalence. A person of ordinary skill would recognize that the referenced gap extension is not meant to capture a meaningful extension product, but correct for the possibility that the probes are too far apart to be ligated directly. In contrast, So-456's extension/ligation is an intentional step designed to create the template for isothermal amplification by specifically capturing the mutation to be studied with the extension reaction. Thus, a person of ordinary skill would view the disclosures as meaningfully different, and would not see the '689 Patent's "gap extension" as the same as So-456's "extension/ligation."

72. Therefore, So-456 does not disclose forming ligated probes. Dr. Spellman's annotations amount to little more than creating disclosure out of thin air and are not tethered to the actual disclosures in So-456.

B. GROUNDS 2 AND 3: CLAIMS 1-30 WOULD NOT HAVE BEEN OBVIOUS OVER SO-456 [AND STAHL]

73. Ground 2 of the Petition argues that Claims 1-30 are rendered obvious by So-456 alone. Ground 3 of the Petition again uses So-456 as the base reference, but argues that a combination with Stahl renders claims 9-10 and 24-25 obvious. Claims 9, 10, 24, and 25 are dependent claims that depend from claims 1 and 16. In my opinion, So-456 fails to render obvious claims 1 and 16, and therefore

Grounds 2 and 3 both fail. Because Petitioner only relied on Stahl for the further limitations in dependent claims, I do not independently address Ground 3, but opine that Ground 3 fails for the same reasons Ground 2 fails.

1. So-456 Does Not Render The Claimed Extension Steps Obvious

74. Petitioner argued, and Dr. Spellman opined, only on the extension steps recited in Claims 1(d)-(e) and Claims 16(e)-(f) are obvious under So-456. A person of ordinary skill in the art, however, would not have found it obvious to apply an extension step to add a spatial barcode, as recited by the '689 Patent's claims, based on So-456.

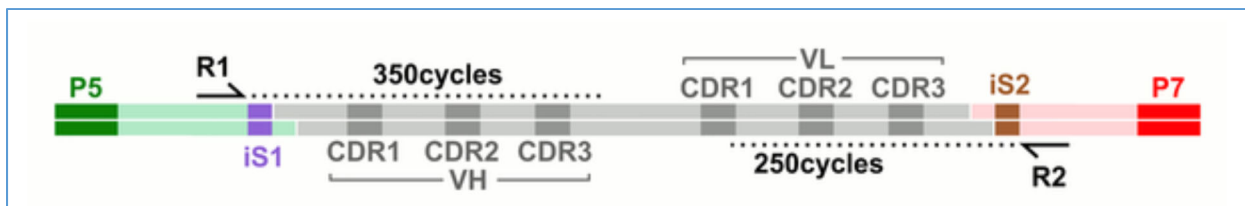
75. Petitioner and Dr. Spellman rely on many of the same disclosures as the basis for Petitioner's obviousness argument. For the reasons I have detailed above, these disclosures do not include an extension step that incorporates a spatial barcode. I disagree that with Dr. Spellman's conclusory assertion that "a POSA would have known that So-456's methods incorporate a spatial barcode in the target nucleic acids by tagging the nucleic acids with a probe containing a spatial barcode." EX1002 ¶ 220. In fact, the process of adding a spatial barcode by extension is, in my opinion, an inventive step that was a nonobvious improvement over the prior art.

76. First, I reiterate my opinions above that there is no disclosure that the extension step in So-456 is bi-directional, nor is there any evidence that the capture

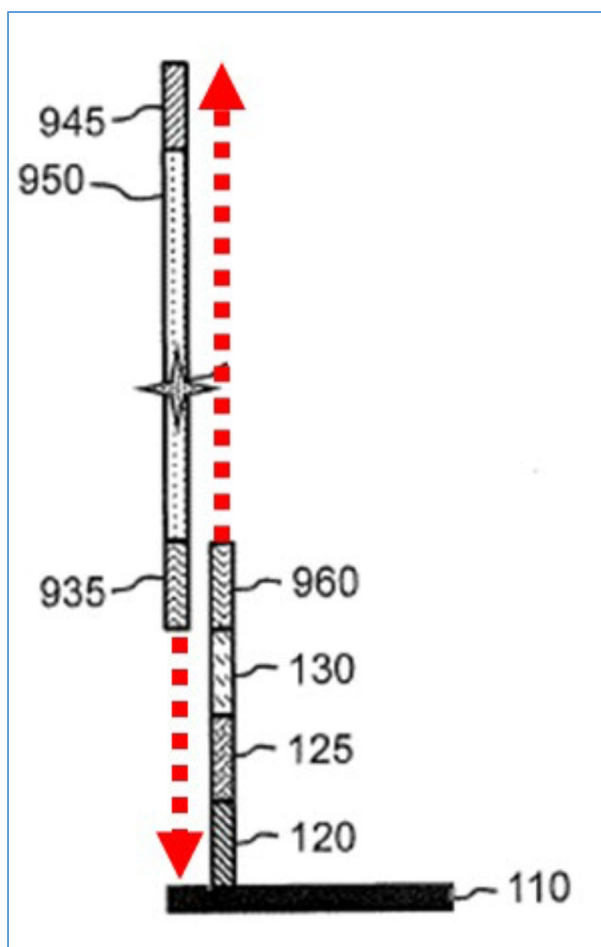
probe contains a spatial address region. Therefore, beginning with the disclosures in So-456, Petitioner's argument is deficient.

77. Second, Dr. Spellman's discussion of the use of SBS primers fails to fill the logical gap that Petitioner attempts to argue. Dr. Spellman argues that So-456 teaches using sequencing by synthesis (SBS), a method of sequencing developed by Illumina that uses a pair of SBS primers and fluorescently labeled reversible terminator nucleotides. *Id.* ¶ 224. According to Dr. Spellman, because SBS platforms use paired end sequencing, So-456 must in turn suggest the type of bidirectional amplification that would incorporate a spatial barcode to the capture probe. *Id.* ¶¶ 228-231. Dr. Spellman is incorrect.

78. The paired-end sequencing ensures that both strands of a DNA molecule can be used as a template for SBS, because DNA synthesis occurs in a 5' to 3' direction—i.e., is unidirectional. This paired-end sequencing is why, for example, both of Dr. Spellman's figures emphasize that SBS occurs in an "inward" direction between two paired-end primers:



Id. ¶¶ 225-226. To contrast, I have reproduced Dr. Spellman's of Figure 9:



Id. ¶¶ 230-231. As is evident, Dr. Spellman’s annotation confusingly showcases bidirectional extension happening in *opposite directions*, as opposed to the 5’ to 3’ directions made evident by Illumina’s documentation regarding paired end primers.

79. Therefore, Dr. Spellman fails to address the lack of disclosure in So-456, and So-456 does not teach nor suggest the claimed extension reaction. Furthermore, it is my opinion that Dr. Spellman and Petitioner failed to show any motivation to modify the methods described.

80. Petitioner argues that a person of ordinary skill would have been motivated for no reason other than it was “well-known, obvious, and routine” to

incorporate a spatial barcode as recited by the claims. Paper 2 at 42. Dr. Spellman provides little clarification on how it was well-known, obvious, and routine, as he relies only on the use of SBS forward and reverse primers and opines that because So-456 teaches the use of such primers, it must be obvious that the method could be modified to use an extension reaction that adds a spatial barcode. EX1002 ¶¶ 234-236.

81. As I detail above, the extension of a capture probe would, unidirectionally, incorporate the target sequence and the necessary second SBS primer sequence onto the capture probe from the captured probe. In other words, the *captured* probe is the amplicon that features the target sequence, with a mutation, and a universal capture sequence. The *capture* probe is the sequence that is affixed to the array and extended.

82. If Petitioner's theory that the capture probe contains a spatial barcode were correct (which it is not), the extension of the capture probe would generate a single molecule that includes both primers necessary for sequencing and the spatial barcode. Therefore, there is no motivation for bi-directional extension, nor is there any motivation generally to modify the method. The purported end result, under Petitioner's theory, is already accomplished without the need for any modification.

83. My opinions are wholly consistent with the Board's findings that

Petitioner does not allege or provide evidence to support that the skilled artisan would have found it obvious based on the teachings of So-456 to collect the cleaved identifier oligonucleotides rather than the genomic amplicons as alleged. In addition, we share Patent Owner's view that Petitioner, on this record, does not sufficiently show a motivation for the ordinarily skilled artisan to have included a spatial barcode in the capture probes of So-456.

Paper 14 at 31.

C. GROUND 4: CHELL DOES NOT ANTICIPATE THE CLAIMS OF THE '689 PATENT

84. Petitioner's ground 4 is based on the alleged anticipation of the '689 Patent claims by the Chell reference. I understand that the Chell reference was published on July 1, 2021. EX1006 code (43). I also understand that the '689 Patent claims priority to the '487 Application, which was filed on February 11, 2019. EX1001 code (63). Accordingly, I understand from counsel that Chell is not prior art to the '689 patent because the '487 application predates Chell.

85. Nonetheless, I understand Petitioner contends that the '689 patent should not be entitled to the priority date of the '487 application because the '487 application lacks written description support for the claims of the '689 Patent. Accordingly, I understand it is Petitioner's contention that the '689 Patent should have a priority date of September 16, 2021, when the corresponding application was filed, so that Chell would be prior art to the '689 Patent.

86. As explained below, it is my opinion that there is written description in the '487 application for the '689 Patent claims, contrary to Petitioner's contention. For this reason, it is my understanding that the '689 Patent is entitled to a priority date of February 11, 2019, when the '487 application was filed.

87. Also as explained below, it is my opinion that even if Chell is considered prior art to the '689 patent, Chell does not anticipate the '689 Patent.

1. The '487 application contains adequate written description

88. It is my understanding that Petitioner contends two features claimed in the '689 patent are missing or not disclosed in the '487 application. As a shorthand, I refer to these allegedly missing features as “whole probes” and “on-analyte ligation.” As I explain below, a POSA would have understood both of these features to be disclosed and understood that the inventors of the '689 patent possessed these features.

89. I refer back to my description of a POSA. *See* Section V. It is my understanding that Dr. Spellman agrees with me that for the purposes of this proceeding, a POSA is highly skilled and well-versed in a number of techniques. For example, in addition to being highly educated in molecular biology and having specific experience in laboratory techniques and methods involving the manipulation and spatial analysis of nucleic acids, proteins, tissue samples. This is supported by the '689 Patent itself, which notes that the invention improves over

“standard immunohistochemical or in situ hybridization methods.” EX1001 at 16:30-43. This is also supported by Dr. Spellman, who noted that a POSA would possess basic competencies including the “field of spatial detection of target analytes—specifically, mRNAs or DNA...involving microscopy, pathological imaging, tissue section, and related technologies,” “broad-scale understanding of the genome as a whole, the structure of the genome,” “a whole set of other competencies,” “multiplex components of the data...[including] image processing, image segmentation, image analysis, multianalyte interpretation, multiplex measurements,” “high-throughput sequencing, oligonucleotide binding, hybridization, microarray experience,” and “all host of molecular biology approaches of that flavor,” such as “tagging with barcodes and using ligation of specificity.” EX2007 at 46:17-49:30. I agree with Dr. Spellman that a POSA would have a strong understanding of at least these areas and approaches.

90. I understand that written description must be evaluated from the perspective of a POSA and taking into account the POSA’s background knowledge, which is extensive when evaluating the ’487 application and ’689 patent. I understand the inquiry to be whether a POSA would understand the ’487 application in light of their background knowledge that the inventors possessed the invention as claimed. As I explain below, it is my opinion that the ’689 patent has written description support from the ’487 application.

a. Collecting “Whole Probes” Is Disclosed

91. I understand Petitioner’s position to be that collecting “whole probes” is not disclosed in the ’487 application. As I understand Petitioner’s argument, the ’689 patent claims require “collecting the nucleic acid probes, or portions thereof, bound to the at least one target analyte” in claim 1(b) and 1(c) and “collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte” in claim 16(c) and 16(d). Accordingly, “probes, or portions thereof” must refer to both an entire probe and a portion of a probe. I agree with this interpretation that the ’689 patent claims cover both collecting an entire probe and a portion of a probe. I understand it is Petitioner’s contention that the ’487 application discloses only collecting a portion of a probe but not an entire probe.

92. I have reviewed the ’487 application and, in my opinion, the specification does not require a portion of a probe to be released by cleaving the probe. While the specification includes numerous embodiments in which it is described that a probe consists of a “target binding domain” and an “identifier oligonucleotide” and a “cleavable linker” between the two, the specification also explicitly states that this is a non-limiting example or words to that effect. For example, the ’487 application uses language similar to the following: “In a non-limiting example, in aspects in which the probe comprises a photo-cleavable linker between the identifier oligonucleotide and the target binding domain, a region-of-

interest is excited with light of a sufficient wavelength capable of cleaving the photo-cleavable linker.” EX1009 ¶ 107; *see also, e.g., id.* ¶¶ 106-107, 123-127, 131, 140-145, 159, 176, 180, 184, 200-201, 211, 221, 236, 246, 315-317. In my opinion, a POSA would have understood such explicit wording to mean that some probes have “a photo-cleavable linker between the identifier oligonucleotide and the target binding domain,” but not all.

93. In my opinion, a POSA would also have considered all of the disclosure in the '487 Application and understood that the cleavable linker, as well as its position “between the identifier oligonucleotide and the target binding domain,” reflect various options that a POSA can consider when practicing the '689 Patent. For example, the '487 Application states that the cleavable portion can be a photo-cleavable linker, an enzymatically cleavable linker, or a chemically cleavable linker, among other types of linkers. *Id.* ¶¶ 18, 131, 137. In addition, the '487 application refers to other sequences that can contain a cleavable section, such as a “[t]he constant nucleic acid sequence can also contain a cleavable moiety.” *Id.* ¶¶ 18, 131. As I understand the '487 Application, the “constant nucleic acid sequence” refers to a sequence that can be ligated to an identifier oligonucleotide. *See, e.g., id.* ¶¶ 14-15. The “constant nucleic acid sequence” can be part of a number of other sequences, as the '487 Application teaches that “[a]ny of these features can also be flanked by regions comprising constant nucleic acid sequences

of about 1 nucleotide to about 10 nucleotides.” *Id.* ¶ 199. In my opinion, a POSA would have understood from this broad disclosure that a “cleavable moiety” is not fixed between an identifier oligonucleotide and a target binding domain of a probe and can be in other locations and be part of other sequences in the method.

94. In my opinion, a POSA would also have understood that the ’487 Application is not limited to cleavage as a means of releasing a probe. One well-known alternative is denaturation. Denaturation is simply separating two nucleic acid strands that are hybridized to each other through base pairing rules. I understand that during cross-examination Dr. Spellman provided testimony when asked whether a POSA would know “how to denature a probe that’s attached to a target of interest” that “as an abstract concept, denaturation is well-known to a POSA.” EX2007 at 113:25-114:7. I agree with Dr. Spellman that denaturation is well-known. It was well understood long before 2018/2019 that double-stranded nucleic acid can be separated into single strands by disrupting the hydrogen bonds between the two strands. This process can be generally be referred to as denaturation. Denaturation removes high-level structures that may form from double-stranded nucleic acids and results in individual (e.g. single) strands of nucleic acids. In my opinion, a POSA would be well-versed with this concept of denaturation because it is a basic concept in genetics and molecular biology. For example, during DNA replication, denaturation occurs so that two daughter strands

can be generated from a parental strand. As another example, PCR is a common laboratory technique that has been used for decades and relies on repeated cycles of separating double-stranded DNA and synthesizing new double-stranded DNA to rapidly generate copies of sequences of interest—e.g., amplicons.

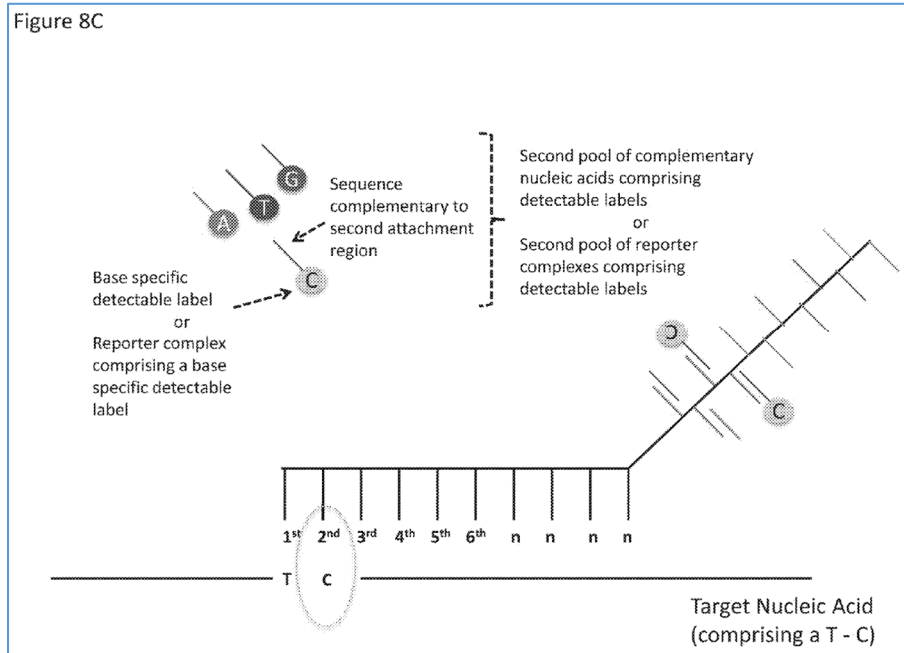
95. The '487 Application explicitly discusses the concept of denaturation. For example, the application discusses that the identifier oligonucleotide can be fully or partially double-stranded and that denaturation can be used to separate the strands. EX1009 ¶ 240. A POSA would have understood that a double-stranded sequence can be formed when an identifier oligonucleotide binds completely or partially to another sequence, such as the target sequence. The '487 Application also teaches that “[s]uitable denaturing conditions will be apparent to the skilled reader with reference to standard molecular biology protocols.” *Id.* ¶ 285. This confirms that denaturation is a standard technique that can be performed using “standard molecular biology protocols.” In addition, in my opinion, a POSA would have understood that a probe can be released using denaturation, a standard technique that is specifically referenced in the '487 Application.

96. The '487 Application also includes specific examples of entire probes that are released from binding to a target analyte using denaturation. It is my understanding that U.S. Patent Application No. 14/946,386 is incorporated by reference into the '487 application. *Id.* ¶¶ 64, 216, 227. I understand this to mean

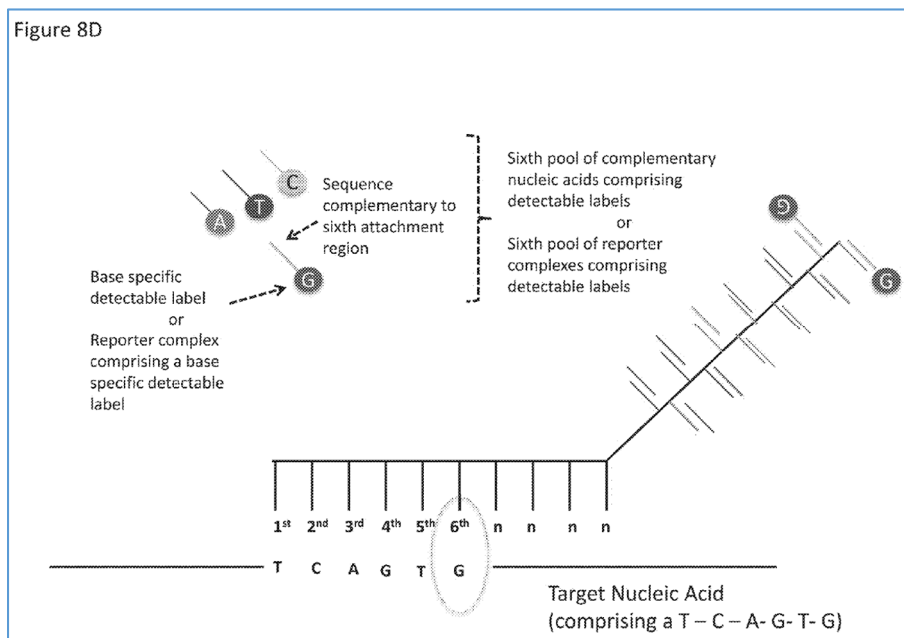
that because the '386 Application is specifically referenced in the '487 application to be incorporated by reference in its entirety, all of the disclosure in the '386 Application is to be treated as being part of the '487 Application. Thus, in my opinion, a POSA would understand that the disclosures in the '386 Application to disclose concepts in the '487 Application, such as denaturation.

97. The '386 Application is a patent application directed to methods for “enzyme-free, amplification-free, library-free nucleic acid sequencing that has long-read-lengths with low error rate.” EX2008 Abstract. A generalized series of steps of the method is as follows: hybridizing a first sequencing probe to a target nucleic acid via target binding domain, binding complementary reporters to a first attachment region that allow identification of the first position of the target binding domain to identify the nucleotide, removing the reporters and attaching sequences without reporters to the first region, repeat binding reporters for each attachment region that correspond to a position in the target binding domain to identify the nucleotide, dehybridize (e.g. denature) the entire sequencing probe and hybridize a second sequencing probe to the target nucleic acid, and repeat the process until the desired sequencing coverage has been reached. *Id.* ¶¶ 10-12. Specifically, the '386 Application teaches “hybridizing at least one sequencing probe...to an target nucleic acid”, “de-hybridizing each sequencing probe of the first population from

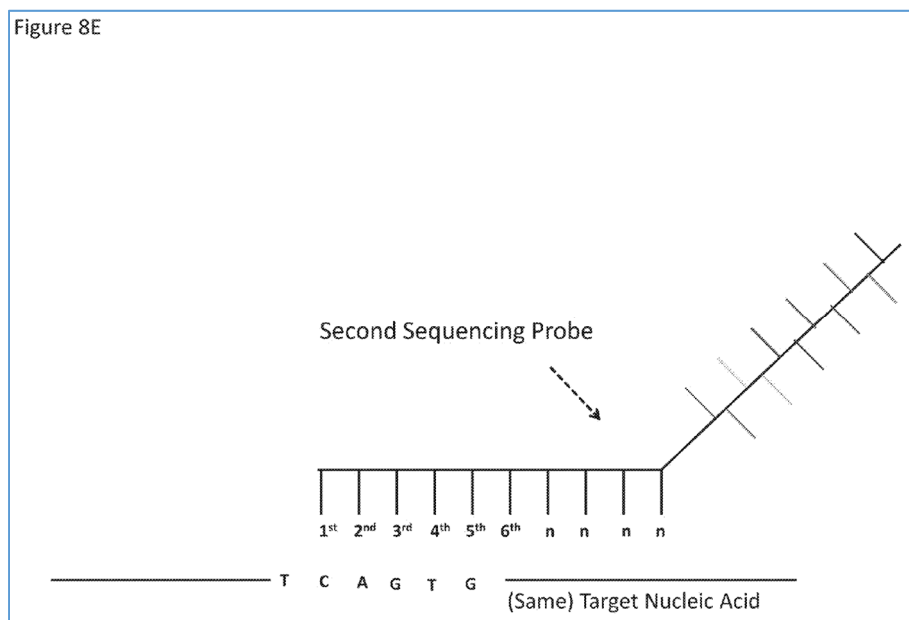
Id. Fig. 8B (depicting a complementary sequence with no detectable label effectively “blocking” a first attachment region on the probe);



Id. Fig. 8C (depicting a reporter hybridizing to a second attachment region corresponding to a second base in the target binding domain of the probe);



Id. Fig. 8D (depicting the process repeating to identify each base in the target nucleic acid);



Id. Fig. 8E (depicting a second sequencing probe hybridizing to the same target nucleic acid after the first sequencing probe has de-hybridized).

98. The '386 Application also teaches conditions that can be adjusted so that sequencing probes can reversibly hybridize and de-hybridize, such as “level of chaotropic agents, temperature, salt concentration, pH, and hydrodynamic forces.”

Id. ¶ 11. The '386 application also teaches that the design of the probes can affect hybridization and de-hybridization when it teaches designing probes “to control the likelihood of hybridization and/or de-hybridization and the rates at which these occur.” *Id.* ¶ 86.

99. The '386 application also includes working examples of probes hybridizing and de-hybridizing from a target nucleic acid. For example, Example

1 discloses how to implement the sequencing probes in an experiment. *Id.* ¶¶ 174-194. Of particular relevance is the disclosure in the example of “[b]inding a population of probes to an immobilized target nucleic acid” and “[p]robes of the first population are de-hybridized.” *Id.* ¶¶ 177, 183. This teaching correlates to the using denaturation to release an entire probe from a target analyte.

100. A POSA would have understood the disclosure in the ’386 Application to teach denaturing an entire probe from a target nucleic acid. A POSA would have understood that there is no practical difference between the probe in the ’386 application and the ’487 Application, as they are both nucleic acid probes that hybridize to a target nucleic acid. Probe design is a well-known skill and, given the teachings in the ’386 Application, a POSA would have understood how to design probes that can reversibly hybridize to a target nucleic acid so that the probe can completely de-hybridize via denaturation. In addition, because the ’487 Application teaches that “[a]ny probe or set of probes described herein may be used in methods and kits of the present disclosure,” EX1009 ¶ 259, a POSA would have understood that any probe disclosed in the ’487 Application, which includes those in the ’386 application, can be applied and used. Based on a POSA’s background, that individual would have understood that there is no difficulty to de-hybridizing an entire probe from a target nucleic acid.

101. In my opinion, the '487 Application also contemplates releasing entire probes from a target nucleic acid, which may be by denaturation. The '487 application discloses how to perform negative purification to reduce background noise:

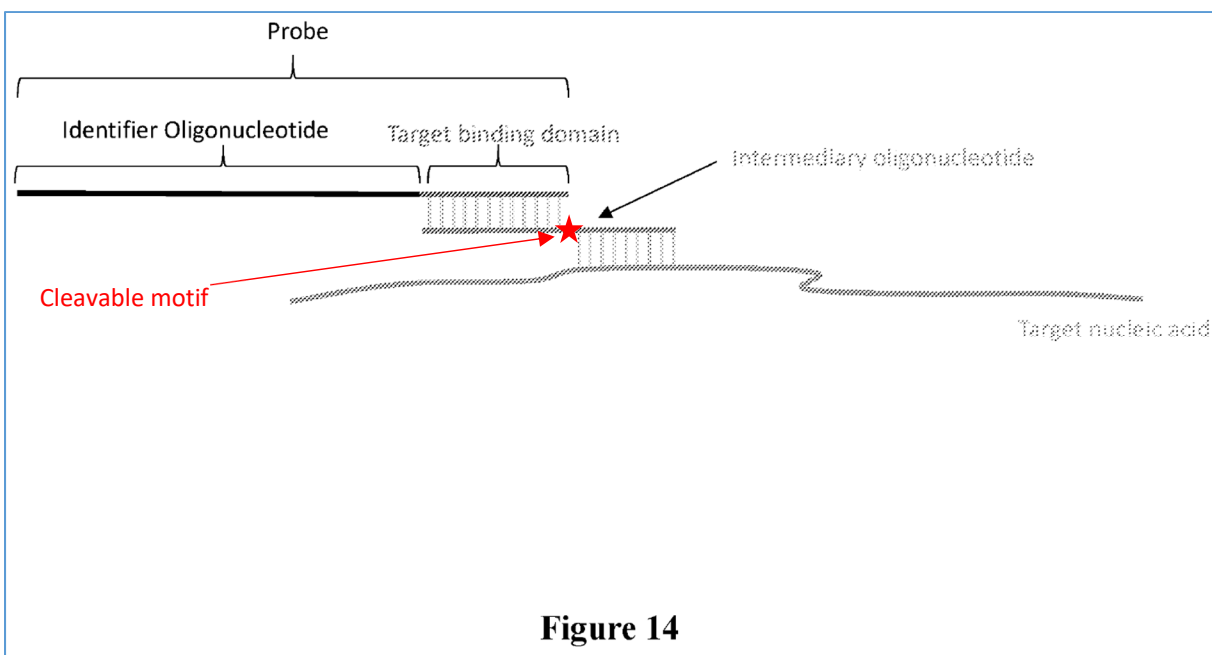
Background noise, during nucleic acid detection, can be reduced by performing a negative purification of the intact probe molecule. This can be done by conducting an affinity purification of the target binding domain or photo-cleavable linker after collection of eluate from a region of interest. Normally, released signal oligonucleotides will not be pulled out of solution. To assist in the negative purification, a universal purification sequence may be included in a probe, e.g., in the target binding domain.

Id. ¶ 252. A POSA would have understood from this disclosure that entire probes, as noted by “intact probe molecule,” are being purified to separate probes that bound to their target analyte and those that did not. A POSA would also have understood that this purification step takes place after an identifier oligonucleotide has been released. This is because the disclosure teaches that “released signal oligonucleotides will not be pulled out of solution.” *Id.* In addition, disclosures of “affinity purification” elsewhere in the '487 Application describe the step as taking place after the identifier oligonucleotide has been released and before sequencing of extension products. *See, e.g., id.* ¶¶ 22-23, 153-154. Thus, a POSA would have understood that the inventors envisioned the identifier oligonucleotide

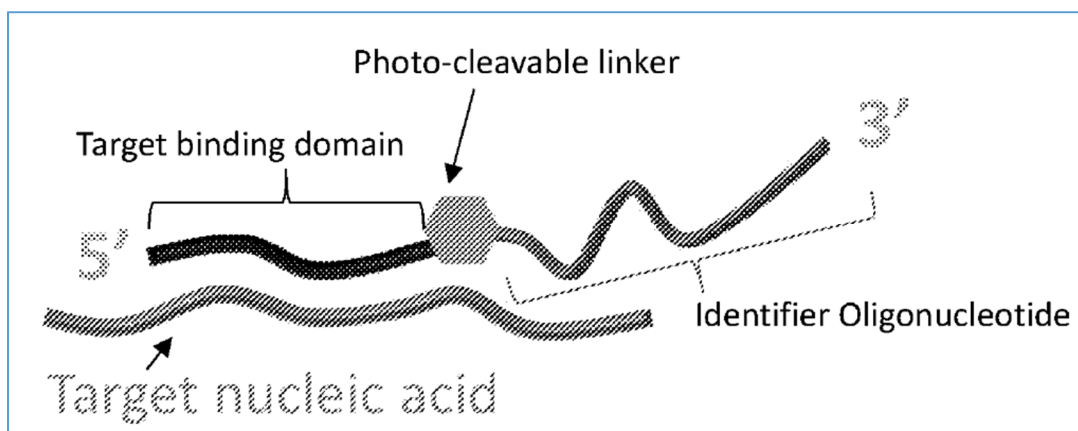
to be released in a way other than by cleaving the probe, because the cleavage would have occurred with both probes that bound to a target analyte and those that did not bind to a target analyte equally. In such a scenario, the probes being purified would also be portions of probe and not an entire probe. The '487 application, however, teaches that it is an “intact probe molecule” that is being purified. As such, a POSA would have understood that the '487 Application discloses releasing an entire probe, the “whole probe,” so that unbound “whole probes” are purified.

102. Additionally, a POSA would have understood that the described regions of a probe, the “target binding domain” and the “identifier oligonucleotide,” can overlap partially or completely. The '487 Application uses the term “target binding domain” and “identifier oligonucleotide” to describe characteristics of those sequences. For example, a “target binding domain” is described as a sequence that “can directly or indirectly hybridize to a target nucleic acid of interest” and an “identifier oligonucleotide” is described as a sequence “which identifies the target analyte bound to the target binding domain.” EX1009 ¶¶ 106, 126, 144, 158, 183, 199. The '487 Application does not teach that the two cannot overlap or that the same sequence cannot serve both functions. Thus, in my opinion, a POSA would have understood that a probe wherein the probe’s sequence serves as both the “target binding domain” and the “identifier oligonucleotide” can be released completely via a technique such as denaturation.

103. As another disclosure of releasing “whole probes,” the ’487 Application teaches that a probe can indirectly hybridize to a target analyte. In describing different embodiments, the ’487 Application teaches that probes can “directly or indirectly” bind to a target analyte. *See, e.g., id.* ¶¶ 106, 310. Specifically, the application teaches that “the probes of the present disclosure can indirectly hybridize to a target nucleic acid present in a sample (via an intermediary oligonucleotide).” *Id.* ¶ 233. The ’487 application also teaches “the intermediary oligonucleotide is a probe, as defined herein, since it comprises a nucleic acid backbone and is capable of binding a target nucleic acid.” *Id.* ¶ 233. Thus, a POSA would have understood that a probe can bind to a target nucleic acid even when it is done so indirectly and also that an “intermediary oligonucleotide” that is part of the indirect binding is a probe and can thus have the attributes of any probe described in the ’487 application. The ’487 application further discloses that “[a] probe of the present disclosure can include a region which permits the release of an identifier oligonucleotide following the application of a suitable force. In one non-limited example, the region is a cleavable motif (e.g., a restriction enzyme site or cleavable linker).” *Id.* ¶ 246. Thus, a POSA would have understood that an “intermediary oligonucleotide” can contain “a region which permits the release of an identifier oligonucleotide,” including “a cleavable motif.” *Id.* Such an arrangement can be as depicted as follows:



Id. Fig. 14 (annotated). In the figure, a “cleavable motif” is located on an “intermediary oligonucleotide,” such that cleavage would allow release of the entire bound probe. The location of the “cleavable motif” in the annotated figure is analogous to other figures included in the ’487 Application, in which the “cleavable motif” is located on a bound probe directly adjacent to where the probe hybridizes to a target nucleic acid:



Id. Fig. 13. As such, a POSA would have understood that the '487 Application discloses what is depicted in the annotated figure and that it would lead to release of an entire probe.

104. For the reasons discussed above, such as the disclosures in the '487 Application in the context of the high skill level and knowledge of a POSA, a POSA in my opinion would have understood that the '487 application at least adequately discloses the feature of a “whole probe.”

b. “On-Analyte Ligation” Is Disclosed

105. I understand Petitioner’s position to be that “on-analyte ligation” is not disclosed in the '487 application. As I understand it, Petitioner’s arguments is that there is a lack of disclosure of a feature in claims 16-30 of the '689 patent, which require “forming ligated probes by ligating each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are bound to each of identical molecules from the at least one target analyte.” EX1001 claim 16(b). Specifically, Petitioner contends that that this forming step requires (1) probes that hybridize to a target analyte and also (2) probes that are ligated together while hybridized, and Petitioner contends that both of these concepts are not disclosed in the '487 application. As a shorthand, Petitioner refers to these concepts as “on-analyte ligation.” On the other hand, Petitioner refers to the '487 Application’s disclosure as “off-analyte

ligation.” In my opinion, both of the parts of “on-analyte ligation” are disclosed. I take each of these allegations in turn.

(i) Petitioner’s Product Need Not Be Described

106. I understand that in making its argument that Petitioner uses terms including primary, secondary, on-analyte, and off-analyte. I have reviewed the ’487 Application and do not find any of these terms. I therefore conclude that these terms are not part of the ’487 Application.

107. I understand that Petitioner produces a product called Visium FFPE, which is a product accused of infringing the ’689 patent in an ongoing litigation in district court. I understand that the Visium FFPE product guide includes two steps, which are probe hybridization and probe ligation:

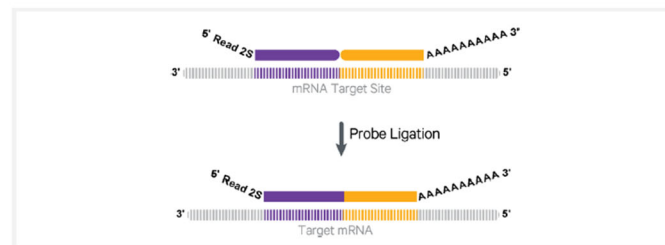
Step 1
Probe Hybridization

The human or mouse whole transcriptome probe panel, consisting of a pair of specific probes for each targeted gene, is added to the deparaffinized, stained, and decrosslinked tissues. Together, probe pairs hybridize to their complementary target RNA.



Step 2
Probe Ligation

After hybridization, a ligase is added to seal the junction between the probe pairs that have hybridized to RNA, forming a ligation product.



EX1020 at 83. As the caption reads, the probe hybridization step involves a pair of probes that hybridize to a target RNA and the probe ligation step involves adding a ligase to ligate the probe pair together. In my opinion, these steps are an example of what Petitioner argues is not disclosed in the '487 Application regarding “on-analyte ligation”—hybridization and ligation.

(ii) The '487 Application Discloses Probes That Bind To The Target

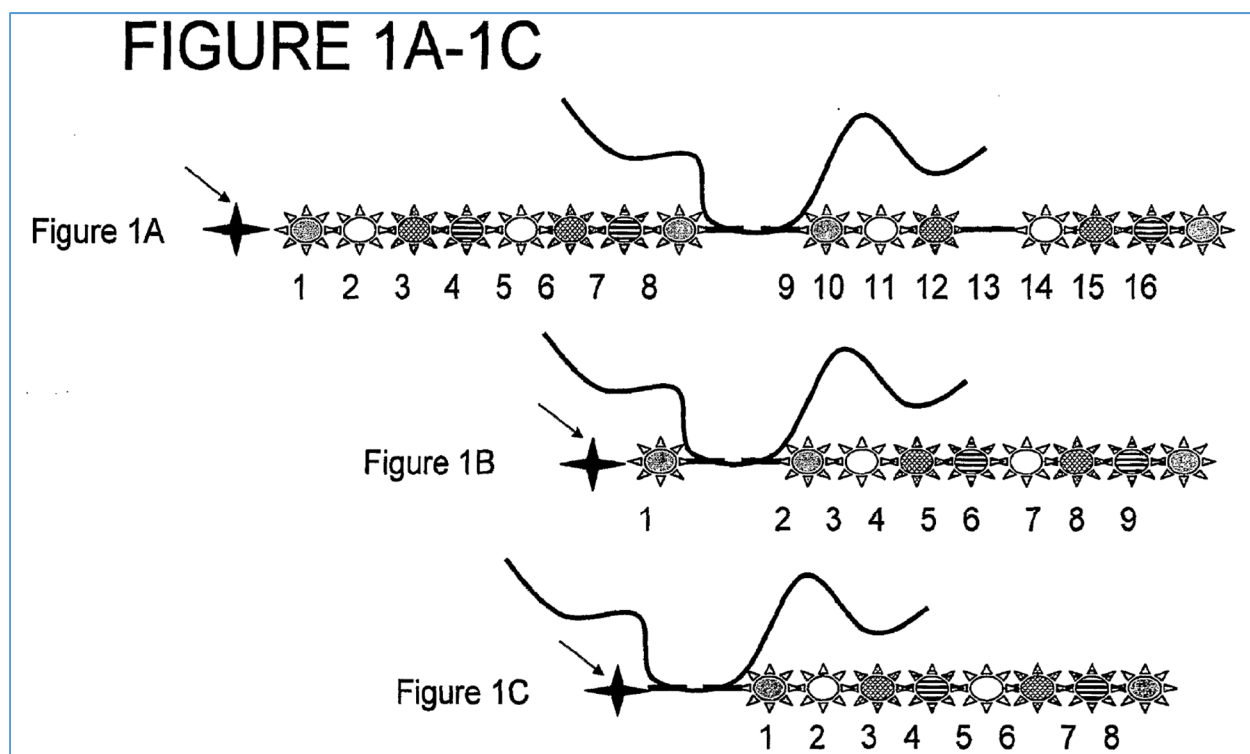
108. Regarding hybridization of probes to a target analyte, it is my opinion that a POSA would have understood this to be disclosed by the '487 Application.

109. As an initial matter, Petitioner states that the '487 Application discloses the “*in situ* hybridization of cleavable ‘probes’ comprising a target-binding domain and an identifier oligonucleotide.” Paper 2 at 55. Thus, Petitioner agrees that the '487 Application discloses probes that hybridize to a target analyte. In my opinion, a POSA would have known how to design probes with specific target-binding sequences and known that a pair of probes can likewise be designed so as to hybridize to the same target nucleic acid. This is particularly so because a POSA would be well aware of *in situ* hybridization, which the '487 Application discloses as a prior method that the '487 Application is specifically improving upon.

110. The '487 application also discloses specific examples of probe pairs that bind directly to a target nucleic acid. U.S. Patent Application Publication No. 2010/0015607 is a reference that is entirely incorporated by reference into the '487 application. EX1009 ¶ 232. I have been informed that the broad language in the '487 Application that the '607 Publication “is incorporated herein by reference in its entirety” means that any disclosure in the '607 Publication is to be treated as being part of the '487 Application. In particular, I understand that the '487 Application references the '607 Publication to disclose “[p]robes and method for binding and identifying a target nucleic acid.” *Id.*

111. The '607 Publication teaches that a probe pair that can hybridize to a target nucleic, wherein a first probe comprises “a first target-specific sequence

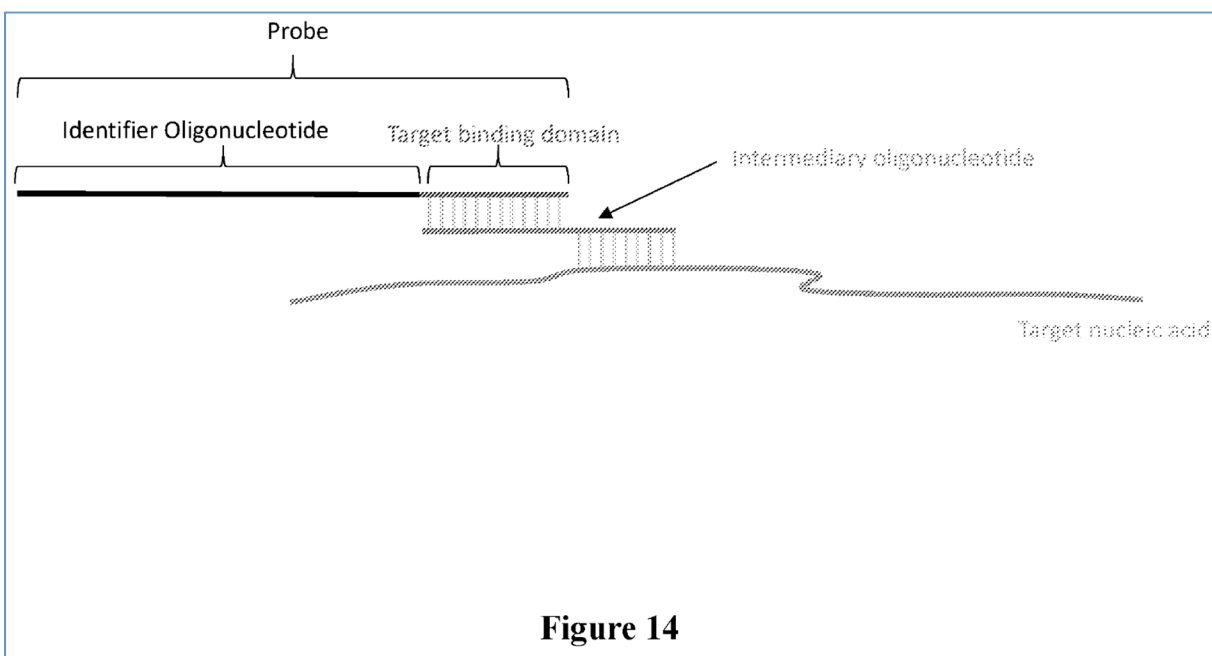
attached to the first molecule” and a second probe comprises a “second target-specific sequence.” EX2009 ¶ 13. The ’607 Publication teaches that these “target-specific sequences are predetermined nucleotide sequences.” *Id.* ¶ 21. A POSA would have understood this to mean that the sequence of the target nucleic acid is known, such that probes can be designed with complementary sequences that can hybridize to the target nucleic acid. Examples of probes disclosed by the ’607 Publication are depicted below:



Id. Fig. 1A-1C. As depicted, each probe pair hybridizes to a target nucleic acid in a manner such that the target-specific sequences on the probe pair do not overlap. Although not directly relevant to the issue in this proceeding, the three different probe pairs in the figure each have different sequences, with the star indicating the

possibility of an affinity molecule on one of the probes in each pair. *Id.* ¶ 71. In my opinion, a POSA would have understood from at least the '607 Publication that the '487 Application discloses probe pairs that hybridize directly to a target nucleic acid.

112. In my opinion, the '487 Application also teaches that probes can hybridize to a target analyte indirectly via an indirect target binding domain. This is due to the '487 Application's explicit teaching that "the probes of the present disclosure can indirectly hybridize to a target nucleic acid present in a sample." EX1009 ¶ 233. A POSA would have understood that a probe pair can indirectly hybridize to a target nucleic acid via an indirect target binding domain. An example of this concept is depicted in Figure 14:



Id. Fig. 14. A POSA would have understood from the '487 Application that a probe can indirectly bind to a target nucleic acid. Thus, a POSA would have understood that the '487 Application's disclosure of probe pairs that bind to a probe comprising an identifier oligonucleotide that the probe pairs bind to a target analyte, as required by the claim that "each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are bound to each of identical molecules from the at least one target analyte." EX1001 claim 16(b). In short, a POSA would have understood that the probe pairs in the '487 Application are bound to a target analyte.

113. To reiterate, a POSA would be familiar with a number of techniques, including in situ hybridization. A POSA would also know how to design oligonucleotides and understand base pairing rules to design probes that hybridize to a nucleic sequence of interest. These techniques are rather fundamental and basic to the field of molecular biology. It is my understanding that Petitioner's expert does not offer a differing opinion. When asked whether a POSA would be able to practice the '689 Patent claims with just a POSA's background knowledge, Petitioner's expert answered in the affirmative. EX2007 at 72:15-73:3, 92:23-93:3. Thus, in my opinion, a POSA would surely be able to practice the claims in light of the disclosure in the '487 Application. Petitioner's expert's contrary opinion defies logic. With a POSA's background knowledge in context, a POSA would have

understood from the disclosure in the '487 Application that hybridizing probes to a target analyte is disclosed.

(iii) The '487 Application Discloses Ligation Of Probes

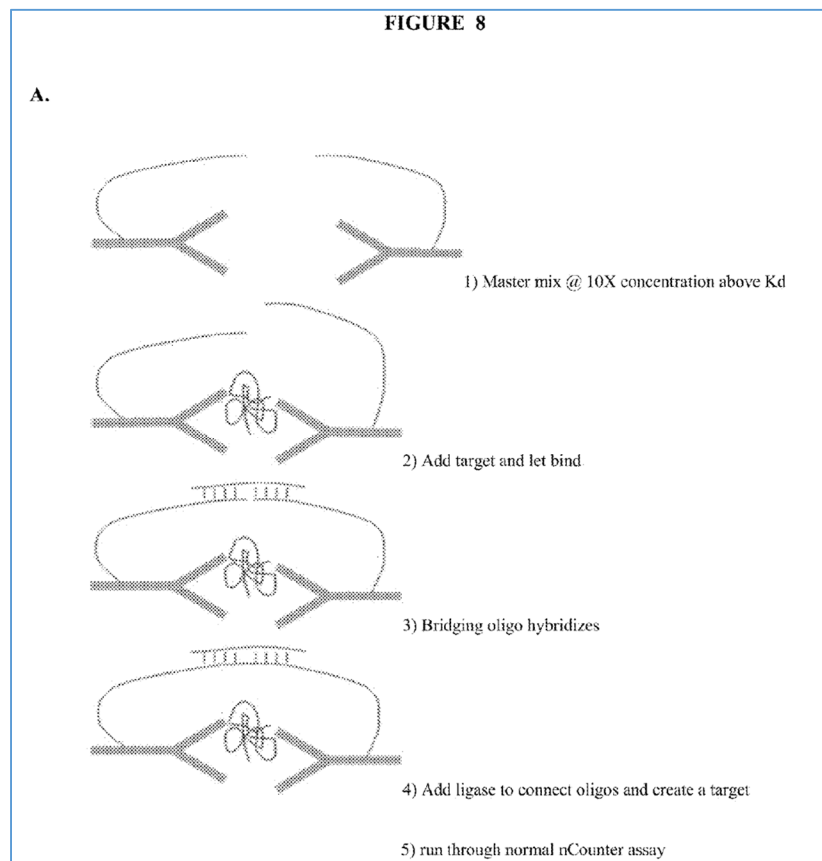
114. Regarding ligation of probes bound to a target analyte, it is my opinion that a POSA would have understood this to be disclosed.

115. As discussed above, the '487 Application specifically teaches that probes can be indirectly bound to a target analyte. A POSA would have understood that the probe pairs in the '487 Application are indirectly bound to a target analyte in this manner. This is because, as discussed, the '487 Application discloses a probe pair that binds to another probe that binds to a target analyte. As such, when the probe pair is ligated, a POSA would have understood this to take place while the probe pair is bound to a target analyte.

116. To the extent Petitioner argues that the ligation of probe pairs disclosed in the '487 Application takes place after the probe comprising the identifier oligonucleotide has already been released and no longer bound to the target analyte, a POSA would have likewise understood that the '487 Application discloses the ligation of probes bound to a target analyte. This is because the probe comprising the identifier oligonucleotide is clearly bound to the target analyte. In addition, a POSA would have understood that the ligation of probe pairs can take place prior to the identifier oligonucleotide's release. Nothing prevents such ligation from

occurring and the '487 Application does not require that any of the steps disclosed in any of the embodiments must take place in the order listed.

117. In addition, the '487 application teaches two nucleic acids that both bind to a third and ligating the two nucleic acids. In U.S. Patent Application Publication No. 2011/0086774, which I have been informed is entirely incorporated by reference, a first oligonucleotide and a second oligonucleotide both hybridize to a third oligonucleotide called a “bridging oligo.” EX2010 ¶ 37. A ligase is subsequently added to ligate the two oligonucleotides together. *Id.* This is depicted in the figure below:



Id. Fig. 8A. In my opinion, a POSA would have understood from this disclosure that two oligonucleotides, as a pair, can jointly be designed to hybridize to a third nucleic acid and, while hybridized, the two oligonucleotides can be ligated together. A POSA would further have understood that this is analogous to a probe pair, such that a probe pair can be designed to hybridize to a nucleic acid, the target analyte. A POSA would have understood that such a design is viable and would have understood the '487 Application to have disclosed this.

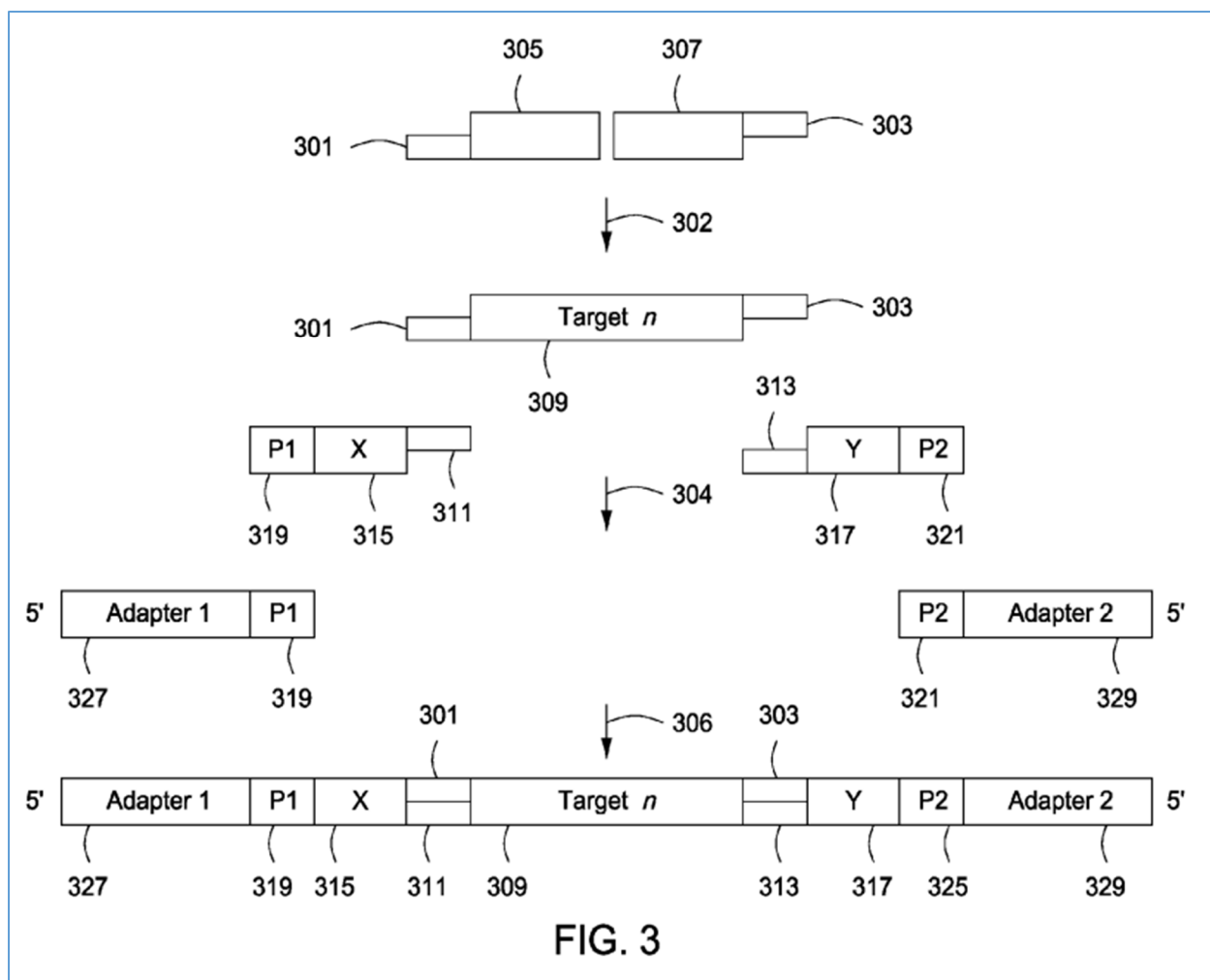
118. In addition, a POSA would have understood the disclosures in the '487 Application in the context of the POSA's background knowledge. During cross-examination, Dr. Spellman was asked whether a POSA performing the claimed method in the '689 Patent would have "within their basic competency the ability to perform ligation off-analyte for barcoding." EX2007 at 76:6-9. I understand Petitioner's expert to have offered an opinion that the '487 application discloses "off-analyte ligation." EX1002 ¶ 285. During cross-examination, however, Petitioner's expert responded that "[o]utside of the claim, a POSA would know how to perform ligation" and that ligation is "a simple skill." EX2007 at 76:11-15. I disagree with Dr. Spellman that a POSA would be unable to perform ligation that is disclosed in the '487 Application.

119. Ligation is a basic skill well within the competency of a POSA. I agree with Dr. Spellman that ligation is a simple skill and well within the basic

competencies of a POSA. *Id.* I also agree with Dr. Spellman that “a person of ordinary skill in the art would be familiar with ligation as a broad concept, extremely broad, and how and when it can be utilized effectively” and that a “POSA has a set of competencies, things they know how to do—ligation being a common one, for example.” *Id.* at 49:6-9, 75:6-8.

120. Ligation involves little more than adding the proper ligase in the proper conditions, which a POSA would have understood. As such, a POSA would have been able to perform a simple ligation step to ligate a probe pair as claimed.

121. I understand that this simple concept is disclosed even in a patent publication from 2011 that Petitioner acquired. U.S. Patent Publication No. 2011/0245111 is a reference published eight years prior to the '487 application's filing date and I understand was in an Information Disclosure Sheet during prosecution of the '689 patent. EX2011 at 208. In the '111 Publication, which is disclosed by the '689 patent, “two oligonucleotide probes are provided” that each “comprises a target-specific region.” EX2012 ¶ 54. The two oligonucleotide probes “are allowed to hybridize to a target nucleic acid,” after which the two probes ligated together. *Id.* This process is depicted in the first two steps of the figure below:



Id. Fig. 3. In my opinion, a POSA would have understood this reference to disclose ligating a probe pair hybridized to a target analyte. Thus, the simple concept of ligating a probe pair hybridized to a target analyte is well within a POSA's basic knowledge.

122. A POSA, in view of the knowledge to ligate a probe pair hybridized to a target analyte, would have understood that such a feature is part of the '487 Application's disclosure.

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.

Dated: August 26, 2024

By: David H. Herman