IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10x GENOMICS, INC.
Petitioner,

v.

NANOSTRING TECHNOLOGIES, INC.
Patent Owner.

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

DECLARATION OF PAUL T. SPELLMAN, PH.D.

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I, Paul T. Spellman, hereby declare as follows:

I. INTRODUCTION

1. I am over the age of 18 and competent to make this declaration.

2. I have been retained as an expert witness on behalf of 10x Genomics, Inc. ("10x") for the above-captioned *inter partes* review (IPR) against NanoString's U.S. Patent No. 11,377,689 ("the 689 patent") (EX1001). I understand that this Declaration accompanies 10x's Petition for the IPR. I am being compensated for my time in connection with this IPR.

3. I understand that the 689 patent resulted from U.S. Patent Application No. 17/476,707 ("the 707 application"), filed on September 16, 2021, which is a continuation of U.S. Patent Application No. 16/272,487 ("487 application"; EX1009), filed on February 11, 2019, which claims benefit to the following provisional applications:

- Provisional Appl. No. 62/771,212 ("the 212 application"; EX1010), filed November 26, 2018; and
- Provisional Appl. No. 62/629,180 ("the 180 application"; EX1011), filed February 12, 2018.

4. I understand February 12, 2018, is the 689 patent's earliest alleged priority date. I refer to this date throughout this Declaration.

5. In preparing for this Declaration, I have reviewed the 689 patent (EX1001) and each of the documents cited herein, in light of the general knowledge in the art before February 12, 2018.

6. In formulating my opinions, I have relied upon my experience, education, and knowledge in the relevant art. In formulating my opinions, I have also considered the viewpoint of a person of ordinary skill in the art ("POSA"), as defined below in Section IV.a, before February 12, 2018.

II. SUMMARY OF MY OPINIONS

7. I have been asked to consider whether, before February 12, 2018, WO 2017/019456 ("So-456"; EX1005) anticipated the challenged claims of the 689 patent. I understand that So-456 was both filed and published before February 12, 2018, and thus it qualifies as prior art. For the reasons I explain under Ground 1, in view of the state of the art before February 12, 2018, So-456 teaches every element of claims 1-8, 11-23, and 26-30, arranged as claimed. I understand that So-456 is presumed enabled. Thus, for the reasons detailed under Ground 1, So-456 anticipated claims 1-8, 11-23, and 26-30 of the 689 patent under NanoString's interpretation.

8. I have been asked to consider whether, before February 12, 2018, So-456 would have rendered the challenged claims of the 689 patent obvious. For the reasons I explain under Ground 2, in view of the state of the art before February

12, 2018, So-456 teaches or suggests all elements of the methods of claims 1-30. Further, as I explain under Ground 2, a POSA would have been motivated to perform the claimed methods with a reasonable expectation of success. Thus, for the reasons detailed under Ground 2, So-456 would have rendered claims 1-30 of the 689 patent obvious under NanoString's interpretation.

9. I have been asked to consider whether, before February 12, 2018, So-456 together with Stahl *et al.*, "Visualization and analysis of gene expression in tissue selections by spatial transcriptomics," *Science* 353(6294):78-82 (July 1, 2016) ("Stahl 2016") would have rendered the challenged claims of the 689 patent obvious. For the reasons I explain under Ground 3, in view of the state of the art before February 12, 2018, So-456 and Stahl 2016 teach or suggest all elements of methods of claims 9, 10, 24, and 25. Further, as I explain under Ground 2, a POSA would have been motivated to perform the claimed methods with a reasonable expectation of success. Thus, for the reasons detailed under Ground 3, So-456 would have rendered claims 1-30 of the 689 patent obvious under NanoString's interpretation.

10. I have been asked to consider whether, before September 16, 2021, WO 2021/133849 ("Chell"; EX1006) anticipated the challenged claims of the 689 patent. As I explain below, the priority applications of the 689 patent do not provide written description support for the full scope of the 689 patent claims, as

required by NanoString. I understand that, as a result, the 689 patent claims are not entitled to a filing date before the patent's filing date of September 16, 2021. I understand that Chell was both filed and published before September 16, 2021, and thus it qualifies as prior art. For the reasons I explain under Ground 4, in view of the state of the art before September 16, 2021, Chell teaches every element of claims 1-30, arranged as claimed. Further, I understand that Chell is presumed enabled. Thus, for the reasons detailed under Ground 4, Chell anticipated claims 1-30 of the 689 patent under NanoString's interpretation.

III. MY BACKGROUND AND QUALIFICATIONS

11. I am a Professor of Medicine and Human Genetics in the David Geffen School of Medicine at the University of California, Los Angeles.

12. I have over 20 years of experience in the field of genetics and genomics, including approaches for detecting or monitoring human diseases, such as spatial detection of target molecules in a biological sample. I also have over 15 years of experience in cancer genomics.

13. My current research focuses on computational biology, cancer biology, and cancer genomics. My lab develops technologies and approaches to understand the process by which cancer develops, monitor disease, and identify therapeutic strategies. Within this broad area of research, I have specific experience in using population genetics to help determine who is at risk for cancer, how to

computationally analyze genomic data to identify early changes in cancers, and developing new genomic methodologies including those focused on analyzing single cells and the use of DNA barcodes that help identify individual molecules.

14. I have provided a copy of my *curriculum vitae* as EX1003.

15. I graduated with a Bachelor's degree in Biology from the Massachusetts Institute of Technology in 1995. I then received my Ph.D. in Genetics from Stanford University Medical School in 2000. My Ph.D. thesis topic was "Generating and Analyzing Genome Scale Data."

16. From 2000-2003, I was a postdoctoral fellow in the Department of Molecular and Cellular Biology at the University of California, Berkeley. As a postdoctoral fellow, I was selected for the prestigious National Science Foundation Biocomputing Fellowship, which covered both my stipend and significant research costs.

17. After my postdoctoral fellowship, I became a Staff Scientist at the Life Science Division of Lawrence Berkeley Lab (2007-2011) and served as a Special Assistant to the Deputy Director of the National Cancer Institute (2011). Subsequently, I served as an Associate Professor (2011-2013) and as a Full Professor with Tenure (2013-2023) in the Department of Molecular and Medical Genetics at Oregon Health & Science University. In 2023, I was recruited to the

University of California, Los Angeles as a Professor of Medicine and Human Genetics.

18. I have authored more than 115 peer-reviewed publications that discuss methods of DNA manipulation and analysis, including DNA sample preparation for sequencing, amplification (*e.g.*, PCR), methods of DNA sequencing (including NGS and related sequencing methods), and bioinformatics methods for raw data analysis and interpretation.

19. Key among these papers are work on developing the field of gene expression profiling (Spellman *et al*., *Molecular Biology of The Cell*, 1998; and Eisen *et al*., *Proceedings of the National Academy of Sciences*, 1998, which together have been cited in more than 20,000 other works), fabricating DNA microarrays (George, Wooley, and Spellman, *Genome Research*, 2001), ligation-based approaches to detecting and quantifying DNA and DNA methylation (Wang *et al.*, *Genome Biology*, 2008; and Nautiyal *et al*., *Proceedings of the National Academy of Sciences*, 2011), histopathological classification by gene expression and imaging (*e.g.*, Chang *et al.*, *BMC Bioinformatics*, 2011), and the use of barcodes to identify unique molecules in multiplexed samples (Butler *et al*., *Cold Spring Harbor Molecular Case Studies*, 2019).

20. In addition, I have presented over 100 invited lectures or conference presentations regarding these topics. I have also supervised five completed Ph.D.

theses and have taught graduate-level courses at OHSU in the area of sequencing technologies, computational analysis, and genetics.

21. I have received over $16 million in federal grants for my research in the area of cancer genomics and cancer precision medicine. I am currently one of 30 principal investigators for the Genome Data Analysis Network, sponsored by the National Cancer Institute. The Genome Data Analysis Network is a consortium of computational researchers from across the United States focused on developing the framework for relating the genomics and outcomes of patients in cancer clinical trials.

22. In 2017, I was named the inaugural holder of the Penny and Phil Knight Endowed Professorship for Cancer Research Innovation. In 2020, I was one of the recipients of the American Association for Cancer Research (AACR) Team Science award for my leadership roles in the Cancer Genome Atlas.

IV. MY UNDERSTANDING OF THE RELEVANT LEGAL PRINCIPLES

23. In formulating my opinions set forth in this Declaration, I have applied the following legal principles.

a. Person of Ordinary Skill in the Art ("POSA")

24. I understand that patent law analyses are performed from the viewpoint of a POSA. I understand that a POSA is a hypothetical person who is presumed to be aware of all pertinent art, thinks along conventional wisdom in the art, and is a

person of ordinary creativity. A POSA, with respect to the 689 patent, would have possessed a strong understanding of genetics and genomics approaches for detecting or monitoring human diseases, such as spatial detection of a target analyte (*e.g.*, mRNA or DNA) in a tissue section. A POSA would typically have a Ph.D. in a relevant field (*e.g.*, molecular biology, genetics, and bioinformatics) with two years of experience in the use and development of genetics and genomics approaches for detecting and monitoring human diseases, such as spatial detection of a target analyte (*e.g.*, mRNA or DNA) in a tissue section.

25. I understand that NanoString stated during prosecution of the 689 patent that "the skill level of a [POSA] in the context of the instant application is high, at least that of a PhD in biology, if not higher)." EX1004, 268.

b. Claim Construction

26. I understand that in an IPR, patent claim terms are given their ordinary and customary meaning as understood by a POSA at the time of the invention, in view of the patent's specification and its prosecution history. I also understand that when a patent explicitly defines a claim term in the specification, the patent's definition controls.

27. I understand that claims 1 and 16 are independent claims in the 689 patent, and that claims 2-15 and 17-30 are dependent claims. I understand that claims 2-15 depend directly or indirectly from claim 1, and claims 17-30 depend

directly or indirectly from claim 16. I understand that a dependent claim contains all the elements of the claim from which it depends. Thus, I understand that claims 2-15 contain all the elements of claim 1 and claims 17-30 contain all the elements of claim 16.

c. Obviousness

28. I understand that an obviousness analysis involves comparing a claim to the prior art to determine whether the claimed subject matter would have been obvious to a POSA in view of the prior art and general knowledge of the prior art. I understand that obviousness must be assessed from the viewpoint of a POSA at the time of the invention. I further understand that to show obviousness a party must perform the following factual inquiries: (a) determining the scope and content of the prior art; (b) ascertaining the differences between the claimed invention and the prior art; (c) resolving the level of skill in the art; and (d) considering any known objective evidence of non-obviousness.

29. I also understand that, for a finding of obviousness, a POSA needs to have (i) a reason or motivation to modify or combine the prior art to arrive at the claimed subject matter and (ii) a reasonable expectation of doing so successfully.

30. I understand that the reason to combine prior-art references can come from a variety of sources, not just the prior art itself or the specific problem the patentee was trying to solve. And I understand that the references themselves need

not provide a specific hint or suggestion of the alteration needed to arrive at the claimed invention; the analysis may include recourse to logic, judgment, and common sense available to a person of ordinary skill that does not need to be explicit in any reference.

31. I understand that a "reasonable expectation of success" is assessed in view of the prior art and general knowledge of the art from the viewpoint of a POSA, and it does not require an absolute certainty of success.

32. I also understand that the objective evidence of non-obviousness can include evidence of commercial success attributable to the claimed invention, evidence of industry praise for the claimed invention, evidence of a long-felt need that was solved by the claimed subject matter, evidence that others copied the claimed subject matter, or evidence that the claimed subject matter achieved an unexpected, superior result relative to the closest prior art. I understand that such evidence must have a nexus, or causal relationship, to the claimed subject matter beyond what was available in the prior art, and must be commensurate in scope with the patent claim(s) at issue.

d. Anticipation

33. I understand that anticipation analysis involves comparing a claim to a single prior art reference to determine whether the claimed subject matter is novel to a POSA in view of the prior art and general knowledge in the prior art. I

understand that anticipation must be assessed from the viewpoint of a POSA at the time of the invention.

34. I understand that anticipation analysis requires comparing the claim language to the prior art on an element-by-element basis. I understand that a prior art reference anticipates a claim if all of the claim's elements are expressly or inherently disclosed in a single prior art reference, arranged as in the claim. I understand that the disclosure in a prior art reference does not have to use the same words as the claim, but all of the requirements of the claim must be expressly or inherently disclosed in the prior art reference.

35. I understand that a prior art reference anticipates a patent claim when the claim element not expressly found in that reference are nonetheless inherent in it. I understand that express disclosure means that the subject matter is clearly described in the prior art reference.

36. I understand that an element or the entire invention is inherent and in the public domain if it is the natural result flowing from the explicit disclosure of the prior art. I understand that for a claimed element to be inherently present within a prior art document, it must be necessarily present. That is, the prior art reference must necessarily function in accordance with or include the claimed element. I understand that inherency may not be established by probabilities or possibilities—

the mere fact that a certain thing may result from a given set of circumstances is not sufficient.

37. I understand that in some cases, the inherent property corresponds to a claimed new benefit or characteristic of a purported invention otherwise in the prior art. I understand that in such circumstances, the new realization alone does not render the old invention patentable. I further understand that when a prior art method is at issue, the inherent anticipation examines the natural and inherent results in that method without regard to the full recognition of those benefits or characteristics within the art field at the time of the prior art disclosure.

e. Written Description

38. I understand that written description of a subject matter is assessed from the viewpoint of a POSA. I understand that to satisfy written description, the disclosures in the application must convey to a POSA that the inventors had "possession" of the claimed subject matter. I understand that the inventor need not physically possess the described subject matter to have "possession" in the context of written description. I understand that possession for written description purposes is determined by the sufficiency of the disclosure in the application taken together, and in the context of, the state of the art at the time of the filing. I understand that the state of the art at the time of filing is determined by the general knowledge of a POSA, as evidenced by the published literature at the time.

39. I also understand that a patent application need not disclose a working example of the subject matter to satisfy the written description requirement. I further understand that a subject matter may be fully described in a patent application even if separate parts of it are described in separate parts of the application, as long as there is sufficient direction in the application to guide a reader towards the subject matter.

V. TECHNICAL TERMINOLOGY

40. I use "primary probe" for a molecule comprising a target-binding domain that directly binds to a target analyte (*e.g.*, DNA or RNA).

41. I use "secondary probe" for a molecule that binds to a primary probe or its portion.

42. I use "spatial barcode" or "positional barcode" for a nucleic acid sequence that is associated with a specific location on an array surface and provides information as to the location of an analyte within a biological (*e.g.*, tissue) sample.

43. I use "array probe" or "capture probe" for a probe that is immobilized on a solid surface.

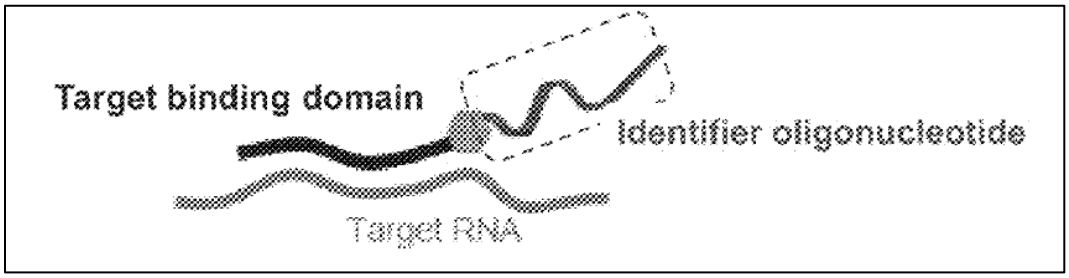
VI. THE 689 PATENT AND ITS CLAIMS

a. The 689 patent

44. The 689 patent relates to spatially detecting and quantifying target analytes (*e.g.*, nucleic acids) in a "user-defined region of a tissue, user-defined cell, and/or user-defined subcellular structure within a cell." EX1001, Abstract.

45. The 689 patent's methods depend on *cleavable* primary probes (referred to as "probes" in the patent) that bind to target analytes in a tissue sample and are cleaved when a "force" is applied at specific, user-defined locations in the tissue sample. EX1001, 16:51-17:3-9, 18:43-49, 19:5635-62, 21:10-15, 21:66-22:18-24, 23:13-19, 23:65-24:15-21, 28:66-29:16-22, 31:5633-62, 32:28-35, 34:16-22, 33:64-34:22, 34:54-61, 35:4323-49, 36:20-26, 39:11-26, 49:11-21, FIG. 11.

46. The patent's cleavable "probes" comprise two regions: (1) a "target binding domain" (a portion that binds to the target analyte in the sample), and (2) an "identifier oligonucleotide" (a portion that is cleaved off from the probe when a force is applied and is collected for further processing). EX1001, 37:14-18, 38:29-33, FIG. 11. I have reproduced a schematic depiction of an exemplary cleavable probe against a target nucleic acid (RNA) from Figure 11 of the patent.



EX1001, FIG. 11.

47. Thus, in every disclosure throughout the 689 patent, the methods require the following features:

(1) The methods use cleavable primary probes (referred to as "probes" in the patent) having a "target binding domain" and an "identifier oligonucleotide." These cleavable probes hybridize to the target analytes in the tissue sample and are discussed in detail below.

(2) The methods apply a "force" at a user-defined "region of interest" (ROI) in the tissue sample to cleave the "probes" within that ROI and release the "identifier oligonucleotide" portion of the probes, collect the released identifier oligonucleotides separately from each ROI, and tag the collected identifier oligonucleotides with a spatial barcode preassigned to the user-defined ROI for analysis.

See, *e.g.*, EX1001, 1:46-67, 2:3-25, 2:28-54, 3:24-47, 3:50-4:9, 4:36-5:2, 5:5-37, 5:46-6:8, 6:11-43, 6:46-7:9, 7:12-44, 8:20-52, 8:55-9:23, 9:26-58, 9:61-10:29, 11:3-25, 11:28-52, 11:55-12:4, 12:7-28, 12:31-57, 12:60-13:8, 16:52-17:32, 18:43-49, 19:36-20:20, 21:10-15, 21:67-22:52, 23:13-19, 23:66-24:47, 27:9-27, 28:3-20, 28:67-29:22, 29:25-44, 31:33-32:21, 32:28-35, 33:65-34:33, 34:54-61, 35:24-36:12, 39:11-26, 49:11-21, 49:43-52, FIGs. 1, 2, 3, 11, 12, 13, 20, 26.

48. In other words, the 689 patent's methods require a user to collect proxies (*i.e.*, the cleaved off identifier oligonucleotides) for the target analytes from within a particular, user-defined region of interest of the tissue sample while maintaining the target analyte's spatial information. *See, e.g.,* EX1001, FIGs. 1, 2, 3, 11, 12, 13,18, 20, 26, 1:46-67, 2:3-25, 2:28-54, 3:24-47, 3:50-4:9, 4:36-5:2, 5:5-37, 5:46-6:8, 6:11-43, 6:46-7:9, 7:12-44, 8:20-52, 8:55-9:23, 9:26-58, 9:61-10:29, 11:3-25, 11:28-52, 11:55-12:4, 12:7-28, 12:31-57, 12:60-13:8, 16:52-17:32, 19:36-20:20, 21:67-22:52, 23:66-24:47, 27:9-27, 28:3-20, 28:67-29:22, 29:25-44, 31:33-32:21, 33:65-34:33, 35:24-36:12.

49. Below I provide further details of the components and workflow of the patent's methods.

i. Components of the 689 patent's methods

50. **"Probes"**. The patent's methods use "*probes* for the compositions and methods of spatially detecting at least one target analyte in a sample" that comprise "a *target binding domain* and an *identifier oligonucleotide*." EX1001, 37:14-16 (emphasis added), 1:46-51, 2:3-9, 2:28-34, 3:26-32, 3:50-56, 4:36-42, 5:5-11, 5:46-51, 6:11-17, 6:46-52, 7:12-18, 8:20-25, 8:55-61, 9:26-32, 9:61-67, 11:3-11, 11:28-35, 11:55-61, 12:7-13, 12:31-36, 12:60-13:1, 16:52-67, 19:36-53, 21:67-22:15, 23:66-24:12, 27:9-23, 28:3-16, 28:67-29:13, 31:33-53, 33:65-34:13, 35:24-40. A "target binding domain" is "a region of the probe that specifically binds to at least

one target analyte in a sample." EX1001, 37:16-18. If the target analyte is a nucleic acid (for example, an mRNA), "the target binding domain can be a target nucleic acid-binding region" comprising a nucleotide sequence. EX1001, 37:19-32.

51. **"Identifier oligonucleotide"**. An "identifier oligonucleotide is a nucleic acid molecule that identifies the target analyte bound to the target binding domain." EX1001, 38:29-33. It "comprises a unique nucleic acid sequence that identifies the target analyte bound to the target binding domain of the probe." EX1001, 38:29-33. Importantly, the identifier oligonucleotide is separable or releasable from the rest of the probe upon application of a "force" and the released identifier oligonucleotide is collected and analyzed. EX1001, 1:51-67, 2:9-25, 2:34-54, 3:32-47, 3:56-4:9, 4:42-5:2, 5:11-37, 5:52-6:8, 6:17-43, 6:52-7:9, 7:18-44, 8:26-52, 8:61-9:23, 9:32-58, 9:67-10:29, 11:11-25, 11:35-52, 11:61-12:4, 12:14-28, 12:37-57, 13:1-8, 17:3-18:18, 19:56-20:64, 22:18-23:4, 24:15-26:14, 27:9-67, 28:3-60, 28:67-31:5, 31:56-32:26, 34:16-46, 35:43-36:12, 39:11-31, 49:1-42, 49:43-53.

52. The patent's preferred embodiment of a "force" is ultraviolet light that cleaves a photo-cleavable linker between the target-binding domain and identifier oligonucleotide. EX1001, 18:45-49, 21:12-15, 23:15-19, 32:31-35, 34:58-61, 36:23-26, 36:67-37:2, 39:26-31, 76:26-28, 76:62-65, 77:17-19, 77:36-39, 80:18-20.

53. **"Region of interest" or "ROI"**. A "region of interest" or "ROI" is a "specific location within a sample." EX1001, 46:51-54. Each ROI has a predetermined spatial barcode sequence assigned to it, allowing identifier oligonucleotides collected from the same ROI to be identified by their shared spatial barcode (*i.e.*, a "nucleic acid sequence which identifies the specific location of the tissue sample from which the identifier oligonucleotide was released"). EX1001, 17:26-32, 18:31-40, 20:65-21:7, 32:11-21, 46:51-54. The collected identifier oligonucleotides are subsequently tagged with a predetermined spatial barcode for their corresponding ROI. EX1001, 17:26-32, 18:31-40, 20:65-21:7, 32:11-21, 46:51-54. Thus, identifier oligonucleotides collected from the same ROI are identified by their shared spatial barcode. EX1001, 17:26-32, 18:31-40, 20:65-21:7, 32:11-21, 46:51-54.

54. **"Nucleic acid probes"**. In addition to the cleavable "probes" that bind to the target analyte, the patent also discloses a different class of oligonucleotides: "nucleic acid probes." *See, e.g.*, EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:47, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38. These nucleic acid probes are oligonucleotides that are used in the methods that include a ligation step (ligation-based methods), such as the method illustrated in Figure 18 (discussed below). *See, e.g.*, EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57,

23:65-24:47, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38, FIGs. 16,18. By using the "probes" and "nucleic acid probes" in different steps of the method and naming them differently, the patent reinforces the two as distinct entities:

> The present disclosure provides a method for spatially detecting at least one target analyte in at least one cell from a tissue sample comprising: (1) contacting at least one target analyte in at least one cell in a tissue sample with at least one *probe* comprising a *target binding domain* and an identifier oligonucleotide, wherein the identifier oligonucleotide comprises a unique nucleic acid sequence which identifies the target analyte bound to the target binding domain; (2) providing a force to a location of the tissue sample sufficient to release the identifier oligonucleotide; (3) collecting the released identifier oligonucleotide; (4) hybridizing to the released identifier oligonucleotide *a first nucleic acid probe and a second nucleic acid probe*, wherein the first nucleic acid probe comprises a nucleic acid complementary to a portion of the identifier oligonucleotide . . . and wherein the second nucleic acid probe comprises a nucleic acid complementary to a portion of the identifier oligonucleotide . . . thereby spatially detecting the at least one target analyte in the at least one cell in a tissue sample.

EX1001, 5:44-6:8 (emphasis added).

55. Indeed, the patent's methods use the "probes" and the first and second "nucleic acid probes" differently in every embodiment and disclosure of its ligation-based methods. *See, e.g.*, EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:47, 25:40-50, 25:58-26:14, 26:54-27:6, 27:24-47, 28:17-40, 29:14-38, FIGs. 16, 17, 18.

56. The "probes" are *primary* probes that contain a target-binding domain and hybridize to a target analyte in the sample (an "on-analyte" hybridization). EX1001, 1:46-51, 2:3-9, 2:28-34, 3:26-32, 3:50-56, 4:36-42, 5:5-11, 5:46-51, 6:11-17, 6:46-52, 7:12-18, 8:20-25, 8:55-61, 9:26-32, 9:61-67, 11:3-11, 11:28-35, 11:55-61, 12:7-13, 12:31-36, 12:60-13:1, 16:52-67, 19:36-53, 21:67-22:15, 23:66-24:12, 27:9-23, 28:3-16, 28:67-29:13, 31:33-53, 33:65-34:13, 35:24-40.

57. In contrast, the "nucleic acid probes" are pairs of *secondary* probes that do not contain a target-binding domain and are never bound to the target analyte. EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:47, 25:40-50, 25:58-26:14, 26:54-27:6, 27:24-47, 28:17-40, 29:14-38, FIGs. 16, 18, 18. Instead, nucleic acid probes are oligonucleotides that hybridize as pairs to the identifier oligonucleotides that are cleaved from the "probes" (not in the presence of the target analyte) via a sequence complementary to the identifier oligonucleotides. EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44,

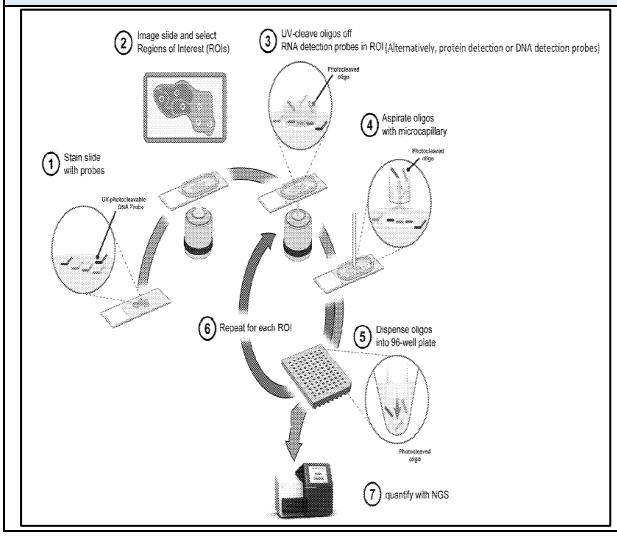
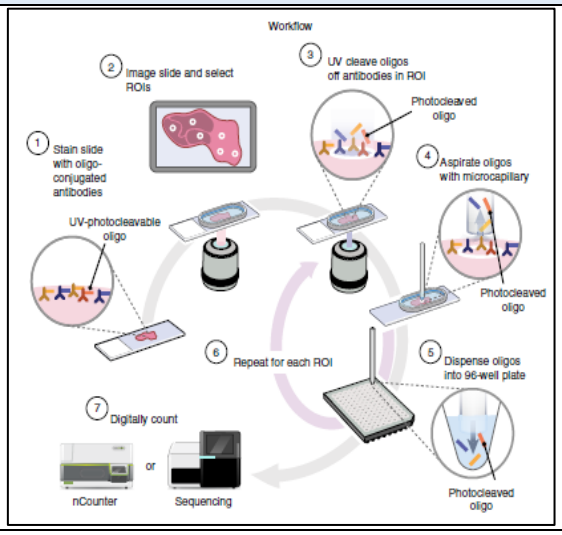
8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:47, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38.

58. Table 1 below summarizes the oligonucleotide entities "probes" and "nucleic acid probes" as disclosed in the specification of the 689 patent (excluding the claims).

TABLE 1: Oligonucleotides disclosed in the 689 patent	
Oligonucleotide	**Disclosure in the specification (excluding the claims)**
"Probes"	Primary probes having a target-binding domain that binds to the target analytes in a tissue sample (on-analyte binding) "The present disclosure provides *probes* comprising a *target binding domain* and an *identifier oligonucleotide*." EX1001, 37:14-16 (emphasis added).
"Nucleic acid probes"	Secondary probes: pairs of oligonucleotides that hybridize to the identifier oligonucleotide cleaved off from the "probes" (off-analyte binding) "[H]ybridizing to the released identifier oligonucleotide *a first nucleic acid probe and a second nucleic acid probe*, wherein the first nucleic acid probe comprises a nucleic acid complementary to a portion of the identifier oligonucleotide . . . and wherein the second nucleic acid probe comprises a nucleic acid complementary to a portion of the identifier oligonucleotide…." EX1001, 5:44-6:8 (emphasis added).

ii. Workflow of the 689 patent's methods

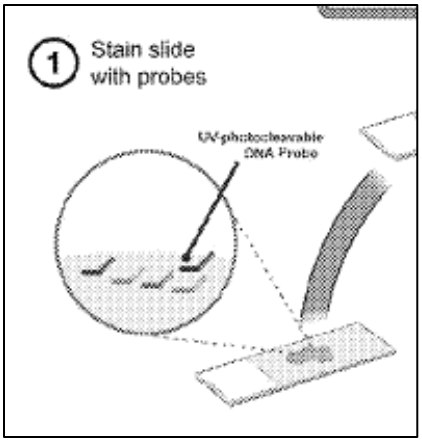
59. The 689 patent's methods, including the ligation-based methods, use cleavable "probes" that hybridize to the target analytes (described above) and involve steps that occur on the target analyte ("on-analyte" steps) and off (or away from) the target analyte ("off-analyte" steps). The overall workflow of the 689 patent's methods is nearly identical to that of NanoString's GeoMx workflow, as illustrated in the Figures reproduced below.

689 Patent	GeoMx
	

EX1001, FIG. 20; EX1013, FIG. 1A.

(a) On-analyte steps

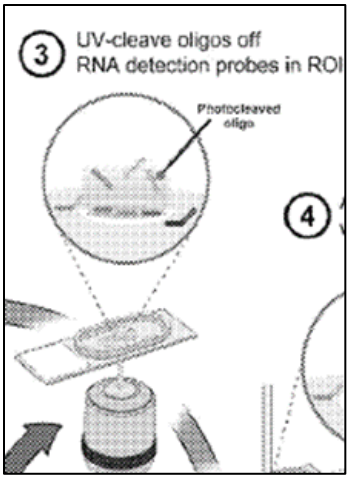
60. The method begins with adding the cleavable primary probes ("probes") to a slide containing a biological sample (*e.g.*, a tissue sample). The 689 patent refers to this as "stain[ing]" the slide with "UV-photocleavable DNA probe" in its overview Figure 20, Step 1:



EX1001, FIG. 20, 36:63-65. This hybridization step is an on-analyte step, which takes place *on* the target analyte in the tissue sample (*in situ*). EX1001, FIG. 20, 36:63-65.

61. After hybridization of DNA probes (i.e., primary probes), a region of interest (ROI) within the sample is selected (FIG. 20, Step (2)), and a "force" (*e.g.*, UV light) is applied to the ROI to selectively cleave oligonucleotide fragments off the primary probes in the ROIs (FIG. 20, Step (3)). EX1001, FIGs. 16, 18, 20, 18:45-49, 21:12-15, 23:15-19, 32:31-35, 34:58-61, 36:23-26, 36:67-37:11, 39:26-31, 76:26-28, 76:62-65, 77:17-19, 77:36-39, 80:18-20.

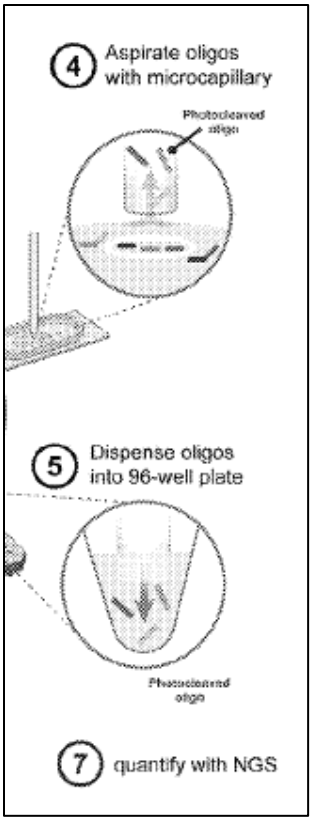
62. The patent's preferred embodiment of a "force" is UV light that cleaves a photo-cleavable linker between the target binding domain and identifier oligonucleotide, similar to the GeoMx workflow. EX1001, 18:45-49, 21:12-15, 23:15-19, 32:31-35, 34:58-61, 36:23-26, 36:67-37:2, 39:26-31, 76:26-28, 76:62-65, 77:17-19, 77:36-39, 80:18-20; EX1013, FIG. 1A. The 689 patent refers to this as "UV-cleave oligos off" in its overview Figure 20, Step 3:



EX1001, FIG. 20.

(b) Off-analyte steps

The released identifier oligonucleotides are then collected from the selected ROI (step 4, "Aspirate oligos") and analyzed (steps 5, "Dispense oligos into 96-well plate" and 7, "quantify with NGS"):

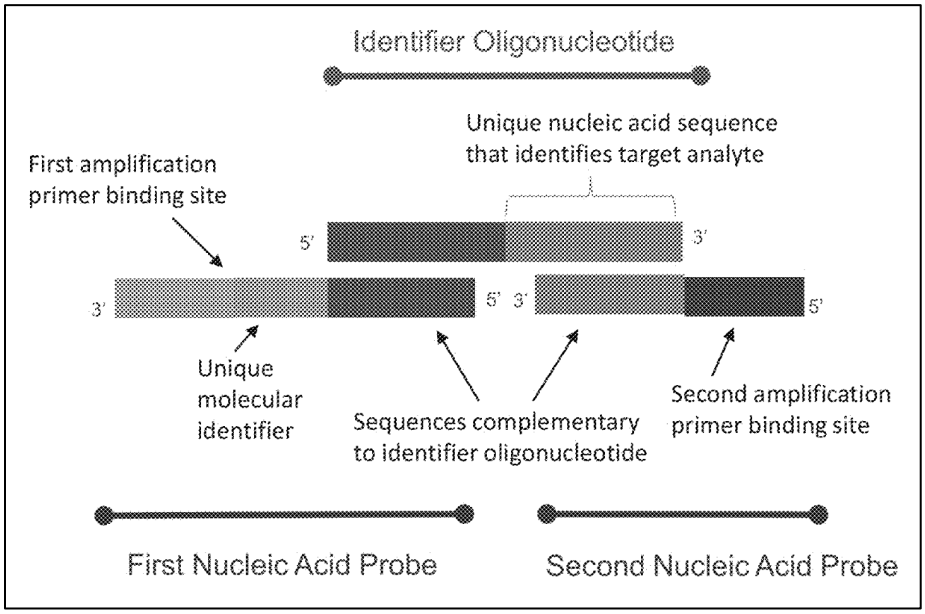


EX1001, FIG. 20. These collection and analysis steps are off-analyte steps, taking place *off* the target analyte. The 689 patent's methods do not use or discuss spatially barcoded arrays for capturing or processing the target analytes or their proxies. *See, e.g.,* EX1001, FIGs. 18, 20.

63. The patent's ligation-based methods follow the above-described workflow and include a ligation step during the processing of the released identifier oligonucleotide. *See, e.g.*, EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:47, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38 , FIGs. 16,18.

64. As discussed above, the ligation-based methods also use the "nucleic acid probes" in addition to the "probes." *See, e.g.*, EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:47, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38.

65. Figure 18 (reproduced below) exemplifies a ligation-based method that uses the nucleic acid probe pairs detailed in Figure 17 (reproduced below).



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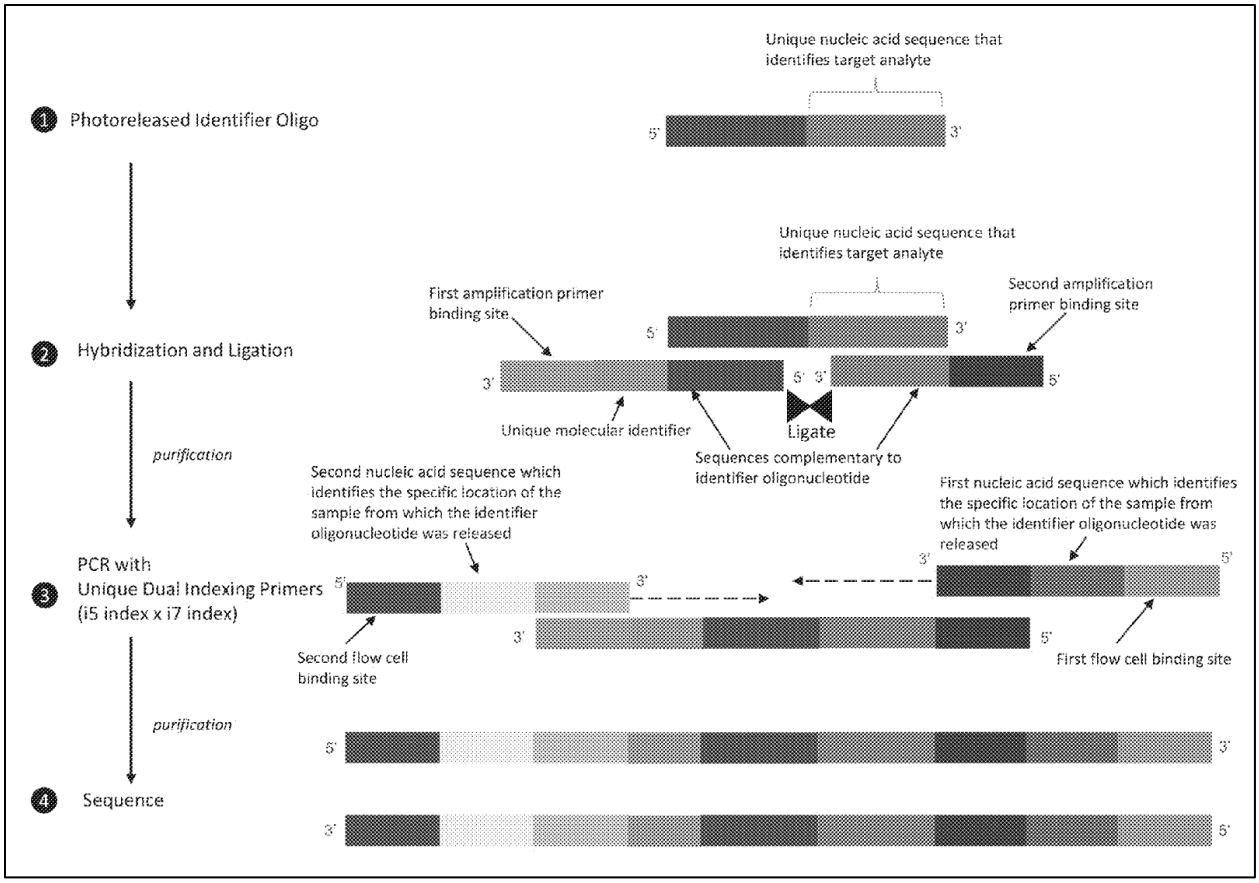
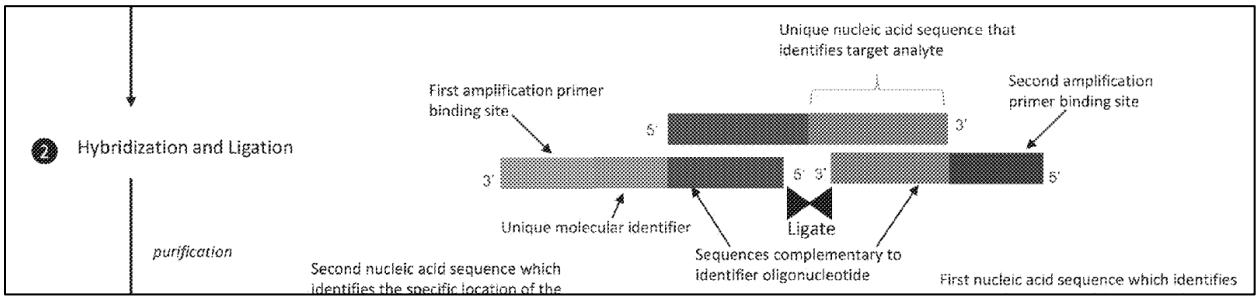


EX1001, FIGs. 17, 18, 28:1-60.

66. The ligation-based method begins with an "on-analyte" step wherein, "at least one target analyte in a sample is contacted with at least one *probe* of the present disclosure," comprising a "target binding domain" and an "identifier oligonucleotide" (*i.e.*, primary probe). EX1001, 28:3-16 (emphasis added).

67. Following contacting, "a force is then provided to a location of the sample sufficient to release the identifier oligonucleotide" and the "identifier oligonucleotide is collected following release" (depicted as "[p]hotoreleased identifier Oligo" in Figure 18). EX1001, 28:17-21, FIG. 18.

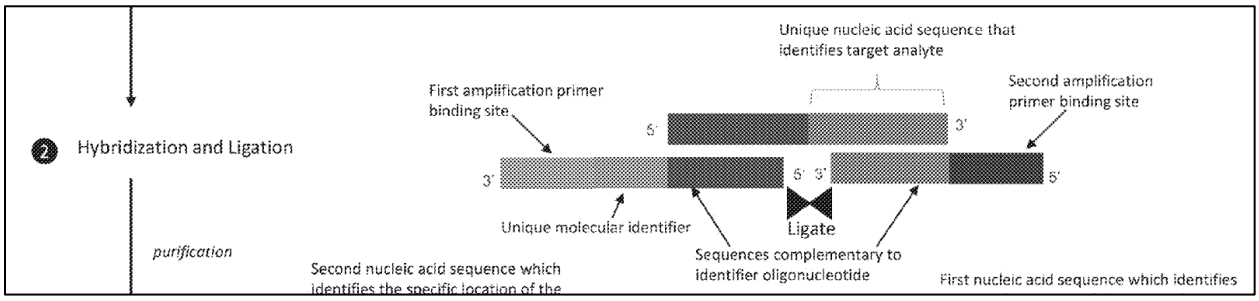
68. Figure 18 illustrates the off-analyte steps that take place after the released identifier oligonucleotide is collected for further processing. "[T]he released identifier oligonucleotide is [] hybridized to a *first nucleic acid probe* and a *second nucleic acid probe*." EX1001, 28:22-24 (emphasis added), FIG. 18. Both nucleic acid probes of the pair comprise "a nucleic acid sequence complementary to a portion of the identifier oligonucleotide" and "hybridize to the identifier oligonucleotide." EX1001, 28:24-38. The nucleic acid probes also comprise an "amplification primer binding site." EX1001, 28:24-48, FIG. 18. The pairs of nucleic acid probes may hybridize to the identifier oligonucleotide such that they are "adjacent and are not overlapping" or "not adjacent and are not overlapping." EX1001, 25:51-57, 28:22-38, FIGs. 17, 18. This hybridization step is depicted in Step 2 of Figure 18 (reproduced below) where the nucleic acid probe pairs are hybridized adjacent and not overlapping on the released identifier oligonucleotide.



EX1001, FIG. 18.

69. Following hybridization, "the first and second probe are ligated together." EX1001, 28:38-40, FIG. 18. Ligation in the 689 patent's methods

includes (i) ligating nucleic acid probe pairs that "are adjacent and are not overlapping" using a "nick repair reaction"; and (ii) ligating nucleic acid probe pairs that "are not adjacent and are not overlapping" using a "gap extension reaction and a nick repair reaction." EX1001, 25:58-26:6, 28:34-40. This ligation step is depicted in Step 2 of Figure 18 (copied below).



EX1001, FIG. 18.

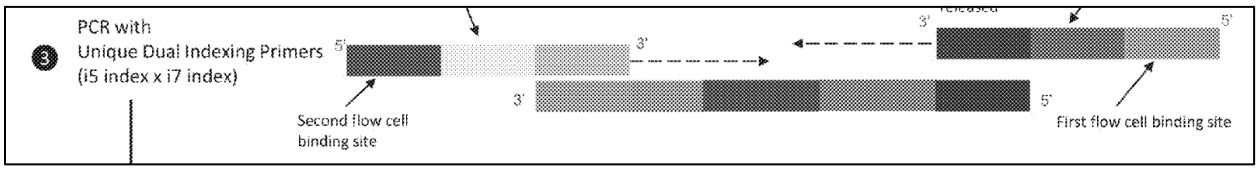
70. In every embodiment and disclosure, the "nucleic acid probes" that hybridized to the released and collected identifier oligonucleotides are ligated in an off-analyte reaction. EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:36, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38, 30:49-64, FIGs. 16, 18.

71. In other words, the patent's ligation-based methods do not include on-analyte ligation of probes that had hybridized to target analytes in the tissue sample. Instead, the patent's methods only include off-analyte ligation, on the identifier oligonucleotide after it is released from the tissue sample. EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57,

234:65-245:36, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38, 30:49-64, FIGs. 16, 18.

72. Following ligation, the ligation product is tagged with a spatial barcode and processed to obtain the spatial information of the target analyte. EX1001, 28:41-60, FIG. 18. Specifically, "the ligation product is amplified via PCR using amplification primers that hybridize to the first and second amplification primer binding sites." EX1001, 28:41-44. The amplification primers comprise a "nucleic acid sequence which identifies the specific location of the sample from which the identifier oligonucleotide was released," *i.e.*, the spatial barcode. EX1001, 28:51-57.

73. The ligation products corresponding to the identifier oligonucleotides collected from the same location of the tissue sample are tagged with the same spatial barcode, and those collected from different locations of the tissue sample are tagged with different spatial barcodes. EX1001, 28:41-60. This amplification step is depicted in Step 3 of Figure 18 (copied below).



EX1001, FIG. 18.

74. Next, the PCR products generated from the amplification are "sequenced to identify the released oligonucleotide, thereby spatially detecting the at least one target analyte in the sample." EX1001, 28:57-60.

75. Thus, the patent's ligation-based method includes the following general steps:

(1) Contacting a target analyte with a probe comprising a target binding domain and an identifier oligonucleotide;

(2) Providing a force to release the identifier oligonucleotide;

(3) Collecting the released oligonucleotide;

(4) Hybridizing a first and second nucleic acid probe to the released identifier oligonucleotide;

(5) Ligating the first and second nucleic acid probes together to create a ligation product;

(6) Amplifying the ligation product using primers that containing a spatial barcode; and

(7) Sequencing the amplified ligation product that contains the spatial barcode to identify the released identifier oligonucleotide and determining the location from which it was derived.

EX1001, 5:44-6:8, 6:9-43, 6:44-7:9, 7:10-44, 8:18-52, 8:53-9:23, 9:24-58, 9:59-10:29, 12:29-57, 23:65-24:36, 25:58-26:14, 27:24-47, 28:17-40, 29:14-38, 30:49-64, FIGs. 16, 18.

b. The 689 patent claims

76. I understand that the 689 patent issued with 30 claims. EX1001, 145:51-150:17. I understand that claims 1 and 16 are the independent claims, and claims 2-15 and 17-30 depend from claim 1 and claim 16, respectively. I understand that the dependent claims incorporate the elements of their respective independent claims.

77. In my opinion, the 689 patent claims are drawn to spatial detection methods that are different from the methods described in the patent specification. For example, the patent claims require (1) *on-analyte* ligation of the *primary* probe pairs while the probes are bound to the target analytes in the tissue sample, and (2) releasing and collecting the *whole* ligated probes formed after the on-analyte ligation. EX1001, 145:51-150:17. In contrast, the patent specification only describes (1) *off-analyte* ligation of *secondary* first and second nucleic acid probe pairs that hybridized to the collected identifier oligonucleotides that were cleaved off from the probes that bound to the target analytes in the tissue sample; and (2) releasing and collecting the identifier oligonucleotide portion *cleaved off from the probes* that bound to the target analytes in the tissue sample. *See above* Section VI.a.

78. As shown in Table 2 below, independent claims 1 and 16 both recite a "method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample," and require the contacting, collecting, extension, and sequencing steps. EX1001, 145:51-147:11, 147:54-148:47. Claim 16 differs from claim 1 in that claim 16 requires that the tissue sample is contacted with *probe pairs* (a "first plurality of nucleic acid probes" and a "second plurality of nucleic acid probes"). Claim 16 also specifies a ligation step in which the probe pairs bound to the same target analyte molecule are ligated to form "ligated probes," which are subsequently collected.

TABLE 2	
Claim 1	**Claim 16**
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[1],	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

[1] I understand that "wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte" reflects the correction made to claim language after the 689 patent issued.

TABLE 2	
Claim 1	**Claim 16**
wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte;	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;
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 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;
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) *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;
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	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

TABLE 2	
Claim 1	**Claim 16**
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;	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;
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) 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
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.	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, 145:51-147:11, 147:54-148:47 (emphasis added).

79. The dependent claims 2-15 and 17-30 further specify various elements of the independent claims, *e.g.*, the tissue sample type, sequencing method, and library amplification steps. EX1001, 147:12-53, 148:48-150:17.

c. The 689 patent prosecution history

80. I have reviewed the 689 patent's prosecution history (EX1004). NanoString filed the 707 application (that led to the 689 patent) on September 16, 2021. EX1004. I understand from the file history that after initially rejecting the claims, the patent examiner eventually allowed the claims—which issued as the 689 patent claims. I understand that the examiner did not raise any rejections against the claims based on prior art. EX1004.

VII. PRIOR ART DISCLOSES THE CLAIMED METHODS

81. The 689 patent's claims relate to spatial analysis technology in life sciences for determining the locations of target analytes (*e.g.*, DNA or RNA) in biological samples, such as tissue samples.

82. As early as 2011, Stahl *et al*. developed a pioneering spatially barcoded array paradigm for determining the locations of target mRNAs in a biological sample. The scientists disclosed this foundational technology in a patent application filed in 2011 (WO 2012/140224; EX1017) and subsequently published it in a 2016 article in Stahl *et al.*, "Visualization and analysis of gene expression in tissue selections by spatial transcriptomics," *Science* 353(6294):78-82 (July 1, 2016), and associated supplementary material ("Stahl 2016"; EX1016). This technology was also applied in the spatial detection products by Spatial Transcriptomics, Inc. (ST). Subsequently, numerous publications, such as WO

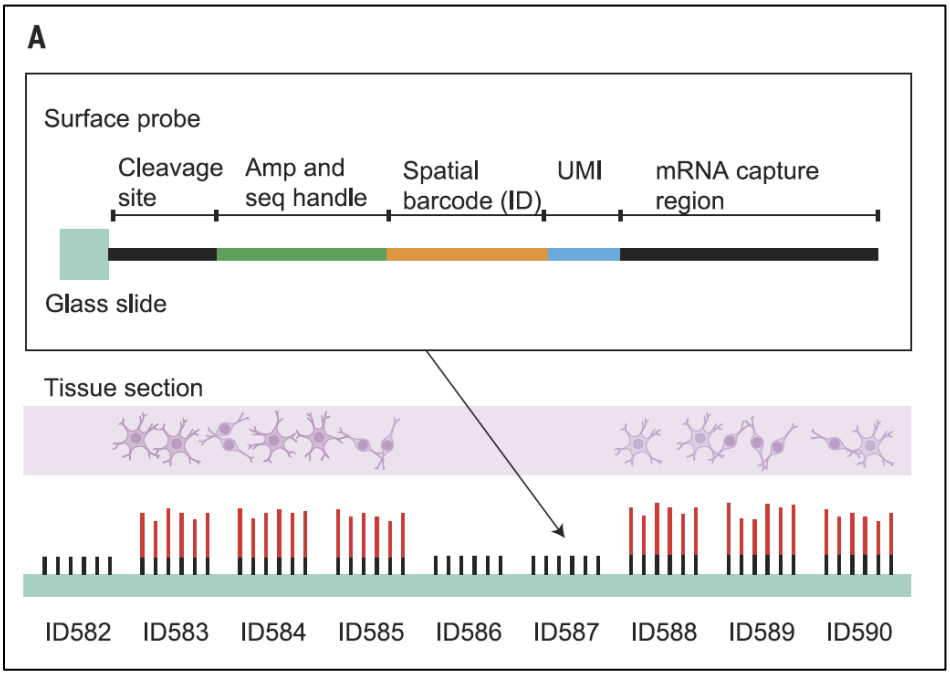
2017/019456 ("So-456") and 10x's WO 2021/133849 ("Chell"), combined Stahl 2016's spatially barcoded array paradigm with ligation chemistries for detecting target analytes in biological samples. EX1005; EX1006; EX1025; EX1026; EX1027; EX1028.

a. Stahl 2016 disclosed methods using spatially barcoded arrays before February 12, 2018

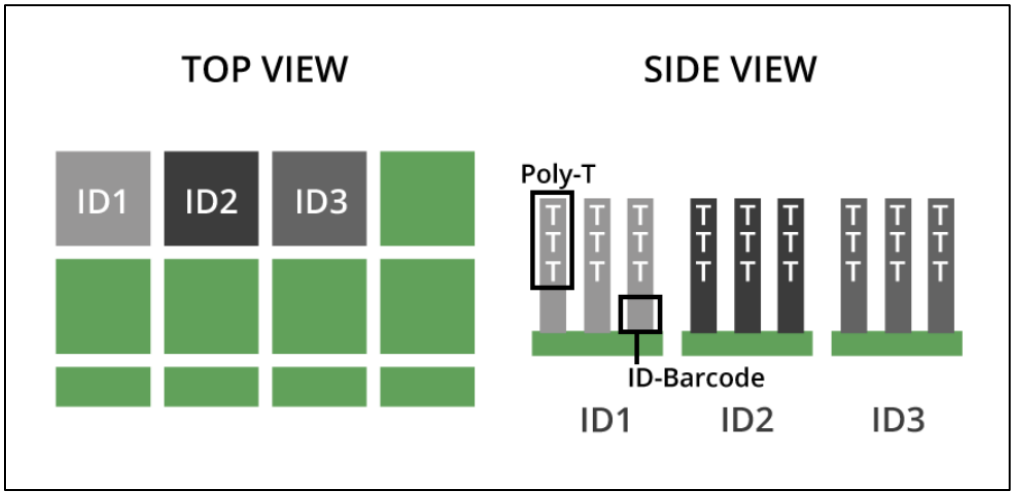
83. Stahl 2016 details a new methodology called "spatial transcriptomics" that allowed for "visualization and quantitative analysis" of the entire transcriptome with "spatial resolution" in individual tissue sections. EX1016, Abstract. A key aspect of this method are unique DNA sequences called "positional barcodes" or "spatial barcodes" that specifically identify the location of a target mRNA molecule in the tissue section. EX1016, Abstract (noting "positioning histological [tissue] sections on arrayed reverse transcription primers with unique positional barcodes"). These spatial barcodes are similar in concept to latitude and longitude, which uniquely identifying a specific location on the map. In Stahl 2016, the spatial barcodes uniquely identify a location on the array that was in contact with a tissue sample.

84. Stahl 2016's method utilizes oligonucleotides (called "surface" or "capture" probes) containing these spatial barcodes, which are immobilized on a solid surface (*e.g.*, a glass slide) in a two-dimensional array of "spots" or "features." EX1016, 78 (middle col. and right col.), FIG. 2A. I have reproduced

Figure 2A from Stahl 2016 showing an array (green glass slide) containing immobilized array probes ("surface probe"; black vertical lines) and a diagram from ST's website showing a top- and a side- view of the array.



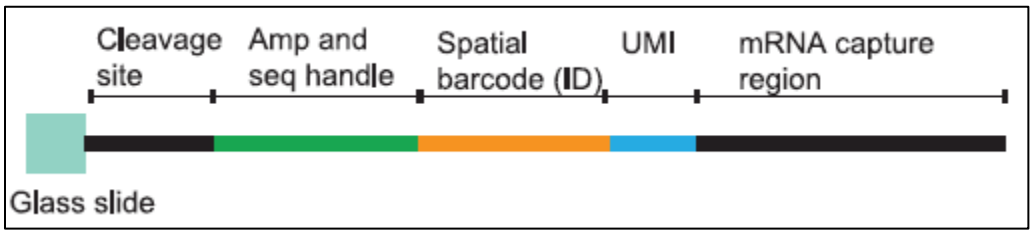
EX1016, FIG. 2A.



EX1018, 3.

85. As depicted above, each spot (identified by a different "ID") on the solid surface contains a cluster of array probes (vertical rectangular bars in the ST diagram; vertical black lines in Stahl 2016's figure) comprising a unique "spatial barcode" ("ID-Barcode"; "ID") having a nucleic acid sequence specific to that spot. EX1016, Abstract, 78, FIG. 2A. All the array probes in one spot on the array share the same spatial barcode sequence, which is different from the spatial barcode sequence in the array probes in other spots. EX1016, Abstract, 78, FIG. 2A. The spatial barcodes act as "tags" for locations of their corresponding array probes on the solid surface, which were contacted to the locations on the tissue sample and allow one to differentiate biological molecules based on their spatial location on the array surface and therefore in the sample. EX1016, Abstract, 78, FIG. 2A.

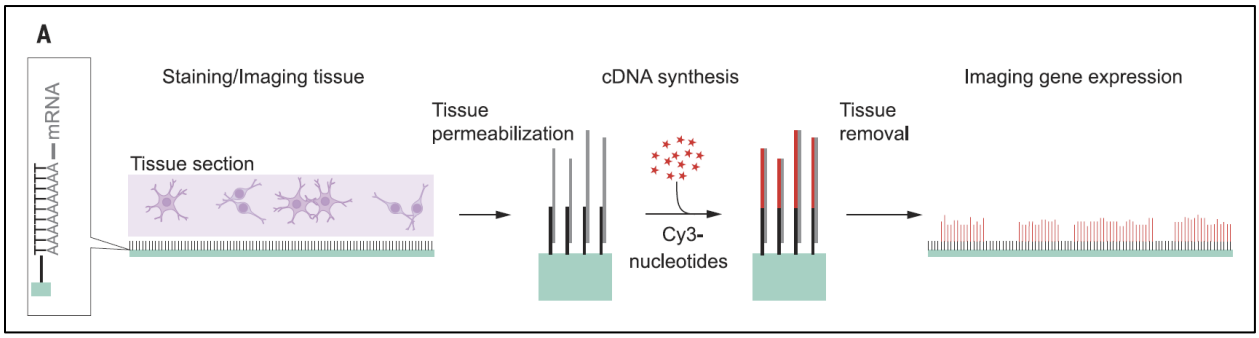
86. In addition to the spatial barcode, each array probe contains a cleavage site, an amplification and sequence handle, a spatial barcode, a unique molecular identifier ("UMI"), and an mRNA capture region. EX1016, FIG. 2A. I have reproduced below a figure from Stahl 2016 depicting an array probe.



EX1016, FIG. 2A.

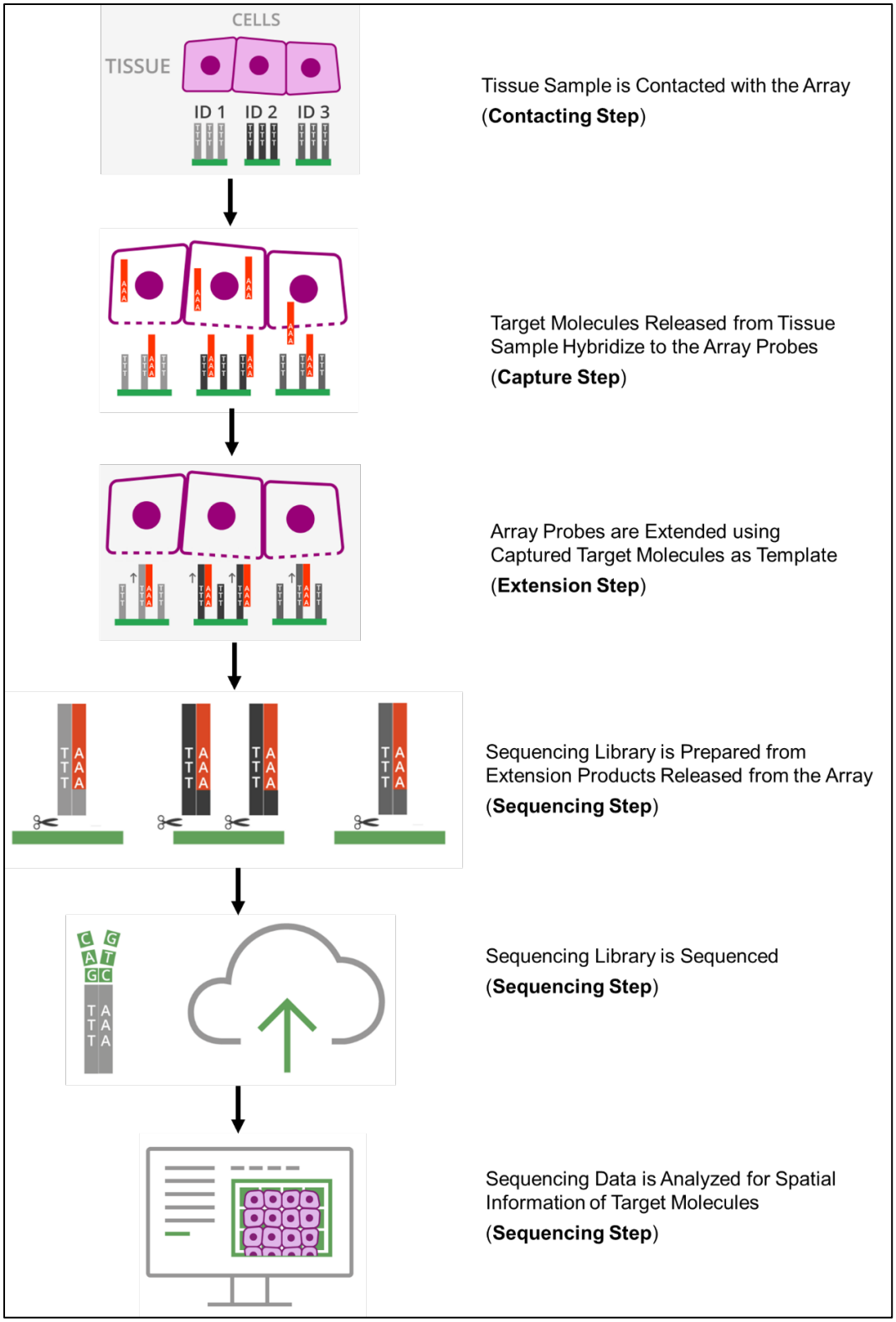
87. The "mRNA capture region" contains a nucleic acid sequence that is complementary to, and hybridizes to, a region of the target mRNA in the tissue sample. For example, an mRNA capture region may contain a poly-T sequence that binds to its complementary poly-A sequence in the target mRNA. EX1016, FIG. 2A, 80. The UMI ("Unique Molecular Identifier") is a random nucleic acid sequence that is unique to each array probe and is used to identify post-amplification molecules that are copies of the same original molecule. EX1016, FIG. 2A; EX1019, FIG. 1A, 72. UMIs were commonly used in sequencing workflows before 2018 with statistical approaches to provide accurate counts of target molecules. EX1019, FIG. 1A, 72.

88. The above-described barcoded arrays are used in the spatial analysis workflow that ultimately detects the signal by imaging (as shown in Stahl 2016's Figure 1A reproduced below) or sequencing (as shown in the figure reproduced below from ST's website, with my annotated text on the right).



EX1016, FIG. 1A.

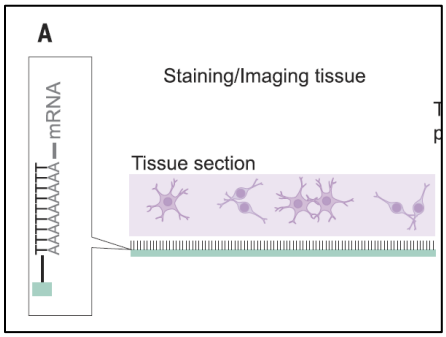
***



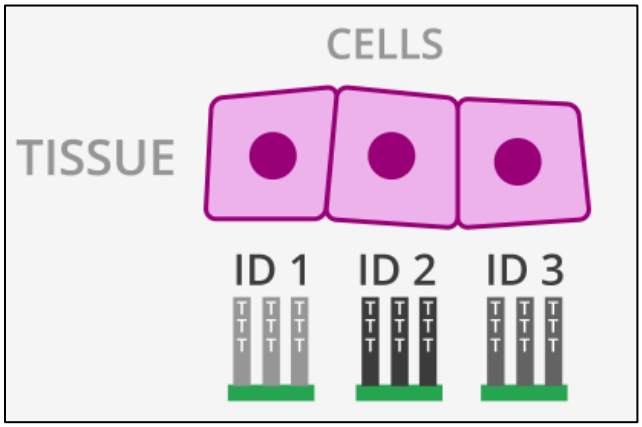
EX1018, 4-9 (annotated).

89. The workflow depicted above includes (1) contacting a tissue section with a barcoded array (contacting), (2) releasing the mRNAs from the tissue section and capturing the released mRNAs via hybridization to the barcoded array probes (release and capture), (3) extending the array probes using the hybridized mRNAs as a template (probe extension), and (4) analyzing the extended probes (imaging or sequencing).

90. **<u>CONTACTING</u>:** A tissue section is placed on the surface of glass slides containing immobilized, barcoded array probes. *See also* EX1016, 78 (middle col. and right col.), FIG. 1A. The figures below show the contacting step.

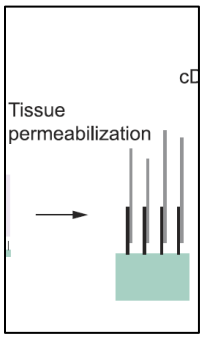


EX1016, FIG. 1A.

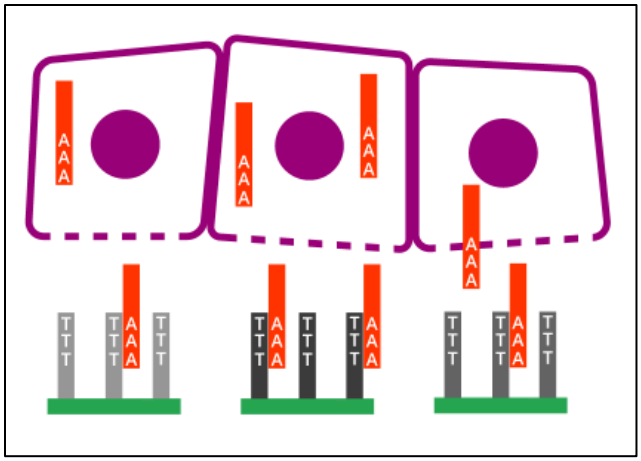


EX1018, 4.

91. **<u>CAPTURE</u>:** The overlaid tissue section is permeabilized, which creates holes in cell membranes and mRNAs diffuse away from the tissue onto the array. EX1016, 78 (middle col.), FIGs. 1A, 2A, Suppl. Materials, 2. The mRNAs are then captured via hybridization to the array probes immobilized in spots on the array surface in proximity to where the mRNAs were found in the tissue. EX1016, 78 (middle col.). This localizes the mRNAs from the tissue sample in the spatial position they were found in the cells. The figures below show the target mRNA diffusing out of the cell and hybridizing to the array probes on the surface of the glass slide in the capture step.

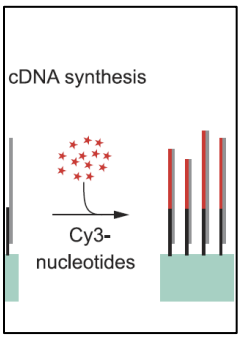


EX1016, FIG. 1A.

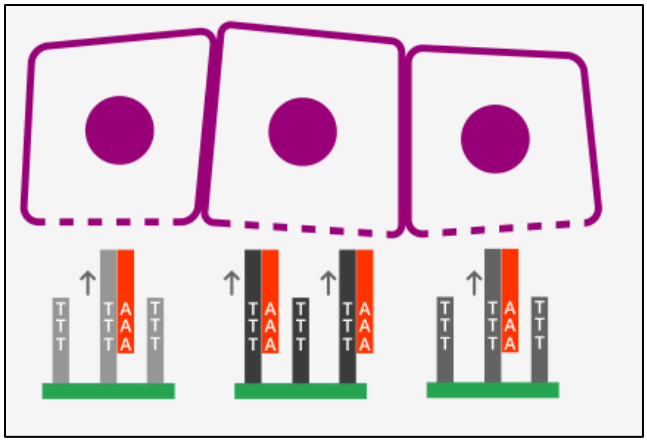


EX1018, 5.

92. **EXTENSION**: After capturing the mRNAs, the barcoded array probes are extended using the array probes as primers and the mRNAs hybridized to the array probes as templates. To achieve this, reverse-transcription reagents are added to the tissue to generate complementary DNAs (cDNAs) that are complementary to the target mRNAs hybridized to the array probes and contain the spatial barcode sequence of the array probes. EX1016, 78 (middle col.). The figures below show the extension step.



EX1016, FIG. 1A.



EX1018, 6.

93. **<u>ANALYSIS</u>:** After extension, the tissue section is enzymatically digested, leaving the extended DNA molecules hybridized to the array probes on the slide. EX1016, 78 (right col.), FIGs. 1A, 2A, Suppl. Materials, 2. For analysis by sequencing, the extended DNA molecules are processed to generate sequencing libraries that are read out using next-generation sequencing devices. EX1016, 78 (middle col. and right col.), FIGs. 1A, 2A, Suppl. Materials, 2.

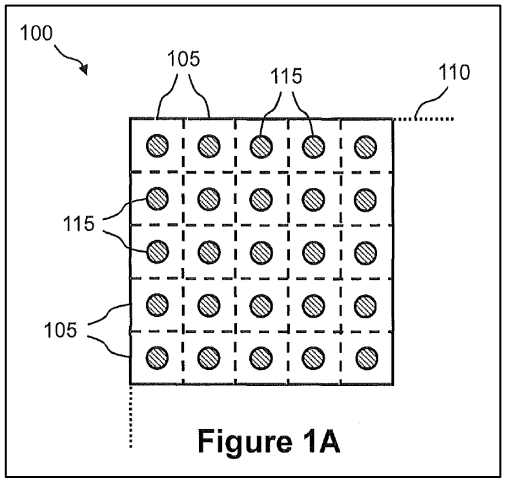
94. The data generated from sequencing the extended DNA molecules are further sorted and aligned to the corresponding barcoded array spots on the array based on the spatial barcodes to determine the location-specific identities of each of the target molecules of the tissue sample. EX1016, 78 (middle col. and right col.), FIGs. 1A, 2A, Suppl. Materials, 2. The data generated from analyzing the extended DNA molecule captures both the genetic information of the target molecule, as well as the spatial location of the target molecule. EX1016, 78 (middle col. and right col.), FIGs. 1A, 2A, Suppl. Materials, 2.

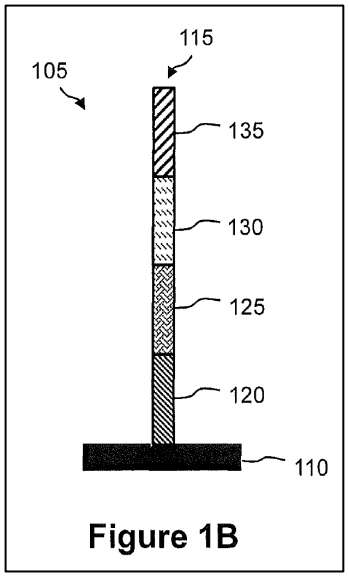
b. WO 2017/019456 (So-456) disclosed methods using spatially barcoded arrays and probe ligation by 2016

95. I understand that in July 2016, Illumina, Inc., filed a PCT application entitled "Spatial Mapping Of Nucleic Acid Sequence Information," which published as WO 2017/019456 ("So-456") on February 2, 2017. EX1005, Face Page. I understand that So-456 is prior art to the 689 patent because it was filed and published before February 12, 2018.

96. So-456 teaches methods for spatial detection of nucleic acids in a tissue sample that apply Stahl 2016's barcoded array and workflow, along with a probe ligation strategy. EX1005, ¶¶[0018]-[0019], [00156], [00177]-[00183], FIGs. 1, 2, 8, 9. Specifically, So-456's methods comprise hybridizing probe pairs to target nucleic acids, ligating the probe pairs hybridized on adjacent sites of the same nucleic acid molecule, amplifying and capturing the ligation products via the barcoded capture probes immobilized on the array, extending the capture probes using the captured ligation products as templates and extending the ligation products using the capture probes as templates (during sequencing library preparation), sequencing the extension products, and analyzing the sequencing data to determine the location of the target nucleic acids in the tissue sample. EX1005, ¶¶[0018]-[0019], [00141], [00156], [00177]-[00183], FIGs. 1, 2, 8, 9.

97. So-456 methods use a "capture array" (100) that comprises an array of "capture sites" (105), depicted in Figures 1A and 1B (reproduced below). EX1005, ¶[00141].





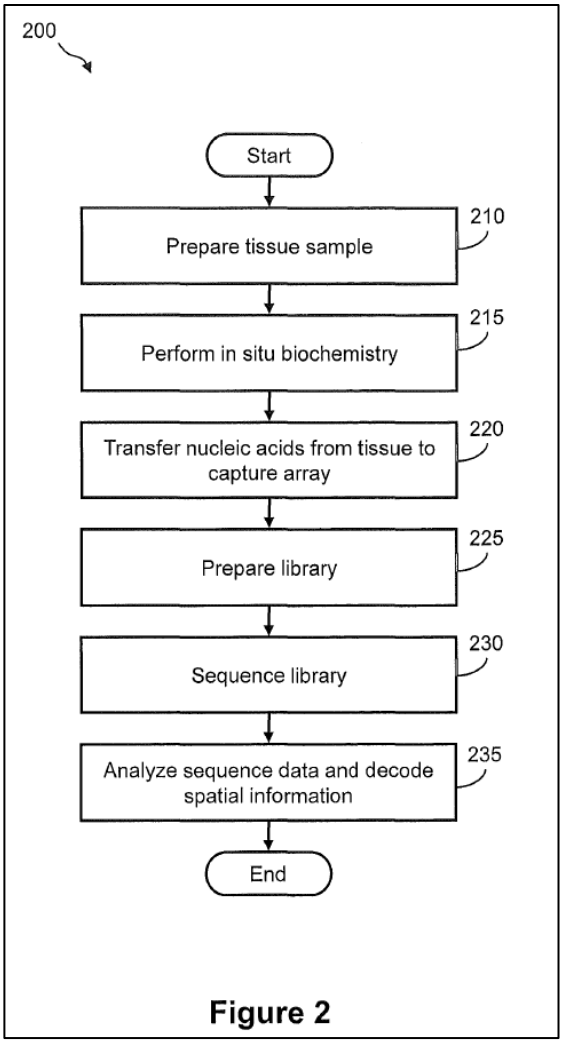
EX1005, FIGs. 1A, 1B.

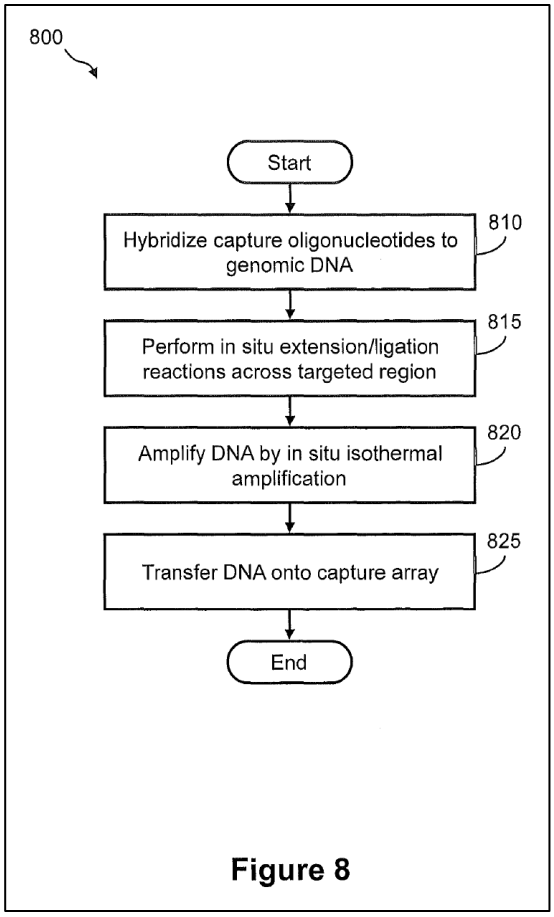
98. So-456's capture sites (105) comprise at least one "capture probe" (*i.e.,* array probe; 115) for capturing the nucleic acids present in a tissue sample, wherein the capture probe is immobilized on a solid support (110). EX1005, ¶[00141], FIG. 1B. So-456 explains that any number of capture probes (115) can be immobilized on the solid support at each capture site (105). EX1005, ¶[00141], FIG. 1B. According to So-456, the capture probes (115) comprise the following sequences: "a cleavable sequence in a cleavable region 120, an SBS primer sequence in a SBS primer binding site 125, a spatial address sequence in a spatial

address region 130, and a capture sequence in a capture region 135." EX1005, ¶[00141], FIG. 1B.

99. So-456 further teaches that the spatial address region (130) (*i.e.*, the spatial barcode) "corresponds to the position of [the] capture probe [] in [the] capture array," which in turn "can be correlated with a position in a tissue sample." EX1005, ¶[00141], FIG. 1B. The SBS primer region (125) is used for sequencing-by-synthesis (SBS) or amplification to generate a sequence library. EX1005, ¶[00141], FIG. 1B. The capture region (135) is used for capturing the nucleic acids released from the tissue sample, which can be a "universal (general) capture region," such as a poly-T oligonucleotide to capture total mRNA in a tissue sample, or a "gene-specific or target specific capture region" for capturing a specific nucleic acid in a tissue sample. EX1005, ¶¶[00142]-[00143].

100. The method depicted in So-456's Figure 8—which follows the overall workflow in So-456's Figure 2—exemplifies the ligation-based spatial detection methods disclosed in So-456. EX1005, ¶¶[0018], [00177]-[00181]. I have reproduced So-456 Figures 2 and 8 below.





EX1005, FIGs. 2, 8.

101. So-456's Figure 9 (reproduced below) further details the steps of the ligation-based method of Figure 8. EX1005, ¶¶[0019], [00182]-[00183].

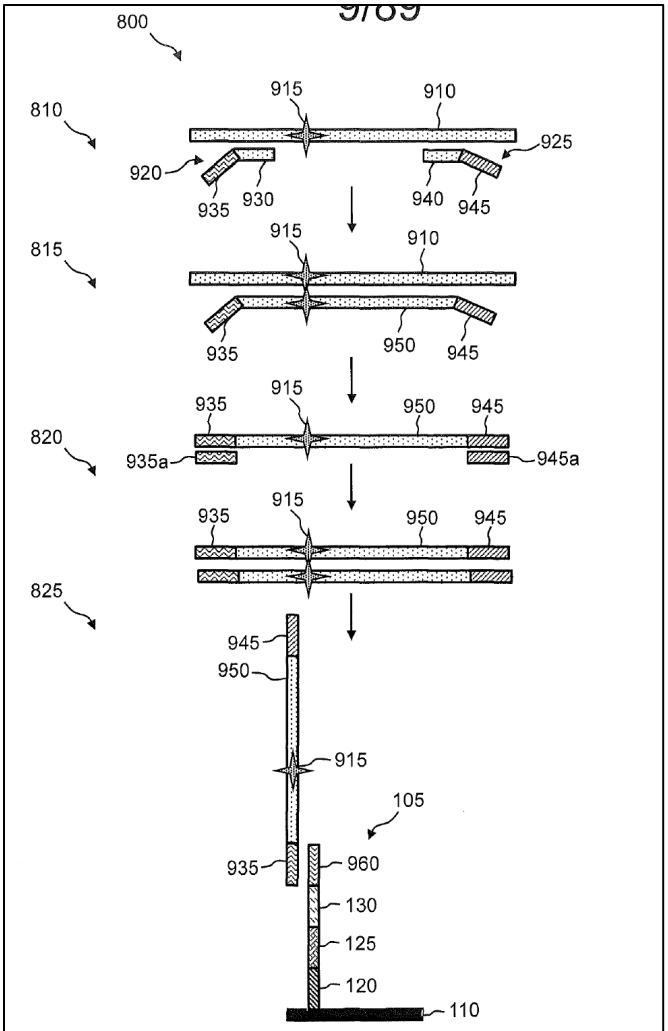


Figure 9

EX1005, FIG. 9.

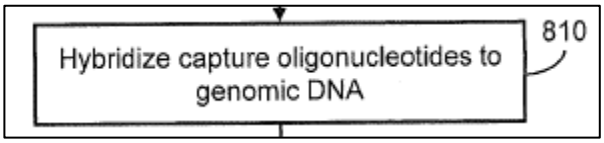
102. As shown above, the method (800) of Figures 8-9 uses two types of oligonucleotide probes: (1) *primary probe pairs* ("capture oligonucleotides" 920 and 925), which bind to the target genomic DNA (910) and are ligated to each other across a region of interest on the same target DNA molecule inside the tissue sample (*in situ*) to form ligation products ("DNA product" 950); and (2) *secondary*

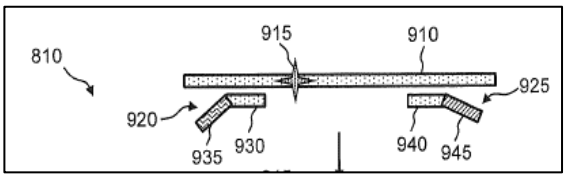
probes ("capture probes" 115) containing a spatial barcode ("spatial address" 130) and are immobilized on a glass slide (110). EX1005, ¶[00182].

103. So-456's capture oligonucleotides (920, 925) comprise a target-binding domain ("gene-specific regions" 930, 940) that hybridizes to a complementary site on a target genomic DNA molecule (910). EX1005, ¶¶[00141], [00182]. One capture oligonucleotide of the pair also comprises a "universal capture region" (935) that hybridizes to the capture region (960) of the capture probes, and the other capture oligonucleotide of the pair also comprises an SBS primer region (945) that can be used for sequencing-by-synthesis (SBS) or amplification to generate a sequence library. EX1005, ¶¶[00141], [00182].

104. The So-456's method of Figures 8-9 (following the overall workflow of Figure 2) includes the following steps:

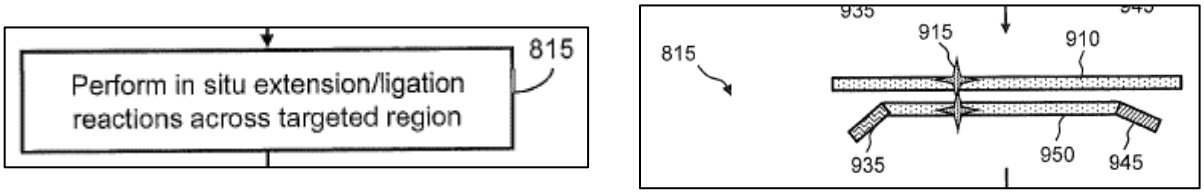
(1) Hybridizing the pairs of "capture oligonucleotides" (920, 925) via their "gene-specific regions" (930, 940) to their complementary sites on the target genomic DNA (910) in the tissue sample. EX1005, ¶[00182], [00178]. This is shown in Step 810 of Figures 8-9, reproduced below.





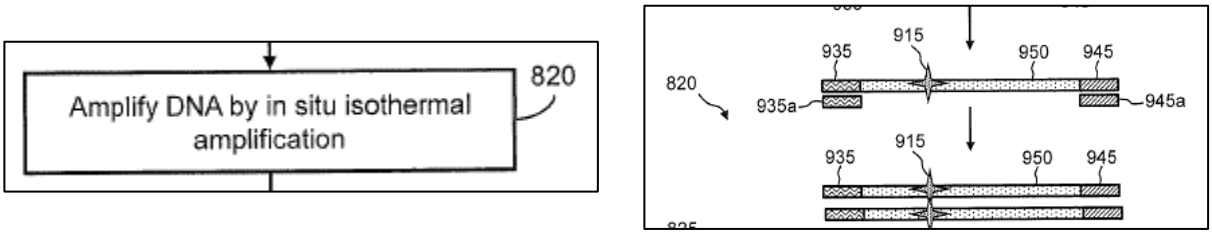
EX1005, FIGs. 8, 9.

(2) Extending/ligating *in situ* the pairs of capture oligonucleotides bound to the same target DNA molecule across the region of interest to form ligation products ("DNA molecules" 950). EX1005, ¶[00182], [00179]. This is shown in Step 815 of Figures 8-9, reproduced below.



EX1005, FIGs. 8, 9.

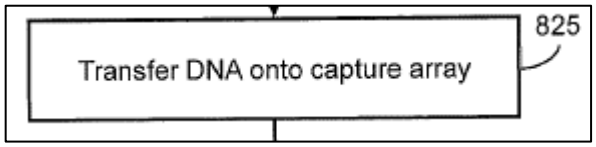
(3) Amplifying the ligation products by isothermal amplification to generate "amplicons" (950) that are released from the target analyte during amplification. EX1005, ¶[00182], [00180]. This is shown in Step 820 of Figures 8-9, reproduced below.

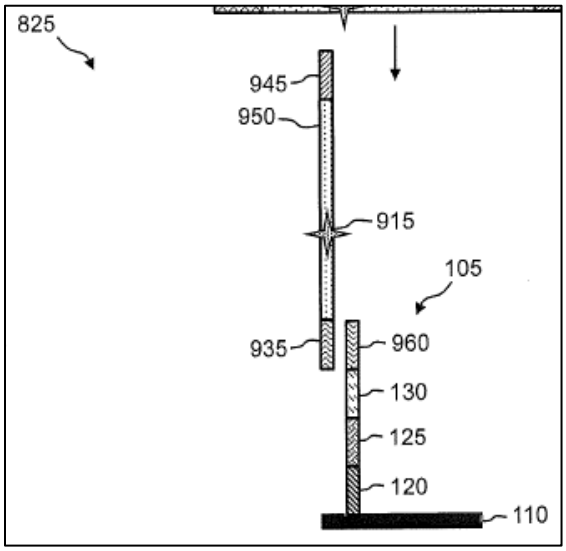


EX1005, FIGs. 8, 9.

(4) Capturing the released amplicons (950) on the capture probes (115) in the capture sites (105) on the array surface that are in proximity to where the target DNAs were found in the tissue sample by hybridizing the released amplicon's "universal capture region" (935) and capture probe's "capture region" (960).

EX1005, ¶[00182], [00181]. This is shown in Step 825 of Figures 8-9, reproduced below.



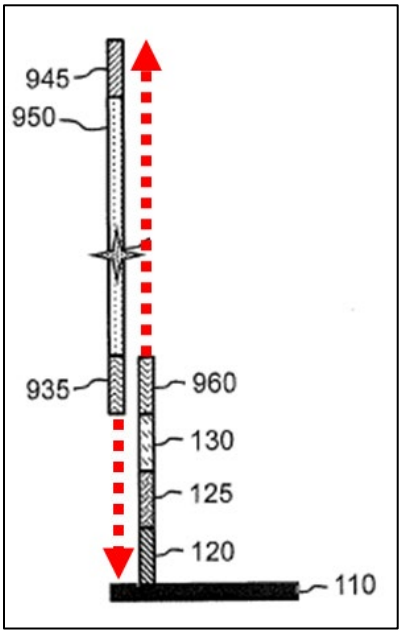


EX1005, FIGs. 8, 9.

(5) Preparing a barcoded sequencing library that includes extending the capture probes and the amplicons (i.e., ligation products) hybridized to the capture probes to incorporate the spatial barcode of the capture probes into the corresponding amplicon sequence. So-456's Figure 2 specifies that after the nucleic acids have been transferred onto the array, a "sequencing library is prepared" (Step 225). EX1005, ¶[00156], FIG. 2. Because the captured amplicons have not yet been spatially tagged, Figures 8-9's method incorporates a spatial barcode into the ligation products during the sequencing library preparation. So-456 explains that the "*capture primers* or amplification primers can be immobilized such that a *3'-end is available for enzymatic extension*." EX1005, ¶[00109] (emphasis added). So-456 also explains that the extension reactions in its

methods can occur in both directions—*i.e.*, an extension from the 3'-end of the capture probe, and an extension from the 3' end of the captured amplicons—such that "[b]oth extension products can be used for downstream library generation." EX1005, ¶[00173], Fig. 6.

So-456's capture probes comprise a "spatial address region 130" containing a "spatial address sequence" (*i.e.*, spatial barcode). EX1005, ¶[00141], FIGs. 1, 9. Thus, during sequencing library preparation, So-456's method extends the 3' end of the capture probes using the captured ligated products (950) as templates and extends the 3' end of the captured ligated products using the capture probes as templates to incorporate a spatial address region (130) of the capture probes into the ligated products (950). I have illustrated this extension reaction in the annotated excerpt of Figure 9.



EX1001, FIG. 9 (annotated).

Indeed, So-456 explained that the nucleic acids are "*tagged by probes* including location-specific sequence information (a '*spatial address*')," which results in the spatial origin information being "*encoded in the nucleic acid[s]*." EX1005, ¶[0089] (emphasis added).

(6) Sequencing the library (*e.g.*, by SBS), followed by analyzing the sequencing data and decoding the spatial information based on the spatial barcodes to determine the location of genomic DNA molecules in the tissue sample (Steps 230-235). EX1005, [00156]-[00158], FIG. 2.

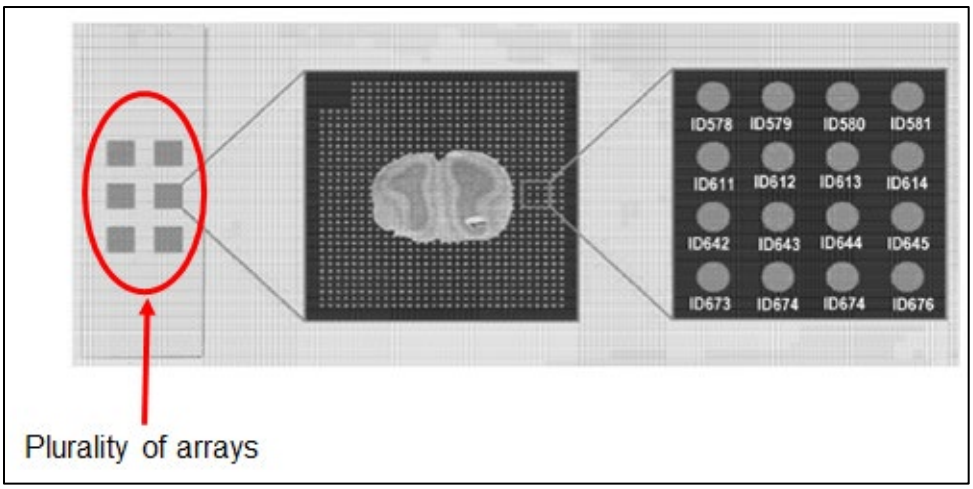
c. WO 2021/133849 (Chell) disclosed methods using spatially barcoded arrays and probe ligation by December 2020

105. I understand that in December 2020, 10x filed a PCT application entitled "Methods for Spatial Analysis Using RNA-Templated Ligation," which published as Chell *et al*. (WO 2021/133849) on July 1, 2021 ("Chell"). EX1006. As I explain below in Section XII.a, the 689 patent's priority applications do not provide written description support for its claimed methods. I understand that, as a result, the 689 patent claims are not entitled to a filing date before the patent's filing date of September 16, 2021. I understand that Chell is prior art because it was filed and published before September 16, 2021.

106. As I explain in detail below, Chell's method of Example 1 (depicted in Figures 1 and 13) comprises hybridizing probe pairs to target mRNAs, ligating the

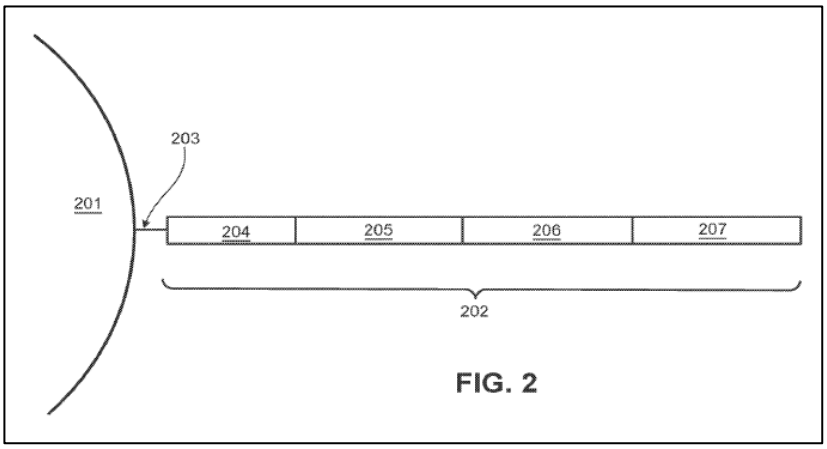
probe pairs hybridized on adjacent sites on the same nucleic acid molecule, releasing and capturing the ligation products on arrays via barcoded array probes, extending the array probes, sequencing the extension products, and analyzing the sequencing data to determine the location of the target nucleic acids in the tissue sample. EX1006, 7:29, 8:5-8, 8:22, 23:30-24:13, 80:14-85:3, FIGs. 1, 6-7, 13-20.

107. Chell's barcoded array is in Figure 5 (reproduced below with my annotation in red).



EX1006, FIG. 5 (annotated).

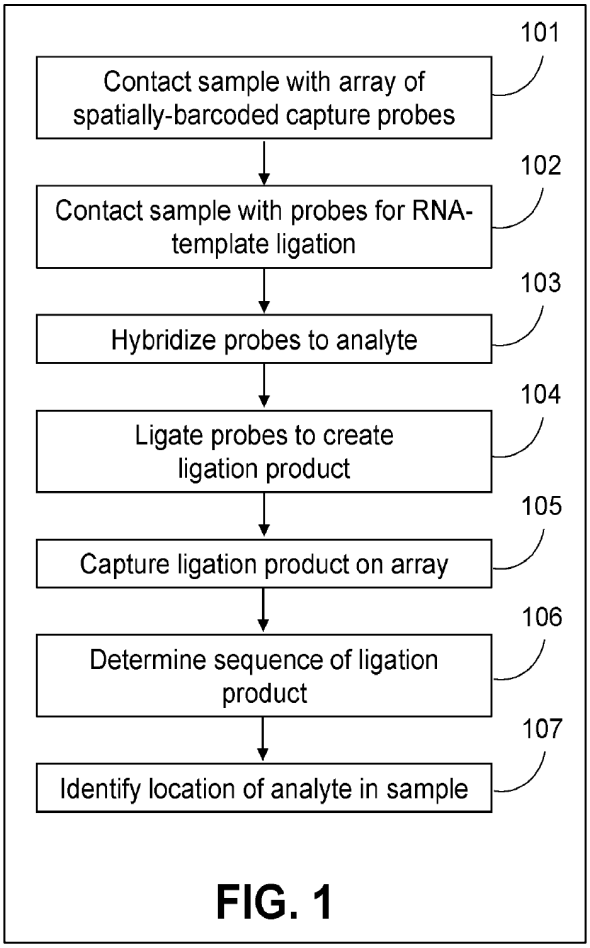
108. Chell's array (shown as 6 square arrays on a slide on the left and one array with an overlaid tissue sample in the center) comprises a "grid of barcoded features" (labeled as ID578, ID579, ID560, etc.) (shown on the right). EX1006, FIG. 5, 19:12-16. Each feature (201) of the array comprises "capture probes" (202) immobilized on the array surface via a cleavable domain (203) (shown in Figure 2 reproduced below).

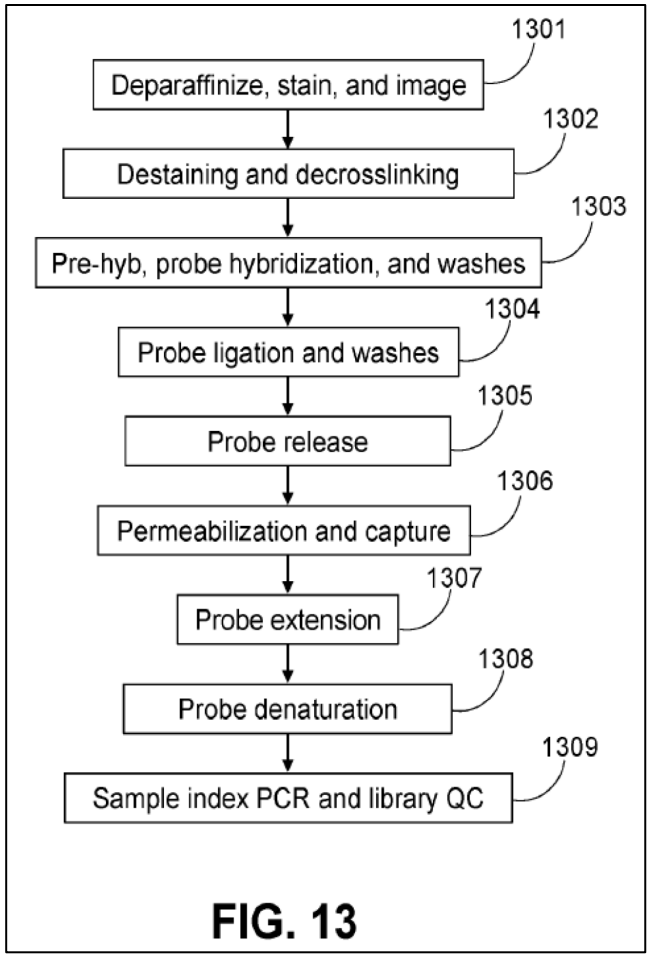


EX1006, FIG. 2, 15:29-30, 16:11-13.

109. Chell's capture probe is a "molecule capable of capturing (directly or indirectly) and/or labelling an analyte (e.g., an analyte of interest) in a biological sample." EX1006, 12:10-12, 15:12-13. The capture probes (202) comprise a "spatial barcode" (206), a "capture domain" (207) and sequences (204 and 205) for subsequent processing (such as, generation of a sequencing library). EX1006, 15:30-16:2, 23:13-29. Chell explains that a spatial barcode is "a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample" and a capture domain is a nucleic acid sequence "capable of binding to an analyte (e.g., a protein and/or a nucleic acid) produced by and/or present in a cell." EX1006, 10:6-10.

110. Chell's Example 1 (illustrated in Figure 13) discloses a ligation-based spatial detection method and follows the overall workflow in Chell's Figure 1. I have reproduced Chell's Figures 1 and 13 below.





EX1006, FIGs. 1, 13.

111. Chell's Figure 6 (reproduced below) further details the steps of the method of Example 1 (Figure 13). EX1006, 8:5-6, 24:4-13, FIG. 6.

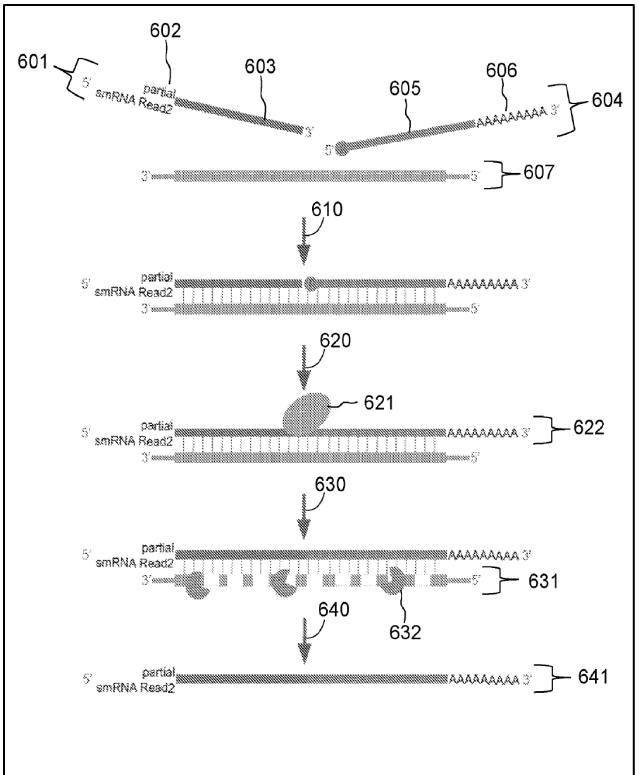


FIG. 6

EX1006, FIG. 6.

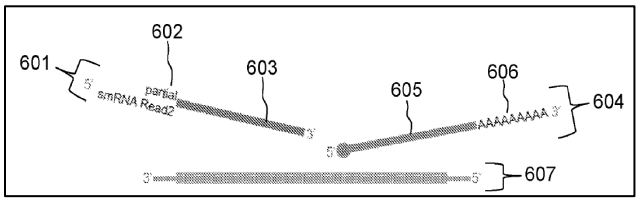
112. As shown above, Chell's method uses two types of oligonucleotide probes:

(1) *Primary probe pairs* (a "first probe" and a "second probe" 601, 604) that hybridize to the target analytes (607) and are ligated to each other when hybridized to the same target molecule to generate "ligation products" (622); and

(2) *Secondary probes* ("capture probes") containing a "spatial barcode" (206) that are immobilized on a glass slide. EX1006, 16:1-2, 24:4-13, 33:19-21.

113. The probe pairs (601, 604) comprise a target-binding domain ("target-hybridization sequence" 603, 605) that hybridizes to a complementary site on a

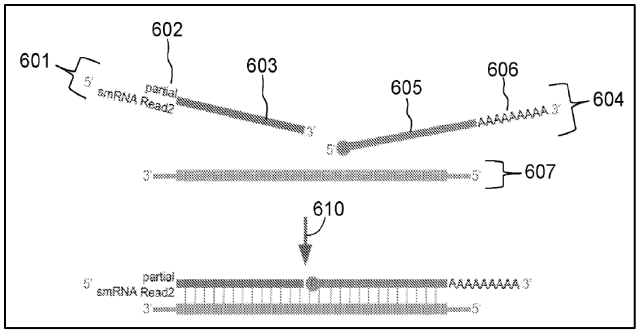
target analyte (607), and one probe of the pairs comprises a capture domain ("capture probe capture domain" 606) that binds "specifically to a capture domain of a capture probe." EX1006, 23:18-22, 24:4-13, 32:27-33, FIG. 6. I have reproduced a portion of Figure 6 showing the probe pairs (601, 604) hybridizing to the target analyte (607).



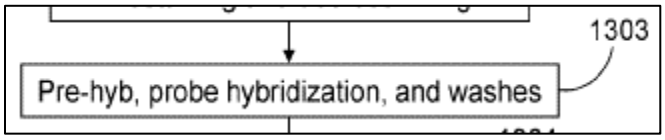
EX1006, FIG. 6.

114. Chell's method of Example 1 (Figures 6 and 13) teaches that a "biological sample is deparaffinized, stained, and imaged" (Step 1301) and then contacted with a plurality of spatially-barcoded capture probes immobilized on an array surface (Step 101). EX1006, 23:13-33. Chell's method of Example 1 (Figures 6 and 13) includes the following steps:

(1) Adding the probe pairs (RHS and LHS DNA probes, 601, 604) to the tissue sample and hybridizing the probe pairs to adjacent complementary sequences in the same target analyte molecule (607) (Step 1303), after destaining and decrosslinking (Step 1302; Step 610; Steps 102-103):

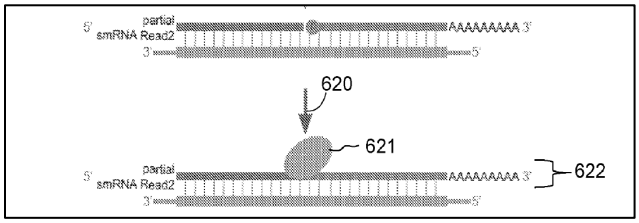


EX1006, FIG. 6.

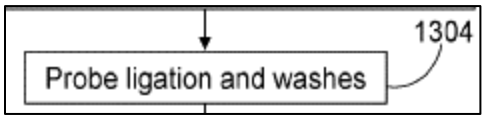


EX1006, FIG. 13.

(2) Ligating *in situ* the probe pairs (601, 604) using a ligase (621) to form the ligation products (622) (Step 1305; Step 620; Step 104):



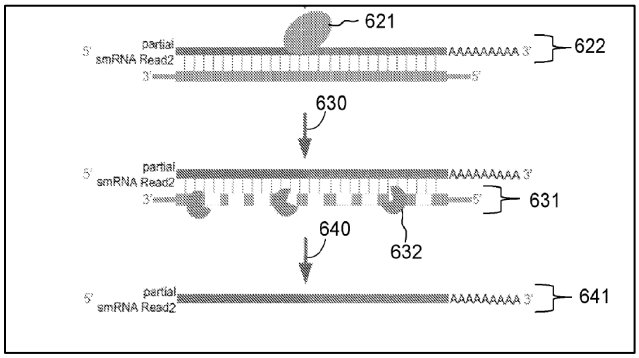
EX1006, FIG. 6.



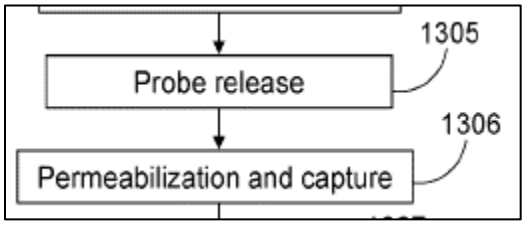
EX1006, FIG. 13.

(3) Releasing the ligation products from the target analyte (631) by digesting the target analyte using an endonuclease (632), permeabilizing the tissue sample,

and capturing the released ligation products via the capture probes comprising a spatial barcode on the array surface (Steps 1305-1306; Steps 630-640; Step 105):

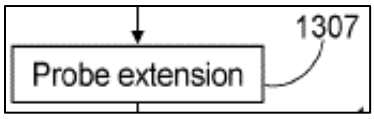


EX1006, FIG. 6.



EX1006, FIG. 13.

(4) Extending the captured ligation products using a polymerase to generate extension products (Step 1307):



EX1006, FIG. 13.

(5) Denaturing the extended capture probes, generating a sequencing library, and sequencing to determine the abundance *and location* of the target analyte (Steps 1308-1309; Steps106-107). EX1006, 7:29, 23:13-24:13, 30:8-14, 33:22-24, 80:14-85:3, 85:32-86:2.

115. The results of Example 1 were shown in Figures 14-21. EX1006, 84:12-85:4.

VIII. CLAIM CONSTRUCTION

116. I understand that NanoString has alleged in related litigation that 10x's Visium FFPE infringes the 689 patent claims. Solely for the purposes of this IPR, I have been asked to apply NanoString's interpretations according to its infringement positions.

IX. GROUND 1: CLAIMS 1-8, 11-23, AND 26-30 ARE ANTICIPATED BY WO 2017/019456 (SO-456)

117. In my opinion, claims 1-8, 11-23, and 26-30 of the 689 patent are anticipated by So-456 under NanoString's interpretation of the claims. Specifically, So-456 discloses a method meeting all the elements of the claimed methods. I understand that So-456 is presumed enabled. I understand that So-456 was both filed and published before February 12, 2018, and thus it qualifies as prior art to the 689 patent.

a. Claim 16

118. As I discuss below, So-456 anticipates claim 16 under NanoString's interpretation. *See also* Appendix A.

i. Preamble: "A method for spatially detecting [in an ROI] at least one target analyte in a first location and a second location of a tissue sample comprising"

119. Claim 16 recites "[a] method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample." EX1001, 147:54-56. I understand that this is the preamble of claim 16. I understand that a preamble may be relevant in understanding the scope of the claim. To the extent, claim 16's preamble is relevant in understanding its scope, So-456 teaches the claimed methods for spatially detecting at least one target analyte in a tissue sample under NanoString's interpretation.

120. So-456 discloses a "method for *spatial detection* and analysis of *nucleic acids* in a sample." EX1005, ¶[00150] (emphasis added). Specifically, So-456's Figure 2 illustrates an overall workflow for its methods for "*spatial detection* and analysis of a *nucleic acid* in a *tissue sample*." EX1005, ¶[00151] (emphasis added), FIG. 2. Following the workflow of Figure 2, So-456 discloses a ligation-based method in Figures 8-9, wherein "Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8." EX1005, ¶¶[0018], [0019], [00177]-[00183]. As I explain below, the method of So-456's Figures 8-9 includes the contacting (Step 16(a)), ligating (Step 16(b)), collecting (Steps 16(c) and 16(d)), extending (Steps 16(e) and 16(f)) and sequencing (Step 16(g)) of claim 16. Thus, So-456 discloses each element of claim 16 under NanoString's interpretation.

ii. "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,

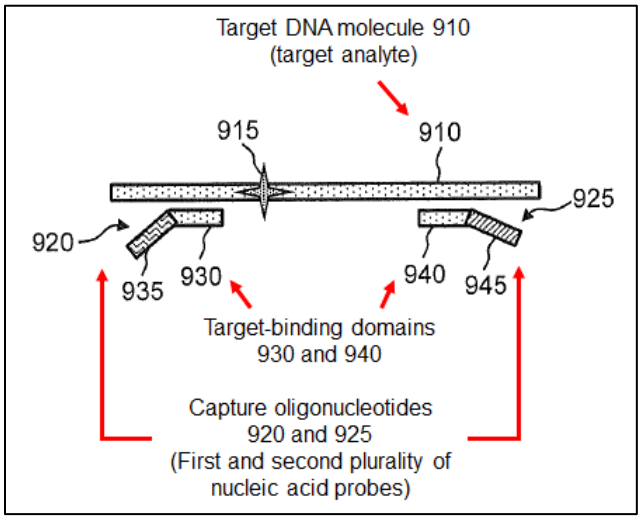
wherein each of the nucleic acid probes in the second plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte . . ."

121. In my opinion, So-456's method includes this contacting step.

122. In Step 810, So-456's method of Figures 8-9 contacts "a tissue section … compris[ing] a *target genomic DNA molecule 910*" (*i.e.*, "at least one target analyte" of the claim) with "a first gene-specific capture oligonucleotide 920" and "a second gene-specific capture oligonucleotide 925" (*i.e.*, "a first [and second] plurality of nucleic acid probes" of the claim). EX1005, ¶¶[00182] (emphasis added), [00178], FIGs. 8, 9. The first and second capture oligonucleotides (920, 925) comprise a "gene-specific region" (930, 940) (*i.e.*, "target binding domain" of the claim) that hybridize to complementary sequences flanking a region of interest in the target DNA molecule (910). Thus, So-456's first and second "capture oligonucleotides" are the plurality of first and second "nucleic acid probes" required in the claim.

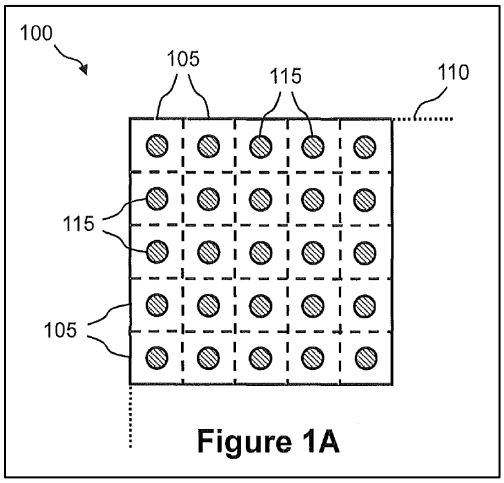
123. The annotated excerpt of Figure 9 below illustrates that So-456's method includes hybridizing capture oligonucleotides (920, 925) (*i.e.*, nucleic acid probes of the claim) comprising gene-specific region (930, 940) (*i.e.*, target-binding

domains of the claim) with a target DNA molecule (910) (*i.e.*, target analyte) in the tissue sample.



EX1005, FIG. 9 (annotated).

124. Further, So-456's methods "facilitate the characterization of transcriptomes and/or genomic variation in tissues" using a "capture array" (100) comprising an array of "capture sites" (105) with immobilized capture probes (115), as shown in Figure 1A, below.



EX1005, FIG. 1A, ¶¶[0002], [0003], [0085], [00154]. Thus, So-456 teaches contacting a plurality of capture oligonucleotide pairs at multiple locations in a tissue sample—*i.e.*, a first plurality and second plurality of nucleic acid probes.

> **". . . 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"**

125. So-456's method also includes treating the tissue sample to facilitate the binding of the primary probe pairs to the target analyte, as claimed. For example, So-456 teaches that the "*tissue sample is prepared* for analysis" and that "in situ *biochemistry is performed* on the tissue section to *facilitate manipulation* of a nucleic acid in the sample" to allow hybridization of the capture oligonucleotides (*i.e.*, nucleic acid probes of the claim) to the target analytes. EX1005, ¶¶[00152]-[00153] (emphasis added).

126. Further, So-456's Figure 2 specifies performing "in situ biochemistry" (Step 215) on the tissue sample that facilitates probe binding to the target analyte because Figure 8 depicts "[h]ybridiz[ing] capture oligonucleotides to genomic DNA" (*i.e.*, binding). EX1005, FIGs. 2, 8, 9, ¶¶[00153], [00182]. Because the capture oligonucleotides hybridized to the target analytes and were processed according to the subsequent steps, So-456's method treated the tissue sample to facilitate binding of the first and second plurality of capture oligonucleotides (*i.e.*,

nucleic acid probes of the claim) to the target DNA molecules (i.e., target analytes).

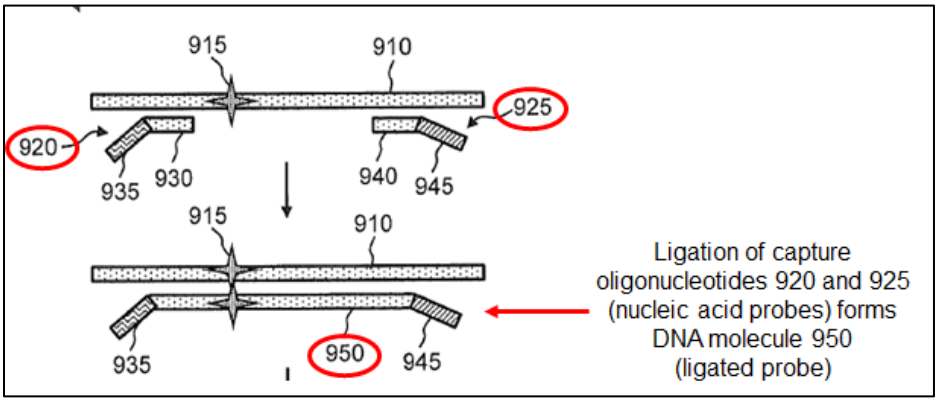
127. Accordingly, So-456's method includes contacting a tissue sample with a first and second plurality of nucleic acid probes comprising target-binding domains, wherein the tissue sample is treated to facilitate binding of the probe to a target analyte. Thus, in my opinion, So-456's method includes claim 16's contacting step.

iii. "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"

128. In my opinion, So-456's method includes this ligation step. The 689 patent discloses "ligating" probe pairs that are "not adjacent and are not overlapping" by "performing a gap extension reaction and a nick repair reaction." EX1001, 25:66-26:6, 6:66-7:4, 7:34-40, 9:48-54, 10:19-24, 30:59-65, 68:66-69:3, 70:40-44.

129. So-456's method of Figures 8-9 specifies that "an *extension/ligation* reaction is performed in situ between the flanking capture oligonucleotides 920 and 925 across the region of interest" to form "DNA molecule 950" (*i.e.*, ligated probes of the claim) (Step 815). EX1005, ¶¶[00182] (emphasis added), [00179], FIGs. 8, 9. As shown below in the annotated excerpt of Figure 9, So-456's method includes

forming DNA molecules (950) (*i.e.*, ligated probes of the claim) by ligating capture oligonucleotide pairs (920, 925) (*i.e.*, nucleic acid probes of the claim) that had hybridized to the identical molecule of the target analyte.



EX1005, FIG. 9 (annotated).

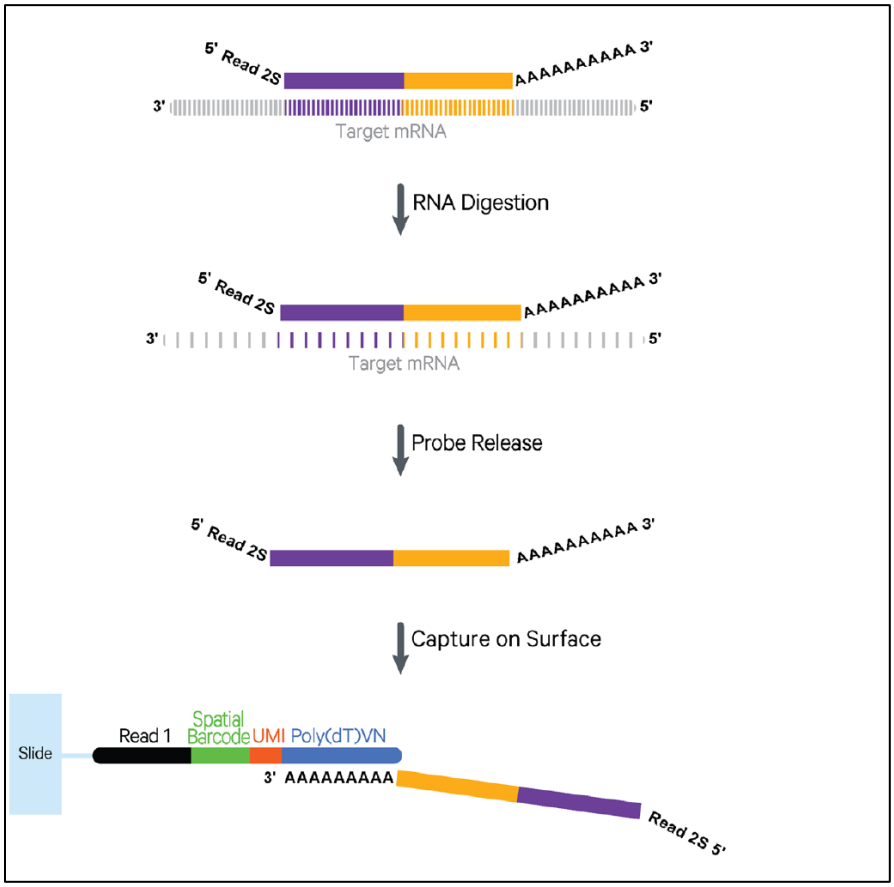
130. Accordingly, So-456's method includes forming ligated probes by ligating nucleic acid probe pairs that are bound to the identical molecule of the target analyte. Thus, in my opinion, So-456's method includes claim 16's ligation step.

iv. "c) [and (d)] 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 [(d): second location] of the tissue sample under conditions that release the ligated probes, or portions thereof, from the first location [(d): second location] of the tissue sample"

131. In my opinion, So-456's method includes these collecting steps under NanoString's interpretation.

132. I understand that NanoString has alleged in related litigation that 10x's Visium FFPE infringes the 689 patent claims. EX1020. Under NanoString's

interpretation, the collecting steps of claim 16 (and claim 1) encompass certain of the steps of 10x's Visium FFPE workflow. EX1020, 53-54. I have reviewed the 10x user guide for Visium FFPE that NanoString identifies in its contentions. In Visium FFPE, *whole* ligation products (not a portion) generated from ligated primary probe pairs are diffused from and captured across the *whole* tissue sample, all at the same time. These steps of Visium FFPE are depicted in the figure below.



EX1008, 20.

133. Thus, under NanoString's interpretation, the collecting steps of claim 16 (and claim 1) encompass diffusion and capture of *whole* probes or *whole* oligonucleotides across the *whole* tissue sample, all at the same time.

134. So-456's method of Figures 8-9 specifies that the DNA molecules (950) formed in the previous step by ligating primary probe pairs (capture oligonucleotides) are "amplified by in situ isothermal amplification to generate multiple copies . . . of the targeted region of interest, *i.e.*, multiple genomic *amplicons.*" (Step 820). EX1005, ¶¶[00182] (emphasis added), [00180], FIGs. 8, 9. The amplicons generated by isothermal amplification are free from (*i.e.*, not bound to) the target nucleic acid molecules.

135. These amplicons (950) (*i.e.*, ligated probes of the claim) are "captured onto the capture site 105" of a spatially barcoded array, such as an array in Figure 1A. EX1005, ¶¶[00182], [00180], [00181], FIGs. 1, 8, 9. The annotated excerpts of Figures 9 and 1A below depict these steps in So-456's method.

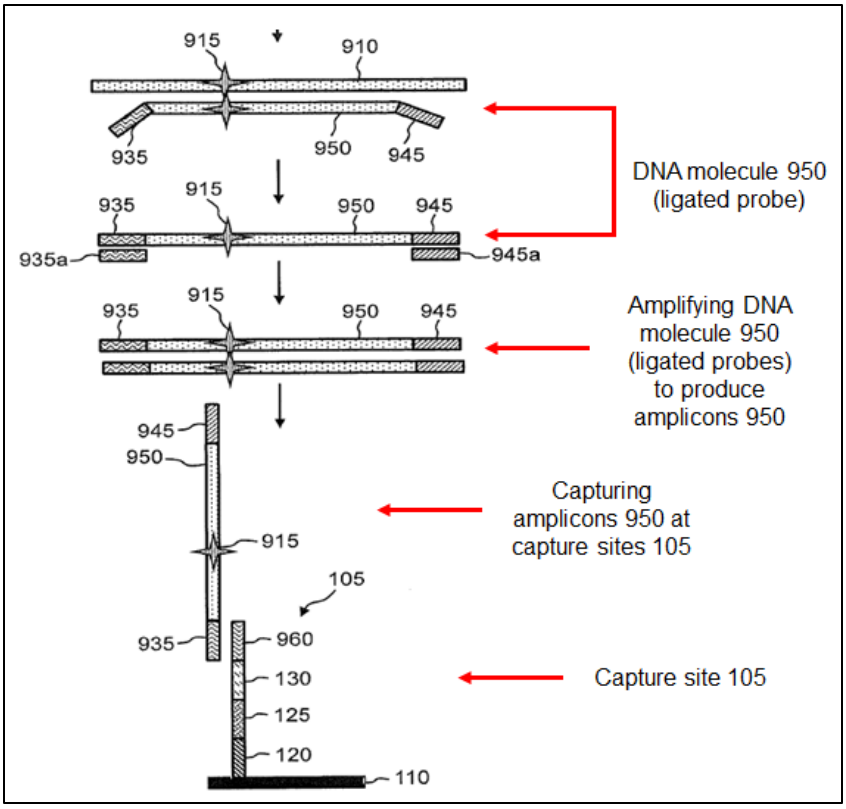


FIG. 9

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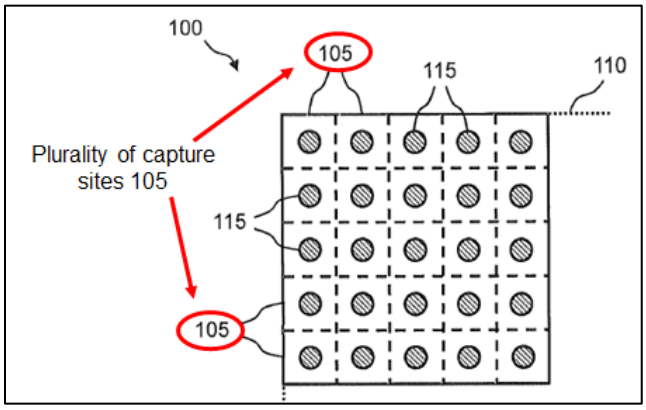


FIG. 1A

EX1005, FIGs. 1A, 9 (annotated).

136. So-456's "capture array 100" comprises "an arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110." EX1005, ¶[00141], FIG. 1A. A capture site (105) comprises "at least one capture probe 115" immobilized on the solid support (110). EX1005, ¶[00141], FIGs. 9, 1B. In fact, "any number of capture probes 115 can be immobilized on solid support 110 at each capture site 105." EX1005, ¶[00141], FIGs. 9, 1A. So-456's workflow is also carried out simultaneously at multiple locations of the tissue sample and consequently at multiple capture sites of the array. EX1005, ¶¶[00141], [00182], [00183], [00165], FIGs. 1A, 9.

137. Accordingly, So-456's method includes capturing *whole* ligated probes (formed by ligating the primary probe pairs) that diffuse from and are captured across the *whole* tissue sample, all at the same time. Thus, in my opinion, So-456's method includes claim 16's collecting steps under NanoString's interpretation.

v. "e) [and (f)] performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first [and second] location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (c) [and (d)],

thereby forming a first [(f): second] plurality of extension products that comprise the ligated probes, or portions thereof, collected in step (c) [and (d)] and the at least one nucleic acid sequence that identifies the first [(f): second] location of the tissue sample"

138. In my opinion, So-456's method includes these extension steps.

139. A "nucleic acid sequence that identifies the first [and second] location of the tissue sample" in the claim is a spatial barcode (as used in this declaration). Thus, the claim requires an extension reaction that incorporates a spatial barcode into the ligated probes collected in the previous step, forming extension products that comprise the ligated probes and the spatial barcode. Even though So-456's method of Figures 8-9 does not expressly perform the claimed extension step, it does so inherently to achieve spatially barcoded nucleic acids for analysis.

140. So-456 teaches that its methods are based on a "realization that information related to the spatial origin of a nucleic acid in a tissue sample can be *encoded in the nucleic acid[s]* in the process of *preparing* the nucleic acid for sequencing." EX1005, ¶[0089] (emphasis added). To accomplish this, So-456 teaches that the "nucleic acids from a tissue sample can be tagged by probes including location-specific sequence information (a 'spatial address')." EX1005, ¶[0089]. Indeed, So-456 states that, in some instances, two or three "spatial address sequences are incorporated into a nucleic acid during preparation of a sequencing library." EX1005, ¶¶[00131], [00132]. Thus, So-456's methods require incorporating a spatial barcode into the target nucleic acids by tagging the nucleic acids with a probe containing a spatial barcode.

141. So-456's Figure 2 (which illustrates the overall workflow of So-456's methods) specifies that after the nucleic acids have been captured on the array

(Step 220), a "sequencing library is prepared" (Step 225) for use in a subsequent sequencing step (Step 230) and an analysis and decoding step (Step 235). EX1005, ¶[00156]. Thus, So-456's methods require (1) encoding the "spatial origin of a nucleic acid in a tissue sample" in "the process of preparing the nucleic acid for sequencing," and (2) performing sequencing to obtain data that is "analyzed . . . and the spatial information is decoded." EX1005, ¶¶[0089], [00131], [00132], [00158].

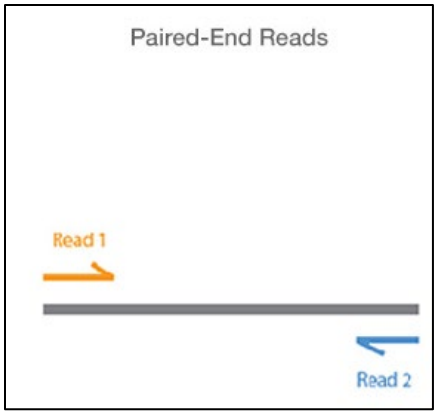
142. However, So-456's amplicons (950) captured on the arrays in the previous step had not yet been tagged with a spatial barcode and do not include the functional sequences (such as, a pair of SBS primer-binding sites) necessary for performing sequencing (such as, sequencing-by-synthesis (SBS)) in the subsequent steps. Therefore, during sequencing library preparation (Step 225)—which is the only step remaining before the sequencing step (Step 230)—So-456's method must generate molecules containing a spatial barcode and sequencing primer-binding sites, such that the molecules are ready for sequencing, analysis, and decoding in the subsequent steps (Steps 230, 235). EX1005, ¶¶[00156]-[00158], FIG. 2.

143. So-456's capture probes comprise a unique "spatial address region 130" containing a "spatial address sequence" (*i.e.*, spatial barcode). EX1005, ¶[00141], FIGs. 1, 9. So-456 explains that the spatial address region (130) "corresponds to the position of capture probe 115 in capture array 100," which in turn "can be

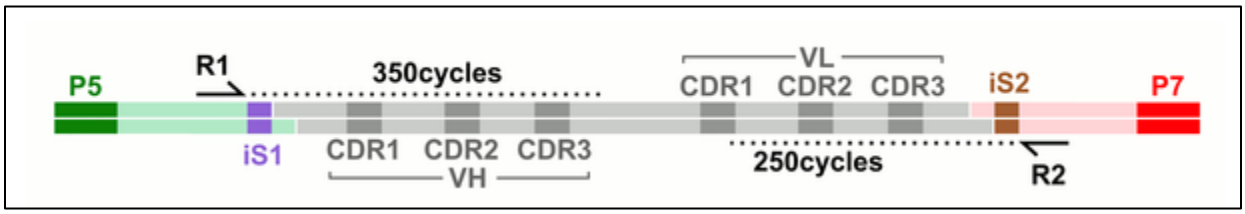
correlated with a position in the tissue sample." EX1005, ¶[00141]; *see also* EX1005, ¶[0099] ("spatial address" means "an address, tag or index encoding spatial information related to the region or location of origin of an addressed, tagged, or indexed nucleic acid in a tissue sample").

144. Additionally, So-456 (which was filed by Illumina) teaches using SBS (sequencing-by-synthesis), *e.g.*, "developed by [] Illumina, Inc." EX1005, ¶¶[00156]-[00157], [00158], FIG. 2. The components and methods for sequencing by Illumina method and for generating the corresponding sequencing libraries were well known prior to February 2018. Illumina's SBS method utilizes a pair of SBS primers that initiate the incorporation of fluorescently labeled reversible terminator nucleotides into the molecules generated for sequencing. EX1030; EX1005, ¶¶[00141], [00199], [00201], [00211], [00229], [00496]-[00497].

145. SBS platforms standardly allow for paired-end sequencing. Paired-end sequencing allows the users to sequence from both ends of a sequencing library molecule in order to generate more accurate reads and to detect error-prone regions such as genomic rearrangements and repetitive sequence elements. EX1007. In paired-end sequencing, the molecules include primer-binding sites (commonly referred to as "read sequences") "read 1" (R1) and "read 2" (R2) at its opposite ends:



146. For SBS to allow for paired-end sequencing, it utilizes a pair of particular sequencing primers, *e.g.*, "SBS12" and "SBS3," which are complementary to primer-binding sites, R1 and R2, as shown in the figure below.



EX1030 ("The forward read (R1) uses SBS3 sequencing primer (Illumina), while the reverse read (R2) uses SBS12 primer (Illumina).").

147. The method of Figures 8-9 uses the Illumina SBS platform for sequencing (reflected by the inclusion of the SBS primer-binding sites (SBS3 and SBS12) in the captured amplicons (950) and the capture probes). Specifically, the amplicons (950) captured on the array in So-456's method include the binding site for one SBS primer ("SBS primer region 945 (e.g., SBS12)"). EX1005, ¶¶[00182], [00141]. And the capture probes immobilized on the array include the binding site for the second SBS primer ("SBS primer region [] SBS3"). *See*, *e.g.*, EX1005,

¶¶[0141] ("SBS primer region 125 [] SBS3"), [00182] ("SBS primer region 130 [] SBS3"), FIG. 1, FIG. 9.

148. Thus, for tagging the oligonucleotides captured on the array with the spatial barcode of the capture probes and for incorporating both SBS primer-binding sites in the same molecule, So-456's method must extend the capture probes (using the captured ligated probes as template) and the captured ligated probes (using the capture probes as template). This generates a molecule comprising the captured amplicon (containing the first primer-binding site) and the capture probe (containing the spatial barcode and the second primer-binding), which can then be used in the subsequent sequencing, decoding, and analyzing steps.

149. So-456 discloses that the "[o]ligonucleotides to be used as *capture primers* . . . can be immobilized such that a *3'-end is available for enzymatic extension*." EX1005, ¶[00109] (emphasis added). So-456 does not disclose any blocking or other modifications to the 3'-end of the captured oligonucleotides that are extended in its methods[2], allowing extension of both the 3'-end of the captured

[2] In contrast, So-456's other disclosures specify modifying the 3' end of a nucleic acid, *e.g.*, to prevent 3'-end ligation or to prevent further nucleotide

amplicons and 3'-end of the capture probes in the method of Figures 8-9. EX1005, ¶¶[00109], [00182]-[00183], FIGs. 8, 9. This is because extension enzymes, such as DNA polymerase, automatically extend all available 3'-ends. Thus, due to the nature of the extension enzymes and the presence of unblocked and unmodified 3'-ends, So-456's extension reaction necessarily proceeds in *both* directions—extending the 3'-end of the capture probe in one direction and the 3'-end of the captured ligated probe in the other direction. EX1005, ¶¶[00109], [00182]-[00183], FIG. 9. Indeed, So-456 confirms that when the capture probe is "used as a primer," the captured "DNA molecule [] is copied," and when the captured DNA molecule is "used as a primer," the "spatial address region 130" is copied. EX1005, ¶[00173], FIG. 6. So-456 confirms that "*[b]oth extension products* can be used for *downstream library generation*." EX1005, ¶[00173] (emphasis added), FIG. 6.

150. I have illustrated the extension reaction of So-456's method in the annotated excerpt of Figure 9 below.

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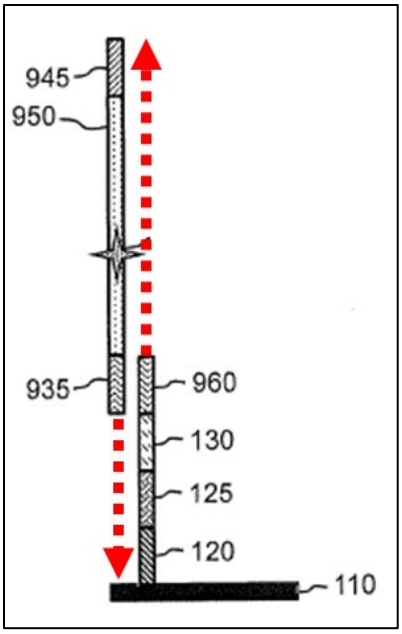
incorporation during sequencing. *See e.g.,* EX1005, ¶¶[00484], [00491], [00601], FIGs. 48-49.



EX1001, FIG. 9 (annotated).

151. As shown above in the figure, So-456's extension reaction forms extension products comprising the sequences of the captured ligated probe (950), spatial barcode (130), and the two primer-binding sites (945, 125)necessary for sequencing. EX1005, ¶¶[00141], [00182], FIG. 9.

152. Accordingly, So-456's method performs an extension reaction during sequencing library preparation that incorporates a spatial address region (130) (*i.e.*, spatial barcode) into amplicons (950) (*i.e.*, ligated probes of the claim), thereby forming extension products that comprise the captured amplicon and the spatial barcode. Thus, in my opinion, So-456's method includes claim 16's extension steps.

vi. "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."

153. In my opinion, So-456's method includes this sequencing step under NanoString's interpretation. I understand that NanoString agrees that "spatially detecting" means "identifying the presence of a specific target analyte within a specific region of interest in a sample." EX1024, 31.

154. So-456's Figure 2 (which illustrates the overall workflow of So-456's methods) specifies that after sequencing library preparation, "the library is sequenced" using "any sequencing technique" (Step 230). EX1005, ¶[00157]. "Examples of suitable sequencers include those available from, or being developed by, Illumina, Inc., F. Hoffmann-La Roche AG, Life Technologies, Inc., Beckman Coulter, Inc., Pacific Biosciences, Inc., Oxford Nanopore, Inc., and/or their affiliates." EX1005, ¶[00157].

155. So-456's Figure 2 further specifies that "the sequence data is analyzed . . . and the spatial information is decoded" (Step 235). EX1005, ¶[00158]. The decoded spatial information is "used to provide information as to the location of the nucleic acid in the tissue section" (*i.e.*, spatially detecting at least one target analyte in a first and second location of the tissue sample, as claimed). EX1005,

¶¶[0089], [00158]. To achieve this, the "sequence-identical nucleic acid molecules originating from *different* regions in a tissue sample can be distinguished based on their spatial address and can be *mapped onto their regions of origin* in the tissue sample." EX1005, ¶[0089]. So-456 further teaches that its methods "enable the identification of *the location of a cell or a cell cluster* in a tissue" sample. EX1005, ¶[0002], [0088].

156. Accordingly, So-456's method of Figures 8-9 includes sequencing the extension products to determine their sequence and sorting the sequencing data to determine the location of the target mRNAs in the tissue sample. As such, So-456's method identifies the extension products by sequencing them, thereby spatially detecting the target analytes in the tissue sample. Thus, in my opinion, So-456's method includes claim 16's sequencing step.

157. For the reasons I discuss above, So-456 teaches every element of claim 16 under NanoString's interpretation, arranged as claimed. I understand that So-456's methods are presumed enabled. Thus, in my opinion, So-456 anticipates claim 16 under NanoString's interpretation under NanoString's interpretation.

b. Claim 1

158. For the reasons I explain above for claim 16, So-456 also anticipates claim 1. *See also* Appendix B.

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

159. Claim 1's preamble is the same as claim 16's preamble and recites "[a] method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample." EX1001, 145:51-53. For the same reasons as I explain for claim 16 above, So-456's method of Figures 8-9 (following the overall workflow of Figure 2) discloses the preamble of claim 1 under NanoString's interpretation.

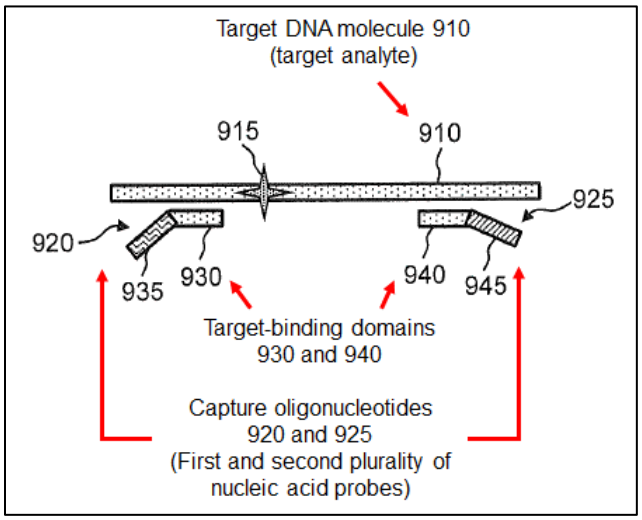
ii. "a) contacting the tissue sample with a plurality of nucleic acid probes,

wherein each of the nucleic acid probes comprise a target binding domain that binds to the at least one target analyte . . ."

160. In my opinion, So-456's method includes this contacting step. For the reasons I explain above for claim 16, So-456's method of Figures 8-9 includes the contacting step of claim 1.

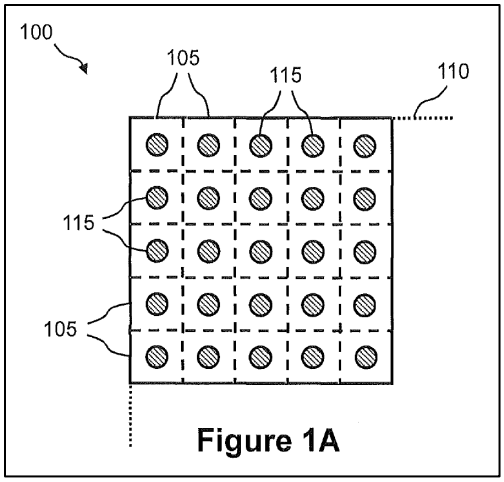
161. As I explain above for claim 16, So-456's method contacts "a tissue section . . . compris[ing] a *target genomic DNA molecule*" (*i.e.*, at least one target analyte) with probe pairs, "first [and second] *gene-specific capture oligonucleotide*," comprising a "gene-specific region" (*i.e.*, target-binding domain) that hybridizes to the target genomic DNA. EX1005, ¶¶[00182] (emphasis added), [00141], FIGs. 1B, 2, 8, 9. The annotated excerpt of Figure 9 below illustrates that

So-456's method includes hybridizing capture oligonucleotides (920, 925) (*i.e.*, nucleic acid probes of the claim) comprising gene-specific region (930, 940) (*i.e.*, target-binding domains of the claim) with a target DNA molecule (910) (*i.e.*, target analyte) in the tissue sample.



EX1005, FIG. 9 (annotated).

162. Further, So-456's methods "facilitate the characterization of transcriptomes and/or genomic variation in tissues" using a "capture array" (100) comprising an array of "capture sites" (105) with immobilized capture probes (115), as shown in Figure 1A, below.



163. EX1005, FIG. 1A, ¶¶[0002], [0003], [0085], [00154]. Thus, So-456 teaches contacting a plurality of capture oligonucleotide pairs at multiple locations in a tissue sample—*i.e.*, a first plurality and second plurality of nucleic acid probes.

". . . wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte"

164. As I explain above for claim 16, So-456 discloses 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 in the sample" to allow hybridization of the capture oligonucleotides (*i.e.*, nucleic acid probes of the claim) to the at least one target analyte (in Step 210/810). EX1005, ¶¶[00152]-[00153] (emphasis added). Further, So-456's Figure 2 specifies performing "in situ biochemistry" (Step 215) on the tissue sample which facilitates probe binding because Figure 8 depicts "[h]ybridiz[ing] capture oligonucleotides to genomic DNA" (*i.e.*, binding). EX1005, FIGs. 2, 8, 9, ¶¶[00153], [00182]. Because the capture oligonucleotides hybridized to the target analytes and were processed

according to the subsequent steps, So-456's method treated the tissue sample to facilitate binding of the first and second plurality of capture oligonucleotides (*i.e.*, nucleic acid probes) to the target analytes.

165. Accordingly, for these reasons, So-456's method includes contacting a tissue sample with nucleic acid probes comprising target binding domains, wherein the tissue sample is treated to facilitate binding of the probe to a target analyte. Thus, in my opinion, So-456's method includes claim 1's contacting step.

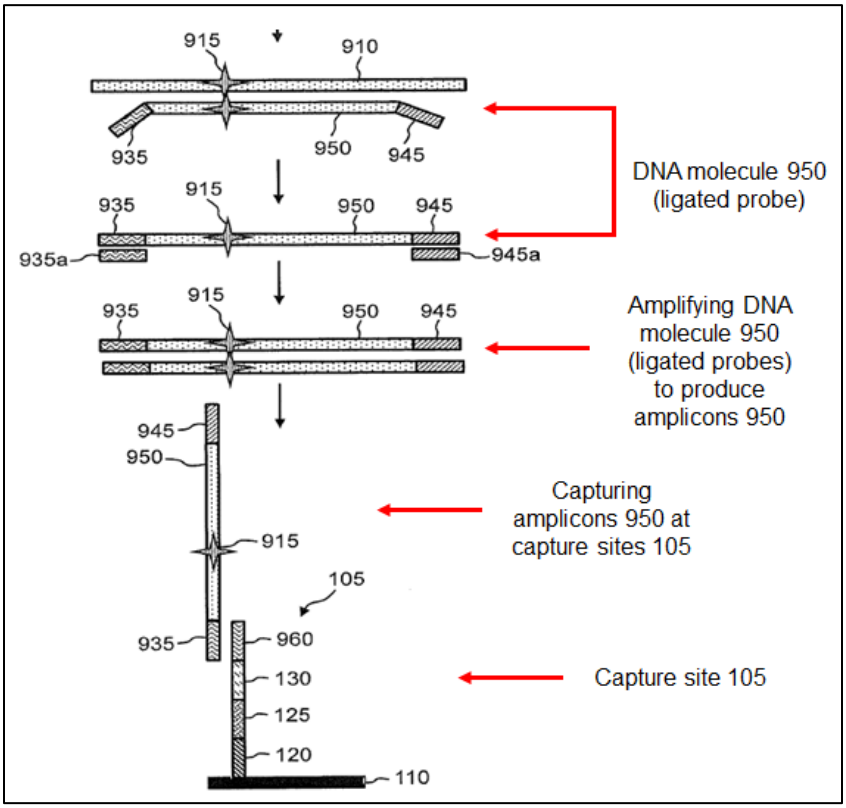
iii. "b) [and (c)] collecting the nucleic acid probes, or portions thereof, bound to the at least one target analyte in a first location [(c): second location] of the tissue sample under conditions that release the nucleic acid probes, or portions thereof, from the first location [(c): second location] of the tissue sample"

166. In my opinion, So-456's method includes these collecting steps under NanoString's interpretation. For the reasons I explain above for claim 16, So-456's method of Figures 8-9 includes the collecting steps of claim 1 under NanoString's interpretation.

167. I understand that NanoString has alleged in related litigation that 10x's Visium FFPE infringes the 689 patent claims. EX1020. For the reasons I explain above, the collecting steps of claim 1 encompass the "capture" step of 10x's Visium FFPE workflow under NanoString's interpretation. EX1020, 53-54.

168. As I explain above for claim 16, So-456's method of Figures 8-9 amplifies the DNA molecules (950) (*i.e.*, ligated probes of the claim) formed by

ligating the capture oligonucleotides (*i.e.*, nucleic acid probes of the claim) (Step 820). EX1005, ¶¶ [00182], FIGs. 1, 8, 9. The method next captures the amplicons (950) generated during amplification (*i.e.*, ligated probes of the claim) onto the capture probes immobilized on an array. EX1005, ¶¶ [00182]-[00183], FIGs. 1, 8, 9. This is illustrated in the annotated excerpt of Figure 9 below.



EX1005, FIG. 9 (annotated).

169. As I explain for claim 16, So-456's method includes capturing *whole* ligated probes (formed by ligating the primary probe pairs) that diffuse from and are captured across the *whole* tissue sample, all at the same time. Accordingly, for

these reasons (and those explained in detail for claim 16), So-456's method includes collecting *whole* ligated probes that were previously formed in the ligation step under conditions that release them from the *whole* tissue sample at the same time. Thus, in my opinion, So-456's method includes claim 1's collecting steps under NanoString's interpretation.

iv. "d) [and (e)] performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first [(e): second] location of the tissue sample into each of the nucleic acid probes, or portions thereof, collected in step (b) [and (c)],

thereby forming a first [(e): second] plurality of extension products that comprise the nucleic acid probes, or portions thereof, collected in step (b) [and (c)] and the at least one nucleic acid sequence that identifies the first [(e): second] location of the tissue sample"

170. In my opinion, So-456's method includes these extension steps.

171. A "nucleic acid sequence that identifies the first [and second] location of the tissue sample" in the claim is a spatial barcode (as used in this declaration). For the reasons I explain above for claim 16, So-456's method of Figures 8-9 includes the extension steps of claim 1.

172. So-456 teaches that its methods are based on a "realization that information related to the spatial origin of a nucleic acid in a tissue sample can be *encoded in the nucleic acid[s]* in the process of *preparing* the nucleic acid for sequencing." EX1005, ¶[0089] (emphasis added). To accomplish this, So-456

teaches that the "nucleic acids from a tissue sample can be tagged by probes including location-specific sequence information (a 'spatial address')." EX1005, ¶[0089]. Indeed, So-456 states that, in some instances, two or three "spatial address sequences are incorporated into a nucleic acid during preparation of a sequencing library." EX1005, ¶¶[00131], [00132]. Thus, So-456's methods require incorporating a spatial barcode into the target nucleic acids by tagging the nucleic acids with a probe containing a spatial barcode.

173. So-456's Figure 2 (which illustrates the overall workflow of So-456's methods) specifies that after the nucleic acids have been captured on the array (Step 220), a "sequencing library is prepared" (Step 225) for use in a subsequent sequencing step (Step 230) and an analysis and decoding step (Step 235). EX1005, ¶[00156]. Thus, So-456's methods require (1) encoding the "spatial origin of a nucleic acid in a tissue sample" in "the process of preparing the nucleic acid for sequencing," and (2) performing sequencing to obtain data that is "analyzed . . . and the spatial information is decoded." EX1005, ¶¶[0089], [00131], [00132], [00158].

174. However, So-456's amplicons (950) captured on the arrays in the previous step had not yet been tagged with a spatial barcode and do not include the functional sequences (such as, a pair of SBS primer-binding sites) necessary for performing sequencing (such as, sequencing-by-synthesis (SBS)) in the subsequent

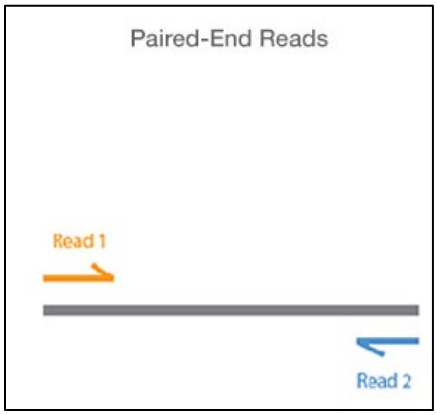
steps. Therefore, during sequencing library preparation (Step 225)—which is the only step remaining before the sequencing step (Step 230)—So-456's method must generate molecules containing a spatial barcode and sequencing primer-binding sites, such that the molecules are ready for sequencing, analysis, and decoding in the subsequent steps (Steps 230, 235). EX1005, ¶¶[00156]-[00158], FIG. 2.

175. So-456's capture probes comprise a unique "spatial address region 130" containing a "spatial address sequence" (*i.e.*, spatial barcode). EX1005, ¶[00141], FIGs. 1, 9. So-456 explains that the spatial address region (130) "corresponds to the position of capture probe 115 in capture array 100," which in turn "can be correlated with a position in the tissue sample." EX1005, ¶[00141]; *see also* EX1005, ¶[0099] ("spatial address" means "an address, tag or index encoding spatial information related to the region or location of origin of an addressed, tagged, or indexed nucleic acid in a tissue sample").

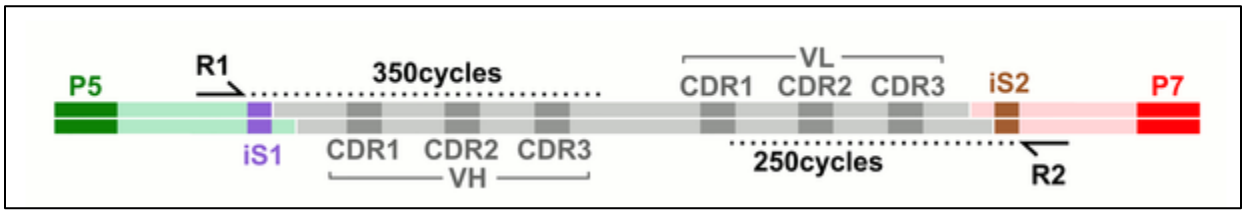
176. Additionally, So-456 (which was filed by Illumina) teaches using SBS (sequencing-by-synthesis), *e.g.*, "developed by [] Illumina, Inc." EX1005, ¶¶[00156]-[00157], [00158], FIG. 2. The components and methods for sequencing by Illumina method and for generating the corresponding sequencing libraries were well known prior to February 2018. Illumina's SBS method utilizes a pair of SBS primers that initiate the incorporation of fluorescently labeled reversible terminator

nucleotides into the molecules generated for sequencing. EX1030; EX1005, ¶¶[00141], [00199], [00201], [00211], [00229], [00496]-[00497].

177. SBS platforms standardly allow for paired-end sequencing. Paired-end sequencing allows the users to sequence from both ends of a sequencing library molecule in order to generate more accurate reads and to detect error-prone regions such as genomic rearrangements and repetitive sequence elements. EX1007. In paired-end sequencing, the molecules include primer-binding sites (commonly referred to as "read sequences") "read 1" (R1) and "read 2" (R2) at its opposite ends:



178. For SBS to allow for paired-end sequencing, it utilizes a pair of particular sequencing primers, *e.g.*, "SBS12" and "SBS3," which are complementary to primer-binding sites, R1 and R2, as shown in the figure below.



EX1030 ("The forward read (R1) uses SBS3 sequencing primer (Illumina), while the reverse read (R2) uses SBS12 primer (Illumina).").

179. The method of Figures 8-9 uses the Illumina SBS platform for sequencing (reflected by the inclusion of the SBS primer-binding sites (SBS3 and SBS12) in the captured amplicons (950) and the capture probes). Specifically, the amplicons (950) captured on the array in So-456's method include the binding site for one SBS primer ("SBS primer region 945 (e.g., SBS12)"). EX1005, ¶¶[00182], [00141]. And the capture probes immobilized on the array include the binding site for the second SBS primer ("SBS primer region [] SBS3"). *See*, *e.g.*, EX1005, ¶¶[0141] ("SBS primer region 125 [] SBS3"), [00182] ("SBS primer region 130 [] SBS3"), FIG. 1, FIG. 9.

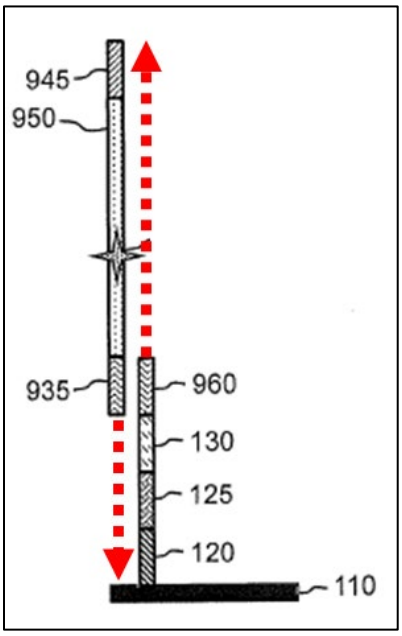
180. Thus, for tagging the oligonucleotides captured on the array with the spatial barcode of the capture probes and for incorporating both SBS primer-binding sites in the same molecule, So-456's method must extend the capture probes (using the captured ligated probes as template) and the captured ligated probes (using the capture probes as template). This generates a molecule comprising the captured amplicon (containing the first primer-binding site) and the capture probe (containing the spatial barcode and the second primer-binding), which can then be used in the subsequent sequencing, decoding, and analyzing steps.

181. So-456 discloses that the "[o]ligonucleotides to be used as *capture primers* . . . can be immobilized such that a *3'-end is available for enzymatic extension*." EX1005, ¶[00109] (emphasis added). So-456 does not disclose any blocking or other modifications to the 3'-end of the captured oligonucleotides that are extended in its methods[3], allowing extension of both the 3'-end of the captured amplicons and 3'-end of the capture probes in the method of Figures 8-9. EX1005, ¶¶[00109], [00182]-[00183], FIGs. 8, 9. This is because extension enzymes, such as DNA polymerase, automatically extend all available 3'-ends. Thus, due to the nature of the extension enzymes and the presence of unblocked and unmodified 3'-ends, So-456's extension reaction necessarily proceeds in *both* directions—extending the 3'-end of the capture probe in one direction and the 3'-end of the captured ligated probe in the other direction. EX1005, ¶¶[00109], [00182]-[00183], FIG. 9. Indeed, So-456 confirms that when the capture probe is "used as a primer," the captured "DNA molecule [] is copied," and when the captured DNA molecule is "used as a primer," the "spatial address region 130" is copied. EX1005,

[3] In contrast, So-456's other disclosures specify modifying the 3' end of a nucleic acid, *e.g.*, to prevent 3'-end ligation or to prevent further nucleotide incorporation during sequencing. *See e.g.,* EX1005, ¶¶[00484], [00491], [00601], FIGs. 48-49.

¶[00173], FIG. 6. So-456 confirms that "*[b]oth extension products* can be used for *downstream library generation*." EX1005, ¶[00173] (emphasis added), FIG. 6.

182. I have illustrated the extension reaction of So-456's method in the annotated excerpt of Figure 9 below.



EX1001, FIG. 9 (annotated).

183. As shown above in the figure, So-456's extension reaction forms extension products comprising the sequences of the captured ligated probe (950), spatial barcode (130), and the two primer-binding sites (945, 125)necessary for sequencing. EX1005, ¶¶[00141], [00182], FIG. 9. EX1005, ¶¶[00141], [00182], FIG. 9.

184. Accordingly, So-456's method performs an extension reaction during sequencing library preparation that incorporates a spatial address region (130) (*i.e.*,

spatial barcode) into amplicons (950) (*i.e.*, ligated probes of the claim), thereby forming extension products that comprise the captured amplicon and the spatial barcode. Thus, in my opinion, So-456's method includes claim 1's extension steps.

v. "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."

185. In my opinion, So-456's method includes the sequencing step under NanoString's interpretation. The sequencing step of claim 1 is the same as the sequencing step of claim 16. For the reasons I explain above for claim 16, So-456's method of Figures 8-9 includes the sequencing step of claim 1 under NanoString's interpretation.

186. So-456's Figure 2 (which illustrates the overall workflow of So-456's methods) specifies that after sequencing library preparation, "the library is sequenced" using "any sequencing technique" (Step 230). EX1005, ¶[00157]. "Examples of suitable sequencers include those available from, or being developed by, Illumina, Inc., F. Hoffmann-La Roche AG, Life Technologies, Inc., Beckman Coulter, Inc., Pacific Biosciences, Inc., Oxford Nanopore, Inc., and/or their affiliates." EX1005, ¶[00157].

187. So-456's Figure 2 further specifies that "the sequence data is analyzed . . . and the spatial information is decoded" (Step 235). EX1005, ¶[00158]. The decoded spatial information is "used to provide information as to the location of the nucleic acid in the tissue section" (*i.e.*, spatially detecting at least one target analyte in a first and second location of the tissue sample, as claimed). EX1005, ¶¶[0089], [00158]. To achieve this, the "sequence-identical nucleic acid molecules originating from *different* regions in a tissue sample can be distinguished based on their spatial address and can be *mapped onto their regions of origin* in the tissue sample." EX1005, ¶[0089]. So-456 further teaches that its methods "enable the identification of *the location of a cell or a cell cluster* in a tissue" sample. EX1005, ¶[0002], [0088].

188. Accordingly, So-456's method of Figures 8-9 includes sequencing the extension products to determine their sequence and sorting the sequencing data to determine the location of the target mRNAs in the tissue sample. As such, So-456's method identifies the extension products by sequencing them, thereby spatially detecting the target analytes in the tissue sample. Thus, in my opinion, So-456's method includes claim 1's sequencing step under NanoString's interpretation.

189. For the reasons I discuss above, So-456 teaches every element of claim 1 under NanoString's interpretation, arranged as claimed. I understand that So-456's

methods are presumed enabled. Thus, in my opinion, So-456 anticipates claim 1 under NanoString's interpretation.

c. Claims 2-8, 11-15, 17-23, and 26-30

190. Claims 2-8, 11-15, 17-23, and 26-30 depend directly or indirectly from claim 1 or claim 16, and further specify the elements of claim 1 or claim 16. I understand that the dependent claims incorporate all the elements of the claims from which they depend.

i. Claims 2, 3, 17, and 18

191. **Claims 2 and 17** depend from claims 1 and 16, respectively, and further require that "the tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample." EX1001, 147:12-13, 148:48-49. **Claims 3 and 18** depend from claims 1 and 16, respectively, and further require that "the tissue sample is immobilized onto a microscope slide." EX1001, 147:14-15, 148:50-51. So-456 discloses the elements of claims 2, 3, 17, and 18 under NanoString's interpretation.

192. So-456 discloses that a tissue sample "refers to a piece of tissue that has been obtained from a subject, fixed, sectioned, and mounted on a planar surface, e.g., a *microscope slide*." EX1005, ¶[00125] (emphasis added). So-456 also discloses that a tissue sample can be "a *formalin-fixed paraffin-embedded (FFPE)* tissue sample." EX1005, ¶[00125] (emphasis added). In describing Figure 2's method, So-456 explains that in Step 210, "the tissue sample is a *FFPE tissue*

sample that is sectioned onto a *slide*." EX1005, ¶[00152] (emphasis added). Thus, So-456 discloses the elements of claims 2, 3, 17, and 18 under NanoString's interpretation.

ii. Claims 4 and 19

193. Claims 4 and 19 depend from claims 3 and 18, respectively, and further require that "the microscope slide comprises a plurality of primers immobilized on the microscope slide." EX1001, 147:16-18, 148:52-54. So-456 discloses the elements of claims 4 and 19 under NanoString's interpretation.

194. So-456 explains that in the method of Figures 8-9, "the tissue sample is contacted with capture probe 115 that is *fixed on the surface* of capture site 105." EX1005, ¶[00183] (emphasis added). So-456 further discloses that "*any number* of capture probes 115 can be *immobilized* on solid support 110 at each capture site 105" and that the solid support has "an array of capture sites, such as capture sites 105 of capture array 100 shown in Figure 1A and Figure 1B" include "a plurality of capture probes." EX1005, ¶¶[00141] (emphasis added), [00154], FIG. 1. Thus, So-456 discloses the elements of claims 4 and 19 under NanoString's interpretation.

iii. Claims 5 and 20

195. Claims 5 and 20 depend from claims 4 and 19, respectively, and further require that "the plurality of primers is immobilized on the microscope slide at

their 5' ends." EX1001, 147:20-21, 148:55-57. So-456 discloses the elements of claims 5 and 20 under NanoString's interpretation.

196. So-456 discloses that the "[o]ligonucleotides to be used as *capture primers* . . . can be *immobilized* such that a *3'-end is available* for enzymatic extension." EX1005, ¶[00109] (emphasis added). Because the 3' end of the capture primers is free to allow an extension reaction at that end, a POSA would understand that the primers can be immobilized on the surface at their other end, *i.e.*, the 5' end. Thus, So-456 discloses the elements of claims 5 and 20 under NanoString's interpretation.

iv. Claims 6 and 21

197. Claims 6 and 21 depend from claims 5 and 20, respectively, and further require that the extension steps "comprise performing a solid-phase amplification reaction" on the "microscope slide using the plurality of primers immobilized on the microscope slide." EX1001, 147:22-26, 148:58-62. So-456 discloses the elements of claims 6 and 21 under NanoString's interpretation.

198. So-456 discloses that the oligonucleotides immobilized on the microscope scope slide can be used as "capture primers or *amplification* primers." EX1005, ¶[00109] (emphasis added). A POSA would understand this to mean that the capture probes immobilized on the array contain a primer sequence that can be used for an amplification reaction on the array surface. So-456 similarly specifies

that the "[l]ibrary preparation may be accomplished *on the capture array substrate*." EX1005, ¶[00156] (emphasis added). Indeed, So-456's capture probes (115) contain an "SBS primer region 125" that "can also be used in an amplification reaction to generate a sequencing library." EX1005, ¶[00141]. Accordingly, So-456's method includes a solid-phase amplification. Thus, So-456 discloses the elements of claims 6 and 21 under NanoString's interpretation.

v. Claims 7 and 22

199. Claims 7 and 22 depend from claims 1 and 16, respectively, and further require that "the sequencing step is performed using a next generation sequencing reaction." EX1001, 147:27-28, 148:63-64. So-456 discloses the elements of claims 7 and 22 under NanoString's interpretation.

200. So-456 discloses that in its method the "spatially-tagged DNA molecules are released from the array and analyzed, for example, by high throughput *next generation sequencing* (NGS), such as sequencing-by-synthesis (SBS)." EX1005, ¶[00127] (emphasis added). Accordingly, So-456's method includes using next-generation sequencing. Thus, So-456 discloses the elements of claims 7 and 22 under NanoString's interpretation.

vi. Claims 8 and 23

201. Claims 8 and 23 depend from claims 1 and 16, respectively, and further require "amplifying a library using the first plurality of extension products and the

second plurality of extension products as templates." EX1001, 147:29-31, 148:65-67. So-456 discloses the elements of claims 8 and 23 under NanoString's interpretation.

202. So-456 discloses that its capture probes comprising a "spatial address region 130" also comprise an "SBS [sequencing-by-synthesis] *primer binding site 125.*" EX1005, ¶¶[00141] (emphasis added), [00182]. So-456 further specifies that an SBS primer binding site "can also be used in an *amplification reaction* to generate a sequencing library." EX1005, ¶[00141] (emphasis added). Particularly, the "SBS primer region 125 can comprise an SBS primer sequence (e.g., SBS12 or SBS3)" that can be used to amplify the extension product using the extension product as template. EX1005, ¶[00141]. Accordingly, So-456's method amplifies a sequencing library using the extension products as template. Thus, So-456 discloses the elements of claims 8 and 23 under NanoString's interpretation.

vii. Claims 11, 12, and 27

203. **Claims 11 and 12** depend from claim 1, and **claim 27** depends from claim 16, and further require that the "nucleic acid sequence" that "identifies the first location" (claim 11), "second location" (claim 12), and "first location," "second location," or "both" first and second locations (claim 27) of the tissue sample comprise "at least one amplification primer binding site." EX1001, 147:40-47, 149:18-150:9. A POSA would understand that these claims require that the

oligonucleotides comprising a spatial barcode (that identifies a location of the tissue sample) also comprise "at least one amplification primer binding site." So-456 discloses the elements of claims 11, 12, and 27 under NanoString's interpretation.

204. So-456 discloses that the capture probes immobilized on the array surface containing a spatial barcode (*i.e.*, a sequence that identifies the location of the tissue sample) also contain an "SBS primer binding site" that can be used as an amplification primer binding site for amplifying a sequencing library:

> *Capture probe 115* may optionally comprise a cleavable sequence in a cleavable region 120, *an SBS primer sequence in a SBS primer binding site 125*, a spatial address sequence in a spatial address region 130, and a capture sequence in a capture region 135. . . . *SBS primer region 125* can also be *used in an amplification reaction to generate a sequencing library* as described in more detail with reference to Figure 12 and Figure 13.

EX1005, ¶[00141] (emphasis added).

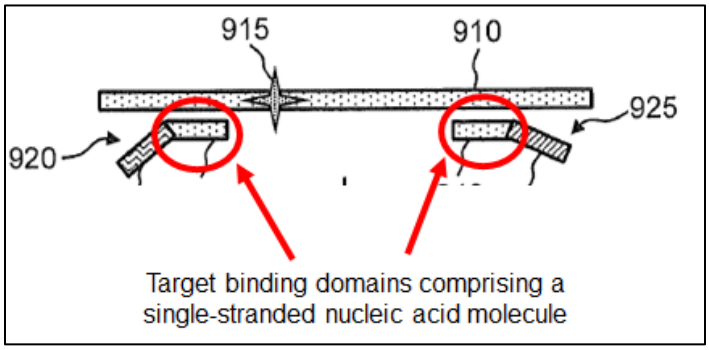
205. Accordingly, So-456 discloses the elements of claims 11, 12, and 27 under NanoString's interpretation.

viii. Claims 13 and 28

206. Claims 13 and 28 depend from claims 1 and 16, respectively, and require that "the target binding domains" of the nucleic acid probes (claim 13) and each of

the first and second plurality of nucleic acid probes (claim 28) "comprise[s] a single-stranded nucleic acid molecule." EX1001, 147:48-49, 150:10-13. So-456 discloses the elements of claims 13 and 28 under NanoString's interpretation.

207. As I discuss above for claims 1 and 16, So-456's method uses pairs of gene-specific capture oligonucleotide probes comprising gene-specific regions (*i.e.*, target-binding domains) that bind to their complementary sites on the target DNA. A POSA would understand that in order for So-456's probe pairs to hybridize to the target DNA, the target-binding domains of the probes must comprise a single-stranded region. Accordingly, So-456's method uses probes comprising a single-stranded target-binding domain, as illustrated in the annotated excerpt from Figure 9 below.



EX1005, FIG. 9 (annotated).

208. Thus, So-456 discloses the elements of claims 13 and 28 under NanoString's interpretation.

ix. Claims 14 and 29

209. Claims 14 and 29 depend from claims 1 and 16, respectively, and further require that the collecting steps (b)-(c) (claim 14) and (c)-(d) (claim 29) are "performed simultaneously." EX1001, 147:50-51, 150:14-15. So-456 discloses the elements of claims 14 and 20 under NanoString's interpretation.

210. So-456 teaches that its "[c]apture array 100 comprises an arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110." EX1005, ¶[00141]. So-456 specifies simultaneously capturing (*i.e.*, collecting) at different locations the amplified ligated probes (amplicons 950) when the probes are "transferred *onto an array and captured* by hybridization to universal capture regions on *the array*." EX1005, ¶[00181] (emphasis added); *see also* EX1005, ¶[00182] ("DNA amplicons 950 are captured on the capture site 105"). A POSA would understand this to mean that So-456 performs the steps of the method simultaneously at different capture sites of the array, which correspond to different locations (*i.e.*, a first and second location) of a tissue sample. Thus, So-456 discloses the elements of claims 14 and 29 under NanoString's interpretation.

x. Claims 15 and 30

211. Claims 15 and 30 depend from claims 1 and 16, respectively, and further require that extension steps (d)-(e) (claim 15) and (e)-(f) (claim 30) are "performed

simultaneously." EX1001, 147:52-53, 150:16-17. So-456 discloses the elements of claims 15 and 30 under NanoString's interpretation.

212. So-456 discloses that the "capture primers" immobilized at the capture sites on an array are "available for *enzymatic extension*." EX1005, ¶[00109] (emphasis added), [00141], FIG. 1. So-456 further specifies that the spatial barcodes "can be *encoded in the nucleic acid* in the process of preparing the nucleic acid for sequencing." EX1005, ¶[0089] (emphasis added). Thus, So-456's method performs the extension step at multiple capture sites on the array. Accordingly, So-456 discloses the elements of claims 15 and 30 under NanoString's interpretation.

xi. Claim 26

213. Claim 26 depends from claim 16, and further requires that the "nucleic acid probes" in the "first plurality," "the second plurality," or "both" "comprise an amplification primer binding site." EX1001, 149:9-17. So-456 discloses the elements of claim 26 under NanoString's interpretation.

214. So-456 discloses that the pairs of capture oligonucleotide pairs (920, 925) (*i.e.*, nucleic acid probes of the claim) comprising a gene-specific region (930 or 940) (*i.e.*, target binding domain of the claim) used in Figures 8-9's method also comprise "universal capture region 935" or an "SBS primer region 945 (e.g., SBS12)." EX1005, ¶[00182].

215. So-456 explains that the amplicons (950) (generated by ligating the capture oligonucleotides) bound to the capture probes of the array are "amplified by in situ isothermal amplification to generate multiple copies (multiple amplicons) of the targeted region of interest," and that the isothermal amplification "can be performed, for example**,** using a *primer region 935a* that is complementary to universal capture region 935 and a *primer region 945a* that is complementary to SBS primer region 945." EX1005, ¶[00182] (emphasis added). Accordingly, the universal capture region and SBS primer region in So-456's capture oligonucleotide pairs (*i.e.*, nucleic acid probes of the claim) comprise an amplification primer binding site. Thus, So-456 discloses the elements of claim 26 under NanoString's interpretation.

216. For these reasons, So-456 teaches every element of claims 2-8, 11-15, 17-23, and 26-30, arranged as claimed, under NanoString's interpretation. I understand that So-456's methods are presumed enabled. Thus, in my opinion, So-456 anticipates claims 2-8, 11-15, 17-23, and 26-30 under NanoString's interpretation.

X. GROUND 2: CLAIMS 1-30 WOULD HAVE BEEN OBVIOUS OVER WO 2017/019456 (SO-456)

217. As I explain above, So-456 teaches every element of claims 1 and 16 under NanoString's interpretation, including the extension steps 1(d)-1(e) and 16(e)-16(f), and thus So-456 anticipates the claims. However, even assuming that

So-456 does not teach the extension steps, claims 1 and 16 and their dependent claims 2-15 and 17-30 would have been obvious over So-456 under NanoString's interpretation for the reasons I explain below.

a. Claims 1 and 16

218. A "nucleic acid sequence that identifies the first [and second] location of the tissue sample" in the claim is a spatial barcode (as used in this declaration). Thus, the claims require an extension reaction that incorporates a spatial barcode into the ligated probes collected in the previous step, forming extension products that comprise the ligated probes and the spatial barcode.

219. So-456 discloses that its methods are based on a "realization that information related to the spatial origin of a nucleic acid in a tissue sample can be *encoded in the nucleic acid[s]* in the process of *preparing* the nucleic acid for sequencing." EX1005, ¶[0089] (emphasis added). To accomplish this, So-456 discloses that the "nucleic acids from a tissue sample can be tagged by probes including location-specific sequence information (a 'spatial address')." EX1005, ¶[0089]. Indeed, So-456 discloses that, in some instances, two or three "spatial address sequences are incorporated into a nucleic acid during preparation of a sequencing library." EX1005, ¶¶[00131], [00132].

220. Thus, 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. In other words, a POSA would have known that in So-456's methods both the spatial barcode (of the capture probe) and the sequence corresponding to the target nucleic acid must be incorporated into the same nucleic acid molecule for decoding the spatial information from the sequence data.

221. So-456's Figure 2 (which illustrates the overall workflow of So-456's methods) specifies that after the nucleic acids have been captured on the array (Step 220), a "sequencing library is prepared" (Step 225) for use in a subsequent sequencing step (Step 230) and an analysis and decoding step (Step 235). EX1005, ¶[00156]. Thus, a POSA would have known that So-456's methods require (1) encoding the "spatial origin of a nucleic acid in a tissue sample" in "the process of preparing the nucleic acid for sequencing," and (2) performing sequencing to obtain data that is "analyzed . . . and the spatial information is decoded." EX1005, ¶¶[0089], [00131], [00132], [00158].

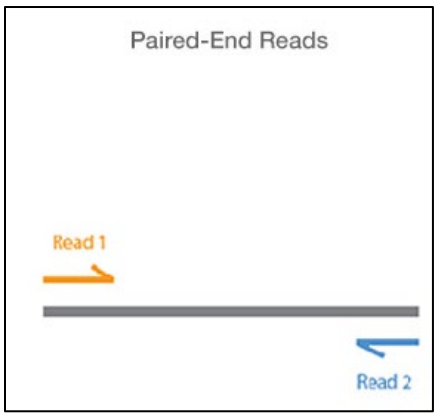
222. However, So-456's amplicons (950) captured on the arrays in the previous step had not yet been tagged with a spatial barcode and do not include the functional sequences (such as, a pair of SBS primer-binding sites) necessary for performing sequencing (such as, sequencing-by-synthesis (SBS)) in the subsequent steps. Thus, a POSA would have known that during sequencing library preparation (Step 225) (which is the only remaining step before the sequencing step (Step 230)), So-456's method generates molecules containing the target nucleic acid

sequence, a spatial barcode, and sequencing primer-binding sites that are ready for sequencing, analysis, and decoding in the subsequent steps (Steps 230, 235). EX1005, ¶¶[00156]-[00158], FIG. 2.

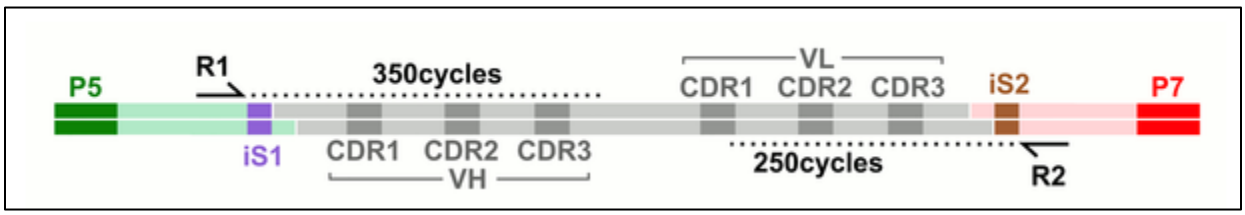
223. So-456's capture probes comprise a unique "spatial address region 130" containing a "spatial address sequence" (*i.e.*, spatial barcode). EX1005, ¶[00141], [0099], FIG. 1. The spatial address region (130) "corresponds to the position of capture probe 115 in capture array 100," which in turn "can be correlated with a position in the tissue sample." EX1005, ¶[00141]; *see also* EX1005, ¶[0099] ("spatial address" means "an address, tag or index encoding spatial information related to the region or location of origin of an addressed, tagged, or indexed nucleic acid in a tissue sample").

224. Additionally, So-456 (which was filed by Illumina) teaches using SBS (sequencing-by-synthesis), *e.g.*, "developed by [] Illumina, Inc." EX1005, ¶¶[00156]-[00157], [00158], FIG. 2. The components and methods for sequencing by Illumina method and for generating the corresponding sequencing libraries were well known prior to February 2018. Illumina's SBS method utilizes a pair of SBS primers that initiate the incorporation of fluorescently labeled reversible terminator nucleotides into the molecules generated for sequencing. EX1030; EX1005, ¶¶[00141], [00199], [00201], [00211], [00229], [00496]-[00497].

225. SBS platforms standardly allow for paired-end sequencing. Paired-end sequencing allows the users to sequence from both ends of a sequencing library molecule in order to generate more accurate reads and to detect error-prone regions such as genomic rearrangements and repetitive sequence elements. EX1007. In paired-end sequencing, the molecules include primer-binding sites (commonly referred to as "read sequences") "read 1" (R1) and "read 2" (R2) at its opposite ends:



226. For SBS to allow for paired-end sequencing, it utilizes a pair of particular sequencing primers, *e.g.*, "SBS12" and "SBS3," which are complementary to primer-binding sites, R1 and R2, as shown in the figure below.



EX1030 ("The forward read (R1) uses SBS3 sequencing primer (Illumina), while the reverse read (R2) uses SBS12 primer (Illumina).").

227. The method of Figures 8-9 uses the Illumina SBS platform for sequencing (reflected by the inclusion of the sequencing primer-binding sites in the captured amplicons (950) and the capture probes). However, the amplicons (950) captured on the array in So-456's method include the binding site for only one SBS primer ("SBS primer region 945 (e.g., SBS12)"). EX1005, ¶¶[00182], [00141]. The other primer-binding site ("SBS primer region [] SBS3") is included in the capture probes immobilized on the array. *See*, *e.g.*, EX1005, ¶¶[0141] ("SBS primer region 125 [] SBS3"), [00182] ("SBS primer region 130 [] SBS3"), FIG. 1, FIG. 9.

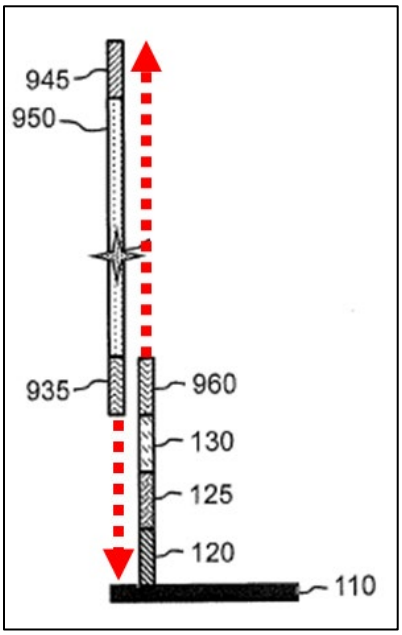
228. Thus, a POSA would have known that for tagging the oligonucleotides captured on the array with the spatial barcode of the capture probes and for incorporating both SBS primer-binding sites in the same molecule, So-456's method must extend the capture probes (using the captured ligated probes as template) and the captured ligated probes (using the capture probes as template). A POSA would have known that this generates a molecule comprising the captured amplicon (containing the first primer-binding site) and the capture probe (containing the spatial barcode and the second primer-binding), which can then be used in the subsequent sequencing, decoding, and analyzing steps.

229. So-456 discloses that the "[o]ligonucleotides to be used as *capture primers* . . . can be immobilized such that a *3'-end is available for enzymatic*

extension." EX1005, ¶[00109] (emphasis added). So-456 does not disclose any blocking or other modifications to the 3'-end of the captured oligonucleotides that are extended in its methods[4], allowing extension of both the 3' end of the captured amplicons and 3' end of the capture probes in the method of Figures 8-9. EX1005, ¶¶[00109], [00182]-[00183], FIGs. 8, 9. This is because extension enzymes, such as DNA polymerase, automatically extend from all available 3'-ends. Thus, due to the nature of the extension enzymes and the presence of unblocked and unmodified 3' ends, So-456's extension reaction necessarily proceeds in *both* directions—extending the 3'-end of the capture probe in one direction and the 3'-end of the captured ligated probe in the other direction. EX1005, ¶¶[00109], [00182]-[00183], FIG. 9. Indeed, So explains that when the capture probe is "used as a primer," the captured "DNA molecule [] is copied," and when the captured DNA molecule is "used as a primer," the "spatial address region 130" is copied. EX1005, ¶[00173], FIG. 6. So-456 confirms that "*[b]oth extension products* can be used for *downstream library generation*." EX1005, ¶[00173] (emphasis added).

[4] In contrast, So-456's other disclosures specify modifying the 3' end of a nucleic acid, e.g., to prevent 3' end ligation or further nucleotide incorporation during sequencing. *See e.g.,* EX1005, ¶¶[00484], [00491], [00601], FIGs. 48-49.

230. Thus, So-456 suggests the enzymatic extension of the 3'-end of the capture probes and the 3'-end of the ligated probe to incorporate a spatial barcode, a pair of SBS primer-binding sites, and the ligated probes into the same molecule during library preparation. I have illustrated this extension reaction in the annotated excerpt of Figure 9.



EX1005, FIG. 9 (annotated).

231. As shown above in the figure, So-456's extension reaction forms extension products comprising the sequences of the captured ligated probe (950), spatial barcode (130), and the two primer-binding sites (945, 125)necessary for sequencing. EX1005, ¶¶[00141], [00182], FIG. 9. EX1005, ¶¶[00141], [00182], FIG. 9.

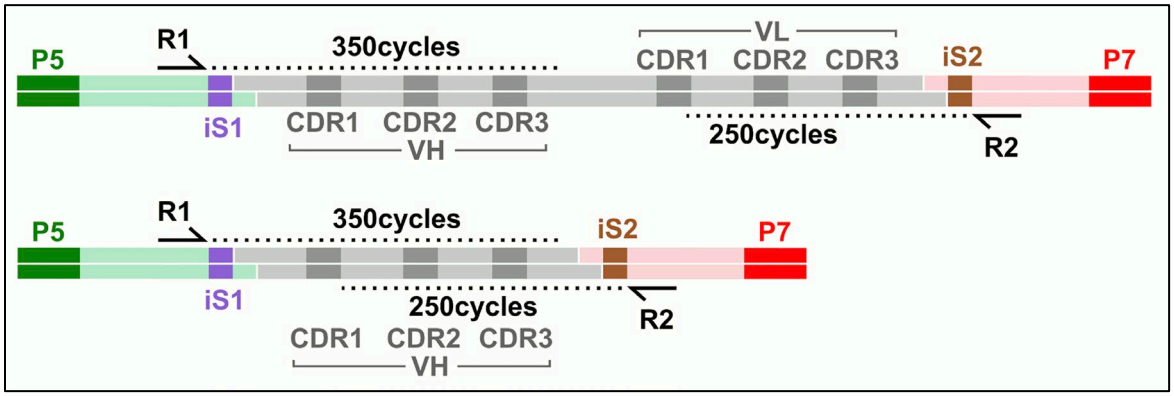
232. Thus, even if So-456 does not disclose performing an extension step (it does) in its method, So-456 still suggests doing so, thereby forming extension products comprising the sequences of the ligated probe, the spatial barcode, and the two sequencing primer-binding sites required to prepare a sequencing library ready for SBS. Accordingly, So-456 teaches or suggests all the elements of claims 1 and 16.

233. Further, a POSA would have been motivated to make obvious changes to So-456's method to perform the claimed extension reaction steps with a reasonable expectation of success.

i. Motivation

234. In my opinion, a POSA would have been motivated to practice the claimed extension step in So-456's method in Figures 8-9. This is because So-456's methods create a sequencing library of molecules that are (i) tagged with spatial barcodes and (ii) include the necessary pair of sequencing primer-binding sites for SBS. EX1005, ¶¶[0002], [0089], [0092], [00137], [00141]. The use of an extension reaction to extend So-456's captured amplicons (950) or capture probes was a well-known, obvious, and routine technique for incorporating the spatial barcode (and necessary second sequencing primer) into the resulting extension reaction products to produce the desired finished sequencing library. EX1005, ¶[00173].

235. So-456 teaches the use of a pair of Illumina SBS forward and reverse sequencing primers—SBS3 and SBS12. EX1005, ¶¶[00141], [00199], [00201], [00211], [00229], [00496]-[00497]. A POSA would know that SBS3 and SBS12 are the commercially available forward and reverse (respectively) sequencing primers used in Illumina SBS method. As I discuss above, this is confirmed by art from the time:



EX1030, Fig. 1 ("The forward read (R1) uses SBS3 sequencing primer (Illumina), while the reverse read (R2) uses SBS12 primer (Illumina).").

236. So-456 also teaches the use of commercially available Illumina sequencing library preparation kits, tools, and SBS sequencers for use in So-456's disclosed methods. EX1005, ¶¶[00127], [00156]-[00157], [00611]. It was known that to sequence a library on an Illumina sequencer (e.g., MiSeq, EX1005, ¶[00611]), the sequences in that library have to each include the pair of Illumina sequencing primers (SBS3 and SBS12). EX1037, 5-6 (discussing benefits of Illumina's paired-end sequencing and the need for a pair of both forward and

reverse primers). Thus, So-456 teaches including a first sequencing primer-binding site (945, *e.g*. SBS12) on the captured amplicon (i.e., ligated probe of the claims) and a second sequencing primer-binding site of the pair (125, *e.g*. the complement of SBS3) on the capture probe—so that an extension reaction will incorporate both primer-binding sites (and the spatial barcode) into the products of the extension reaction. EX1005, FIG. 9. So-456 emphasizes the importance of incorporating the pair of sequences into finished library so it can be sequenced. EX1005, *e.g*., ¶¶[00199], [00201], [00211], [00229], [00266], [00496]-[00497], [00611].

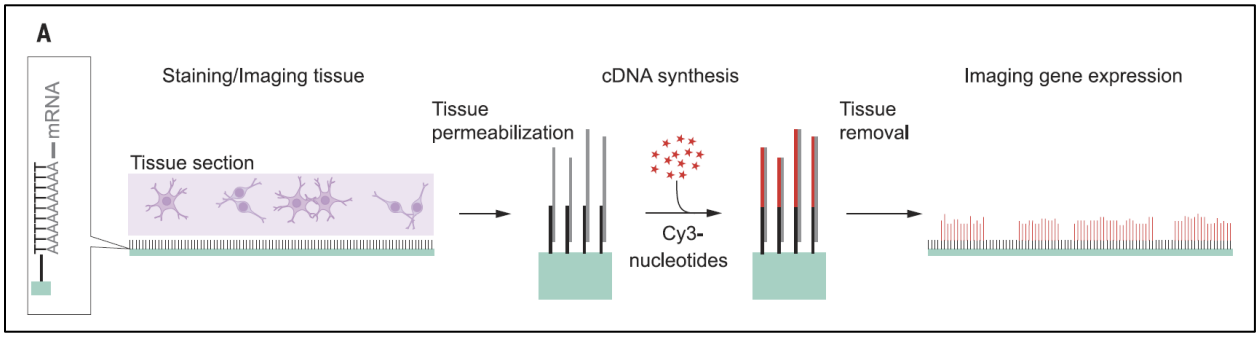
ii. Reasonable expectation of success

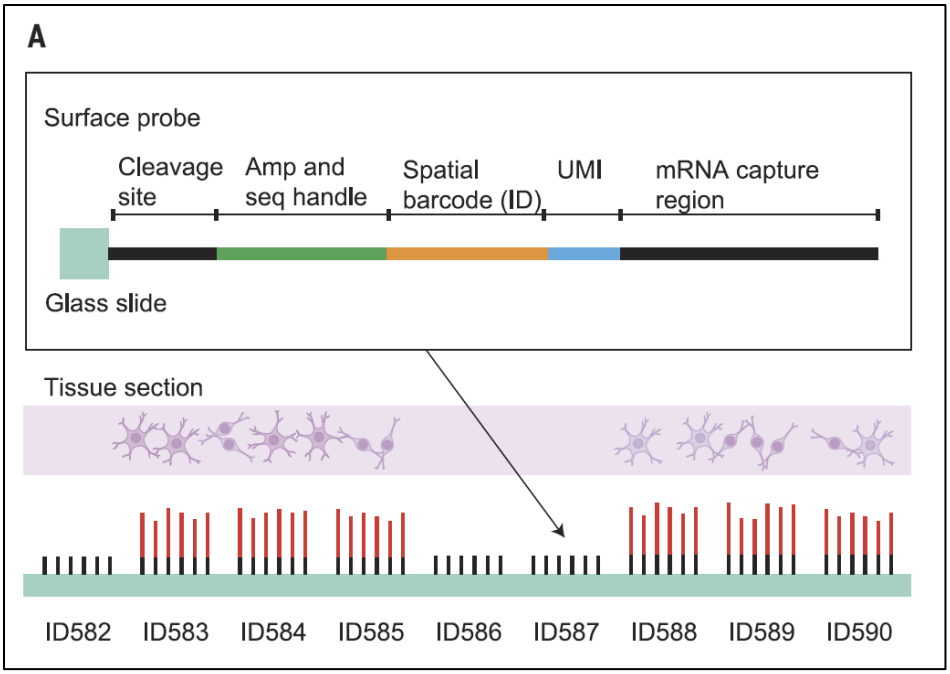
237. In my opinion, a POSA would have had a reasonable expectation of successfully practicing the methods of claim 1 and 16 by including the claimed extension steps in So-456's method. This is because (i) So-456 provides ample guidance and direction as I explain above, and (ii) the use of extension reactions to extend the captured amplicons (950) (i.e., ligated probes of the claims) and the capture probes in So-456 to create the spatially tagged sequencing library was common in the art and required only routine skill.

238. So-456 confirms extension reactions were known, including for preparation of SBS sequencing libraries. EX1005, ¶¶[00109] ("capture primers . . . available for enzymatic extension"), [00595]. So-456 also teaches that Illumina provided multiple commercially available products for conducting sequencing

library preparation reactions and subsequent sequencing. EX1005, ¶¶[00127], [00137], [00156]-[00157], [00611]. By the time of So-456 (and the 689 patent), Illumina sequencers were the most well-known and widely used SBS solutions in the art. The use of laboratory protocols (or commercial solutions) for such library preparation extension reactions was routine for a POSA.

239. Stahl 2016 also confirms that extension reactions using immobilized capture probes as primers had been successfully performed in the prior art (further confirming a reasonable expectation of success of performing extension reaction steps using So-456's method). EX1016, FIGs. 1A, 2A. Stahl 2016's "spatial resolution" method included an extension reaction (referred to as "cDNA synthesis" in the figure below) to tag captured target nucleic acids with a spatial barcode:





EX1016, FIGs. 1A, 2A, Abstract.

240. Thus, a POSA would have been motivated to perform the methods of claims 1 and 16 with a reasonable expectation of success. I understand that NanoString has not alleged any objective evidence of non-obviousness. Thus, in my opinion, claims 1 and 16 would have been obvious over So-456 under NanoString's interpretation.

b. Claims 2-8, 11-15, 17-23, and 26-30

241. As I explain above, So-456 discloses each and every element of claims 2-8, 11-15, 17-23, and 26-30 under NanoString's interpretation. For the reasons I explain above for claims 1 and 16, a POSA would have been motivated to practice the methods of claims 2-8, 11-15, 17-23, and 26-30 with a reasonable expectation

of success. Thus, in my opinion, claims 2-8, 11-15, 17-23, and 26-30 would have also been obvious over So-456 under NanoString's interpretation.

c. Claims 9, 10, 24, and 25

242. **Claims 9-10** depend from claim 1, and **claims 24-25** depend from claim 16. Claims 9-10 and 24-25 further require that "the at least one nucleic acid sequence that identifies the first location" (claims 9 and 24) or "second location" (claims 10 and 25) "of the tissue sample comprises *at least one unique molecular identifier*." EX1001, 147:32-38, 149:1-8 (emphasis added). For the reasons I explain below, claims 9, 10, 24 and 25 would have been obvious over So-456 under NanoString's interpretation.

243. A "nucleic acid sequence that identifies the first [or second] location of the tissue sample" in the claims is a spatial barcode (as used in this declaration). Thus, claims 9, 10, 24, and 25 require a nucleic acid sequence comprising both a spatial barcode and "at least one unique molecular identifier."

244. So-456's capture probes comprise a "[s]patial address region 130 [that] corresponds to the position of capture probe 115 in capture array 100." EX1005, ¶[00141], FIG. 9. So-456 also suggests including a unique molecule identifier (UMI) in its capture probes.

245. Although the method of Figures 8-9 does not use capture probes that include a unique molecular identifier (UMI), its "capture oligonucleotides" (920,

925) (*i.e.*, nucleic acid probe pairs of the claims that bound to the target analyte) include a "UMI region." EX1005, ¶[00182]. Moreover, So-456 discloses other methods that include a UMI in the capture probes comprising a spatial barcode. For example, So-456's method of Figures 23A and 23B uses capture probes that "include … a spatial address region 2325, *a unique molecular identifier (UMI)* 2330, and a capture region 2335." EX1005, ¶[00302] (emphasis added), FIG. 23. Thus, So-456 suggests using a UMI in its methods, and it suggests using capture probes comprising both a UMI and a spatial barcode.

i. Motivation

246. A POSA would have been motivated to modify So-456's method of Figures 8-9 to include a UMI in the capture probes. ***First***, the use of UMIs was generally well known. *See, e.g.,* EX1019, Fig. 1A, 72; EX1016, Fig. S5; EX1031; EX1032; EX1033; EX1034; EX1035; EX1036. It was well known to a POSA that UMIs incorporate a unique barcode onto each molecule within a given sample library and by using UMIs on each original DNA fragment, variant alleles present in the original sample (true variants) can be distinguished from errors introduced during library preparation, target enrichment, or sequencing.

247. ***Second***, So-456 already discloses that UMI sequences can be incorporated during two separate workflow steps so that it is incorporated into the final product to be sequenced: (1) as part of the primary probe (in the method of

Figures 8-9) or (2) as a part of the capture probe (along with the spatial barcode) as described in Figures 23A and 23B (capture probes that "include … a spatial address region 2325, *a unique molecular identifier (UMI)* 2330, and a capture region 2335"). EX1005, ¶[00302], FIG. 23. Thus, So-456 expressly teaches including UMIs in capture probes comprising a spatial barcodes in other methods (other than the method of Figures 8-9) for spatially detecting target analytes.

248. ***Third***, So-456 discloses that its methods can be modified, stating that "[o]ther embodiments having different structures and operations do not depart from the scope of the present disclosure" and "various details of the present disclosure can be changed without departing from the scope of the disclosed embodiments." EX1005, ¶[00612]. Thus, a POSA would have understood that the incorporation of the UMI in different examples was intended to be exemplary and was flexible.

249. Additionally, So-456 recognized that "[t]he present disclosure is further based, in part, on the realization that the robustness and data quality of spatial transcriptomics experiments can be enhanced by facilitating the transfer of nucleic acids from a tissue sample onto a capture array, e.g., a capture array of spatially addressed capture probes."). EX1005, ¶[0093]. A POSA reading So-456 interested in spatial transcriptomics would have been aware of Stahl 2016's spatial

transcriptomics method, including that the UMI was incorporated in the capture array probes in Stahl 2016.

250. Because the use of UMIs was well known in the art, So-456 discloses the possibility of UMIs in only two locations: (1) in the capture probes adjacent to the spatial barcodes and (2) within the primary probe. A POSA interested in spatial transcriptomics would have been aware of a spatial transcriptomics article published in Science where UMIs were included in the capture probes (Stahl 2016; EX1016), it would have been obvious to try to incorporate the UMI in the array probe containing the spatial barcode like Stahl 2016.

251. Thus, a POSA would have been motivated to include a UMI with a spatial barcode in the capture probes.

ii. Reasonable expectation of success

252. A POSA would have reasonably expected to successfully modify So-456's method of Figures 8-9 to include a UMI and a spatial barcode in the capture probe. This is because So-456 discloses other embodiments that successfully used the same approach. EX1005, ¶[00302], FIG. 23. Additionally, the spatial detection methods in the prior art successfully included a UMI with a spatial barcode sequence in the immobilized capture probes. EX1016, FIG. 1A. For example, Stahl 2016 explained that the UMI in the capture probes enabled successful

"amplification duplicate removal" via "UMI-filtering." EX1016, Suppl. Materials, 21, FIG. S5.

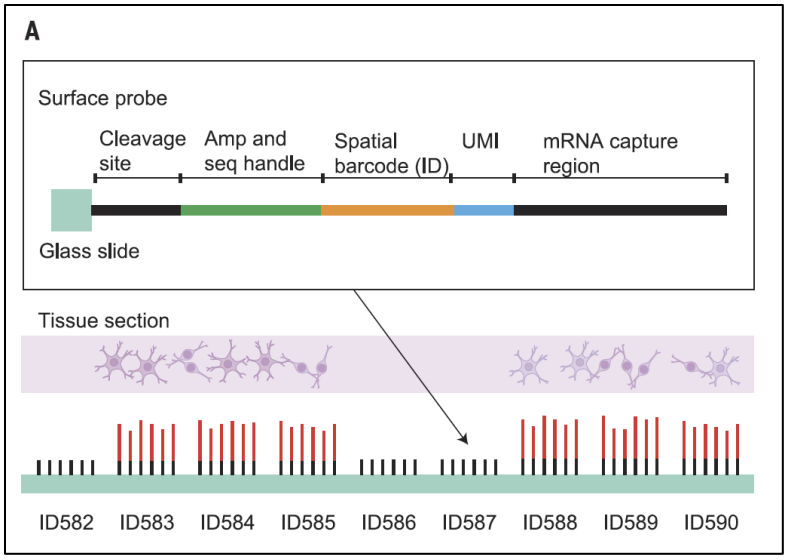
253. Thus, a POSA would have been motivated to perform the methods of claims 9, 10, 24, and 25 with a reasonable expectation of success.

254. I understand that NanoString has not alleged any objective evidence of non-obviousness. Thus, in my opinion, claims 9, 10, 24, and 25 would have been obvious over So-456 under NanoString's interpretation.

XI. GROUND 3: CLAIMS 9-10 AND 24-25 WOULD HAVE BEEN OBVIOUS OVER WO 2017/019456 (SO-456) AND STAHL 2016

255. As discussed above for Grounds 1 and 2, So-456 either anticipates claims 1-8, 11-15, 17-23, and 26-30 or it renders obvious claims 1-30, including claims 9-10 and 24-25. Even assuming So-456 alone does not render claims 9-10 and 24-25 obvious, it renders them obvious in combination with Stahl 2016.

256. So-456 and Stahl 2016 together teach or suggest including a UMI with a spatial barcode in the capture probes of So-456's method of Figures 8-9s. As I explain above, Stahl 2016's "spatial resolution" method uses arrays of capture probes in spots or features. EX1016, 78, FIG. 2A. Each capture probe on Stahl 2016's array contains a spatial barcode and a UMI as shown in Figure 2A below. EX1016, 78, FIG. 2A.



EX1016, FIG. 2A.

257. After reverse transcription (cDNA synthesis), the spatial barcodes and UMIs are incorporated into the extension products (cDNA), and the barcoded extension products are then amplified, generated into a sequencing library, and then sequenced. Once sequenced, the spatial barcodes provide the information to identify the corresponding spots from where the gene transcript came. And the UMIs provide a sequence to identify how many true unique transcripts there are in each spot to "provide quantitative gene expression data." EX1016, Abstract.

258. Stahl 2016 discloses the results of its transcriptomics method, such as, Figure S5 that provides a comparison of gene expression data by ST's method to that in an ISH database and includes in (A) "distributions of unique transcripts captured per feature (UMI-filtered) under the tissue during an experiment on the

Spatially barcoded array." EX1016, FIG. S5. Stahl 2016's Figures S4 and S6 also provide the average number of "unique transcripts." EX1016, FIG. S4, S6.

a. Motivation

259. A POSA would have been motivated to modify the method of So-456's Figures 8-9 to include a UMI sequence in the capture probe as in Stahl 2016's capture probe.

260. ***First***, the use of UMIs was generally well known. *See, e.g.,* EX1019, Fig. 1A, 72; EX1016, Fig. S5; EX1031; EX1032; EX1033; EX1034; EX1035; EX1036. It was well known to a POSA that UMIs incorporate a unique barcode onto each molecule within a given sample library and by using UMIs on each original DNA fragment, variant alleles present in the original sample (true variants) can be distinguished from errors introduced during library preparation, target enrichment, or sequencing.

261. ***Second***, So-456 already discloses that UMI sequences can be incorporated during two separate workflow steps so that it is incorporated into the final product to be sequenced: (1) as part of the primary probe (in the method of Figures 8-9) or (2) as a part of the capture probe (along with the spatial barcode) as described in Figures 23A and 23B (capture probes that "include … a spatial address region 2325, *a unique molecular identifier (UMI)* 2330, and a capture region 2335"). EX1005, ¶[00302], FIG. 23. Thus, So-456 expressly teaches

including UMIs in capture probes comprising a spatial barcodes in other methods (other than the method of Figures 8-9) for spatially detecting target analytes.

262. ***Third***, So-456 discloses that its methods can be modified, stating that "[o]ther embodiments having different structures and operations do not depart from the scope of the present disclosure" and "various details of the present disclosure can be changed without departing from the scope of the disclosed embodiments." EX1005, ¶[00612]. Thus, a POSA would have understood that the incorporation of the UMI in different examples was intended to be exemplary and was flexible.

263. Additionally, So-456 recognized that "[t]he present disclosure is further based, in part, on the realization that the robustness and data quality of spatial transcriptomics experiments can be enhanced by facilitating the transfer of nucleic acids from a tissue sample onto a capture array, e.g., a capture array of spatially addressed capture probes."). EX1005, ¶[0093]. A POSA reading So-456 interested in spatial transcriptomics would have been aware of Stahl 2016's spatial transcriptomics method, including that the UMI was incorporated in the capture array probes in Stahl 2016.

264. Because the use of UMIs was well known in the art, So-456 discloses the possibility of UMIs in only two locations: (1) in the capture probes adjacent to the spatial barcodes and (2) within the primary probe. A POSA interested in spatial

transcriptomics would have been aware of a spatial transcriptomics article published in Science where UMIs were included in the capture probes (Stahl 2016), it would have been obvious to try to incorporate the UMI in the array probe containing the spatial barcode like Stahl 2016.

265. Thus, a POSA would have been motivated to include a UMI with a spatial barcode in the capture probes.

b. Reasonable expectation of success

266. A POSA would have reasonably expected to modify successfully So-456's method of Figures 8-9 to include a UMI and a spatial barcode in the capture probe because So-456 discloses embodiments that successfully used the same approach (EX1005, ¶[0302], Fig. 23) and, as explained above, spatial detection methods in the prior art successfully included a UMI with a spatial barcode sequence in capture probes immobilized on the array.

267. Thus, a POSA would have been motivated to perform the methods of claims 9, 10, 24, and 25 with a reasonable expectation of success.

268. I understand that NanoString has not alleged any objective evidence of non-obviousness. Thus, in my opinion, claims 9-10 and 24-25 would have been obvious.

XII. GROUND 4: CLAIMS 1-30 ARE ANTICIPATED BY WO 2021/133849 (CHELL)

269. In my opinion, claims 1-30 of the 689 patent are anticipated by Chell under NanoString's interpretation. Specifically, Chell discloses a method meeting all the elements of the claimed methods and I understand that its presumed enabled.

270. I understand that the 689 patent claims priority benefit to the following applications: the 487 application (EX1009); the 212 application (EX1010); and the 180 application (EX1011). I understand that these applications are the "priority applications" of the 689 patent. As I explain below, the priority applications of the 689 patent do not provide written description support for the full scope of the 689 patent claims under NanoString's interpretation. I understand that, as a result, the 689 patent claims are not entitled to a filing date before the patent's filing date of September 16, 2021. I understand that Chell was filed and published before September 16, 2021, and thus it qualifies as prior art.

a. The 689 patent's priority applications do not provide written description support for the claimed methods

271. The priority applications of the 689 patent do not provide written description support for the full scope of the 689 patent claims under NanoString's interpretation.

272. The priority applications to the 689 patent disclose methods that use cleavable "probes" that hybridize to the target analytes (same as described above) and involve steps that occur on the target analyte ("on-analyte" steps) and off (or away from) the target analyte ("off-analyte" steps). *See, e.g.,* EX1009, ¶¶[0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00119], [00123], [00126]-[00138], [00140], [00144]-[00153], [00155], [00158]-[00170], [00183]-[00196], [00199]-[00204], [00210]-[00215], [00217], [00220]-[00226], [00228], [00246], [00317]; EX1010, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00117], [00121], [00124]-[00136], [00138], [00142]-[00151], [00153], [00156]-[00168], [00181]-[00194], [00197]-[00202], [00208]-[00213], [00215], [00218]-[00224], [00226], [00244]; EX1011, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0045], [0076]-[0089], [0093], [0096]-[00108], [00110], [00114]-[00123], [00125], [00128]-[00138], [00141]-[00154], [00157]-[00162], [00165]-[00170], [00172], [00175]-[00181], [00183], [00200]. These *cleavable* probes comprise (1) a target-binding domain ("target binding domain") that specifically hybridizes to a target analyte in a tissue sample, and (2) an oligonucleotide tag that identifies the target analyte ("identifier oligonucleotide"). *See, e.g.,* EX1009, ¶¶[0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00107], [00123],

[00126]-[00127], [00140], [00144]-[00145], [00155], [00158]-[00159], [00174]-[00175], [00178]-[00179], [00183]-[00184], [00199]-[00200], [00210]-[00211], [00220]-[00221], [00228], [00231]-[00232], [00235]-[00236], [00246], [00310]-[00312], [00315], [00317], [00409], [00411], [00413], [00415]; EX1010, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00105], [00121], [00124]-[00125], [00138], [00142]-[00143], [00153], [00156]-[00157], [00172]-[00173], [00176]-[00177], [00181]-[00182], [00197]-[00198], [00208]-[00209], [00215], [00218]-[00219], [00226], [00228]-[00230], [00233]-[00234], [00244], [00377], [00379], [00388]; EX1011, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032], [0033],-[0035], [0042]-[0045], [0076]-[0077], [0093], [0096]-[0097], [00110], [00114]-[00115], [00125], [00128]-[00129], [00141]-[00142], [00157]-[00158], [00165]-[00166], [00172], [00175]-[00176], [00186], [00189], [00190], [00200].

273. The on-analyte step of the methods of the priority applications requires hybridizing these *singular cleavable* probes comprising a target-binding domain to the target analytes in the tissue sample. *See, e.g.,* EX1009, ¶¶[0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00107], [00123], [00126]-[00127], [00140], [00144]-[00145], [00155], [00158]-[00159], [00174]-[00175], [00178]-[00179], [00183]-[00184], [00199]-[00200], [00210]-[00211], [00220]-[00221], [00228], [00231]-[00232], [00235],

[00310]-[00312], [00315], [00317]; EX1010, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00105], [00121], [00124]-[00125], [00138], [00142]-[00143], [00153], [00156]-[00157], [00172], [00176], [00181]-[00182], [00197]-[00198], [00208]-[00209], [00215], [00218]-[00219], [00226], [00229]-[00230], [00233]; EX1011, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032], [0033],-[0035], [0042]-[0045], [0076]-[0077], [0093], [0096]-[0097], [00110], [00114]-[00115], [00125], [00128]-[00129], [00141]-[00142], [00157]-[00158], [00165]-[00166], [00172], [00175]-[00176], [00185]-[00186], [00189], [00190].

274. After on-target hybridization of the probes, the identifier oligonucleotides are cleaved off from the probes within a specific region of the tissue sample by applying a force (such as, UV light). *See, e.g.,* EX1009, [0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00107], [00123], [00126]-[00127], [00140], [00144]-[00145], [00155], [00158]-[00159], [00174]-[00175], [00178]-[00179], [00183]-[00184], [00199]-[00200], [00210]-[00211], [00220]-[00221], [00228], [00231]-[00232], [00235]-[00236], [00246], [00310]-[00312], [00315], [00317], [00409], [00411], [00413], [00415]; EX1010, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00105], [00121], [00124]-[00125], [00138], [00142]-[00143], [00153], [00156]-[00157], [00172]-[00173], [00176]-[00177],

[00181]-[00182], [00197]-[00198], [00208]-[00209], [00215], [00218]-[00219], [00226], [00228]-[00230], [00233]-[00234], [00244], [00377], [00379], [00388]; EX1011, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032], [0033],-[0035], [0042]-[0045], [0076]-[0077], [0093], [0096]-[0097], [00110], [00114]-[00115], [00125], [00128]-[00129], [00141]-[00142], [00157]-[00158], [00165]-[00166], [00172], [00175]-[00176], [00186], [00189], [00190], [00200].

275. Once the identifier oligonucleotides are cleaved off from the probes, the released identifier oligonucleotides are collected from the specific region of the tissue sample and analyzed off-analyte (*i.e.*, away from the target analyte). *See, e.g.,* EX1009, ¶¶[0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00119], [00126]-[00138], [00144]-[00153], [00158]-[00170], [00183]-[00196], [00199]-[00203], [00210]-[00216], [00220]-[00226]; EX1010, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00106], [00124]-[00136], [00142]-[00151], [00156]-[00168], [00181]-[00194], [00197]-[00201], [00208]-[00213], [00218]-[00224]; EX1011, ¶¶[0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0045], [0076]-[0089], [0096]-[00108], [00114]-[00123], [00128]-[00138], [00141]-[00154], [00157]-[00161], [00165]-[00170], [00175]-[00181].

276. The ligation-based methods of the priority applications perform the following off-analyte steps: hybridize in *pairs* a new class of oligonucleotides, called "nucleic acid probes," to the identifier oligonucleotides released and collected from a specific region of the tissue sample; ligate the nucleic acid probe pairs while hybridized to the identical target analyte; incorporate a spatial barcode into the ligated probes and analyze to spatially detect the target analytes. *See, e.g.,* EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161]-[00170], [00174]-[00177], [00178]-[00181], [00183]-[00196]; EX1010, ¶¶[0023]-[0026], [0032]-[0035], [0046], [00159]-[00168], [00174]-[00175], [00178]-[00179], [00184]-[00194]; EX1011, ¶¶[0023]-[0026], [0032]-[0035], [00131]-[00138], [00144]-[00154].

277. These disclosures in the priority applications however fail to provide written description support for the full scope of the claims for at least the two reasons I explain below.

i. The priority applications do not provide written description support for *on-analyte ligation* of probe pairs that had hybridized to the target analytes in the tissue sample

278. As discussed above in Ground 1, the 689 patent claims encompass hybridizing probe pairs comprising a target-binding domain to the target nucleic acids and ligating together the probe pairs while they are hybridized to the identical molecules of the target analyte. These hybridization and ligation steps occur "on-

analyte" in the claims. In other words, the claims require "on-analyte" ligation of the probe pairs that had hybridized to the target analyte in the tissue sample.

279. By giving different names to the *singular* "probes" (that hybridize on-analyte" via their target-binding domains to the target analytes in the tissue sample) and *pairs* of "nucleic acid probes" (that hybridize off-analyte to an identifier oligonucleotide portion that was cleaved off from the "probes"), the priority applications of the 689 patent reinforce two distinct oligonucleotides. *See*, *e.g.*, EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161]-[00170], [00174]-[00181], [00183]-[00186], [00389]; EX1010, ¶¶[0023]-[0026], [0032]-[0035], [0046], [00159]-[00168], [00174]-[00175], [00178]-[00179], [00184]-[00194], [00347], [00357]; EX1011, ¶¶[00127]-[00138], [0023]-[0026], [0032]-[0035], [00141]-[00154], [00293], [00300], [00303].

280. Only singular "probes" undergo "on-target" hybridization to the target analyte in the tissue sample in the methods of the priority applications. *See*, *e.g.*, EX1009, ¶¶[0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00107], [00123], [00126]-[00127], [00140], [00144]-[00145], [00155], [00158]-[00159], [00174]-[00175], [00178]-[00179], [00183]-[00184], [00199]-[00200], [00210]-[00211], [00220]-[00221], [00228], [00231]-[00232], [00235]-[00236], [00246], [00310]-[00312], [00315], [00317], [00409], [00411], [00413], [00415]; EX1010, ¶¶[0004]-[0006], [0013]-[0014],

[0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00105], [00121], [00124]-[00125], [00138], [00142]-[00143], [00153], [00156]-[00157], [00172]-[00173], [00176]-[00177], [00181]-[00182], [00197]-[00198], [00208]-[00209], [00215], [00218]-[00219], [00226], [00228]-[00230], [00233]-[00234], [00244], [00377], [00379], [00388]; EX1011, ¶¶0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032], [0033],-[0035], [0042]-[0045], [0076]-[0077], [0093], [0096]-[0097], [00110], [00114]-[00115], [00125], [00128]-[00129], [00141]-[00142], [00157]-[00158], [00165]-[00166], [00172], [00175]-[00176], [00186], [00189], [00190], [00200].

281. Whereas, the *pairs* of "nucleic acid probes" always undergo "off-target" (away from a tissue) hybridization to an identifier oligonucleotide portion cleaved off from the "probes" in the methods of the priority applications. *See*, *e.g.*, EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161]-[00170], [00174]-[00177], [00178]-[00181], [00183]-[00196]; EX1010, ¶¶[0023]-[0026], [0032]-[0035], [0046], [00159]-[00168], [00174]-[00175], [00178]-[00179], [00184]-[00194]; EX1011, ¶¶[0023]-[0026], [0032]-[0035], [00131]-[00138], [00144]-[00154].

282. Additionally, none of the 689 patent's priority applications disclose a method where probe pairs that are applied to the target analytes in a tissue sample are ligated together while hybridized to the target analyte in the tissue sample. *See*,

e.g., EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161], [00168]-[00169], [00175]-[00177], [00179]-[00180], [00183]-[00186], [00389]; EX1010, ¶¶[0023]-[0026], [0032]-[0035], [0046], [00159], [00166]-[00167], [00174], [00178], [00184], [00192]-[00193], [00347], [00357]; EX1011, ¶¶[00127]-[00131], [0023]-[0026], [0032]-[0035], [00136]-, [00144], [00152], [00290], [00293], [00300], [00303]. That is, none of the priority applications discloses methods where an on-analyte ligation occurs.

283. Thus, in the priority applications, there are no probe *pairs* that undergo "on-analyte" hybridization to the target analytes in a tissue sample, and there is no "on-analyte" ligation of the probe pairs. *See*, *e.g.*, EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161], [00168]-[00169], [00174]-[00180], [00183]-[00186], [00389]; EX1010, ¶¶[0023]-[0026], [0032]-[0035], [0046], [00156]-[00167], [00172]-[00178], [00181]-[00184], [00192]-[00193], [00347], [00357]; EX1011, ¶¶[00127]-[00131], [0023]-[0026], [0032]-[0035], [00136], [00144], [00152], [00290], [00293], [00300], [00303].

284. This is exemplified in the method illustrated in Figure 18. As shown in the excerpt below, the method of Figure 18 contacts a target analyte in a tissue sample to "probe" (not pairs of probes) comprising a "target binding domain" and an "identifier oligonucleotide" for an "on-analyte" hybridization (Step 1), and in a subsequent step, the method hybridizes a different class of oligonucleotides—a

pair of "a first nucleic acid probe and a second nucleic acid probe"—"off-analyte" to the identifier oligonucleotide portion released from the probe:

> The present disclosure provides a method for spatially detecting at least one target analyte in at least one cell from a tissue sample comprising: (1) *contacting* at least one target analyte in at least one cell in a tissue sample with at least one *probe comprising a target binding domain* and an identifier oligonucleotide, wherein the identifier oligonucleotide comprises a unique nucleic acid sequence which identifies the target analyte bound to the target binding domain; (2) providing a force to a location of the tissue sample sufficient to release the identifier oligonucleotide; (3) collecting the released identifier oligonucleotide; (4) *hybridizing* to the released identifier oligonucleotide *a first nucleic acid probe and a second nucleic acid probe*, wherein the first nucleic acid probe comprises a nucleic acid complementary to a portion of the identifier oligonucleotide . . . and wherein the second nucleic acid probe comprises a nucleic acid complementary to a portion of the identifier oligonucleotide,. . . thereby spatially detecting the at least one target analyte in the at least one cell in a tissue sample.

EX1009, ¶¶[0024] (emphasis added), [00178]-[00181]; *see also* EX1010, ¶¶[0023]-[0026], [00176]-[00179], EX1011, ¶¶[0023]-[0026], [00127]-[00138].

285. Table 2 below summarizes the contacting and ligation steps as disclosed in the specification of the priority applications of the 689 patent compared to the 689 patent claims.

TABLE 2		
	Specification of the priority applications (including claims)	**689 patent claims**
Contacting	On-analyte hybridization of *singular* probes comprising target-binding domain to the target analytes in the tissue sample	On-analyte hybridization of *pairs* of probes comprising target-binding domain to the target analytes in the tissue sample
Ligation	*Off-analyte* ligation of pairs of probes hybridized to the identifier oligonucleotide portions cleaved off from the probes bound to the target analytes in the tissue sample	*On-analyte* ligation of pairs of probes hybridized to the target analytes in the tissue sample

286. Thus, because the 689 patent claims include on-analyte hybridization of probe pairs to the target analytes in a tissue sample and on-analyte ligation of those probe pairs while they are hybridized to the target analytes, the priority applications do not provide written description support for the full scope of the claims.

ii. The priority applications do not provide written description support for *collecting whole* probes that had hybridized via their target-binding domain to the target analytes in the tissue sample

287. The patent claims recite collecting "the nucleic acid probes, or portions thereof" (claim 1) or "the ligated probes, or portions" (claim 16) that were "bound to the at least one target analyte" in a "first" and a "second location of the tissue sample." EX1001, 145:60-146:52, 148:11-22. Thus, the claims encompass

collecting *whole* probes containing a target-binding domain that had hybridized to the target analyte.

288. However, none of the 689 patent's priority applications describe collecting *whole* probes containing a target-binding domain that had hybridized to a target analyte. *See*, *e.g.*, EX1009, ¶¶[00174]-[00177], [0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00119], [00126]-[00138], [00144]-[00153], [00158]-[00170], [00183]-[00196], [199]-[00203], [00210]-[00216], [00220]-[00226]; *see also* EX1010, ¶¶[00172]-[00173], [0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00106], [00124]-[00136], [00142]-[00151], [00156]-[00168], [00181]-[00194], [00197]-[00201], [00208]-[00213], [00218]-[00224]; EX1011, ¶¶[00127]-[00130], [0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0045], [0076]-[0089], [0096]-[00108], [00114]-[00123], [00141]-[00154], [00157]-[00161], [00165]-[00170], [00175]-[00181].

289. Instead, at the core, the methods disclosed in the priority applications use cleavable "probes" containing a target-binding domain and collect only a *portion* ("identifier oligonucleotide") of the probe that had hybridized to the target analyte as a proxy for the target analyte. *See*, *e.g.*, EX1009, ¶¶[00174]-[00177], [0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-

[0048], [00106]-[00119], [00126]-[00138], [00144]-[00153], [00158]-[00170], [00183]-[00196], [00199]-[00203], [00210]-[00216], [00220]-[00226]; *see also* EX1010, ¶¶[00172]-[00173], [0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00106], [00124]-[00136], [00142]-[00151], [00156]-[00168], [00181]-[00194], [00197]-[00201], [00208]-[00213], [00218]-[00224]; EX1011, ¶¶[00127]-[00130], [0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0045], [0076]-[0089], [0096]-[00108], [00114]-[00123], [00141]-[00154], [00157]-[00161], [00165]-[00170], [00175]-[00181]. The collected portion does not have a target-binding domain complementary to the target analyte.

290. Thus, each time the priority applications disclose collecting probes from a tissue sample (that had hybridized via their target-binding domains to the target analyte in the tissue sample), they exclusively refer to collecting the identifier oligonucleotide portion released from the hybridized probe —never the *whole* probe. *See*, *e.g.*, EX1009, ¶¶[00174]-[00177], [0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106]-[00119], [00126]-[00138], [00144]-[00153], [00158]-[00170], [00183]-[00196], [00199]-[00203], [00210]-[00216], [00220]-[00226]; *see also* EX1010, ¶¶[00172]-[00173], [0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0047], [00104]-[00106], [00124]-[00136], [00142]-[00151], [00156]-

[00168], [00181]-[00194], [00197]-[00201], [00208]-[00213], [00218]-[00224]; EX1011, ¶¶[00127]-[00130], [0004]-[0006], [0013]-[0014], [0020]-[0021], [0023]-[0026], [0032]-[0035], [0042]-[0045], [0076]-[0089], [0096]-[00108], [00114]-[00123], [00141]-[00154], [00157]-[00161], [00165]-[00170], [00175]-[00181].

291. For example, Figure 18 of the 487 parent application discloses a method that (1) contacts the target nucleic acids in a tissue sample with "probes" comprising a "target nucleic acid-binding region" and an "identifier oligonucleotide" and (2) collects only the identifier oligonucleotide *portion* of the probes that was cleaved off and released from the probes hybridized to the target nucleic acids:

> Figure 18 shows a schematic overview of an exemplary short probe hybridization method of the present disclosure. First, at least one *target analyte* in a sample is *contacted* with at least one *probe* of the present disclosure. . . . In the aspect that the at least one target analyte is a target nucleic acid, the probe can comprise a target nucleic acid-binding region that can directly or indirectly hybridize to a target nucleic acid of interest. The probe further comprises an *identifier oligonucleotide*. . . . *Following contacting* the at least one target analyte with the at least one probe, a force is then provided to a location of the sample sufficient to *release the identifier oligonucleotide. The identifier oligonucleotide is collected following release*, as shown in the top panel of Figure 18.

EX1009, ¶¶[00178]-[00179] (emphasis added); *see also* EX1010, [00176]-[00177]; EX1011, ¶¶[00128]-[00130].

292. Thus, the priority applications do not provide written description support for the full scope of the claims, which encompass collecting *whole* probes comprising a target-binding domain that had hybridized to the target analyte in the tissue sample.

293. Accordingly, in my opinion, for at least the two reasons I explain above, the priority applications do not provide written description support for claims 1-30 of the 689 patent under NanoString's interpretation.

b. Claim 16

294. As I explain below, Chell anticipates claim 16 under NanoString's interpretation. *See also* Appendix C.

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

295. Claim 16's preamble recites "a method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample." EX1001, 147:54-56. I understand that a preamble may be relevant in understanding the scope of the claim. To the extent claim 16's preamble is relevant in understanding its scope, Chell teaches the claimed methods for spatially detecting at least one target analyte in a tissue sample under NanoString's interpretation.

296. Chell discloses "*methods for spatial analysis* using RNA-templated ligation." EX1006, Title (emphasis added). Specifically, Chell discloses "methods

of detecting an analyte of interest to interrogate *spatial gene expression* in a sample using RNA-templated ligation." EX1006, Abstract (emphasis added). Chell's Example 1 (which follows Figure 1's overall workflow) performs "[s]patial gene expression analysis of FFPE-fixed samples using RNA-templated ligation." EX1006, 80:14-15, FIGs. 1, 6, 13. Chell's method of Example 1 is depicted in Figures 6 and 13, which I have reproduced below.

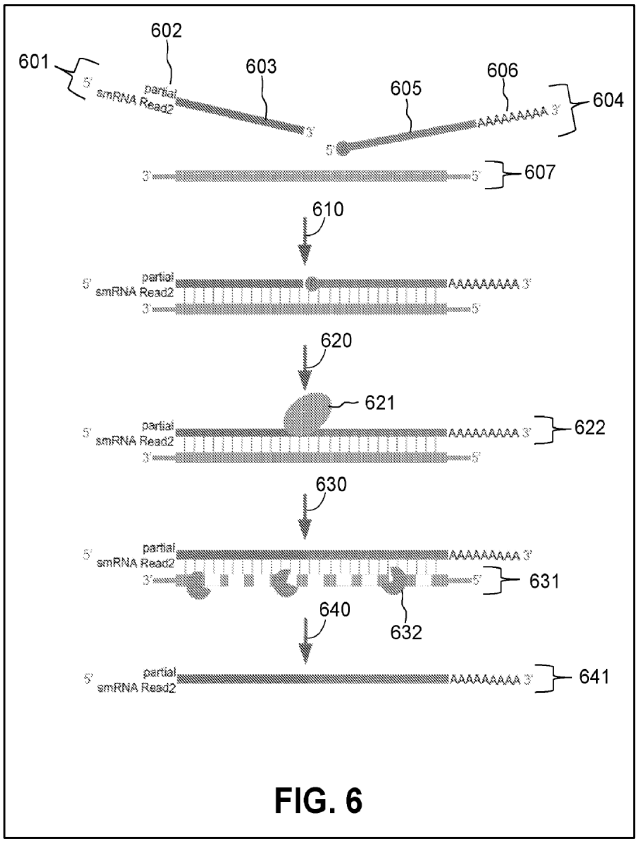


FIG. 6

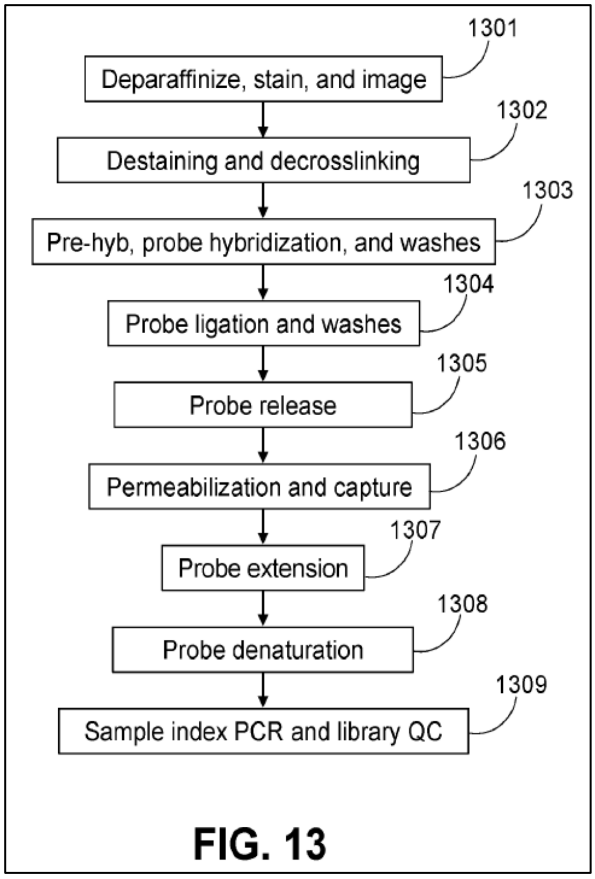


FIG. 13

EX1006, FIGs. 6, 13, 80:14-33.

297. As I explain below, the method of Chell's Example 1 includes the contacting (Step 16(a)), ligating (Step 1(b)), collecting (Steps 16(c) and 16(d)), extending (Steps 16(e) and 16(f)) and sequencing (Step 16(g)) of claim 16. Thus, Chell discloses each element of claim 16 under NanoString's interpretation.

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

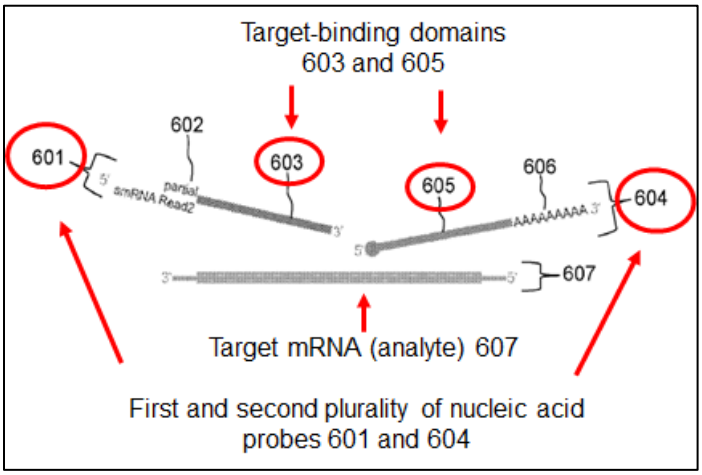
wherein each of the nucleic acid probes in the first plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte,

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 . . ."

298. In my opinion, Chell's method includes this contacting step.

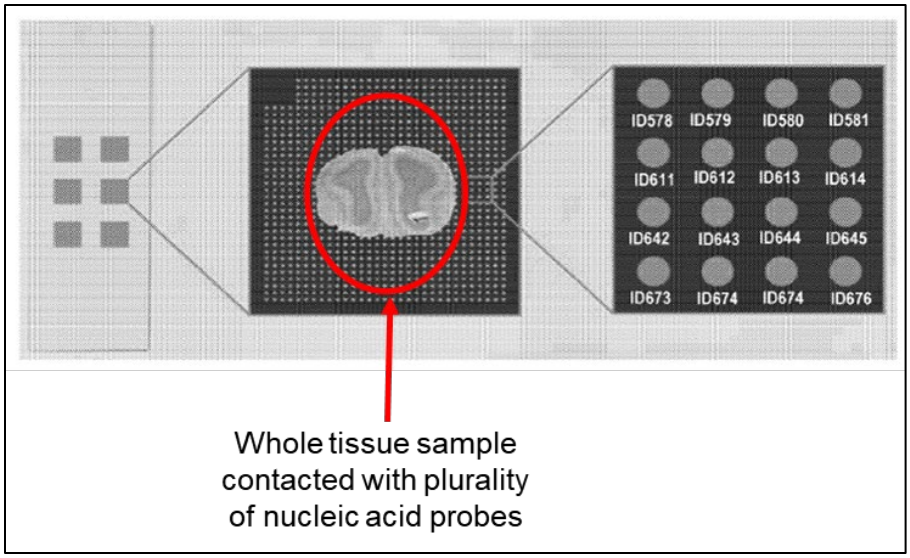
299. Chell's method of Example 1 includes contacting a tissue sample with probe pairs comprising a target-binding domain that binds to target mRNAs in a tissue sample. Specifically, Chell's method uses probe pairs ("RTL probes" comprising "a left-hand side (LHS) probe and a right-hand side (RHS) probe" 601, 604) that "were designed to hybridize to adjacent sequences of each analyte (e.g., mRNA sequence) of interest." EX1006, 81:16-22, 24:4-9, FIG. 6. These RTL probe pairs (601, 604) (*i.e.*, nucleic acid probes of claim 16) comprise a "target-hybridization sequence" (603, 605) (*i.e.*, target binding domain of claim 16) that hybridizes to target mRNAs (*i.e.*, target analyte of claim 16) in the tissue sample. EX1006, 24:4-9, FIG. 6. The annotated excerpt of Figure 6 below shows Chell's

RTL probe pairs (601, 604) comprising a target-binding domain (603, 605) that hybridizes to the target analytes (607) in the tissue sample.



EX1006, FIG. 6 (annotated).

300. In Step 1303 of Chell's method of Example 1 (Figure 13), the pairs of "probes are *added to the [biological] sample* and hybridize to an analyte." EX1006, 23:30-31 (emphasis added); *see also* EX1006, 80:21-26 (contacting probes with FFPE-fixed samples), 81:16-82:7, FIGs. 1, 6, 13. Example 1 further specifies that "20,056 probe pairs (e.g., RHS and LHS probes) were added to each *tissue sample* to capture 19,490 different genes." EX1006, 81:18-19 (emphasis added). And probe pairs "for each analyte were added simultaneously," wherein they "hybridized at adjacent sequences of the target mRNA." EX1006, 81:19-22. Thus, as depicted in the annotated Figure 5, Chell's method contact a plurality of probe pairs at multiple locations in a tissue sample.



EX1006, FIG. 5 (annotated).

> **". . . 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"**

301. Chell also teaches that "the tissue sample is treated to facilitate binding" of the plurality of probe probes to the target mRNAs, as claimed. For example, Chell specifies that the tissue sample is "deparaffinized," "decrosslinked," and then "*treated with pre-hybridization buffer*" before contacting the tissue with the probes. EX1006, 80:21-26 (emphasis added), 23:30-32. These processes allow the solutions (such as those containing the probes) to penetrate the tissue and bind the target analyte. Indeed, Chell teaches that after these treatments, the probe pairs hybridized to the target mRNAs, confirming that the tissue was "treated" as claimed. EX1004, 80:26, FIG. 13.

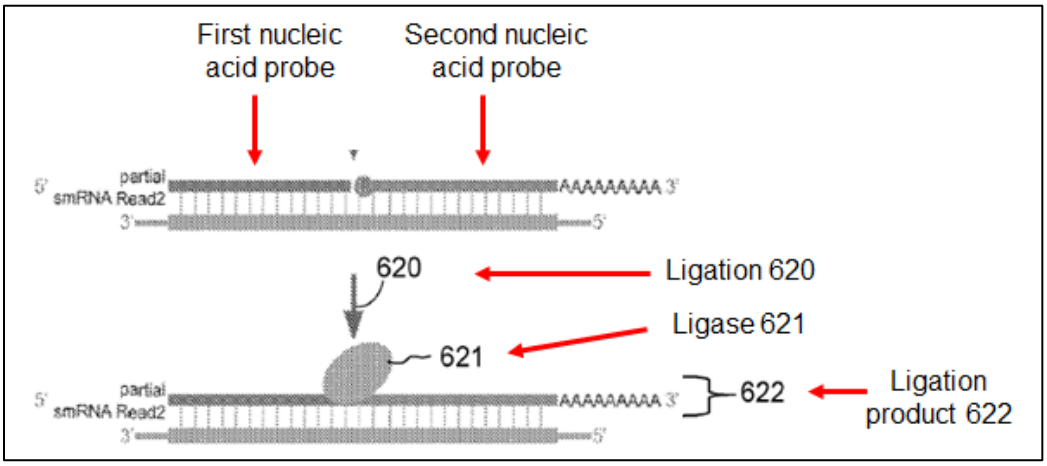
302. Accordingly, Chell's method includes contacting a tissue sample with a first and second plurality of nucleic acid probes comprising target-binding domains, wherein the tissue sample is treated to facilitate binding of the probe to a target analyte. Thus, in my opinion, Chell's method includes claim 16's contacting step.

iii. "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"

303. In my opinion, Chell's method includes this ligation step. The 689 patent discloses "ligating" probe pairs that are "adjacent and are not overlapping" by "performing a nick repair reaction," using, for example, ligase. EX1001, 25:60-65, 70:63-71:6, 5:54-6:8, 6:9-44, 8:28-52, 8:63-9:23, 12:39-57, 27:41-47, 28:34-40, 30:53-58, 30:59-65.

304. In Step 1304 of Chell's method of Example 1 (Figure 13), the "probes are ligated." EX1006, 23:33-34. Chell explains that "[a] ligase 621 *ligates 620 the first probe to the second probe* thereby generating a ligation product 622." EX1006, 24:9-10 (emphasis added); *see also* EX1006, 80:26-27 ("[ligase] was added to the samples to ligate hybridized probes to generate a ligation product"), 82:15-83:3, FIGs. 1, 6, 13. The annotated excerpt of Figure 6 shows Chell's method of forming ligation products (622) (*i.e.*, ligated probes of claim 16) by ligating RTL probe

pairs (*i.e.*, first and second nucleic acid probes of claim 16) that are bound to the identical molecule of the target mRNA (*i.e.*, target analyte of claim 16).



EX1006, FIG. 6 (annotated).

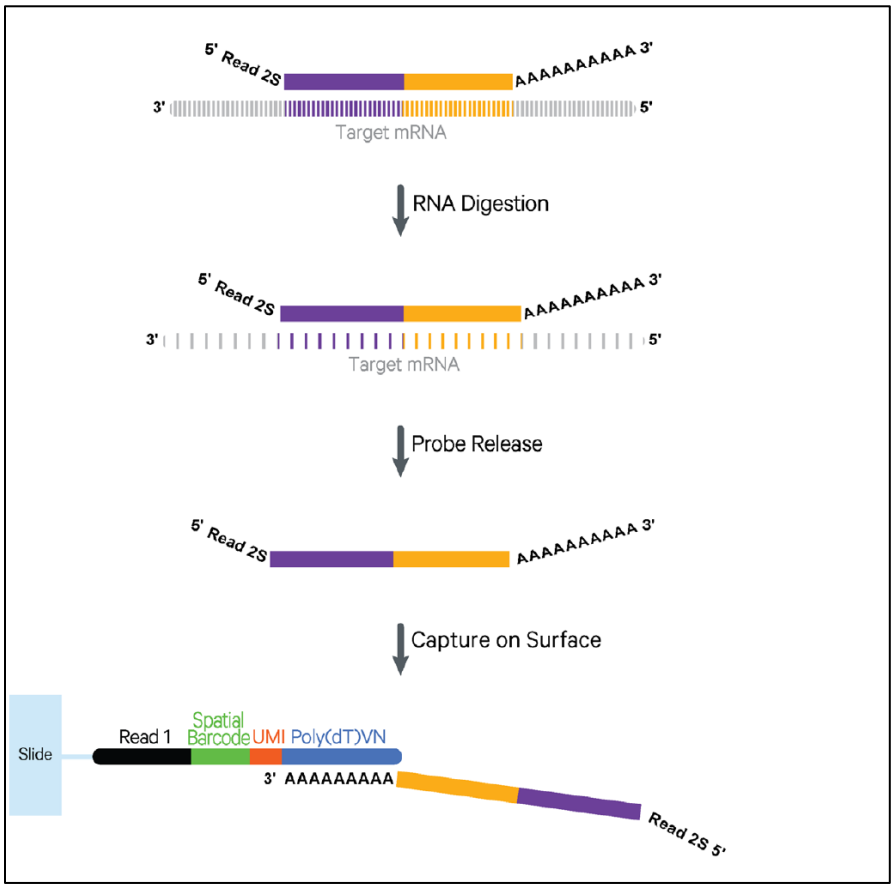
305. Accordingly, Chell's method includes forming ligated probes by ligating nucleic acid probe pairs that are bound to the identical molecule of the target analyte. Thus, in my opinion, Chell's method includes claim 16's ligation step.

iv. "c) [and (d)] 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 [(d): second location] of the tissue sample under conditions that release the ligated probes, or portions thereof, from the first location [(d): second location] of the tissue sample"

306. In my opinion, Chell's method includes these collecting steps under NanoString's interpretation.

307. I understand that NanoString has alleged in related litigation that 10x's Visium FFPE infringes the 689 patent claims. EX1020. Under NanoString's interpretation, the collecting steps of claim 16 (and claim 1) encompass certain of

the steps of 10x's Visium FFPE workflow. EX1020, 53-54. I have reviewed the 10x user guide for Visium FFPE that NanoString identifies in its contentions. In Visium FFPE, *whole* ligation products (not a portion) generated from ligated primary probe pairs are diffused from and captured across the *whole* tissue sample, all at the same time. These steps of Visium FFPE are depicted in the figure below.

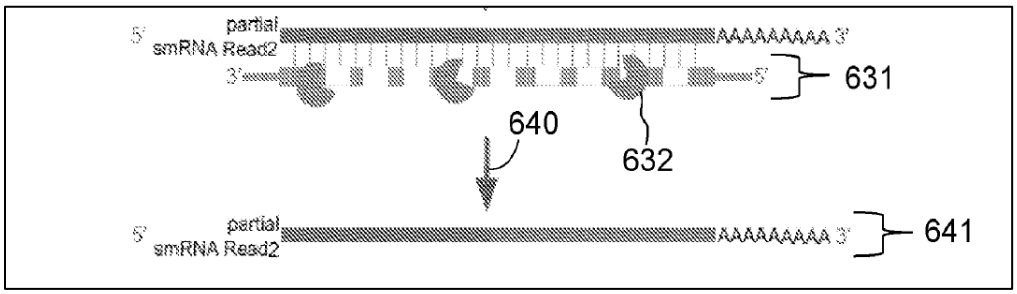


EX1008, 20.

308. Thus, under NanoString's interpretation, the collecting steps of claim 16 (and claim 1) encompass diffusion and capture of *whole* probes or *whole* oligonucleotides across the *whole* tissue sample, all at the same time.

309. In Step 1305 of Chell's method of Example 1 (Figure 13), the "ligation product" (622) (*i.e.*, ligated probes of claim 16) are "*released* from the analyte by contacting the *biological sample* with RNAse H." EX1006, 80:28-29 (emphasis added); *see also* EX1006, 23:33-24:1, 24:9-13, 83:4-15, FIGs. 1, 6, 13. Next, in Step 1306, the tissue sample is "*permeabilized to facilitate capture* of the ligation product by the capture probes on the substrate," wherein the ligation product diffuses on to the substrate. EX1006, 80:29-30 (emphasis added), 83:4-15, 23:34-24:1, 24:11-13.

310. Chell's Example 1 specifies that the ligated probes diffused out from the tissue sample, which "serve[] as a proxy for the target mRNA," are "allowed to hybridize to the capture domain on the capture probe immobilized on the spatial array via the poly-A tail on the 3' end of the RHS probe." EX1006, 83:13-15. The excerpt of Chell's Figure 6 shows that ligation products (*i.e.*, ligated probes of claim 16) are freed from the target mRNAs (631) by digesting the mRNAs using an endoribonuclease (632) and the tissue sample is permeabilized (640), releasing ligation products (641) that are able to hybridize to capture probes on the array.



EX1006, FIG. 6, 8:5-6, 24:4-13, 30:8-11, 33:22-24.

311. Chell's array comprises "spatially-coded" "capture probes" (202) that are immobilized on the array surface for capturing nucleic acids released from the tissue sample. EX1006, 23:13-29, 15:29-30, 16:1-2, FIGs. 1, 2, 5. Chell's Example 1 also specifies that "20,056 probe pairs (e.g., RHS and LHS probes) were *added to each tissue sample* to capture 19,490 different genes," and the "biological sample was permeabilized to release the ligated RTL probes" after the "the sample was incubated . . . in the solution." EX1006, 81:16-82:2, 83:4-12 (emphasis added). Thus, Chell's method releases the ligated probes simultaneously from multiple locations. Chell's Example 1 also specifies that the "*[s]amples* were then permeabilized to *facilitate capture* of the ligation product *by the capture probes* on the substrate." EX1006, 80:29-30 (emphasis added), 83:4-15. Thus, Chell's method includes capturing *whole* ligated probes (formed by ligating the primary probe pairs) that diffuse from and are captured across the *whole* tissue sample, all at the same time.

312. Accordingly, Chell's method includes collecting *whole* ligated probes that were previously formed in the ligation step under conditions that release them from the *whole* tissue sample, all at the same time. Thus, in my opinion, Chell's method includes claim 16's collecting steps under NanoString's interpretation.

v. "e) [and (f)] performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first [and second] location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (c) [and (d)],

thereby forming a first [(f): second] plurality of extension products that comprise the ligated probes, or portions thereof, collected in step (c) [and (d)] and the at least one nucleic acid sequence that identifies the first [(f): second] location of the tissue sample"

313. In my opinion, Chell's method includes these extension steps.

314. A "nucleic acid sequence that identifies the first [or second] location of the tissue sample" in the claim is a spatial barcode (as used in this declaration). After capturing the ligation products (*i.e.*, ligated probes of claim 16),

315. Chell's method of Example 1 (Figure 13) specifies that in Step 1307, the "[l]igation products that hybridized to the capture probes *were then extended*" to generate "*extended capture probes*" (*i.e.*, extension products). EX1006, 80:30-31 (emphasis added), 83:13-84:3, 23:30-24:3, FIG. 13. Chell describes its "extended capture probe" as "a capture probe having additional *nucleotides added to the terminus (e.g., 3' or 5' end)* of the capture probe thereby extending the overall length of the capture probe." EX1006, 17:24-26 (emphasis added). Chell explains that the "extended 3' end" indicates that "additional nucleotides were added to the most 3' nucleotide of the capture probe to *extend* the length of the *capture probe*, for example, by polymerization reactions used to extend nucleic acid molecules

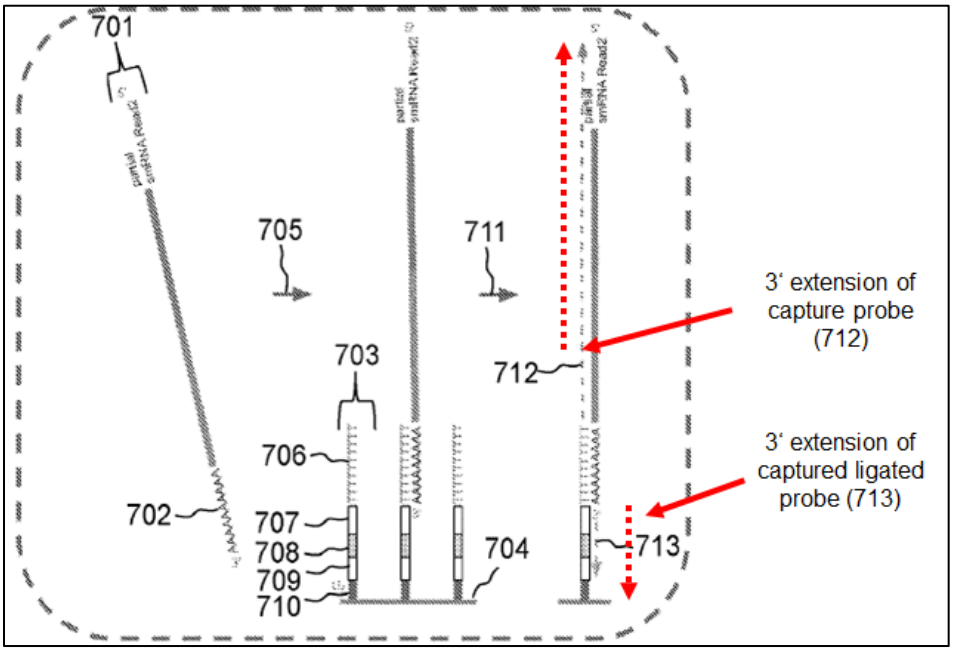
including templated polymerization catalyzed by a polymerase (e.g., a DNA polymerase or a reverse transcriptase)." EX1006, 17:26-30 (emphasis added). Chell also explains that capture probe extension includes "adding to a 3' end of a capture probe *a nucleic acid sequence that is complementary to a nucleic acid sequence of an analyte* . . . ." EX1006, 17:30-33 (emphasis added).

316. Additionally, Chell discloses that the "captured ligated probes were copied, using the *capture probe as a template* and the extension product was released from the spatial array," i.e., captured ligation products (i.e., the ligated probes of the claim) as primers. EX1006, 83:15-17.

317. Chell's "capture probe" (202) comprises a "spatial barcode" (206), which is "a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample." EX1006, 10:6-10, 12:10-19, 15:28-16:14, 23:13-29, 24:25-33. Chell also specifies the "extended capture probes include the sequence of the capture probe and the sequence of the spatial barcode of the capture probe." EX1006, 18:1-2.

318. As depicted in Figure 7 (reproduced below), Chell's method performs bi-directional extension of the ligated probes (701) that were captured in the previous steps on the capture probes (703) containing the spatial barcode (708). EX1006, 24:25-25:8, FIG. 7; EX1002, ¶XX. The 3'-end of the capture probes (703) is extended in one direction to generate extension products 712, and the 3'-end of the

captured ligated probes is extended the other direction to generate the extension products 713.



EX1006, 8:7-8, 24:25-25:8, FIG. 7.

319. Chell discloses that the capture probes' (703) "[i]ncubation with the [reverse transcription] reagents can *extend the capture probes* [Step] 711 to produce *spatially-barcoded full-length cDNA* 712 and 713." EX1006, 25:4-6 (emphasis added), FIG. 7.

320. Accordingly, Chell's method performs an extension reaction that incorporates a spatial barcode (206, 708) into the ligation products (*i.e.*, ligated probes of the claim), thereby forming extension products that comprise the collected ligation products and the spatial barcode. Thus, in my opinion, Chell's method includes claim 16's extension steps.

vi. "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."

321. In my opinion, Chell's method includes this sequencing step under NanoString's interpretation. I understand that NanoString agrees that "spatially detecting" means "identifying the presence of a specific target analyte within a specific region of interest in a sample." EX1024, 31. After extension, Chell's method of Example 1 (Figure 13) specifies that, in Steps 1308 and 1309, the "extended capture probes [are] denatured" and the "[d]enatured, extended capture probes" are "indexed" and "subjected to quality control … before *being sequenced*." EX1006, 80:31-33 (emphasis added), 84:7-9 ("samples were indexed … [n]ucleic acids were then *sequenced and analyzed*." (emphasis added)), 23:13-29, FIGs. 1, 13.

322. Chell also teaches spatially detecting at least one target mRNA in a first and second location of the tissue sample because Chell's method determines "the abundance *and location* of an analyte." EX1006, 24:2-3 (emphasis added), 23:13-24:3, FIG. 1 ("*[d]etermine sequence* of ligation product … *[i]dentify location* of analyte in sample" (emphasis added)), 84:12-85:3, FIGs. 14-20. Accordingly, Chell's method includes sequencing the extension products to determine their

sequence and sorting the sequencing data to determine the location of the target mRNAs in the tissue sample. As such, Chell's method identifies the extension products by sequencing them, thereby spatially detecting the target analytes in the tissue sample. Thus, in my opinion, Chell's method includes claim 16's sequencing step under NanoString's interpretation.

323. For the reasons I discuss above, Chell teaches every element of claim 16 under NanoString's interpretation, arranged as claimed. I understand that Chell's methods are presumed enabled. Thus, in my opinion, Chell anticipates claim 16 under NanoString's interpretation.

c. Claim 1

324. For the reasons I explain above for claim 16, So-456 anticipates claim 1 under NanoString's interpretation. *See also* Appendix D.

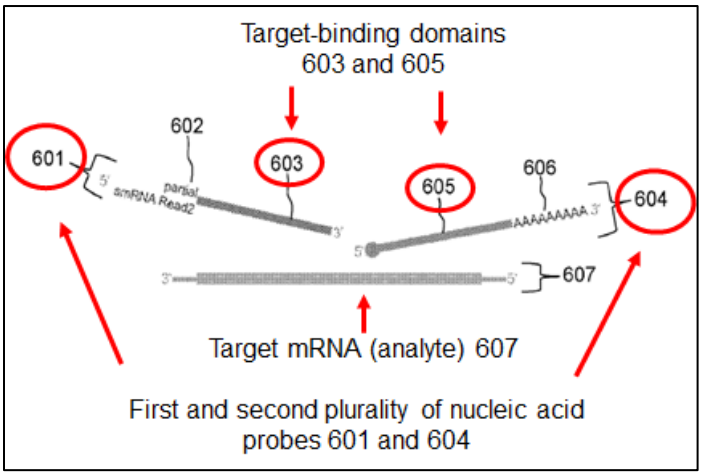
i. Preamble: "A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising"

325. Claim 1's preamble is the same as claim 16's preamble and recites "a method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample." EX1001, 145:51-53. For the same reasons as I explain for claim 16 above, Chell's method of Example 1 (illustrated in Figures 6 and 13 and following the overall workflow of Figure 1) discloses the preamble of claim 1 under NanoString's interpretation.

ii. "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 . . ."

326. For the reasons I explain above for claim 16, Chell's method includes the contacting step of claim 1.

327. As I explain above for claim 16, Chell's method of Example 1 includes contacting probe pairs ("RTL probes" comprising "a left-hand side (LHS) probe and a right-hand side (RHS) probe" 601, 604) to "adjacent sequences of each analyte (e.g., mRNA sequence) of interest." EX1006, 81:16-18, 24:4-9, FIG. 6. Chell's probe pairs (601, 604) (*i.e.*, nucleic acid probes of claim 1) comprise a "target-hybridization sequence" (603, 605) (*i.e.*, target binding domain of claim 1) that hybridizes to the target mRNAs (*i.e.*, target analyte of claim 1) in the tissue sample. EX1006, 24:4-9, FIG. 6. The annotated excerpt of Figure 6 below shows Chell's RTL probe pairs (601, 604) comprising a target-binding domain (603, 605) that hybridizes to the target analytes (607) in the tissue sample.



EX1006, FIG. 6 (annotated), 1:24-30, 17:19-24, 49:34-35, 50:15-18, 50:32-51:5, 52:10-19, 57:23-25, 60:14-16, 62:25-28, 66:4-13, 66:26-28, 75:3-8, 76:8:10, 84:33-85:2, 85:28-31.

328. As I explain above for claim 16, Chell's method contacts a plurality of probe pairs at multiple locations in a tissue sample. EX1006, 23:30-32, 80:21-26, 81:16-82:7, FIGs. 1, 5, 6, 13. For example, Chell's Example 1 specifies that "20,056 probe pairs (e.g., RHS and LHS probes) were added to each *tissue sample* to capture 19,490 different genes." EX1006, 81:18-19 (emphasis added). The probe pairs "for each analyte were added simultaneously" and they "hybridized at adjacent sequences of the target mRNA." EX1006, 81:19-21.

". . . 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"

329. Chell also discloses that "the tissue sample is treated to facilitate binding" of the plurality of probe probes (601, 604) to the at least one target mRNA, as claimed. Chell teaches that the tissue sample is "deparaffinized," "decrosslinked," and then "*treated with pre-hybridization buffer*" before contacting the tissue with the probes. EX1006, 80:21-26 (emphasis added), 23:30-32. These processes allow the solutions (such as those containing the probes) to penetrate the tissue and bind the target analyte. Indeed, Chell teaches that after these treatments,

the probe pairs hybridized to the target mRNAs, confirming that the tissue was "treated" as claimed. EX1004, 80:26, FIG. 13.

330. Accordingly, Chell's method includes contacting a tissue sample with nucleic acid probes comprising target binding domains, wherein the tissue sample is treated to facilitate binding of the probe to a target analyte. Thus, in my opinion, Chell's method includes claim 1's contacting step.

iii. "b) [and (c)] collecting the nucleic acid probes, or portions thereof, bound to the at least one target analyte in a first location [(c): second location] of the tissue sample under conditions that release the nucleic acid probes, or portions thereof, from the first location [(c): second location] of the tissue sample"

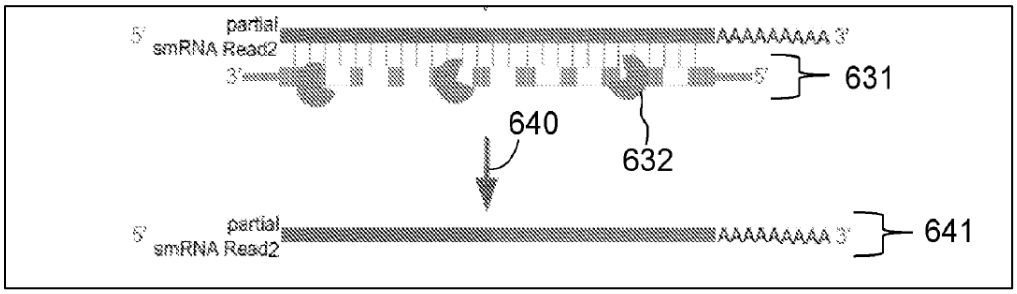
331. For the reasons I explain above for claim 16, Chell's method includes the collecting steps of claim 1 under NanoString's interpretation.

332. I understand that NanoString has alleged in related litigation that 10x's Visium FFPE infringes the 689 patent claims. EX1020. For the reasons I explain above, the collecting steps of claim 1 encompass the "capture" step of 10x's Visium FFPE workflow under NanoString's interpretation. EX1020, 53-54. For the reasons I explain above for claim 16, Chell's method of Example 1 (Figure 13) includes the collecting steps of claim 1.

333. In Step 1305 of Chell's method of Example 1 (Figure 13), the "ligation product" (622) (*i.e.*, ligated probes of claim 16) are "*released* from the analyte by contacting the *biological sample* with RNAse H." EX1006, 80:28-29 (emphasis

added); *see also* EX1006, 23:33-24:1, 24:9-13, 83:4-15, FIGs. 1, 6, 13. Next, in Step 1306, the tissue sample is "*permeabilized to facilitate capture* of the ligation product by the capture probes on the substrate," wherein the ligation product diffuses on to the substrate. EX1006, 80:29-30 (emphasis added), 83:4-15, 23:34-24:1, 24:11-13.

334. Chell's Example 1 specifies that the ligated probes diffused out from the tissue sample, which "serve[] as a proxy for the target mRNA," are "allowed to hybridize to the capture domain on the capture probe immobilized on the spatial array via the poly-A tail on the 3' end of the RHS probe." EX1006, 83:13-15. The excerpt of Chell's Figure 6 shows that ligation products (*i.e.*, ligated probes of claim 16) are freed from the target mRNAs (631) by digesting the mRNAs using an endoribonuclease (632) and the tissue sample is permeabilized (640), releasing ligation products (641) that are able to hybridize to capture probes on the array.



EX1006, FIG. 6, 8:5-6, 24:4-13, 30:8-11, 33:22-24.

335. Chell's array comprises "spatially-coded" "capture probes" (202) that are immobilized on the array surface for capturing nucleic acids released from the

tissue sample. EX1006, 23:13-29, 15:29-30, 16:1-2, FIGs. 1, 2, 5. Chell's Example 1 also specifies that "20,056 probe pairs (e.g., RHS and LHS probes) were *added to each tissue sample* to capture 19,490 different genes," and the "biological sample was permeabilized to release the ligated RTL probes" after the "the sample was incubated . . . in the solution." EX1006, 81:16-82:2, 83:4-12 (emphasis added). Thus, Chell's method releases the ligated probes simultaneously from multiple locations. Chell's Example 1 also specifies that the "*[s]amples* were then permeabilized to *facilitate capture* of the ligation product *by the capture probes* on the substrate." EX1006, 80:29-30 (emphasis added), 83:4-15. Thus, Chell's method includes capturing *whole* ligated probes (formed by ligating the primary probe pairs) that diffuse from and are captured across the *whole* tissue sample, all at the same time.

336. Accordingly, Chell's method includes collecting *whole* ligated probes that were previously formed in the ligation step under conditions that release them from the *whole* tissue sample at the same time. Thus, in my opinion, Chell's method includes claim 1's collecting steps under NanoString's interpretation.

iv. "d) [and (e)] performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first [(e): second] location of the tissue sample into each of the nucleic acid probes, or portions thereof, collected in step (b) [and (c)],

thereby forming a first [(e): second] plurality of extension products that comprise the nucleic acid probes, or portions thereof, collected in step (b) [and (c)] and the at least one nucleic acid sequence that identifies the first [(e): second] location of the tissue sample"

337. For the reasons I explain above for claim 16, Chell's method includes the extension steps of claim 1.

338. A "nucleic acid sequence that identifies the first [and second] location of the tissue sample" in the claim is a spatial barcode (as used in this declaration).

339. As I explain above for claim 16, the patent's methods perform an extension reaction using the ligated probes as templates to extend the hybridized primers, incorporating the spatial barcodes into the ligated probes such that the generated extension products comprise the spatial barcode and the ligated probe. For the reasons I explain above for claim 16, Chell's method of Example 1 (Figure 13) includes the extension steps of claim 1.

340. Chell's method of Example 1 (Figure 13) specifies that in Step 1307, the "[l]igation products that hybridized to the capture probes *were then extended*" to generate "*extended capture probes*" (*i.e.*, extension products). EX1006, 80:30-31 (emphasis added), 83:13-84:3, 23:30-24:3, FIG. 13. Chell describes its "extended

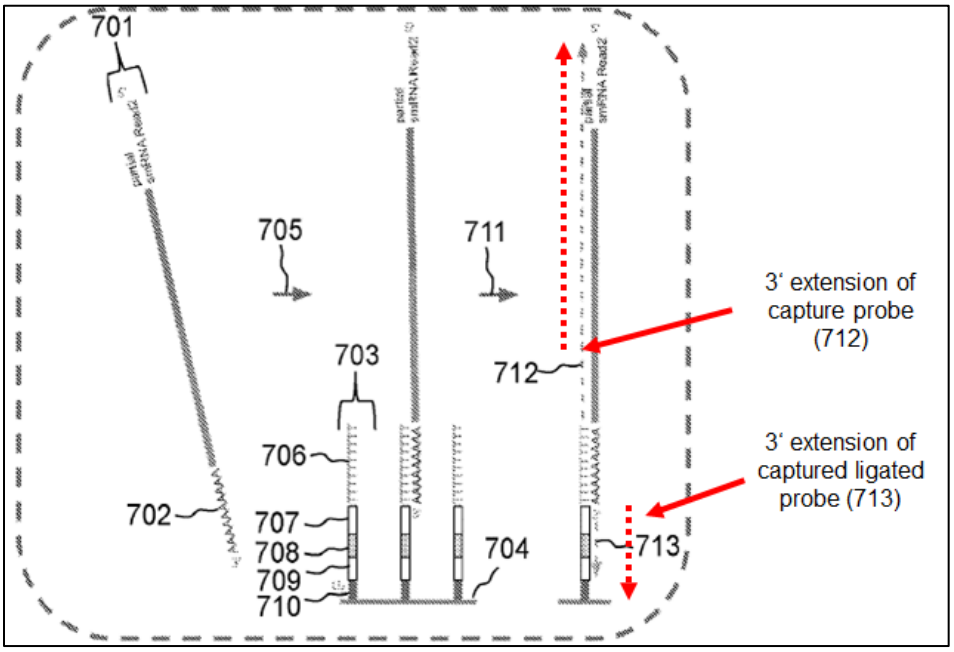
capture probe" as "a capture probe having additional *nucleotides added to the terminus (e.g., 3' or 5' end)* of the capture probe thereby extending the overall length of the capture probe." EX1006, 17:24-26 (emphasis added). Chell explains that the "extended 3' end" indicates that "additional nucleotides were added to the most 3' nucleotide of the capture probe to *extend* the length of the *capture probe*, for example, by polymerization reactions used to extend nucleic acid molecules including templated polymerization catalyzed by a polymerase (e.g., a DNA polymerase or a reverse transcriptase)." EX1006, 17:26-30 (emphasis added). Chell also explains that capture probe extension includes "adding to a 3' end of a capture probe *a nucleic acid sequence that is complementary to a nucleic acid sequence of an analyte* . . . ." EX1006, 17:30-33 (emphasis added).

341. Additionally, Chell discloses that the "captured ligated probes were copied, using the *capture probe as a template* and the extension product was released from the spatial array," i.e., captured ligation products (i.e., the ligated probes of the claim) as primers. EX1006, 83:15-17.

342. Chell's "capture probe" (202) comprises a "spatial barcode" (206), which is "a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample." EX1006, 10:6-10, 12:10-19, 15:28-16:14, 23:13-29, 24:25-33. Chell also specifies the "extended capture probes

include the sequence of the capture probe and the sequence of the spatial barcode of the capture probe." EX1006, 18:1-2.

343. As depicted in Figure 7 (reproduced below), Chell's method performs bi-directional extension of the ligated probes (701) that were captured in the previous steps on the capture probes (703) containing the spatial barcode (708). EX1006, 24:25-25:8, FIG. 7; EX1002, ¶XX. The 3'-end of the capture probes (703) is extended in one direction to generate extension products 712, and the 3'-end of the captured ligated probes is extended the other direction to generate the extension products 713.



EX1006, 8:7-8, 24:25-25:8, FIG. 7.

344. Chell discloses that the capture probes' (703) "[i]ncubation with the [reverse transcription] reagents can *extend the capture probes* [Step] 711 to

produce *spatially-barcoded full-length cDNA* 712 and 713." EX1006, 25:4-6 (emphasis added), FIG. 7.

345. Accordingly, Chell's method performs an extension reaction that incorporates a spatial barcode (206, 708) into the ligation products (*i.e.*, ligated probes of the claim), thereby forming extension products that comprise the collected ligation products and the spatial barcode. Thus, in my opinion, Chell's method includes claim 1's extension steps.

v. "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."

346. For the reasons I explain above for claim 16, Chell's method includes the sequencing step of claim 1 under NanoString's interpretation. The sequencing step of claim 1 is the same as the sequencing step of claim 16. For the reasons I explain above for claim 16, Chell's method of Example 1 (Figure 13) includes the sequencing step of claim 1 under NanoString's interpretation.

347. After extension, Chell's method specifies that in Steps 1308 and 1309, the "extended capture probes were denatured" and the "[d]enatured, extended capture probes" were "indexed" and "subjected to quality control … before *being sequenced*." EX1006, 80:31-33 (emphasis added), 84:7-9 ("samples were indexed

… [n]ucleic acids were then *sequenced and analyzed*" (emphasis added)), 23:13-29, FIGs. 1, 13.

348. Chell also teaches spatially detecting at least one target mRNA in a first and second location of the tissue sample because Chell's method determines "the abundance *and location* of an analyte." EX1006, 24:2-3 (emphasis added), 23:13-24:3, Fig. 1 ("*[d]etermine sequence* of ligation product … *[i]dentify location* of analyte in sample" (emphasis added)), 84:12-85:3, Figs. 14-20. Accordingly, Chell teaches sequencing the extension products to determine their sequence and sorting the sequencing data to determine the location of the target mRNAs in the tissue sample. As such, Chell's method identifies the extension products by sequencing them, thereby spatially detecting the target analytes in the tissue sample. Thus, in my opinion, Chell's method includes claim 1's sequencing step under NanoString's interpretation.

349. For the reasons I discuss above, Chell teaches every element of claim 1 under NanoString's interpretation, arranged as claimed. I understand that Chell's methods are presumed enabled. Thus, in my opinion, Chell anticipates claim 1 under NanoString's interpretation.

d. Claims 2-15 and 17-30

350. Claims 2-15 and 17-30 depend directly or indirectly from claim 1 or claim 16, and they further specify the elements of claim 1 or claim 16. I understand

that the dependent claims incorporate all elements of the claims from which they depend.

i. Claims 2, 3, 17, and 18

351. **Claims 2 and 17** depend from claims 1 and 16, respectively, and further require that "the tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample." EX1001, 147:12-13, 148:48-49. **Claims 3 and 18** depend from claims 1 and 16, respectively, and further require that "the tissue sample is immobilized onto a microscope slide." EX1001, 147:14-15, 148:50-51. Chell discloses the elements of claims 2, 3, 17, and 18 under NanoString's interpretation.

352. Chell's Example 1 describes a method for "[s]patial gene expression analysis of *FFPE-fixed samples* using RNA-templated ligation." EX1006, 80:14-15 (emphasis added), 80:21-23, FIG. 13. Example 1 specifies that the "FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged" and the "FFPE sectioned mouse *brain tissue slides* were deparaffinized, permeabilized with PBST." EX1006, 80:21-23, 81:1-2 (emphasis added). Thus, Chell discloses the elements of claims 2, 3, 17, and 18 and anticipates them under NanoString's interpretation.

ii. Claims 4 and 19

353. Claims 4 and 19 depend from claims 3 and 18, respectively, and further require that "the microscope slide comprises a plurality of primers immobilized on

the microscope slide." EX1001, 147:16-18, 148:52-54. Chell discloses the elements of claims 4 and 19 under NanoString's interpretation.

354. Chell's Example 1 teaches that the "[s]amples were then permeabilized to facilitate capture of the ligation product by the *capture probes on the substrate* 1306." EX1006, 23:13-18, 80:29-30 (emphasis added). Chell also specifies that "[t]he released, ligated DNA probes that served as a proxy for the target mRNA were allowed to hybridize to the capture domain on the *capture probe immobilized on the spatial array*…." EX1006, 83:13-15 (emphasis added). Chell further specifies "contacting a biological sample with [an] *array* of spatially-barcoded capture probes" and that "the array includes a *plurality* of capture probes." EX1006, 23:13-18 (emphasis added). Chell's "plurality of capture probes" constitute the claimed "a plurality of primers" because the Chell's probes are used as primers for polymerase extension reactions in the extension steps. EX1006, 23:13-18, 80:30-31, 83:15-16. Thus, Chell discloses the elements of claims 4 and 19.

iii. Claims 5 and 20

355. Claims 5 and 20 depend from claims 4 and 19, respectively, and further require that "the plurality of primers is immobilized on the microscope slide at their 5' ends." EX1001, 147:20-21, 148:55-57. Chell discloses the elements of claims 5 and 20.

356. Chell's Example 1 specifies that the "[l]igation products that hybridized to the capture probes were then *extended* [in step] 1307" to obtain "extended capture probes." EX1006, 80:30-31 (emphasis added). Chell explains that "an 'extended capture probe' refers to a capture probe having additional nucleotides added to the terminus," and, for example, the "*additional nucleotides were added to the most 3' nucleotide* of the capture probe to extend the length of the capture probe." EX1006, 17:27-28 (emphasis added). Because the 3' end of the capture probes is free to allow the additional nucleotides to be added to that end, a POSA would understand that the probes are immobilized to the array by their other end, *i.e.*, the 5' end. Accordingly, Chell discloses the elements of claims 5 and 20.

iv. Claims 6 and 21

357. Claims 6 and 21 depend from claims 5 and 20, respectively, and further require that the extension steps "comprise performing a solid-phase amplification reaction" on the "microscope slide using the plurality of primers immobilized on the microscope slide." EX1001, 147:22-26, 148:58-62. Chell discloses the elements of claims 6 and 21.

358. Chell discloses that the extended capture probes can be "*amplified* (e.g., in bulk solution or on the *array*) to yield quantities that are sufficient for downstream analysis, e.g., via DNA sequencing." EX1006, 18:3-6 (emphasis added). Chell's array is on a microscope slide. *See*, *e.g.*, 19:11-16, FIG. 5. A POSA

would understand this to mean that an amplification reaction is performed on the array (*i.e.*, the microscope slide) using the plurality of immobilized capture probes as primers. Accordingly, Chell discloses the elements of claims 6 and 21.

v. Claims 7 and 22

359. Claims 7 and 22 depend from claims 1 and 16, respectively, and further require that "the sequencing step is performed using a next generation sequencing reaction." EX1001, 147:27-28, 148:63-64. Chell teaches the elements of claims 7 and 22.

360. Chell teaches that the "methods for sequencing genetic material include, but are not limited to, DNA hybridization methods (e.g., Southern blotting), restriction enzyme digestion methods, Sanger sequencing methods, *next-generation sequencing* methods (e.g., single-molecule real-time sequencing, nanopore sequencing, and Polony sequencing), ligation methods, and microarray methods." EX1006, 79:8-12 (emphasis added), 5:30-31, 16:2-5. Accordingly, Chell discloses the elements of claims 7 and 22.

vi. Claims 8 and 23

361. Claims 8 and 23 depend from claims 1 and 16, respectively, and further require "amplifying a library using the first plurality of extension products and the second plurality of extension products as templates." EX1001, 147:29-31, 148:65-67. Chell teaches the elements of claims 8 and 23.

362. Chell discloses that the "*extended capture probes* (e.g., DNA molecules) act as *templates* for an amplification reaction (e.g., a polymerase chain reaction)." EX1006, 18:5-6 (emphasis added). Chell's Example 1 specifies that the "[d]enatured, *extended captured probes* were indexed and the *amplified libraries* were subjected to quality control 1309 before being sequenced." EX1006, 80:31-33 (emphasis added), FIG. 13 ("[s]ample index *PCR*" (emphasis added)). Accordingly, Chell's method includes amplifying a library using the extended capture probes (*i.e.*, extension products) as templates. Thus, Chell discloses the elements of claims 8 and 23.

vii. Claims 9, 10, 24 and 25

363. **Claims 9-10** depend from claim 1, and **claims 24-25** depend from claim 16. Claims 9-10 and 24-25 further require that "the at least one nucleic acid sequence that identifies the first location" (claims 9 and 24) or "second location" (claims 10 and 25) "of the tissue sample comprises *at least one unique molecular identifier*." EX1001, 147:32-38 (emphasis added), 149:1-8. A "nucleic acid sequence that identifies the first [and second] location of the tissue sample" in the claims is a spatial barcode (as used in this declaration). Thus, claims 9, 10, 24, and 25 require a nucleic acid sequence that comprises both a spatial barcode and "at least one unique molecular identifier." Chell teaches the elements of claims 9, 10, 24, and 25.

364. Chell's "capture probe includes a barcode (e.g., a *spatial barcode and*/or a *unique molecular identifier (UMI)*) and a capture domain." EX1006, 12:12-14 (emphasis added), 15:15-16, 24:31-33 ("[t]he capture probe can also include a *unique molecular identifier (UMI)* 707, a *spatial barcode* 708…."), FIG. 7. Chell further explains that a "spatial barcode" is "a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample." EX1006, 10:6-10. Thus, Chell's capture probes comprise both a spatial barcode and a UMI. In other words, Chell's method uses a nucleic acid sequence that identifies a first or second location of the tissue sample also comprises at least one UMI. Accordingly, Chell discloses the elements of claims 9, 10, 24, and 25.

viii. Claims 11, 12, and 27

365. **Claims 11 and 12** depend from claim 1, and **claim 27** depends from claim 16, and further require that the "nucleic acid sequence" that "identifies the first location" (claim 11), "second location" (claim 12), and "first location," "second location," or "both" first and second locations (claim 27) of the tissue sample comprise "at least one amplification primer binding site." EX1001, 147:40-47, 149:18-150:9. A "nucleic acid sequence that identifies the first [and second] location of the tissue sample" in the claims is a spatial barcode (as used in this declaration). Thus, claims 11, 12, and 27 require a nucleic acid sequence that

comprises both a spatial barcode and "at least one amplification primer binding site." Chell teaches the elements of claims 11, 12, and 27.

366. Chell's "capture probe includes a barcode (e.g., a *spatial barcode* and/or a unique molecular identifier (UMI)) and a capture domain." EX1006, 12:11-14 (emphasis added), 15:15-16, 24:31-33 ("[t]he capture probe can also include a *unique molecular identifier (UMI)* 707, a *spatial barcode* 708…." (emphasis added)), FIG. 7. Chell's "spatial barcode" is "a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample." EX1006, 10:6-10. Thus, Chell's capture probes include a sequence that identifies a location of a tissue sample.

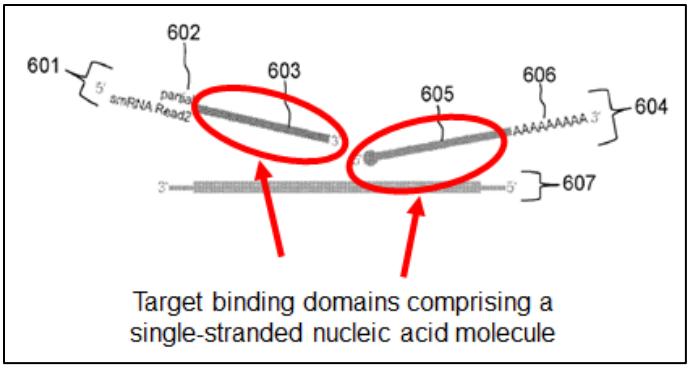
367. Chell's "capture probe can include functional sequences that are useful for subsequent processing," and the "capture probe can include a cleavage domain and/or a functional domain (e.g., a *primer-binding site*, such as for next-generation sequencing (NGS))." EX1006, 15:16-17, 12:14-16 (emphasis added). Indeed, in Chell's Example 1, the extended captured probes were indexed and amplified to generate "*amplified libraries* [that] were subjected to quality control 1309 before being sequenced." EX1006, 80:31-33 (emphasis added), 83:20-84:11, FIG. 13 ("[s]ample index *PCR*" (emphasis added)). Thus, Chell's capture probes comprise both a spatial barcode and an amplification primer binding site. In other words, Chell's method uses a nucleic acid sequence that identifies a first or second

location of the tissue sample also comprises at least one amplification primer binding site. Accordingly, Chell teaches the elements of claims 11, 12, and 27.

ix. Claims 13 and 28

368. Claims 13 and 28 depend from claims 1 and 16, respectively, and require that "the target binding domains" of the nucleic acid probes (claim 13) and each of the probes from the first and second plurality of nucleic acid probes (claim 28) "comprise[s] a single-stranded nucleic acid molecule." EX1001, 147:48-49, 150:10-13. Chell teaches the elements of claims 13 and 28.

369. As I discuss above for claims 1 and 16, Chell's method uses pairs of "RTL probes (a left-hand side (LHS) probe and a right-hand side (RHS) probe (see e.g., FIG. 6)) for each analyte" that are "*hybridized at adjacent sequences of the target mRNA*, forming RNA:DNA duplex structures." EX1006, 81:16-82:2 (emphasis added). Chell further specifies that the probe pairs comprise a "target-hybridization sequence" (*i.e.*, target binding domain) that binds to the target mRNAs. EX1006, 24:4-9, FIG. 6. A POSA would understand that in order for Chell's probe pairs to hybridize to the target mRNAs, their target-binding domains must comprise a single-stranded region. This is illustrated in the annotated excerpt of Figure 6 below.



EX1006, FIG. 6 (annotated).

370. Accordingly, Chell discloses the elements of claims 13 and 28.

x. Claims 14 and 29

371. Claims 14 and 29 depend from claims 1 and 16, respectively, and further require that the collecting steps (b)-(c) (claim 14) and (c)-(d) (claim 29) are "performed simultaneously." EX1001, 147:50-51, 150:14-15. Chell teaches the elements of claims 14 and 29.

372. Chell's Example 1 specifies that "20,056 probe pairs (e.g., RHS and LHS probes) were *added to each tissue sample* to capture 19,490 different genes," and the "biological sample was permeabilized to release the ligated RTL probes" after the "the sample was incubated . . . in the solution." EX1006, 81:16-82:2, 83:4-12 (emphasis added). Thus, Chell's method releases the ligated probes simultaneously from multiple locations. Chell's Example 1 also specifies that the "*[s]amples* were then permeabilized to *facilitate capture* of the ligation product by the capture probes on the substrate." EX1006, 80:29-30 (emphasis added), 83:4-15, 16:23-30, 23:33-24:2. FIG. 13. Thus, Chell's method captures the released ligated probes

from multiple locations of the tissue simultaneously on the capture probes immobilized on the array. Accordingly, Chell's method includes simultaneously collecting the ligated probes from multiple locations of the tissue sample. Thus, Chell discloses the elements of claims 14 and 29.

xi. Claims 15 and 30

373. Claims 15 and 30 depend from claims 1 and 16, respectively, and further require that extension steps (d)-(e) (claim 15) and (e)-(f) (claim 30) are "performed simultaneously." EX1001, 147:52-53, 150:16-17. Chell teaches the elements of claims 15 and 30.

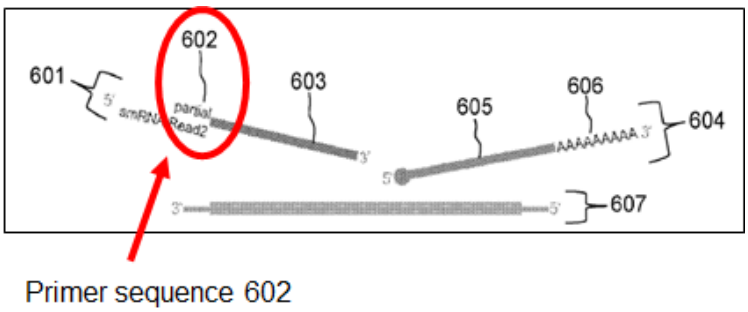
374. Chell specifies that the "*[l]igation products* that hybridized to the capture probes *were then extended*" by incubating "the *tissues* … with a second strand *extension mix*." EX1006, 80:30-31 (emphasis added), 83:17-18 (emphasis added), FIG. 13. A POSA would understand this to mean that Chell performs the extension steps simultaneously at multiple locations of the array, which in turn correspond to multiple locations (*i.e.*, a first and second location) of a tissue sample. Accordingly, Chell discloses the elements of claims 15 and 30.

xii. Claim 26

375. Claim 26 depends from claim 16, and further requires that "the nucleic acid probes" in the "first plurality," "the second plurality," or "both" the first and

second plurality of the nucleic acid probes, "comprise an amplification primer binding site." EX1001, 149:9-17. Chell teaches the elements of claim 26.

376. Chell's probe pairs (*i.e.*, nucleic acid probes of the claim) include a "first probe 601 [having] a target-hybridization sequence 603 and a *primer sequence* 602." EX1006, 81:19-22, 24:4-13 (emphasis added), 30:1-2, FIG. 6. Chell similarly specifies that "a first probe [of the probe pair] includes a functional sequence," and that a "functional sequence includes a *primer sequence*." EX1006, 30:1-2 (emphasis added). This is illustrated in the annotated excerpt of Figure 6 below.



EX1005, FIG. 6 (annotated).

377. Chell's Example 1 performs an amplification reaction on the extended capture probes (*i.e.*, extension product of the claims) to generate "amplified libraries" that are "sequenced." EX1006, 80:31-33, 83:20-84:11, FIG. 13. The primer sequence (602) in Chell's probe is used as a primer binding site during amplification. This is because the primer sequence (602) in Chell's probe is for a primer binding site or the reverse complement primer sequence as they are

functionally interchangeable in this context. Thus, Chell's primary probe pairs (*i.e.*, nucleic acid probes of the claim) comprise an amplification primer binding site. Accordingly, Chell discloses the elements of claim 26.

378. For these reasons, Chell discloses each and every element of claims 2-15 and 17-30, arranged as claimed. I understand that Chell's method is presumed enabled. Thus, in my opinion, Chell anticipates claims 2-8, 11-15, 17-23, and 26-30.

XIII. CONCLUSION

379. I conclude that in view of the state of the art before February 12, 2018, So-456 anticipated claims 1-8, 11-23, and 26-30 (Ground 1) or would have rendered claims 1-30 obvious (Ground 2). So-456 teaches each and every element of claims 1-8, 11-23, and 26-30, arranged as claimed. I understand that So-456 is presumed enabled. Thus, for the reasons detailed under Ground 1, So-456 anticipated claims 1-8, 11-23, and 26-30 of the 689 patent under NanoString's interpretation.

380. So-456 teaches or suggests all elements of the methods of claims 1-30 and a POSA would have been motivated to perform the methods of these claims with a reasonable expectation of success. Thus, for the reasons detailed under Ground 2, So-456 would have rendered claims 1-30 of the 689 patent obvious.

381. So-456 teaches and Stahl 2016 teach or suggest all elements of the methods of claims 1-30 and a POSA would have been motivated to perform the methods of these claims with a reasonable expectation of success. Thus, for the reasons detailed under Ground 3, So-456 and Stahl 2016 would have rendered claims 9, 10, 24, and 25 of the 689 patent obvious under NanoString's interpretation.

382. I conclude that the priority applications of the 689 patent do not provide written description support for the full scope of the 689 patent claims under NanoString's interpretation. Accordingly, I conclude that in view of the state of the art before September 16, 2021, Chell teaches every element of claims 1-30, arranged as claimed. I understand that Chell is presumed enabled. Thus, for the reasons detailed under Ground 4, Chell anticipated claims 1-30 of the 689 patent under NanoString's interpretation.

I hereby 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 U.S. Code.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Respectfully submitted,

Paul T. Spellman, Ph.D.

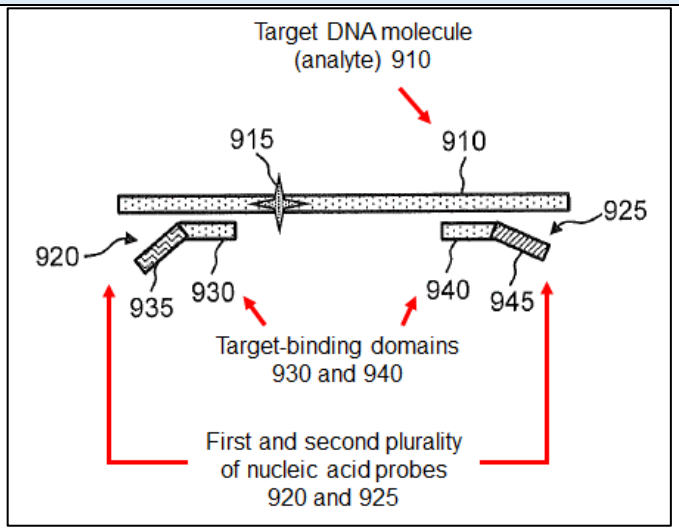
Date: August 16, 2023

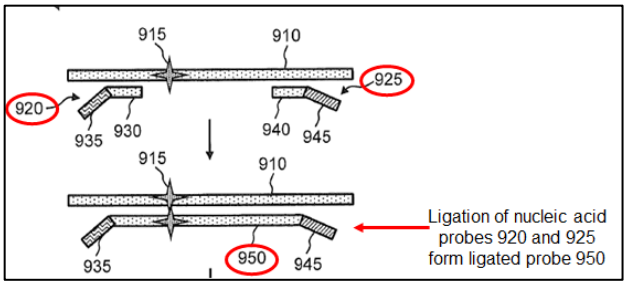
APPENDIX A[1]

Claim 16	So-456 (EX1005)
16. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:	"In another aspect, provided herein is a **method for spatial detection** and analysis of nucleic acids in a sample." (¶[00150]) "Figure 2 illustrates a flow diagram of an embodiment of a **method 200 of spatial detection** and analysis of a nucleic acid in a tissue sample. Method 200 can include, but is not limited to, some or all of the following steps." (¶[00151]) "Figure 8 illustrates a flow diagram of an **exemplary embodiment of a method of capturing DNA amplicons onto an array**, wherein the DNA amplicons are generated by in situ amplification of target nucleic acid." (¶[0018]) "Figure 9 illustrates an exemplary embodiment of the steps of a method of Figure 8." ([¶0019]) "Figure 8 illustrates a flow diagram of an embodiment of a **method 800 of capturing DNA amplicons onto an array (e.g., capture array 100 of Figure 1 A)**, wherein the DNA amplicons are generated by in situ amplification of target nucleic acid. In this embodiment, the amplification reaction is a TSCA-like amplification (TruSeq Custom Amplicon assembly, Illumina). Method 800 comprises, but

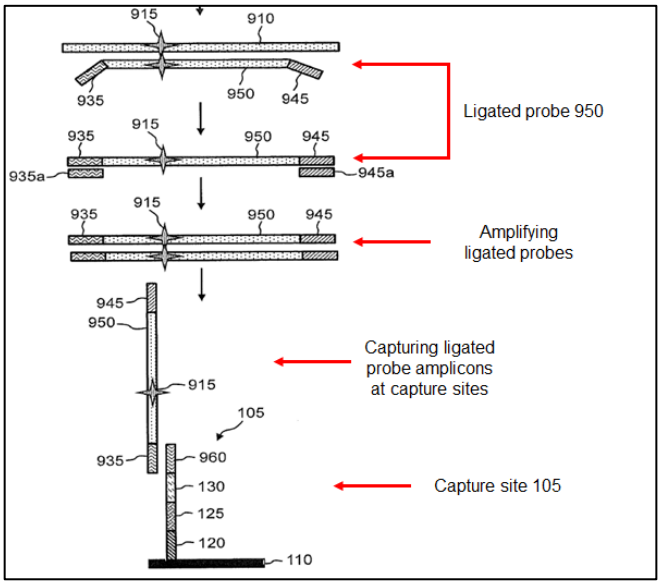
[1] Emphasis added in bold.

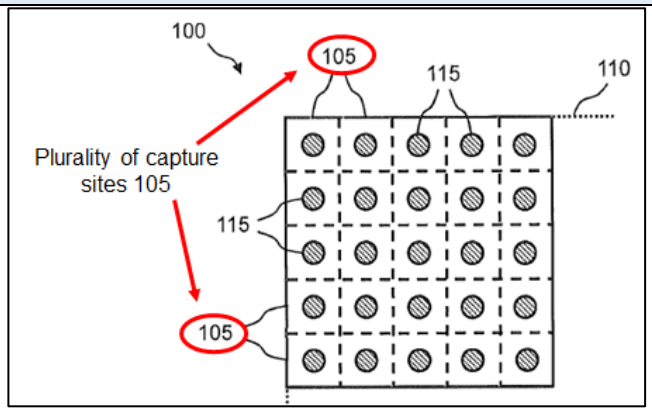
Claim 16	So-456 (EX1005)
	is not limited to, some or all of the following steps." (¶[00177]) *See also* ¶¶[00178]-[00183], FIGs. 2, 8, 9.
a) contacting the tissue sample with a first plurality of nucleic acid probes and a second plurality of nucleic acid probes, wherein each of the nucleic acid probes 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;	"At a step 810, **a pair of gene-specific capture oligonucleotides that flank a region of interest are hybridized in situ to genomic DNA**. For example, a first capture oligonucleotide that hybridizes 5' to a region of interest can comprise a gene-specific sequence and a universal capture sequence. A second capture oligonucleotide that hybridizes 3' to the region of interest can comprise a second gene-specific sequence and an SBS primer sequence (e.g., SBS12)." (¶[00178]) "Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8. Namely, a tissue section (not shown) comprises a **target genomic DNA molecule 910**. Target DNA molecule 910 can include a mutation 915. At step 810, **a first gene-specific capture oligonucleotide 920 and a second gene-specific capture oligonucleotide 925** that flank a region of interest **are hybridized in situ to DNA molecule 910**. Capture oligonucleotide 920 comprises a **gene-specific region 930** and a universal capture region 935. Capture oligonucleotide 920 can also comprise a UMI region (not shown). Capture oligonucleotide 925 comprises a **second gene-specific region 940** and an SBS primer region 945 (e.g., SBS12). . . ." (¶[00182])

Claim 16	So-456 (EX1005)
	 (FIG. 9 (annotated)) "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." (¶[00152]) "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." (¶[00153])

Claim 16	So-456 (EX1005)
	See also FIGs. 2, 8.
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;	"At a step 815, an **in situ extension/ligation reaction** is performed **between the flanking capture oligonucleotides** across the region of interest." (¶[00179]) "Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8. . . . At step 815, **an extension/ligation reaction** is performed in situ **between the flanking capture oligonucleotides 920 and 925** across the region of interest. A **DNA molecule 950 that is formed in the extension/ligation reaction** comprises universal capture region 935 and SBS primer region 945. . . ." (¶[00182])  (FIG. 9 (annotated)) *See also* FIG. 8.
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	"At a 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. Isothermal amplification can be performed, for example, using primer sequences that are complementary to the

Claim 16	So-456 (EX1005)
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;	universal capture sequence and the SBS primer sequence." (¶[00180]) "At a step 825, the **genomic amplicons are transferred onto an array and captured by hybridization to universal capture regions on the array**." (¶[00181]) "Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8. . . . At step 820, **DNA molecule 950 is amplified by in situ isothermal amplification to generate multiple copies** (multiple amplicons) of the targeted region of interest. Isothermal amplification can be performed, for example, using a primer region 935a that is complementary to universal capture region 935 and a primer region 945a that is complementary to SBS primer region 945. 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 comprises a capture region 960 that is complementary to universal capture region 935; SBS primer region 130 [sic] is SBS3. DNA amplicons 950 are captured on the capture site 105 by hybridization of universal capture region 935 to capture region 960." (¶[00182]) "In this example, the tissue sample is contacted with capture probe 115 that is fixed on the surface of capture site 105. **Genomic amplicon 950 is captured onto the array by**

Claim 16	So-456 (EX1005)
	hybridization of universal capture sequence 935 to capture sequence 960. In another example (not shown), the tissue sample is contacted with the array and capture probes 115 are released from capture site 105 into the tissue sample by cleavage of optional cleavable sequence 120." (¶[00183)  (FIG. 9 (annotated)) "Figure 1 A illustrates a plan view of an exemplary embodiment of a capture array for capture of nucleic acid in a tissue sample." (¶[0009])

Claim 16	So-456 (EX1005)
	 (FIG. 1A (annotated)) "Figure 1 A illustrates a plan view of an exemplary embodiment of a **capture array 100 for capture of nucleic acid** in a tissue sample. **Capture array 100 comprises an arrangement (e.g., rows and columns) of capture sites 105** on a solid support 110. In some embodiments, solid support 110 is a planar glass substrate. In some embodiments, solid support 110 is a bead (e.g., see Figure 1C). At least one capture probe 115 is immobilized at each of the capture sites 105 of capture array 100, as shown in Figure IB. Namely, Figure IB illustrates a side view of one capture site 105 of capture array 100, wherein the one capture site 105 comprises at least one capture probe 115 for capture of nucleic acid in a tissue sample. Figure IB shows a **capture probe 115 immobilized on the surface of solid support 110**. In this embodiment, **a single capture probe 115 is shown, but any number of capture probes 115 can be immobilized on solid support 110 at each capture site 105**. Capture probe 115 may optionally comprise a cleavable sequence in a cleavable region 120, an

Claim 16	So-456 (EX1005)
	SBS primer sequence in a SBS primer binding site 125, a spatial address sequence in a spatial address region 130, and a capture sequence in a capture region 135. Cleavable region 120 can be used to release captured nucleic acid from capture array 100 such that spatial address region 130 is included in the released nucleic acid and the nucleic acid is "tagged." SBS primer region 125 can comprise an SBS primer sequence (e.g., SBS12 or SBS3) that can be used in a sequencing-by- synthesis (SBS) process. Alternatively, SBS primer sequence 125 or some portion thereof may be added subsequently, e.g., by ligation or by PCR synthesis. SBS primer region 125 can also be used in an amplification reaction to generate a sequencing library as described in more detail with reference to Figure 12 and Figure 13. Spatial address region 130 corresponds to the position of capture probe 115 in capture array 100. Each capture probe 115 at a capture site 105 comprises a unique spatial address region 130. The position of capture probe 115 in capture array 100 can be correlated with a position in the tissue sample." (¶[00141]) *See also* FIGs. 2, 8.
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	"The present disclosure is based, in part, on the **realization that information related to the spatial origin of a nucleic acid in a tissue sample can be encoded in the nucleic acid in the process of preparing the nucleic acid for sequencing**. For example, **nucleic acids from a tissue sample can be tagged by probes including location-specific sequence**

Claim 16	So-456 (EX1005)
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	**information (a 'spatial address').** Spatially addressed nucleic acid molecules from a tissue sample can then be sequenced in bulk. The sequence-identical nucleic acid molecules originating from different regions in a tissue sample can be distinguished based on their spatial address and can be mapped onto their regions of origin in the tissue sample." (¶[0089]) "In some embodiments, **two spatial address sequences are incorporated into a nucleic acid during preparation of a sequencing library**. A first spatial address can be used to define a certain position (i.e., capture site) in the X dimension on a capture array and a second spatial address sequence can be used define a position (i.e., a capture site) in the Y dimension on the capture array. During library sequencing, both X and Y spatial address sequences can be determined and the sequence information can be analyzed to define the specific position on the capture array." (¶[00131]) "In some embodiments**, three spatial address sequences are incorporated into a nucleic acid during preparation of a sequencing library**. A first spatial address can be used to define a certain position (i.e., capture site) in the X dimension on a capture array, a second spatial address sequence can be used define a position (i.e., a capture site) in the Y dimension on the capture array, and a third spatial address sequence can be used to define a position of a two-dimensional sample section (e.g., the position of a slice of a tissue sample) in a sample (e.g., a tissue biopsy) to provide

Claim 16	So-456 (EX1005)
	positional spatial information in the third dimension (Z dimension) of a sample. During library sequencing, X, Y, and Z spatial address sequences can be determined and the sequence information can be analyzed to define the specific position on the capture array." (¶[00132]) "At a step 225, a **sequencing library is prepared**. In some embodiments, the sequencing library is prepared for sequencing-by-synthesis [SBS]. Library preparation may be accomplished on the capture array substrate, or the nucleic acids may be cleaved from the substrate and pooled, so that library preparation can be accomplished separately, e.g., using a NeoPrep™ Library Prep System (Illumina, Inc., San Diego)." (¶[00156]) "Figure 1 A illustrates a plan view of an exemplary embodiment of a capture array 100 for capture of nucleic acid in a tissue sample. Capture array 100 comprises an arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110. In some embodiments, solid support 110 is a planar glass substrate. In some embodiments, solid support 110 is a bead (e.g., see Figure 1C). At least one capture probe 115 is immobilized at each of the capture sites 105 of capture array 100, as shown in Figure IB. Namely, Figure IB illustrates a side view of one capture site 105 of capture array 100, wherein the one capture site 105 comprises at least one capture probe 115 for capture of nucleic acid in a tissue sample. Figure IB shows a capture probe 115 immobilized on the surface of solid support 110. In this embodiment, a single

Claim 16	So-456 (EX1005)
	capture probe 115 is shown, but any number of capture probes 115 can be immobilized on solid support 110 at each capture site 105**. Capture probe 115 may optionally comprise** a cleavable sequence in a cleavable region 120, an SBS primer sequence in a SBS primer binding site 125, **a spatial address sequence in a spatial address region 130**, and a capture sequence in a capture region 135. Cleavable region 120 can be used to release captured nucleic acid from capture array 100 such that spatial address region 130 is included in the released nucleic acid and the nucleic acid is "tagged." SBS primer region 125 can comprise an SBS primer sequence (e.g., SBS12 or SBS3) that can be used in a sequencing-by- synthesis (SBS) process. Alternatively, SBS primer sequence 125 or some portion thereof may be added subsequently, e.g., by ligation or by PCR synthesis. SBS primer region 125 can also be used in an amplification reaction to generate a sequencing library as described in more detail with reference to Figure 12 and Figure 13. Spatial address region 130 corresponds to the position of capture probe 115 in capture array 100. Each capture probe 115 at a capture site 105 comprises a unique spatial address region 130. The position of capture probe 115 in capture array 100 can be correlated with a position in the tissue sample." (¶[00141]) "As used herein, a '**spatial address**,' 'spatial tag' or 'spatial index,' when used in reference to **a nucleotide sequence, means an address, tag or index encoding spatial information related to the region or location of origin of an**

Claim 16	So-456 (EX1005)
	addressed, tagged, or indexed nucleic acid in a tissue sample." (¶[0099]) "As used herein, the term 'immobilized' when used in reference to a nucleic acid is intended to mean direct or indirect attachment to a solid support via covalent or non-covalent bond(s). In certain embodiments, covalent attachment can be used, but all that is required is that the nucleic acids remain stationary or attached to a support under conditions in which it is intended to use the support, for example, in applications requiring nucleic acid amplification and/or sequencing. **Oligonucleotides to be used as capture primers** or amplification primers can be **immobilized such that a 3'-end is available for enzymatic extension** and at least a portion of the sequence is capable of hybridizing to a complementary sequence. Immobilization can occur via hybridization to a surface attached oligonucleotide, in which case the immobilized oligonucleotide or polynucleotide can be in the 3' -5' orientation. Alternatively, immobilization can occur by means other than base-pairing hybridization, such as the covalent attachment set forth above." (¶[00109]) "In this example, **both capture region 660 and universal capture region 630 can also function as primers for an extension reaction**. When capture region 660 is used as a primer, mutation 615 in DNA molecule 655 is copied. When universal capture region 630 is used as a primer, spatial address region 130 and SBS primer region 125 are copied. **Both extension**

Claim 16	So-456 (EX1005)
	products can be used for downstream library generation." (¶00173]) *See also* FIGs. 2, 6.
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.	"At a step 230, **the library is sequenced**. Sequencing may be accomplished using **any sequencing technique**. Examples of suitable sequencers include those available from, or being developed by, Illumina, Inc., F. Hoffmann-La Roche AG, Life Technologies, Inc., Beckman Coulter, Inc., Pacific Biosciences, Inc., Oxford Nanopore, Inc., and/or their affiliates." (¶[00157]) "At a step 235, **the sequence data is analyzed** (e.g., mutations and/or variant calling) **and the spatial information is decoded**. The spatial information can be used **to provide information as to the location of the nucleic acid in the tissue section**." (¶[00158]) "The present disclosure is based, in part, on the realization that information related to the spatial origin of a nucleic acid in a tissue sample can be encoded in the nucleic acid in the process of preparing the nucleic acid for sequencing. For example, nucleic acids from a tissue sample can be tagged by probes including location-specific sequence information (a "spatial address"). **Spatially addressed nucleic acid molecules from a tissue sample can then be sequenced in bulk. The sequence-identical nucleic acid molecules originating from different regions in a tissue sample can be distinguished based on their spatial address and can be mapped**

Claim 16	So-456 (EX1005)
	onto their regions of origin in the tissue sample." (¶[0089]) *See also* ¶¶[00150]-[00157], [00177]-[00183], [00611], [00100], [00101], [00102], [00103], [00105]-[00108], [00110], [00111], [00114], [00122], [00143], [00221], [00222], [00574], [00598], [00599], [00601], [00602], [00603], [00604], [00606], [00607], [00608], FIGs. 2, 8, 9.

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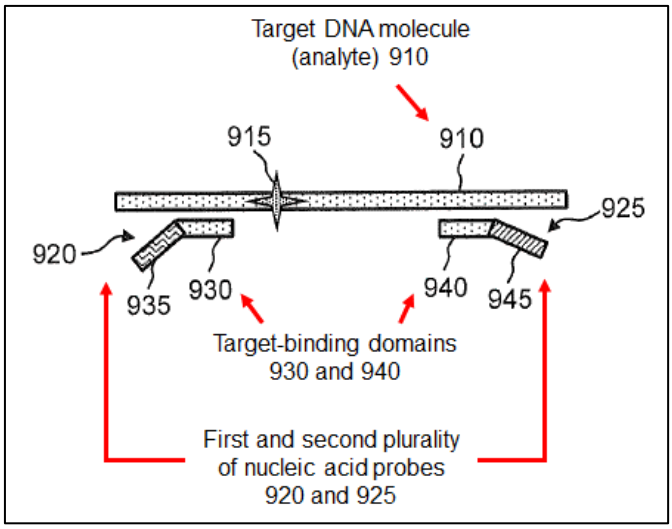
APPENDIX B[1]

Claim 1	So-456 (EX1005)
1. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:	"In another aspect, provided herein is a **method for spatial detection** and analysis of nucleic acids in a sample." (¶[00150]) "Figure 2 illustrates a flow diagram of an embodiment of a **method 200 of spatial detection** and analysis of a nucleic acid in a tissue sample. Method 200 can include, but is not limited to, some or all of the following steps." (¶[00151]) "Figure 8 illustrates a flow diagram of an **exemplary embodiment of a method of capturing DNA amplicons onto an array**, wherein the DNA amplicons are generated by in situ amplification of target nucleic acid." (¶[0018]) "Figure 9 illustrates an exemplary embodiment of the steps of a method of Figure 8." ([¶0019]) "Figure 8 illustrates a flow diagram of an embodiment of a **method 800 of capturing DNA amplicons onto an array (e.g., capture array 100 of Figure 1 A)**, wherein the DNA amplicons are generated by in situ amplification of target nucleic acid. In this embodiment, the amplification reaction is a TSCA-like amplification (TruSeq Custom Amplicon assembly, Illumina). Method 800

[1] Emphasis added in bold.

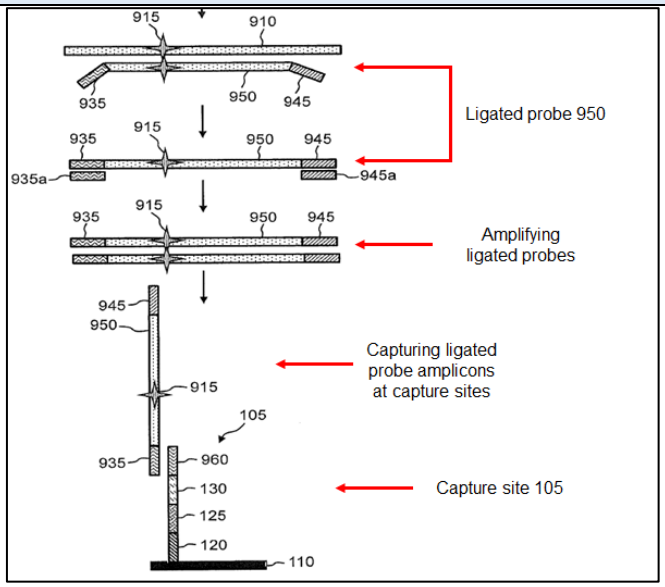
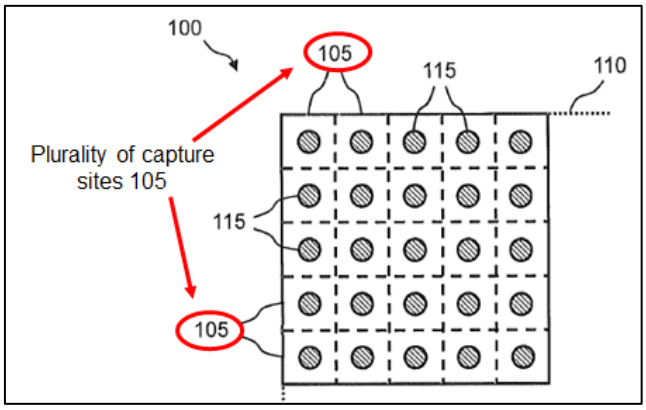
Claim 1	So-456 (EX1005)
	comprises, but is not limited to, some or all of the following steps." (¶[00177]) *See also* ¶¶[00178]-[00183], FIGs. 2, 8, 9.
a) contacting the tissue sample with a plurality of nucleic acid probes, wherein each of the nucleic acid probes comprise a target binding domain that 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[2];	"At a step 810, **a pair of gene-specific capture oligonucleotides that flank a region of interest are hybridized in situ to genomic DNA**. For example, a first capture oligonucleotide that hybridizes 5' to a region of interest can comprise a gene-specific sequence and a universal capture sequence. A second capture oligonucleotide that hybridizes 3' to the region of interest can comprise a second gene-specific sequence and an SBS primer sequence (e.g., SBS12)." (¶[00178]) "Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8. Namely, a tissue section (not shown) comprises a **target genomic DNA molecule 910**. Target DNA molecule 910 can include a mutation 915. At step 810, **a first gene-specific capture oligonucleotide 920 and a second gene-specific capture oligonucleotide 925** that flank a region of interest **are hybridized in situ to DNA molecule 910**. Capture oligonucleotide 920 comprises a **gene-specific region 930** and a universal capture region 935. Capture oligonucleotide 920 can also comprise a UMI region (not

[2] The claim language "wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte" reflects the correction made after the 689 patent issued.

Claim 1	So-456 (EX1005)
	shown). Capture oligonucleotide 925 comprises a **second gene-specific region 940** and an SBS primer region 945 (e.g., SBS12). . . .” (¶[00182])  (FIG. 9 (annotated)) “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.” (¶[00152]) “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

Claim 1	So-456 (EX1005)
	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." (¶[00153]) *See also* FIGs. 2, 8.
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;	"At a 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. Isothermal amplification can be performed, for example, using primer sequences that are complementary to the universal capture sequence and the SBS primer sequence." (¶[00180]) "At a step 825, the **genomic amplicons are transferred onto an array and captured by hybridization to universal capture regions on the array**." (¶[00181]) "Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8. . . . At step 820, **DNA molecule 950 is amplified by in situ isothermal amplification to generate multiple copies** (multiple amplicons) of the targeted region of interest. Isothermal amplification can be performed, for example, using a primer region 935a that is complementary to universal capture region 935 and a primer region 945a that is complementary to SBS primer region 945. At step 825, **genomic amplicons 950 are captured onto the capture site 105**. For

Claim 1	So-456 (EX1005)
	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 comprises a capture region 960 that is complementary to universal capture region 935; SBS primer region 130 [sic] is SBS3. DNA amplicons 950 are captured on the capture site 105 by hybridization of universal capture region 935 to capture region 960." (¶[00182]) "In this example, the tissue sample is contacted with capture probe 115 that is fixed on the surface of capture site 105. **Genomic amplicon 950 is captured onto the array by hybridization of universal capture sequence 935 to capture sequence 960**. In another example (not shown), the tissue sample is contacted with the array and capture probes 115 are released from capture site 105 into the tissue sample by cleavage of optional cleavable sequence 120." (¶[00183)

Claim 1	So-456 (EX1005)
	 (FIG. 9 (annotated)) "Figure 1 A illustrates a plan view of an exemplary embodiment of a capture array for capture of nucleic acid in a tissue sample." (¶[0009])  (FIG. 1A (annotated)) "Figure 1 A illustrates a plan view of an exemplary embodiment of a **capture array 100 for capture of nucleic acid** in a tissue sample. **Capture array 100 comprises an**

Claim 1	So-456 (EX1005)
	arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110. In some embodiments, solid support 110 is a planar glass substrate. In some embodiments, solid support 110 is a bead (e.g., see Figure 1C). At least one capture probe 115 is immobilized at each of the capture sites 105 of capture array 100, as shown in Figure IB. Namely, Figure IB illustrates a side view of one capture site 105 of capture array 100, wherein the one capture site 105 comprises at least one capture probe 115 for capture of nucleic acid in a tissue sample. Figure IB shows a **capture probe 115 immobilized on the surface of solid support 110**. In this embodiment, **a single capture probe 115 is shown, but any number of capture probes 115 can be immobilized on solid support 110 at each capture site 105**. Capture probe 115 may optionally comprise a cleavable sequence in a cleavable region 120, an SBS primer sequence in a SBS primer binding site 125, a spatial address sequence in a spatial address region 130, and a capture sequence in a capture region 135. Cleavable region 120 can be used to release captured nucleic acid from capture array 100 such that spatial address region 130 is included in the released nucleic acid and the nucleic acid is "tagged." SBS primer region 125 can comprise an SBS primer sequence (e.g., SBS12 or SBS3) that can be used in a sequencing-by- synthesis (SBS) process. Alternatively, SBS primer sequence 125 or some portion thereof may be added

Claim 1	So-456 (EX1005)
	subsequently, e.g., by ligation or by PCR synthesis. SBS primer region 125 can also be used in an amplification reaction to generate a sequencing library as described in more detail with reference to Figure 12 and Figure 13. Spatial address region 130 corresponds to the position of capture probe 115 in capture array 100. Each capture probe 115 at a capture site 105 comprises a unique spatial address region 130. The position of capture probe 115 in capture array 100 can be correlated with a position in the tissue sample." (¶[00141]) *See also* FIGs. 2, 8.
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	"The present disclosure is based, in part, on the **realization that information related to the spatial origin of a nucleic acid in a tissue sample can be encoded in the nucleic acid in the process of preparing the nucleic acid for sequencing**. For example, **nucleic acids from a tissue sample can be tagged by probes including location-specific sequence information (a 'spatial address').** Spatially addressed nucleic acid molecules from a tissue sample can then be sequenced in bulk. The sequence-identical nucleic acid molecules originating from different regions in a tissue sample can be distinguished based on their spatial address and can be mapped onto their regions of origin in the tissue sample." (¶[0089]) "In some embodiments, **two spatial address sequences are incorporated into a**

Claim 1	So-456 (EX1005)
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	**nucleic acid during preparation of a sequencing library**. A first spatial address can be used to define a certain position (i.e., capture site) in the X dimension on a capture array and a second spatial address sequence can be used define a position (i.e., a capture site) in the Y dimension on the capture array. During library sequencing, both X and Y spatial address sequences can be determined and the sequence information can be analyzed to define the specific position on the capture array." (¶[00131]) "In some embodiments**, three spatial address sequences are incorporated into a nucleic acid during preparation of a sequencing library**. A first spatial address can be used to define a certain position (i.e., capture site) in the X dimension on a capture array, a second spatial address sequence can be used define a position (i.e., a capture site) in the Y dimension on the capture array, and a third spatial address sequence can be used to define a position of a two-dimensional sample section (e.g., the position of a slice of a tissue sample) in a sample (e.g., a tissue biopsy) to provide positional spatial information in the third dimension (Z dimension) of a sample. During library sequencing, X, Y, and Z spatial address sequences can be determined and the sequence information can be analyzed to define the specific position on the capture array." (¶[00132]) "At a step 225, a **sequencing library is prepared**. In some embodiments, the

Claim 1	So-456 (EX1005)
	sequencing library is prepared for sequencing-by-synthesis [SBS]. Library preparation may be accomplished on the capture array substrate, or the nucleic acids may be cleaved from the substrate and pooled, so that library preparation can be accomplished separately, e.g., using a NeoPrep™ Library Prep System (Illumina, Inc., San Diego)." (¶[00156]) "Figure 1 A illustrates a plan view of an exemplary embodiment of a capture array 100 for capture of nucleic acid in a tissue sample. Capture array 100 comprises an arrangement (e.g., rows and columns) of capture sites 105 on a solid support 110. In some embodiments, solid support 110 is a planar glass substrate. In some embodiments, solid support 110 is a bead (e.g., see Figure 1C). At least one capture probe 115 is immobilized at each of the capture sites 105 of capture array 100, as shown in Figure IB. Namely, Figure IB illustrates a side view of one capture site 105 of capture array 100, wherein the one capture site 105 comprises at least one capture probe 115 for capture of nucleic acid in a tissue sample. Figure IB shows a capture probe 115 immobilized on the surface of solid support 110. In this embodiment, a single capture probe 115 is shown, but any number of capture probes 115 can be immobilized on solid support 110 at each capture site 105**. Capture probe 115 may optionally comprise** a cleavable sequence in a cleavable region 120, an SBS primer sequence in a SBS

Claim 1	So-456 (EX1005)
	primer binding site 125, **a spatial address sequence in a spatial address region 130**, and a capture sequence in a capture region 135. Cleavable region 120 can be used to release captured nucleic acid from capture array 100 such that spatial address region 130 is included in the released nucleic acid and the nucleic acid is "tagged." SBS primer region 125 can comprise an SBS primer sequence (e.g., SBS12 or SBS3) that can be used in a sequencing-by- synthesis (SBS) process. Alternatively, SBS primer sequence 125 or some portion thereof may be added subsequently, e.g., by ligation or by PCR synthesis. SBS primer region 125 can also be used in an amplification reaction to generate a sequencing library as described in more detail with reference to Figure 12 and Figure 13. Spatial address region 130 corresponds to the position of capture probe 115 in capture array 100. Each capture probe 115 at a capture site 105 comprises a unique spatial address region 130. The position of capture probe 115 in capture array 100 can be correlated with a position in the tissue sample." (¶[00141]) "As used herein, a '**spatial address**,' 'spatial tag' or 'spatial index,' when used in reference to **a nucleotide sequence, means an address, tag or index encoding spatial information related to the region or location of origin of an addressed, tagged, or indexed nucleic acid in a tissue sample**." (¶[0099])

Claim 1	So-456 (EX1005)
	"As used herein, the term 'immobilized' when used in reference to a nucleic acid is intended to mean direct or indirect attachment to a solid support via covalent or non-covalent bond(s). In certain embodiments, covalent attachment can be used, but all that is required is that the nucleic acids remain stationary or attached to a support under conditions in which it is intended to use the support, for example, in applications requiring nucleic acid amplification and/or sequencing. **Oligonucleotides to be used as capture primers** or amplification primers can be **immobilized such that a 3'-end is available for enzymatic extension** and at least a portion of the sequence is capable of hybridizing to a complementary sequence. Immobilization can occur via hybridization to a surface attached oligonucleotide, in which case the immobilized oligonucleotide or polynucleotide can be in the 3' -5' orientation. Alternatively, immobilization can occur by means other than base-pairing hybridization, such as the covalent attachment set forth above." (¶[00109]) "In this example, **both capture region 660 and universal capture region 630 can also function as primers for an extension reaction**. When capture region 660 is used as a primer, mutation 615 in DNA molecule 655 is copied. When universal capture region 630 is used as a primer, spatial address region 130 and SBS primer region 125 are copied. **Both extension products**

Claim 1	So-456 (EX1005)
	can be used for downstream library generation." (¶00173]) *See also* FIGs. 2, 6.
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.	"At a step 230, **the library is sequenced**. Sequencing may be accomplished using **any sequencing technique**. Examples of suitable sequencers include those available from, or being developed by, Illumina, Inc., F. Hoffmann-La Roche AG, Life Technologies, Inc., Beckman Coulter, Inc., Pacific Biosciences, Inc., Oxford Nanopore, Inc., and/or their affiliates." (¶[00157]) "At a step 235, **the sequence data is analyzed** (e.g., mutations and/or variant calling) **and the spatial information is decoded**. The spatial information can be used **to provide information as to the location of the nucleic acid in the tissue section**." (¶[00158]) "The present disclosure is based, in part, on the realization that information related to the spatial origin of a nucleic acid in a tissue sample can be encoded in the nucleic acid in the process of preparing the nucleic acid for sequencing. For example, nucleic acids from a tissue sample can be tagged by probes including location-specific sequence information (a "spatial address"). **Spatially addressed nucleic acid molecules from a tissue sample can then be sequenced in bulk. The sequence-identical nucleic acid molecules originating from different regions in a tissue sample can be**

Claim 1	So-456 (EX1005)
	distinguished based on their spatial address and can be mapped onto their regions of origin in the tissue sample." (¶[0089]) *See also* ¶¶[00150]-[00157], [00177]-[00183], [00611], [00100], [00101], [00102], [00103], [00105]-[00108], [00110], [00111], [00114], [00122], [00143], [00221], [00222], [00574], [00598], [00599], [00601], [00602], [00603], [00604], [00606], [00607], [00608], FIGs. 2, 8, 9.

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APPENDIX C[1]

Claim 16	Chell (EX1006)
16. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:	"**Methods for spatial analysis** using rna-templated ligation" (Title) "Provided herein are **methods of detecting an analyte of interest to interrogate spatial gene expression** in a sample using RNA-templated ligation." (Abstract) "FIG. 1 shows an exemplary **spatial analysis** workflow." (7:29) "Example 1. **Spatial gene expression** analysis of FFPE-fixed samples using RNA- templated ligation." (80:14-15) *See also* FIGs. 1, 6, 13, Example 2.
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	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed **as described in FIG. 13**. FFPE-fixed samples were deparaffinized, stained (e.g., H&E

[1] Emphasis added in bold.

Claim 16	Chell (EX1006)
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;	stain), and imaged 1301. **Samples were destained (e.g., using HC1) and decrosslinked 1302**. Following decrosslinking, **samples treated with pre-hybridization buffer** (e.g., hybridization buffer without the first and second probes), **probes were added to the sample, probes hybridized**, and samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306. Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308. Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33) "**RTL probes were designed to hybridize to adjacent sequences of each analyte (e.g., mRNA sequence)** of interest in the genome, including estrogen receptor, progesterone receptor, and ERBB2, also known as HER2. **Here, 20,056 probe pairs (e.g., RHS and LHS probes) were added to each tissue sample to capture 19,490 different genes. Two RTL probes (a left-hand side (LHS) probe and a right-hand side (RHS) probe (see e.g., FIG. 6)) for each analyte were added simultaneously and hybridized at adjacent sequences of the target mRNA**, forming RNA:DNA duplex structures. Following decrosslinking, the PBS-Tween was removed and the DNA probes (1 nM of each probe) were added to the tissue samples in a hybridization buffer for

Claim 16	Chell (EX1006)
	hybridizing the DNA probes to their respective mRNA targets. The hybridization buffer included SSC, formamide or an equivalent, yeast tRNA as carrier and the RHS and LHS DNA probes." (81:16-82:2) "One probe oligonucleotide (e.g., the RHS probe or the 3' probe) comprises a non target functional sequence at its 5' end while the other probe oligonucleotide (e.g., the LHS probe or the 5' probe) comprises a non-target polyA sequence at their 3' ends. The DNA probes were added to the tissue samples and incubated at 50 °C for 2 hours and 30 minutes according to thermal cycler protocol described in Table 3." (82:3-7) "Referring to FIG. 13, in another non-limiting example, a **biological sample** is deparaffinized, stained, and imaged 1301. **After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303**. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. Ligated probes are captured on an array by a capture probe 1306, extended using a polymerase 1307 and denatured 1308. After quality control cleanup 1309, the abundance and location of an analyte is determined." (23:30-24:3)

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	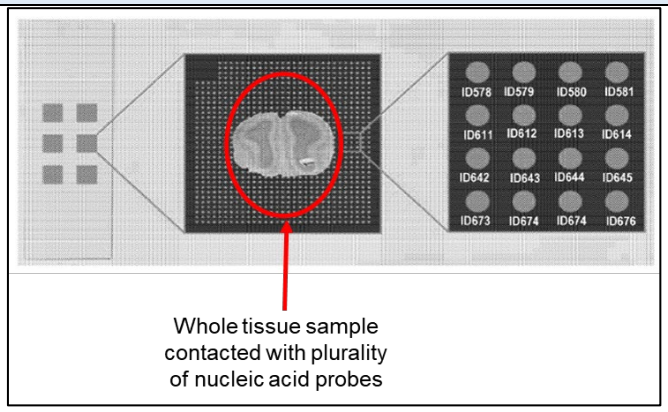 (FIG. 5 (annotated)) "A non-limiting example of the methods disclosed herein is depicted in FIG. 6. After a **biological sample is** contacted with a substrate including a plurality of capture probes and **contacted with (a) a first probe 601 having a target-hybridization sequence 603** and a primer sequence 602 and **(b) a second probe 604 having a target-hybridization sequence 605** and a capture domain (e.g., a poly-A sequence) 606, the first probe 601 and a second probe 604 hybridize 610 to an analyte 607. A ligase 621 ligates 620 the first probe to the second probe thereby generating a ligation product 622. The ligation product is released 630 from the analyte 631 by digesting the analyte using an endoribonuclease 632. The sample is permeabilized 640 and the ligation product 641 is able to hybridize to a capture probe on the substrate." (24:4-13)

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	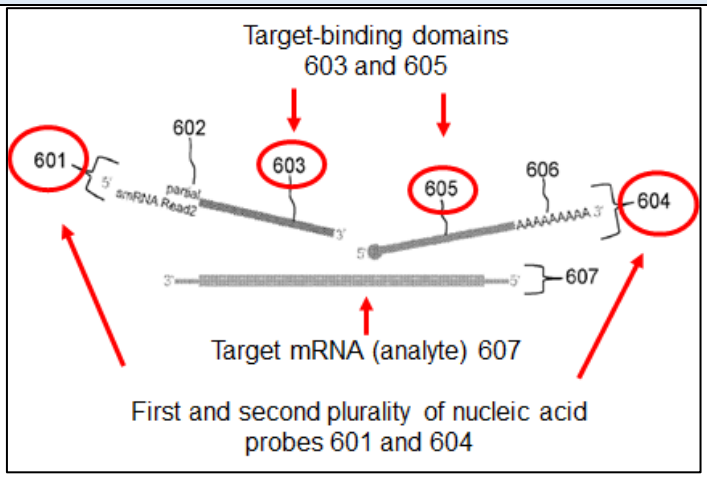 (FIG. 6 (annotated)) "Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 contacting a biological sample with array of spatially -barcoded capture probes. In some instances, the array is on a substrate and the array includes a plurality of capture probes, wherein a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain. **After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte**, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and the ligation product is released from the analyte. The liberated ligation

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	product is then freed 105 to hybridize to the capture domain of a probe on the array. After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample..” (23:13-29) *See also* FIGs. 1, 13, Example 2.
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;	“Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed as described in FIG. 13. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. Samples were destained (e.g., using HC1) and decrosslinked 1302. Following decrosslinking, samples treated with pre-hybridization buffer (e.g., hybridization buffer without the first and second probes), probes were added to the sample, probes hybridized, and samples were washed 1303. **Ligase was added to the samples to ligate hybridized probes to**

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	generate a ligation product and samples were then washed 1304. Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306. Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308. Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33) "**To ligate two diribo probe oligonucleotides that adjacently hybridized on the target mRNA** as described above, each sample was incubated with RNL2 T4 DNA ligase reaction mixture (ligation mix). The ligase reaction mixture included: T4 RNA Ligase Buffer (NEB B0293S), RNL2 ligase and nuclease free water." (82:15-18) "**To ligate two DNA probe oligonucleotides that adjacently hybridized on the target mRNAs** as described above, SplintR (NEB) in a ligation buffer was added to each sample. The ligase reaction mixture included: Tris-HCl (pH 7.5) MnCh ATP, DTT, surrogate fluid, SplintR **ligase** (NEB), and nuclease free water per sample. The ligation and wash steps were performed as described in Table 4." (82:19-23) "Referring to FIG. 13, in another non-limiting example, a biological sample is deparaffinized, stained, and imaged 1301. After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes.

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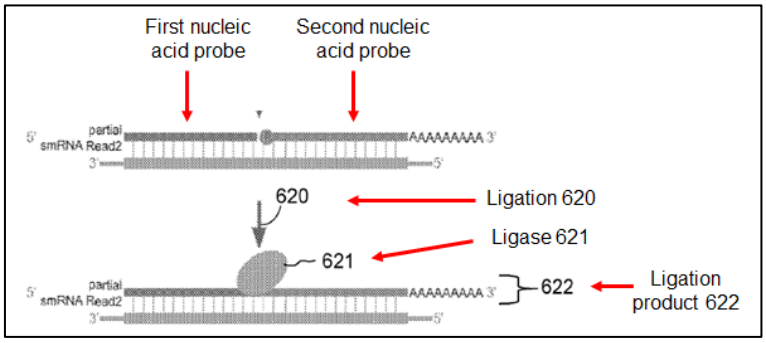
	Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. Ligated probes are captured on an array by a capture probe 1306, extended using a polymerase 1307 and denatured 1308. After quality control cleanup 1309, the abundance and location of an analyte is determined." (23:30-24:3) "A non-limiting example of the methods disclosed herein is depicted in FIG. 6. After a biological sample is contacted with a substrate including a plurality of capture probes and contacted with (a) a first probe 601 having a target-hybridization sequence 603 and a primer sequence 602 and (b) a second probe 604 having a target-hybridization sequence 605 and a capture domain (e.g., a poly-A sequence) 606, the first probe 601 and a second probe 604 hybridize 610 to an analyte 607. **A ligase 621 ligates 620 the first probe to the second probe thereby generating a ligation product 622**. The ligation product is released 630 from the analyte 631 by digesting the analyte using an endoribonuclease 632. The sample is permeabilized 640 and the ligation product 641 is able to hybridize to a capture probe on the substrate." (24:4-13)  (FIG. 6 (annotated))

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	"Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 contacting a biological sample with array of spatially -barcoded capture probes. In some instances, the array is on a substrate and the array includes a plurality of capture probes, wherein a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain. After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. **After hybridization a ligation product comprising the first probe and the second probe 104 is generated**, and the ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array. After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample.." (23:13-29) *See also* FIGs. 1, 6, 13, Example 2.

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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;	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed as described in FIG. 13. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. Samples were destained (e.g., using HC1) and decrosslinked 1302. Following decrosslinking, samples treated with pre-hybridization buffer (e.g., hybridization buffer without the first and second probes), probes were added to the sample, probes hybridized, and samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. **Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306.** Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308. Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33)

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	"**RNase H was added to digest the RNA strand of the hybridized RNA:DNA duplex.** Briefly, the RNA of the DNA:RNA hybrids was digested by incubating the samples with RNase H mix for 30 minutes at 37°C, where the RNAse H mix included: RNAse H buffer and RNaseH. Following the incubation and while the same remained at 37°C, the **biological sample was permeabilized to release the ligated RTL probes** using 1.25mg/mL Proteinase K. In particular, the Proteinase K solution included (per sample): Tris (pH 7.5), MgCh. Sarkosyl, or SDS , Proteinase K (Enzyme), and nuclease free water. **The sample was incubated** at 37°C for at least 5 minutes in the Proteinase K solution. The samples were then washed three times with 2X SSC." (83:4-12) "The **released, ligated DNA probes** that served as a proxy for the target mRNA were allowed to **hybridize to the capture domain on the capture probe immobilized on the spatial array** via the poly A tail on the 3' end of the RHS probe." (83:13-15) "Referring to FIG. 13, in another non-limiting example, a biological sample is deparaffinized, stained, and imaged 1301. After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. **Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. Ligated probes are captured on an array by a capture probe 1306**, extended using a polymerase 1307 and denatured 1308. After quality control

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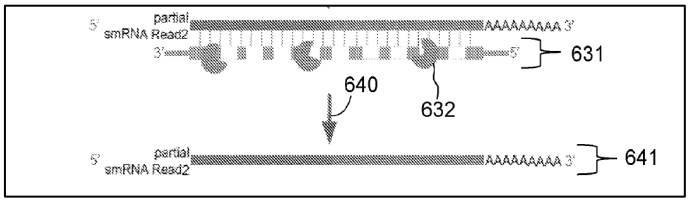
	cleanup 1309, the abundance and location of an analyte is determined." (23:30-24:3) "A non-limiting example of the methods disclosed herein is depicted in FIG. 6. After a biological sample is contacted with a substrate including a plurality of capture probes and contacted with (a) a first probe 601 having a target-hybridization sequence 603 and a primer sequence 602 and (b) a second probe 604 having a target-hybridization sequence 605 and a capture domain (e.g., a poly-A sequence) 606, the first probe 601 and a second probe 604 hybridize 610 to an analyte 607. A ligase 621 ligates 620 the first probe to the second probe thereby generating a ligation product 622. **The ligation product is released 630 from the analyte 631 by digesting the analyte using an endoribonuclease 632. The sample is permeabilized 640 and the ligation product 641 is able to hybridize to a capture probe on the substrate**." (24:4-13)  (FIG. 6) "Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 **contacting a biological sample with array of spatially -barcoded capture probes**. In some

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	instances, the array is on a substrate and the **array includes a plurality of capture probes**, wherein a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain. After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and **the ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array**. After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample..” (23:13-29) “RTL probes were designed to hybridize to adjacent sequences of each analyte (e.g., mRNA sequence) of interest in the genome, including estrogen receptor, progesterone receptor, and ERBB2, also known as HER2. **Here, 20,056 probe pairs (e.g., RHS and LHS probes) were added to each tissue sample to capture 19,490 different genes. Two RTL probes (a left-hand side (LHS) probe and a right-hand side (RHS)**

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	probe (see e.g., FIG. 6)) for each analyte were added simultaneously and hybridized at adjacent sequences of the target mRNA, forming RNA:DNA duplex structures. Following decrosslinking, the PBS-Tween was removed and the DNA probes (1 nM of each probe) were added to the tissue samples in a hybridization buffer for hybridizing the DNA probes to their respective mRNA targets. The hybridization buffer included SSC, formamide or an equivalent, yeast tRNA as carrier and the RHS and LHS DNA probes." (81:16-82:2) *See also* FIGs. 1, 2, 5, 6, 13, Example 2.
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	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed as described in FIG. 13. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. Samples were destained (e.g., using HC1) and decrosslinked 1302. Following decrosslinking, samples treated with pre-hybridization buffer (e.g., hybridization buffer without the first and second probes), probes were added to the sample, probes hybridized, and

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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	samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306. **Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308.** Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33) "The released, ligated DNA probes that served as a proxy for the target mRNA were allowed to hybridize to the capture domain on the capture probe immobilized on the spatial array via the poly A tail on the 3' end of the RHS probe. **The captured ligated probes were copied, using the capture probe as a template and the extension product was released from the spatial array.** Briefly, the tissues were incubated with a second strand extension mix comprising Kapa Hifi DNA polymerase for 25 minutes at 53°C. Following incubation, the second strand extension mix was removed from the tissues and the tissues were washed with 2X SSC. A solution of KOH was added to each of the tissue wells, the tissues were incubated at room temperature for 10 minutes to release the extension product from the spatial array and the supernatant from each tissue well was transferred for quantification, and library preparation. Sample quantification was performed using qPCR and KAPA SYBR FAST qPCR master mix according to the manufacturer's

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	instructions. Briefly, KAPA SYBR master mix was prepared by adding qPCR primer cDNA F and qPCR primer sRNA_R2. The thermal cycler protocol included: 3 minutes at 98°C; 30 cycles with 5 seconds at 98°C, and 30 seconds at 63°C. For library preparation, samples were indexed using an Amp Mix that included dual indexing primers and an Amp Mix. Nucleic acids were then sequenced and analyzed." (83:13-84:9) "Referring to FIG. 13, in another non-limiting example, a biological sample is deparaffinized, stained, and imaged 1301. After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. **Ligated** probes are captured on an array by a capture probe 1306, **extended using a polymerase 1307 and denatured 1308.** After quality control cleanup 1309, the abundance and location of an analyte is determined." (23:30-24:3) "A **'capture probe'** refers to **any molecule capable of capturing (directly or indirectly) and/or labelling an analyte (e.g., an analyte of interest) in a biological sample**. In some embodiments, the capture probe is a nucleic acid or a polypeptide**. In some embodiments, the capture probe includes a barcode (e.g., a spatial barcode** and/or a unique molecular identifier (UMI)) and a capture domain). In some embodiments, a capture probe can include a cleavage domain and/or a functional domain (e.g., a primer-binding site, such as for next- generation

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	sequencing (NGS)). See, e.g., WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Generation of capture probes can be achieved by any appropriate method, including those described in WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663." (12:10-19) "As used herein, an **'extended capture probe' refers to a capture probe having additional nucleotides added to the terminus (e.g., 3' or 5' end) of the capture probe thereby extending the overall length of the capture probe.** For example, **an "extended 3' end" indicates additional nucleotides were added to the most 3' nucleotide of the capture probe to extend the length of the capture probe, for example, by polymerization reactions used to extend nucleic acid molecules including templated polymerization catalyzed by a polymerase (e.g., a DNA polymerase or a reverse transcriptase).** In some embodiments, extending the capture probe includes adding to a 3' end of a capture probe a nucleic acid sequence that is complementary to a nucleic acid sequence of an analyte or intermediate agent specifically bound to the capture domain of the capture probe. In some embodiments, the capture probe is extended using reverse transcription. In some embodiments, the capture probe is extended using one or more DNA polymerases. **The extended capture probes include the sequence of the capture probe and the sequence of the spatial barcode of the capture probe**." (17:24-18:2) "Spatial analysis methodologies and compositions described herein can provide a vast amount of

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	analyte and/or expression data for a variety of analytes within a biological sample at high spatial resolution, while retaining native spatial context. Spatial analysis methods and compositions can include, e.g., the use of a **capture probe including a spatial barcode (e.g., a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample** (e.g., mammalian cell or a mammalian tissue sample) and a capture domain that is capable of binding to an analyte (e.g., a protein and/or a nucleic acid) produced by and/or present in a cell." (10:6-10) "FIG. 2 is a schematic diagram showing an **example of a capture probe**, as described herein. As shown, the capture probe 202 is optionally coupled to a feature 201 by a cleavage domain 203, such as a disulfide linker. The capture probe can include functional sequences that are useful for subsequent processing, such as functional sequence 204, which can include a sequencer specific flow cell attachment sequence, e.g., a P5 or P7 sequence, as well as functional sequence 205, which can include sequencing primer sequences, e.g., a R1 primer binding site. In some embodiments, sequence 204 is a P7 sequence and sequence 205 is a R1 primer binding site. **A spatial barcode 206 can be included within the capture probe for use in barcoding the target analyte.** The functional sequences can generally be selected for compatibility with any of a variety of different sequencing systems, e.g., Ion Torrent Proton or PGM, Illumina sequencing instruments, PacBio, Oxford Nanopore, etc., and the requirements thereof. In some embodiments, functional sequences can be selected for

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	compatibility with non-commercialized sequencing systems. Examples of such sequencing systems and techniques, for which suitable functional sequences can be used, include (but are not limited to) Ion Torrent Proton or PGM sequencing, Illumina sequencing, PacBio SMRT sequencing, and Oxford Nanopore sequencing. Further, in some embodiments, functional sequences can be selected for compatibility with other sequencing systems." (15:28-16:10) "In some embodiments, the **spatial barcode 206**, functional sequences 204 (e.g., flow cell attachment sequence) and 205 (e.g., sequencing primer sequences) can be common to all of the probes attached to a given feature. The spatial barcode can also include a capture domain 207 to facilitate capture of a target analyte." (16:11-14) "Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 contacting a biological sample with array of spatially -barcoded capture probes. In some instances, the array is on a substrate and the **array includes a plurality of capture probes, wherein a capture probe of the plurality includes: (i) a spatial barcode** and (ii) a capture domain. After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to

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	complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and the ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array. After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample.." (23:13-29) "In some embodiments, as shown in FIG. 7, the ligation product 701 includes a capture probe capture domain 702, which can bind to a **capture probe 703 (e.g., a capture probe immobilized, directly or indirectly, on a substrate 704).** In some embodiments, methods provided herein include contacting 705 a biological sample with a substrate 704, wherein the capture probe 703 is affixed to the substrate (e.g., immobilized to the substrate, directly or indirectly). In some embodiments, the capture probe capture domain 702 of the ligated product specifically binds to the capture domain 706. **The capture probe can also include** a unique molecular identifier (UMI) 707, **a spatial barcode 708**, a functional sequence 709, and a cleavage domain 710." (24:25-33) "In some embodiments, methods provided herein include permeabilization of the biological sample such that the capture probe can more easily bind to the captured ligated probe (i.e., compared to no

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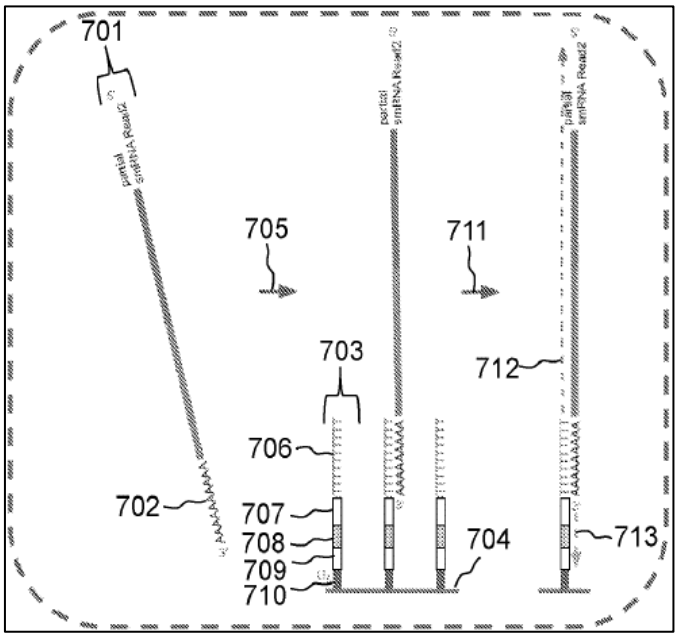
	permeabilization). In some embodiments, reverse transcription (RT) reagents can be added to permeabilized biological samples. **Incubation with the RT reagents can extend the capture probes 711 to produce spatially-barcoded full-length cDNA 712 and 713 from the captured analytes (e.g., polyadenylated mRNA).** Second strand reagents (e.g., second strand primers, enzymes) can be added to the biological sample on the slide to initiate second strand synthesis." (25:1-8)  (FIG. 7) *See also* FIGs. 1, 2, 13, Example 2.
g) identifying the first plurality of extension products and the second plurality of extension products by sequencing the	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45,

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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.	Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed as described in FIG. 13. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. Samples were destained (e.g., using HC1) and decrosslinked 1302. Following decrosslinking, samples treated with pre-hybridization buffer (e.g., hybridization buffer without the first and second probes), probes were added to the sample, probes hybridized, and samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306. Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308. **Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced**." (80:14-33) "The released, ligated DNA probes that served as a proxy for the target mRNA were allowed to hybridize to the capture domain on the capture probe immobilized on the spatial array via the poly A tail on the 3' end of the RHS probe. The

Claim 16	Chell (EX1006)
	captured ligated probes were copied, using the capture probe as a template and the extension product was released from the spatial array. Briefly, the tissues were incubated with a second strand extension mix comprising Kapa Hifi DNA polymerase for 25 minutes at 53°C. Following incubation, the second strand extension mix was removed from the tissues and the tissues were washed with 2X SSC. A solution of KOH was added to each of the tissue wells, the tissues were incubated at room temperature for 10 minutes to **release the extension product from the spatial array and the supernatant from each tissue well was transferred for quantification, and library preparation.** Sample quantification was performed using qPCR and KAPA SYBR FAST qPCR master mix according to the manufacturer's instructions. Briefly, KAPA SYBR master mix was prepared by adding qPCR primer cDNA F and qPCR primer sRNA_R2. The thermal cycler protocol included: 3 minutes at 98°C; 30 cycles with 5 seconds at 98°C, and 30 seconds at 63°C. For library preparation, samples were indexed using an Amp Mix that included dual indexing primers and an Amp Mix. **Nucleic acids were then sequenced and analyzed**." (83:13-84:9) "Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 contacting a biological sample with array of spatially -barcoded capture probes. In some instances, the array is on a substrate and the array includes a plurality of capture probes, wherein a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain. After placing

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	the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and the ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array. **After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample.**" (23:13-29) "Referring to FIG. 13, in another non-limiting example, a biological sample is deparaffinized, stained, and imaged 1301. After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. Ligated probes are captured on an array by a capture probe 1306, extended using a polymerase 1307 and denatured 1308. After quality control cleanup

Claim 16	Chell (EX1006)
	1309, the **abundance and location of an analyte is determined**." (23:30-24:3) "As shown in FIGs. 14-19, spatial expression of ligated probe pairs highlights underlying mouse brain tissue physiology. FIG. 14 shows PCR results demonstrating that a ligation product was captured and amplifiable whether the permeabilization occurred before or after RNase H treatment (positive control=standard, negative control=minus ligase). Arrow 1 points to a PCR band that represents desired ligation products, whereas arrows 2, 3 and 4 represent non-ligation products. FIG. 15 shows non-specific probe detected as a fraction of total reads for each condition. FIGs. 16A-B shows most probe combinations are specific. The arrows indicate RHS probes that had increased background and therefore include some non-specific hybridization. Looking at total counts in FIG. 17, gene-specific LHS+RHS probes overlay with the tissue footprint (see FIGs. 17A-C with black circle indicating tissue footprint)." (84:12-22) "Further, probes specific for various genes (e.g., Grp88, Penk, Plpl, Nptrx, and Mpb2) were added to the sample in Hybridization buffer first for 30 minutes at 60°C, and then for 2 hours at 45°C (See, e.g., FIG. 18 and FIG. 19). When target-specific UMIs were counted, a more detailed expression pattern was observed. See, e.g., FIGs. 19B-19F. This analysis revealed that longer hybridization led to higher sensitivity. Referring to FIGs. 18A-18E, white circles indicate spots reporting expression for the indicated gene." (84:23-28)

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	"An overnight hybridization was performed comparative to a 2 hour hybridization as discussed. Analysis on mouse brain samples using 21,833 probe pairs (e.g., RHS and LHS probes) added to each tissue sample to capture 21,604 different genes revealed that overnight hybridization, thus longer hybridization, led to higher sensitivity compared to a positive control (see FIGs. 20A-20B). The mouse probes were designed from targets derived from Appris (see Rodriguez et ak, Nucleic Acids Research, 46: D213-217, doi: 10.1093/nar/gkx997 (2018), which is herein incorporated by reference in its entirety) and GENCODE. All probe pairs were non-overlapping and include generally about 1 probe pair per gene." (84:29-85:3) *See also* FIGs. 1, 13, 14-21, Example 2, 23:11-24:14, 24:25-33, 80:14-85:3, Figs. 1, 2, 6, 7, 13, 1:24:30, 17:19-24, 49:34-35, 50:15-18, 50:32-51:5, 52:10-19, 57:23-25, 60:14-16, 62:25-28, 66:4-13, 66:26-28, 75:3-8, 76:8:10, 84:33-85:3, 85:28-31.

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APPENDIX D[1]

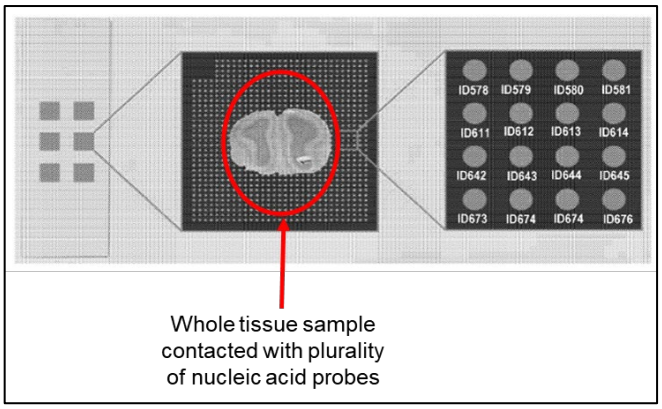
Claim 1	Chell (EX1006)
1. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:	"**Methods for spatial analysis** using rna-templated ligation" (Title) "Provided herein are **methods of detecting an analyte of interest to interrogate spatial gene expression** in a sample using RNA-templated ligation." (Abstract) "FIG. 1 shows an exemplary **spatial analysis** workflow." (7:29) "Example 1. **Spatial gene expression** analysis of FFPE-fixed samples using RNA- templated ligation." (80:14-15) *See also* FIGs. 1, 6, 13, Example 2.
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[2];	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence.

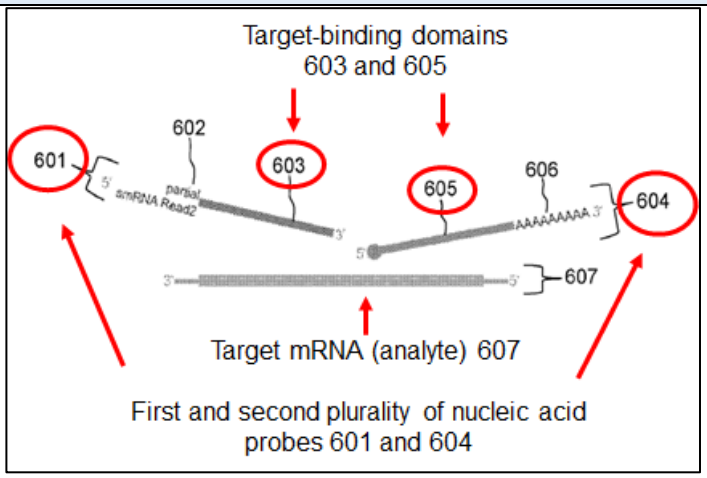
[1] Emphasis added in bold.

[2] The claim language "wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte" reflects the correction made after the 689 patent issued.

Claim 1	Chell (EX1006)
	As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed **as described in FIG. 13**. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. **Samples were destained (e.g., using HC1) and decrosslinked 1302**. Following decrosslinking, **samples treated with pre-hybridization buffer** (e.g., hybridization buffer without the first and second probes), **probes were added to the sample, probes hybridized**, and samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306. Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308. Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33) "**RTL probes were designed to hybridize to adjacent sequences of each analyte (e.g., mRNA sequence)** of interest in the genome, including estrogen receptor, progesterone receptor, and ERBB2, also known as HER2. **Here, 20,056 probe pairs (e.g., RHS and LHS probes) were added to each tissue sample to capture 19,490 different genes. Two RTL probes (a left-hand side (LHS) probe and a right-hand side (RHS) probe (see e.g., FIG.**

Claim 1	Chell (EX1006)
	6)) for each analyte were added simultaneously and hybridized at adjacent sequences of the target mRNA, forming RNA:DNA duplex structures. Following decrosslinking, the PBS-Tween was removed and the DNA probes (1 nM of each probe) were added to the tissue samples in a hybridization buffer for hybridizing the DNA probes to their respective mRNA targets. The hybridization buffer included SSC, formamide or an equivalent, yeast tRNA as carrier and the RHS and LHS DNA probes." (81:16-82:2) "One probe oligonucleotide (e.g., the RHS probe or the 3' probe) comprises a non target functional sequence at its 5' end while the other probe oligonucleotide (e.g., the LHS probe or the 5' probe) comprises a non-target polyA sequence at their 3' ends. The DNA probes were added to the tissue samples and incubated at 50 °C for 2 hours and 30 minutes according to thermal cycler protocol described in Table 3." (82:3-7) "Referring to FIG. 13, in another non-limiting example, a **biological sample** is deparaffinized, stained, and imaged 1301. **After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303**. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. Ligated probes are captured on an array by a capture probe 1306, extended using a polymerase 1307 and denatured 1308. After quality control cleanup 1309, the

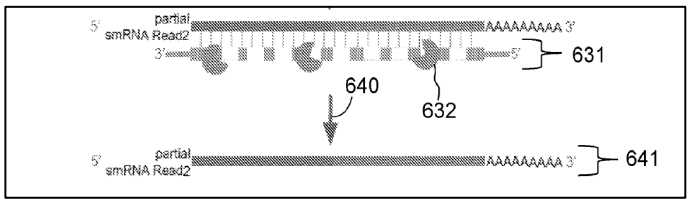
Claim 1	Chell (EX1006)
	abundance and location of an analyte is determined." (23:30-24:3)  (FIG. 5 (annotated)) "A non-limiting example of the methods disclosed herein is depicted in FIG. 6. After a **biological sample is** contacted with a substrate including a plurality of capture probes and **contacted with (a) a first probe 601 having a target-hybridization sequence 603** and a primer sequence 602 and **(b) a second probe 604 having a target-hybridization sequence 605** and a capture domain (e.g., a poly-A sequence) 606, the first probe 601 and a second probe 604 hybridize 610 to an analyte 607. A ligase 621 ligates 620 the first probe to the second probe thereby generating a ligation product 622. The ligation product is released 630 from the analyte 631 by digesting the analyte using an endoribonuclease 632. The sample is permeabilized 640 and the ligation product 641 is able to hybridize to a capture probe on the substrate." (24:4-13)

Claim 1	Chell (EX1006)
	 (FIG. 6 (annotated)) "Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 contacting a biological sample with array of spatially -barcoded capture probes. In some instances, the array is on a substrate and the array includes a plurality of capture probes, wherein a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain. **After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte**, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and the

Claim 1	Chell (EX1006)
	ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array. After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample.." (23:13-29) *See also* FIGs. 1, 13, Example 2.
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;	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed as described in FIG. 13. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. Samples were destained (e.g., using HC1) and decrosslinked 1302. Following decrosslinking, samples treated with pre-hybridization buffer (e.g., hybridization buffer without the first and

Claim 1	Chell (EX1006)
	second probes), probes were added to the sample, probes hybridized, and samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. **Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306.** Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308. Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33) "**RNase H was added to digest the RNA strand of the hybridized RNA:DNA duplex.** Briefly, the RNA of the DNA:RNA hybrids was digested by incubating the samples with RNase H mix for 30 minutes at 37°C, where the RNAse H mix included: RNAse H buffer and RNaseH. Following the incubation and while the same remained at 37°C, the **biological sample was permeabilized to release the ligated RTL probes** using 1.25mg/mL Proteinase K. In particular, the Proteinase K solution included (per sample): Tris (pH 7.5), MgCh. Sarkosyl, or SDS , Proteinase K (Enzyme), and nuclease free water. **The sample was incubated** at 37°C for at least 5 minutes in the Proteinase K solution. The samples were then washed three times with 2X SSC." (83:4-12)

Claim 1	Chell (EX1006)
	"The **released, ligated DNA probes** that served as a proxy for the target mRNA were allowed to **hybridize to the capture domain on the capture probe immobilized on the spatial array** via the poly A tail on the 3' end of the RHS probe." (83:13-15) "Referring to FIG. 13, in another non-limiting example, a biological sample is deparaffinized, stained, and imaged 1301. After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. **Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. Ligated probes are captured on an array by a capture probe 1306**, extended using a polymerase 1307 and denatured 1308. After quality control cleanup 1309, the abundance and location of an analyte is determined." (23:30-24:3) "A non-limiting example of the methods disclosed herein is depicted in FIG. 6. After a biological sample is contacted with a substrate including a plurality of capture probes and contacted with (a) a first probe 601 having a target-hybridization sequence 603 and a primer sequence 602 and (b) a second probe 604 having a target-hybridization sequence 605 and a capture domain (e.g., a poly-A sequence) 606, the first probe 601 and a second probe 604 hybridize 610 to an analyte 607. A ligase 621 ligates 620 the first probe to the second probe thereby generating a ligation product 622. **The ligation product is released 630 from the analyte 631 by digesting the analyte using an**

Claim 1	Chell (EX1006)
	endoribonuclease 632. The sample is permeabilized 640 and the ligation product 641 is able to hybridize to a capture probe on the substrate." (24:4-13)  (FIG. 6) "Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 **contacting a biological sample with array of spatially -barcoded capture probes**. In some instances, the array is on a substrate and the **array includes a plurality of capture probes**, wherein a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain. After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and **the**

Claim 1	Chell (EX1006)
	ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array. After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample.." (23:13-29) "RTL probes were designed to hybridize to adjacent sequences of each analyte (e.g., mRNA sequence) of interest in the genome, including estrogen receptor, progesterone receptor, and ERBB2, also known as HER2. **Here, 20,056 probe pairs (e.g., RHS and LHS probes) were added to each tissue sample to capture 19,490 different genes. Two RTL probes (a left-hand side (LHS) probe and a right-hand side (RHS) probe (see e.g., FIG. 6)) for each analyte were added simultaneously and hybridized at adjacent sequences of the target mRNA**, forming RNA:DNA duplex structures. Following decrosslinking, the PBS-Tween was removed and the DNA probes (1 nM of each probe) were added to the tissue samples in a hybridization buffer for hybridizing the DNA probes to their respective mRNA targets. The hybridization buffer included SSC, formamide or an equivalent, yeast tRNA as carrier and the RHS and LHS DNA probes." (81:16-82:2)

Claim 1	Chell (EX1006)
	See also FIGs. 1, 2, 5, 6, 13, Example 2.
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	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed as described in FIG. 13. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. Samples were destained (e.g., using HC1) and decrosslinked 1302. Following decrosslinking, samples treated with pre-hybridization buffer (e.g., hybridization buffer without the first and second probes), probes were added to the sample, probes hybridized, and samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306. **Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured**

Claim 1	Chell (EX1006)
identifies the second location of the tissue sample; and	**1308.** Denatured, extended capture probes were indexed and the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33) "The released, ligated DNA probes that served as a proxy for the target mRNA were allowed to hybridize to the capture domain on the capture probe immobilized on the spatial array via the poly A tail on the 3' end of the RHS probe. **The captured ligated probes were copied, using the capture probe as a template and the extension product was released from the spatial array.** Briefly, the tissues were incubated with a second strand extension mix comprising Kapa Hifi DNA polymerase for 25 minutes at 53°C. Following incubation, the second strand extension mix was removed from the tissues and the tissues were washed with 2X SSC. A solution of KOH was added to each of the tissue wells, the tissues were incubated at room temperature for 10 minutes to release the extension product from the spatial array and the supernatant from each tissue well was transferred for quantification, and library preparation. Sample quantification was performed using qPCR and KAPA SYBR FAST qPCR master mix according to the manufacturer's instructions. Briefly, KAPA SYBR master mix was prepared by adding qPCR primer cDNA F and qPCR primer sRNA_R2. The thermal cycler protocol included: 3 minutes at 98°C; 30 cycles with 5 seconds at 98°C, and 30 seconds at 63°C. For library preparation, samples were indexed using an Amp Mix that included dual indexing

Claim 1	Chell (EX1006)
	primers and an Amp Mix. Nucleic acids were then sequenced and analyzed." (83:13-84:9) "Referring to FIG. 13, in another non-limiting example, a biological sample is deparaffinized, stained, and imaged 1301. After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. **Ligated** probes are captured on an array by a capture probe 1306, **extended using a polymerase 1307 and denatured 1308.** After quality control cleanup 1309, the abundance and location of an analyte is determined." (23:30-24:3) "A **'capture probe'** refers to **any molecule capable of capturing (directly or indirectly) and/or labelling an analyte (e.g., an analyte of interest) in a biological sample**. In some embodiments, the capture probe is a nucleic acid or a polypeptide**. In some embodiments, the capture probe includes a barcode (e.g., a spatial barcode** and/or a unique molecular identifier (UMI)) and a capture domain). In some embodiments, a capture probe can include a cleavage domain and/or a functional domain (e.g., a primer-binding site, such as for next-generation sequencing (NGS)). See, e.g., WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Generation of capture probes can be achieved by any appropriate method, including those described in WO 2020/176788 and/or U.S. Patent

Claim 1	Chell (EX1006)
	Application Publication No. 2020/0277663." (12:10-19) "As used herein, an **'extended capture probe' refers to a capture probe having additional nucleotides added to the terminus (e.g., 3' or 5' end) of the capture probe thereby extending the overall length of the capture probe.** For example, **an "extended 3' end" indicates additional nucleotides were added to the most 3' nucleotide of the capture probe to extend the length of the capture probe, for example, by polymerization reactions used to extend nucleic acid molecules including templated polymerization catalyzed by a polymerase (e.g., a DNA polymerase or a reverse transcriptase).** In some embodiments, extending the capture probe includes adding to a 3' end of a capture probe a nucleic acid sequence that is complementary to a nucleic acid sequence of an analyte or intermediate agent specifically bound to the capture domain of the capture probe. In some embodiments, the capture probe is extended using reverse transcription. In some embodiments, the capture probe is extended using one or more DNA polymerases. **The extended capture probes include the sequence of the capture probe and the sequence of the spatial barcode of the capture probe**." (17:24-18:2) "Spatial analysis methodologies and compositions described herein can provide a vast amount of analyte and/or expression data for a variety of analytes within a biological sample at high spatial resolution, while retaining native spatial context. Spatial analysis

Claim 1	Chell (EX1006)
	methods and compositions can include, e.g., the use of a **capture probe including a spatial barcode (e.g., a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample** (e.g., mammalian cell or a mammalian tissue sample) and a capture domain that is capable of binding to an analyte (e.g., a protein and/or a nucleic acid) produced by and/or present in a cell." (10:6-10) "FIG. 2 is a schematic diagram showing an **example of a capture probe**, as described herein. As shown, the capture probe 202 is optionally coupled to a feature 201 by a cleavage domain 203, such as a disulfide linker. The capture probe can include functional sequences that are useful for subsequent processing, such as functional sequence 204, which can include a sequencer specific flow cell attachment sequence, e.g., a P5 or P7 sequence, as well as functional sequence 205, which can include sequencing primer sequences, e.g., a R1 primer binding site. In some embodiments, sequence 204 is a P7 sequence and sequence 205 is a R1 primer binding site. **A spatial barcode 206 can be included within the capture probe for use in barcoding the target analyte.** The functional sequences can generally be selected for compatibility with any of a variety of different sequencing systems, e.g., Ion Torrent Proton or PGM, Illumina sequencing instruments, PacBio, Oxford Nanopore, etc., and the requirements thereof. In some embodiments, functional sequences can be selected for compatibility with non-commercialized sequencing systems. Examples

Claim 1	Chell (EX1006)
	of such sequencing systems and techniques, for which suitable functional sequences can be used, include (but are not limited to) Ion Torrent Proton or PGM sequencing, Illumina sequencing, PacBio SMRT sequencing, and Oxford Nanopore sequencing. Further, in some embodiments, functional sequences can be selected for compatibility with other sequencing systems." (15:28-16:10) "In some embodiments, the **spatial barcode 206**, functional sequences 204 (e.g., flow cell attachment sequence) and 205 (e.g., sequencing primer sequences) can be common to all of the probes attached to a given feature. The spatial barcode can also include a capture domain 207 to facilitate capture of a target analyte." (16:11-14) "Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 contacting a biological sample with array of spatially -barcoded capture probes. In some instances, the array is on a substrate and the **array includes a plurality of capture probes, wherein a capture probe of the plurality includes: (i) a spatial barcode** and (ii) a capture domain. After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and wherein the second probe includes a capture probe capture

Claim 1	Chell (EX1006)
	domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and the ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array. After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample..” (23:13-29) “In some embodiments, as shown in FIG. 7, the ligation product 701 includes a capture probe capture domain 702, which can bind to a **capture probe 703 (e.g., a capture probe immobilized, directly or indirectly, on a substrate 704).** In some embodiments, methods provided herein include contacting 705 a biological sample with a substrate 704, wherein the capture probe 703 is affixed to the substrate (e.g., immobilized to the substrate, directly or indirectly). In some embodiments, the capture probe capture domain 702 of the ligated product specifically binds to the capture domain 706. **The capture probe can also include** a unique molecular identifier (UMI) 707, **a spatial barcode 708**, a functional sequence 709, and a cleavage domain 710.” (24:25-33)

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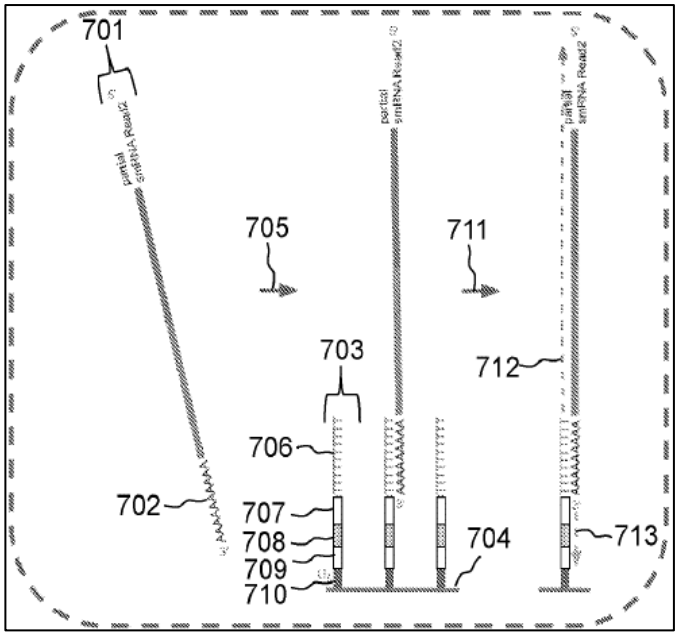
	"In some embodiments, methods provided herein include permeabilization of the biological sample such that the capture probe can more easily bind to the captured ligated probe (i.e., compared to no permeabilization). In some embodiments, reverse transcription (RT) reagents can be added to permeabilized biological samples. **Incubation with the RT reagents can extend the capture probes 711 to produce spatially-barcoded full-length cDNA 712 and 713 from the captured analytes (e.g., polyadenylated mRNA).** Second strand reagents (e.g., second strand primers, enzymes) can be added to the biological sample on the slide to initiate second strand synthesis." (25:1-8)  (FIG. 7) *See also* FIGs. 1, 2, 13, Example 2.

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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.	"Example 1. Spatial gene expression analysis of FFPE-fixed samples using RNA- templated ligation Others have demonstrated in situ ligation. See Credle et ctl, Nucleic Acids Research, Volume 45, Issue 14, 21 August 2017, Page el28, https://doi.org/10.1093/nar/gkx471 (2017). However, the previous approaches have utilized hybridization using poly(A) tails which led to off-target binding. Here, the poly(A) tail on one probe oligonucleotide was switched to another sequence. As an overview, a non-limiting example of RNA-templated ligation on an FFPE-fixed sample was performed as described in FIG. 13. FFPE-fixed samples were deparaffinized, stained (e.g., H&E stain), and imaged 1301. Samples were destained (e.g., using HC1) and decrosslinked 1302. Following decrosslinking, samples treated with pre-hybridization buffer (e.g., hybridization buffer without the first and second probes), probes were added to the sample, probes hybridized, and samples were washed 1303. Ligase was added to the samples to ligate hybridized probes to generate a ligation product and samples were then washed 1304. Probes were released from the analyte by contacting the biological sample with RNAse H 1305. Samples were then permeabilized to facilitate capture of the ligation product by the capture probes on the substrate 1306. Ligation products that hybridized to the capture probes were then extended 1307. The extended capture probes were denatured 1308. **Denatured, extended capture probes were indexed and**

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	the amplified libraries were subjected to quality control 1309 before being sequenced." (80:14-33) "The released, ligated DNA probes that served as a proxy for the target mRNA were allowed to hybridize to the capture domain on the capture probe immobilized on the spatial array via the poly A tail on the 3' end of the RHS probe. The captured ligated probes were copied, using the capture probe as a template and the extension product was released from the spatial array. Briefly, the tissues were incubated with a second strand extension mix comprising Kapa Hifi DNA polymerase for 25 minutes at 53°C. Following incubation, the second strand extension mix was removed from the tissues and the tissues were washed with 2X SSC. A solution of KOH was added to each of the tissue wells, the tissues were incubated at room temperature for 10 minutes to **release the extension product from the spatial array and the supernatant from each tissue well was transferred for quantification, and library preparation.** Sample quantification was performed using qPCR and KAPA SYBR FAST qPCR master mix according to the manufacturer's instructions. Briefly, KAPA SYBR master mix was prepared by adding qPCR primer cDNA F and qPCR primer sRNA_R2. The thermal cycler protocol included: 3 minutes at 98°C; 30 cycles with 5 seconds at 98°C, and 30 seconds at 63°C. For library preparation, samples were indexed using an Amp Mix that included dual indexing primers and an Amp Mix. **Nucleic acids were then sequenced and analyzed**." (83:13-84:9)

Claim 1	Chell (EX1006)
	"Referring to FIG. 1, in an exemplary embodiment of the disclosure, provided are methods for identifying a location of an analyte in a biological sample. In some instances, the methods include 101 contacting a biological sample with array of spatially -barcoded capture probes. In some instances, the array is on a substrate and the array includes a plurality of capture probes, wherein a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain. After placing the biological sample on the array, the biological sample 102 is contacted with a first probe and a second probe, wherein the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and wherein the second probe includes a capture probe capture domain; the first probe and the second probe 103 hybridize to complementary sequences in the analyte. After hybridization a ligation product comprising the first probe and the second probe 104 is generated, and the ligation product is released from the analyte. The liberated ligation product is then freed 105 to hybridize to the capture domain of a probe on the array. **After capture, (i) all or a part of the sequence of the ligation product specifically bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof 106 can be determined, and then one can use the determined sequence of (i) and (ii) 107 to identify the location of the analyte in the biological sample.**" (23:13-29)

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	"Referring to FIG. 13, in another non-limiting example, a biological sample is deparaffinized, stained, and imaged 1301. After destaining and decrosslinking 1302, probes are added to the sample and hybridize to an analyte 1303. In some instances, the probes are DNA probes. In some instances, the probes are diribo-containing probes. Probes are ligated 1304 and then released using an endonuclease such as RNAse H 1305. Ligated probes are captured on an array by a capture probe 1306, extended using a polymerase 1307 and denatured 1308. After quality control cleanup 1309, the **abundance and location of an analyte is determined**." (23:30-24:3) "As shown in FIGs. 14-19, spatial expression of ligated probe pairs highlights underlying mouse brain tissue physiology. FIG. 14 shows PCR results demonstrating that a ligation product was captured and amplifiable whether the permeabilization occurred before or after RNase H treatment (positive control=standard, negative control=minus ligase). Arrow 1 points to a PCR band that represents desired ligation products, whereas arrows 2, 3 and 4 represent non-ligation products. FIG. 15 shows non-specific probe detected as a fraction of total reads for each condition. FIGs. 16A-B shows most probe combinations are specific. The arrows indicate RHS probes that had increased background and therefore include some non-specific hybridization. Looking at total counts in FIG. 17, gene-specific LHS+RHS probes overlay with the tissue footprint (see FIGs. 17A-C with

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	black circle indicating tissue footprint)." (84:12-22) "Further, probes specific for various genes (e.g., Grp88, Penk, Plpl, Nptrx, and Mpb2) were added to the sample in Hybridization buffer first for 30 minutes at 60°C, and then for 2 hours at 45°C (See, e.g., FIG. 18 and FIG. 19). When target-specific UMIs were counted, a more detailed expression pattern was observed. See, e.g., FIGs. 19B-19F. This analysis revealed that longer hybridization led to higher sensitivity. Referring to FIGs. 18A-18E, white circles indicate spots reporting expression for the indicated gene." (84:23-28) "An overnight hybridization was performed comparative to a 2 hour hybridization as discussed. Analysis on mouse brain samples using 21,833 probe pairs (e.g., RHS and LHS probes) added to each tissue sample to capture 21,604 different genes revealed that overnight hybridization, thus longer hybridization, led to higher sensitivity compared to a positive control (see FIGs. 20A-20B). The mouse probes were designed from targets derived from Appris (see Rodriguez et ak, Nucleic Acids Research, 46: D213-217, doi: 10.1093/nar/gkx997 (2018), which is herein incorporated by reference in its entirety) and GENCODE. All probe pairs were non-overlapping and include generally about 1 probe pair per gene." (84:29-85:3) *See also* FIGs. 1, 13, 14-21, Example 2, 23:11-24:14, 24:25-33, 80:14-85:3, Figs. 1, 2, 6, 7, 13, 1:24:30, 17:19-24, 49:34-35, 50:15-18, 50:32-51:5, 52:10-19, 57:23-25, 60:14-16, 62:25-28,

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	66:4-13, 66:26-28, 75:3-8, 76:8:10, 84:33-85:3, 85:28-31.

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