

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CURIO BIOSCIENCE, INC.,
Petitioner

v.

PROGNOSYS BIOSCIENCES INC. AND 10X GENOMICS, INC.,
Patent Owner and Real-Party-In-Interest

Case IPR2024-01289
Patent 10,480,022

DECLARATION OF CHARLES R. CANTOR

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REFERENCES CITED

Exhibit Number	Description
EX1001	U.S. Patent No. 10,480,022 issued to Chee
EX1009	U.S. Provisional Application No. 61/321,124
EX1022	Michael Armani <i>et al.</i> , <i>2D-PCR: a method of mapping DNA in tissue sections</i> , Lab Chip, 2009, 9(24), 3526-3534; NIH Public Access Author Manuscript
EX1031	WO 2009/091934 A1 Cantor
EX1033	WO 2008/075086 A1 Southern <i>et al.</i>
EX1100	Declaration of Joseph D. Puglisi, Ph.D.
EX2004	Web Capture of https://www.10xgenomics.com/products/spatial-gene-expression (December 18, 2024)
EX2005	Khaliq <i>et al.</i> , “Spatial Transcriptomic Analysis of Primary and Metastatic Pancreatic Cancers Highlights Tumor Microenvironmental Heterogeneity,” <i>Nature Genetics</i> 56:2455–2465 (2024)
EX2006	Latham and Peltier, “MELT™ Total Nucleic Acid Isolation System: a new technology for hands-free tissue disruption, RNA preservation and total nucleic acid purification,” <i>Nature Methods</i> 2:i–iii (2005)
EX2007	Griffin <i>et al.</i> , “Pathogenic Human Viruses in Coastal Waters,” <i>Clin. Microbiol. Rev.</i> 16(1):129–143 (2003)
EX2008	Deak <i>et al.</i> , “Molecular Characterization of <i>Yarrowia lipolytica</i> and <i>Candida zeylanoides</i> Isolated from Poultry,” <i>Appl. Env. Microbiol.</i> 66(10):4340–4344 (2000)
EX2009	PCR PRIMER: A LABORATORY MANUAL (Carl W. Dieffenbach and Gabriela S. Dveksler, eds. (2003)), Section 2, “Sample Preparation”

EX2010	PCR PRIMER: A LABORATORY MANUAL (Carl W. Dieffenbach and Gabriela S. Dveksler, eds. (2003)), Chapter 19, “Reverse Transcriptase In Situ PCR”
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I, CHARLES R. CANTOR, hereby declare the following:

1. I have been retained on behalf of Prognosys Biosciences, Inc. (“Prognosys”) and 10x Genomics, Inc. (“10x”) (collectively “Patent Owner”), in an Inter Partes Review (“IPR”) before the Patent Trial and Appeal Board (“PTAB” or “Board”) of U.S. Patent No. 10,480,022 (“the ’022 patent”) (EX1001). The Petitioner in this case is Curio Bioscience, Inc. (“Petitioner” or “Curio”).

2. The ’022 patent is titled “Spatially Encoded Biological Assays.” (’022 Patent, EX1001 at cover page.) It was filed as U.S. Patent Application No. 16/276,235 on February 14, 2019 (“the ’235 application”) and issued as Patent No. 10,480,022 on November 19, 2019. (*Id.*) It claims priority to Provisional Application No. 61/321,124 (“the ’124 provisional,” EX1009), filed on April 5, 2010. (*Id.*) The ’022 patent has 30 claims. (EX1001, 32:64–34:48.) Of those claims, claims 1–7, 11–15, 17, 19, and 27–29 are challenged (the “Challenged Claims”). (Petition, 7.)

3. I have been asked to opine on the validity of the Challenged Claims.

4. My findings, as explained below, are based on my study, experience, and background in the fields discussed below. I have also relied on my review and analysis of the prior art, information provided to me in connection with this case, and information I have independently reviewed.

5. I am being compensated for my work as an expert on this matter, but my compensation is not contingent in any way on the content of my opinions or the outcome of this proceeding.

I. QUALIFICATIONS AND PROFESSIONAL EXPERIENCE

6. I am a founder and retired Chief Scientific Officer at Sequenom, Inc., the leading provider of noninvasive prenatal testing (sold to Labcorp in 2014). I consult for or am a board member of a number of biotech companies including Innomed (China), Apostle (USA, China), Agena Biosciences (USA, sold to Mesa in 2021), Strand Life Sciences (India, sold to Reliance in 2021), ProdermIQ (USA), Armonica (USA), Anchor DX (China), In Silico Medicine (Russia, China, HKG, USA), Seer (USA), GPC (China), Clarapath (USA), ARNA (Russia, USA), Riffit (USA), NeoGenestar (USA), Algorithmic Biologics (India), Healthbadge (USA, India), ProgenomIQ (USA), Celsyntec (USA), Integral Discovery (USA), Iris Kinetics (USA).

7. I am also a founder of Retrotope (USA, bought by BioJiva in 2023), a clinical stage pharmaceutical company.

8. I was educated in chemistry at Columbia College of Columbia University (AB, 1963) and UC Berkeley (PhD, 1966). I became a full professor of chemistry at Columbia University at the age of 29. I have held academic and

leadership positions at Columbia and several other distinguished American universities, but for the past 20 years I have worked mostly with biotechnology companies.

9. I am currently professor emeritus of Biomedical Engineering and of Pharmacology at Boston University, adjunct professor of Molecular Biology at the Scripps Research Institute, distinguished adjunct professor of Physiology and Biophysics at UC Irvine and adjunct professor at the Moscow Institute of Physics and Technology.

10. I have been granted more than 60 US patents and, with Paul Schimmel, wrote a three-volume textbook on biophysical chemistry that has remained the standard in this field for almost 40 years. I have published more than 450 peer-reviewed articles, am a member of the U.S. National Academy of Sciences and a fellow of The National Academy of Inventors.

11. Some of my major scientific accomplishments include the development of pulsed field electrophoresis, immuno-PCR, affinity capture electrophoresis, the earliest uses of FRET to characterize distances in protein complexes and in nucleic acids, the standard methods for assaying and purifying microtubule protein, various applications of nucleic acid mass spectrometry, and methods for noninvasive prenatal diagnostics.

12. I am also considered to be one of the founders of the new field of synthetic biology, and one of the experts in the technology transfer of diagnostic methods.

13. In the past decade, I have focused on developing rational and cost-effective approaches to preventive and personalized medicine. This includes: (1) liquid biopsies (Agena Biosciences, Innomed, Strand Life Sciences, Seer, Anchor Dx, ARNA, Apostle, PrognomIQ) that could detect age-related disease pathology earlier and thus allow for more effective intervention, (2) DNA testing in minutes suitable for use in emergency rooms, operating theaters and DNA testing in large sample pools (Algorithmic Biologics, Healthbadge, Iris Kinetics) suitable for public health crises (Armonica, Star-Array), (3) deuterated lipids (Retrotope) that show great promise at reducing the oxidative damage processes that play a central role in many age-related diseases, and (4) applications of machine learning to reduce the cost of drug discovery and healthcare delivery (In Silico Medicine, Algorithmic Biologics, Clarapath, Riffit). My work prior to my retirement from Sequenom in 2013 is detailed in my curriculum vitae provided as EX2003.

I. MATERIALS CONSIDERED

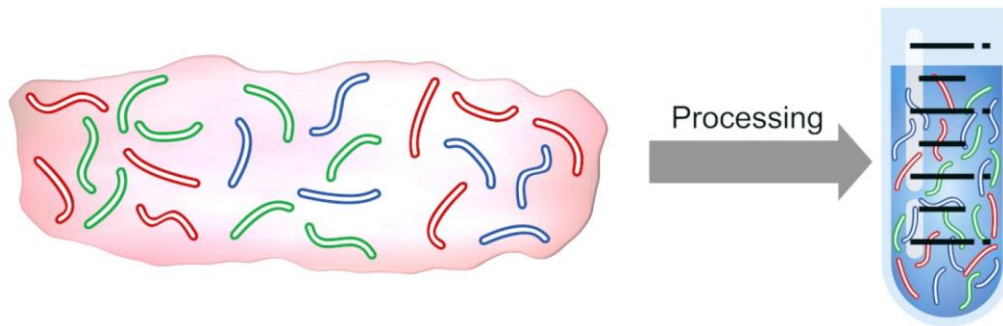
14. I have considered all the documents I cite in this Declaration and all the relevant documents cited in the Petitioner's Petition for Inter Partes Review

(“Petition”) of the ’022 patent in forming my opinions. A list of References Cited is set forth at the beginning of this Declaration.

II. THE ’022 PATENT AND SPATIAL ANALYSIS

15. The ’022 patent relates to spatial analysis assays for analyzing the spatial location of biological molecules in a tissue sample. (EX1001, 1:12–15; 57–62.) Before the ’022 patent, “no practical method exist[ed] to analyze at high resolution the spatial expression patterns of large numbers of genes, proteins, or other biologically active molecules simultaneously.” (EX1001, 1:57–60.) Thus, there was “a need for reproducible, high-resolution spatial maps of biological molecules in tissues.” (EX1001, 1:60–62.)

16. Spatial analysis is distinct from what I will refer to as “bulk-analysis” methods such as those described in Petitioner’s Cantor reference (EX1031), which is my patent application publication (discussed in Section III.A, below). In bulk-analysis methods, a tissue sample is not assayed directly. Rather, it is first processed to extract nucleic acids from the tissue into a mixture (referred to in the Cantor reference as “sample nucleic acid”), as depicted below.



17. Then, the mixture is analyzed to determine **if** a particular nucleic acid was present in the tissue sample or not. They cannot, however, determine **where** the molecule was in the tissue sample. As such, bulk-analysis techniques provide no information about spatial location of biological molecules in tissue samples. This is in contrast to spatial analysis methods such as those claimed in the '022 patent which can determine both **if** a biological molecule was present in a tissue sample and **where** the molecule was in the tissue sample.

18. One can think of a tissue sample, which has many different cells of many different types, each with their own complement of many different nucleic acids (biological molecules), arranged in a spatial pattern, like the fruit in a fruit tart. The fruit tart has many different fruits, intact, with a spatial distribution, like the strawberries, blueberries, and blackberries depicted below:



Fruit Tart

19. For bulk-analysis methods, nucleic acids are extracted from the different types of cells within a tissue sample without preserving their spatial distribution. That is, the sample is homogenized. This is like blending the fruit from the fruit tart into a smoothie:



Fruit Smoothie

20. As a result of this homogenization, with data from a bulk-analysis experiment, one could answer the question: did this fruit tart have any blueberries in it? It could not answer the question: where in the fruit tart were the blueberries

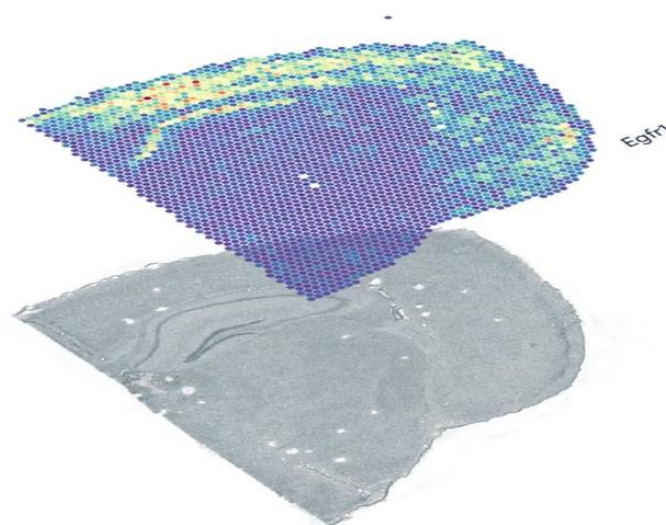
located? The spatial analysis technology described in the '022 patent, however, answers both questions.

21. Indeed, the inventions described in the '022 patent addressed the “need for reproducible, high-resolution spatial maps of biological molecules in tissues.” (EX1001, 1:60-62.) The '022 patent describes assays and assay systems utilizing a spatial encoding scheme that combines molecule-specific binding agents together with coding identifiers to provide a practical and cost-effective determination of information on multiple biological molecules, including specific position information of such molecules in a biological sample, such as a tissue sample. (EX1001, 20:29–50.) This “unique encoding scheme” allows “multiple molecules and their respective multiple locations to be identified in a single system using a unique encoding scheme.” (*Id.*)

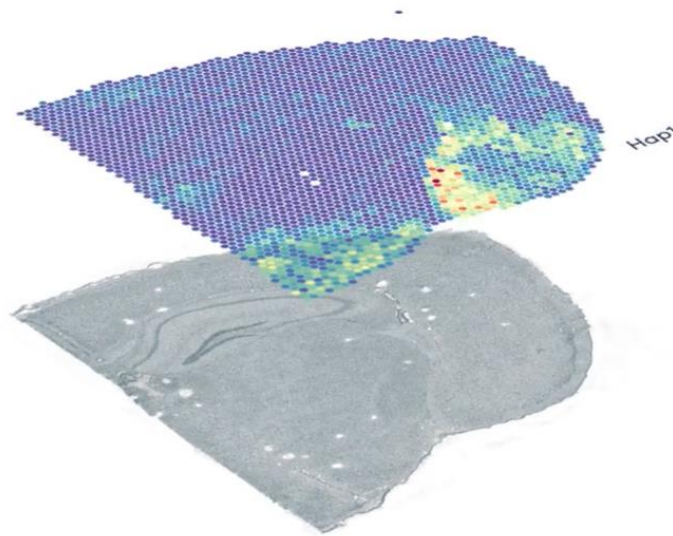
22. The spatial analysis technology claimed in the '022 patent has been commercialized by Patent Owner in the Visium® line of products for spatial analysis of tissue samples. (EX2004.) The Visium® technology allows researchers to assay gene expression patterns across an entire tissue sample for mRNA (e.g., of all of the genes in the genome that are expressed—the “transcriptome”) by capturing polyA sequences.

23. This type of spatial analysis can help researchers to understand things like variation among the different cells within a single tumor (the tumor “microenvironment”), which, in turn, can help them develop new therapeutics. (See, e.g., Khaliq et al., “Spatial Transcriptomic Analysis of Primary and Metastatic Pancreatic Cancers Highlights Tumor Microenvironmental Heterogeneity,” *Nature Genetics* 56:2455–2465, 2455 (2024) (EX2005).)

24. The image below depicts the gene expression pattern across an entire tissue sample for one gene (EGFR1). The image on the top, with colors ranging from cool to warm, is a “heatmap” that depicts EGFR1 expression across the tissue sample. Warmer colors in the heatmap indicate higher EGFR1 expression. Cooler colors indicate lower EGFR1 expression. The grey colored image on the bottom, over which the heatmap is superimposed, is an image of the tissue sample itself.



25. The gene expression pattern across the same tissue sample for a differently expressed gene (HAP1) is depicted below.



26. Utilizing the claimed invention, these types of analyses became possible. But they are not possible using bulk-analysis methods such as those described in the Cantor reference.

III. PETITIONER'S ASSERTED REFERENCES

27. Petitioner asserts that the Challenged Claims would have been obvious over either Cantor (Ground 1) or Cantor and Armani (Ground 2). (Petition, 7.) Petitioner also asserts that if the Challenged Claims are not entitled to the priority date of the '124 provisional, that they are anticipated by Frisen (Ground 3). (*Id.*)

28. Cantor (EX1031) and Armani (EX1022) are described, generally, in this section. Because I conclude that the Challenged Claims are entitled to priority (see Section VI.A), I do not opine on anticipation by Frisen and do not describe or discuss Frisen here.

A. Cantor (EX1031)

29. Cantor (EX1031) is a WIPO Publication (WO 2009/091934) of a PCT Application (PCT/US2009/031169) titled “Single Molecule Nucleic Acid Sequence Analysis Processes and Compositions” which lists Sequenom, Inc. as the Applicant. (EX1031, Cover Page.) I am the only inventor listed on the application. (*Id.*)

1. Overview

30. My application relates “generally to the field of nucleic acid sequence analysis and methodology and components for use in such analysis.” (EX1031, 1, under “Field of the Invention.”) It utilizes “[i]mproved solid supports and methods for analyzing target nucleic acid sequences.” (Page 1, under “Summary.”) The solid supports and methods are for “efficiently preparing nucleic acids that comprise nucleotide sequences identical to or substantially identical to one or more target nucleotide sequences of a sample nucleic acid, or

complement thereof.” (*Id.*) “Efficiently preparing nucleic acids” means generating copies of nucleic acid molecules of interest, which can later be sequenced and analyzed. (*Id.*) This “minimizes the number of steps required by” other prior-art methods for sequence analysis. (*Id.*) To aid in those later sequence and analysis steps, some single-stranded nucleic acid(s) optionally include an “identification sequence,” which is sometimes also referred to in my application as an “identifier sequence.” (EX1031, 15.) I use the terms interchangeably.

31. The technology described in my application does not relate to the analysis of tissue samples as in the '022 patent (i.e., for spatial analysis). Instead, the solid supports and methods described in my patent application are for bulk-analysis, as described above in Section II. A person of ordinary skill in the art (“POSA”) would have understood that the solid supports and methods disclosed in my application would be used to answer the question of **whether** nucleic acid molecules of interest were present in a sample nucleic acid derived from a tissue sample (e.g., a certain pathogen, a gene having a particular mutation, etc.). (EX1031, 5; 30–53.) But a POSA could not have and would not have used my solid supports and methods to answer **where** in a tissue sample those nucleic acid molecules of interest were located.

32. Instead, as discussed throughout my application, the disclosed solid supports and methods relate to preparing and analyzing a “sample nucleic acid.” (EX1031, 1.) As I describe below, a POSA would understand that the “sample nucleic acid” as defined in my application is *not* a tissue sample. Indeed, even if the “sample nucleic acid” was originally derived (prepared) from a tissue sample (rather than one of the many other types of sample sources described in my application), a POSA would understand that the “sample nucleic acid” exists in the form of a homogenized (blended) mixture that does not retain the original spatial distribution of cells or nucleic acids that a tissue sample would have.

33. I describe my solid supports and the steps of my disclosed methods and how they are used to test for one or more nucleic acid molecules of interest within a sample nucleic acid more fully below. Briefly, the methods use probes that hybridize specifically to target nucleotide sequences (i.e., specific gene sequences). The probes are part of an assembly containing single-stranded nucleic acid(s) linked to a solid support (e.g., bead or particle). I refer to this assembly as a “solid phase nucleic acid.” (EX1031, 1; 11.)

34. The sample nucleic acid that is tested could come from many different types of samples or sources—from environmental samples to human tissues. But whatever the source, the sample nucleic acid is derived (prepared) from the sample

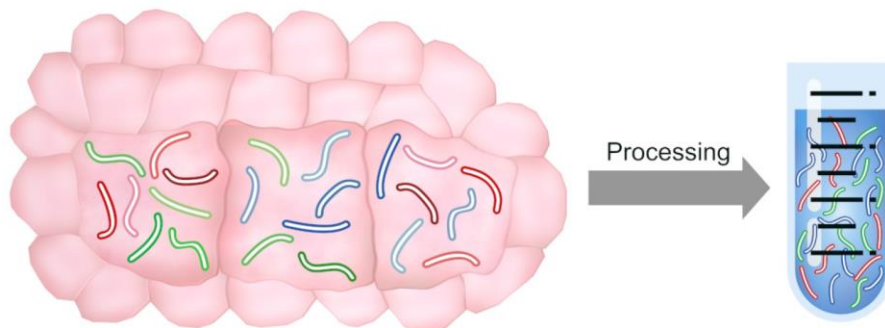
source and then mixed with the solid phase nucleic acid(s). If the nucleic acid molecule of interest is present in the sample nucleic acid, then it will hybridize with the matching probe. Following hybridization, the captured nucleic acid molecule can be copied and analyzed (e.g., by amplification and sequencing) to determine whether a nucleic acid molecule of interest was present in the sample nucleic acid.

2. Preparing sample nucleic acid

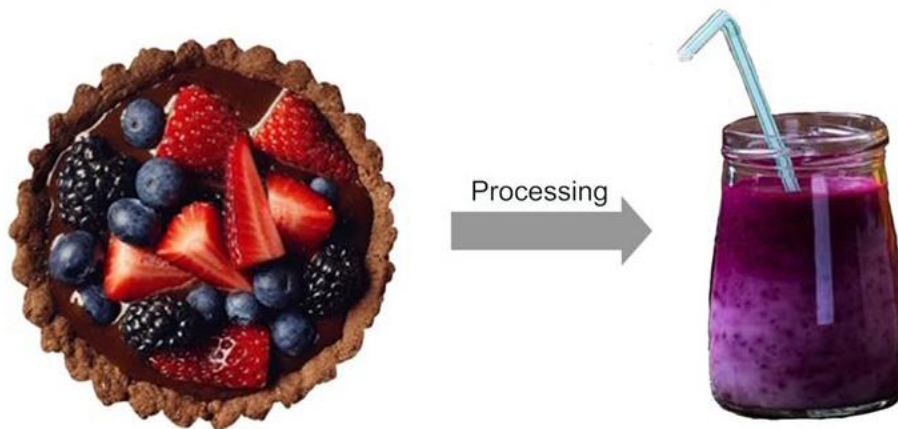
35. A POSA would have understood from my application that the first step in carrying out the disclosed methods is to derive (prepare) the sample nucleic acid from a sample or source (e.g., from a tissue sample). Specifically, as described in my application, “sample nucleic acid” is “derived from one or more samples or sources.” (EX1031, Page 5, last paragraph.) A POSA would have understood from this description that the “sample nucleic acid” analyzed in my methods is not equivalent to a “sample” or a “source.” This is important because Dr. Puglisi’s statement that “Cantor expressly discloses that the biological sample analyzed in its methods may be ‘a solid material such as a tissue [or] cells’” (Puglisi Declaration, ¶124) suggests that my disclosed methods could be used to test tissue samples directly. That is not correct. My application clearly states that

the “sample nucleic acid” is tested, and that the “sample nucleic acid” is itself derived from a sample or source, such as a tissue sample. (EX1031, 5–6.)

36. As described more fully below, the “sample nucleic acid” may come from many different sample sources, including a “biological sample,” which itself may have any form, including a solid form (e.g., tissue, cell, etc.) or fluid form (e.g., urine, blood, etc.). (EX1031, 6.) But the biological sample is **not** the sample nucleic acid assayed in Cantor’s methods. The sample nucleic acid in Cantor is extracted from the sample source (e.g., a biological sample) and mixed together in bulk to create the sample nucleic acid prior to mixing that with the solid phase nucleic acids to capture molecule(s) from the sample nucleic acid. (EX1031, 1; 5–6.) But whatever the source, the sample nucleic acid is in solution. It is not a solid material such as a tissue. This is depicted below, which shows a tissue sample (of which three cells are featured to show the nucleic acids present in the cells), and the mixture that results from processing:



37. Again, this is analogous to the difference between a fruit tart, on the one hand, and a fruit smoothie, on the other:



38. As this figure depicts, the spatial relationship between the nucleic acids in the tissue sample is lost when nucleic acids are extracted from the tissue and mixed to create the sample nucleic acid.

39. My application teaches that the sample nucleic acid may be prepared from a sample or source “by any means commonly known in the art, including but not limited to cell lysis procedures using chemical, physical or electrolytic lysis methods.” (EX1031, 54.) A POSA well understood at the time of my application that preparation of a bulk nucleic acid sample from a sample such as tissue involved at least disrupting (e.g., lysing) the cells—thus liberating nucleic acid to produce the sample nucleic acid used for testing in my methods. Indeed, kits for doing so were commercially available at the time. (See, e.g., Latham and Peltier, “MELT™ Total Nucleic Acid Isolation System: a new technology for hands-free

tissue disruption, RNA preservation and total nucleic acid purification,” *Nature Methods* 2:i–iii (2005) (EX2006).)

40. A POSA would have also understood that different types of samples call for different types of preparation, or “processing” as referred to in my application. (Page 7, first paragraph). For example, an environmental or patient sample with free-floating nucleic acids may be processed only to remove contaminants, or not processed at all. (See, e.g., Griffin et al., “Pathogenic Human Viruses in Coastal Waters,” *Clin. Microbiol. Rev.* 16(1):129–143, 134 (2003) (EX2007), noting presence of both viruses and stable free-floating viral nucleic acids in marine waters.) Yeast cells may have simply been boiled (to disrupt the cell walls). (See, e.g., Deak et al., “Molecular Characterization of *Yarrowia lipolytica* and *Candida zeylanoides* Isolated from Poultry,” *Appl. Env. Microbiol.* 66(10):4340–4344, 4340 (2000) (EX2008)). Thus, when my application states that “sample nucleic acid” may be provided “without processing of the sample(s)” in some embodiments (EX1031, 7), a POSA would have understood those embodiments to mean samples in which the nucleic acid molecules of interest are already accessible.

41. Following the preparation of the sample nucleic acid, further downstream processing steps may also be carried out, such as “shearing, size

fractionation, purification, methylation or demethylation, restriction nuclease treatment, addition of nucleotides or linkers . . . incorporation of detectable labels and the like.” (EX1031, 54.) As my application describes and a POSA would have understood, shearing (or breaking up) of nucleic acids creates nucleic acid fragments. But whatever the downstream processing steps, a POSA would have understood that the first processing step—that is, the preparation of the sample nucleic acid from the sample or source—destroys the spatial organization of cells and nucleic acids within the sample or source.

42. The paragraph referencing “tissue” to which Dr. Puglisi refers (EX1100, ¶124) is on page 6 of my application. That paragraph discusses the differences between “samples,” “biological samples,” and “sample nucleic acid.” For convenience, I reproduce it here, highlighting the different terms with different font colors:

A **sample** may be collected from an organism, mineral or geological site (e.g., soil, rock, mineral deposit, combat theater), forensic site (e.g., crime scene, contraband or suspected contraband), or a paleontological or archeological site (e.g., fossil, or bone) for example. A **sample** may be a “**biological sample**,” which refers to any material obtained from a living source or formerly-living source, for example, an animal such as a human or other mammal, a plant, a bacterium, a fungus, a protist or a virus. The **biological sample** can be in any form, including

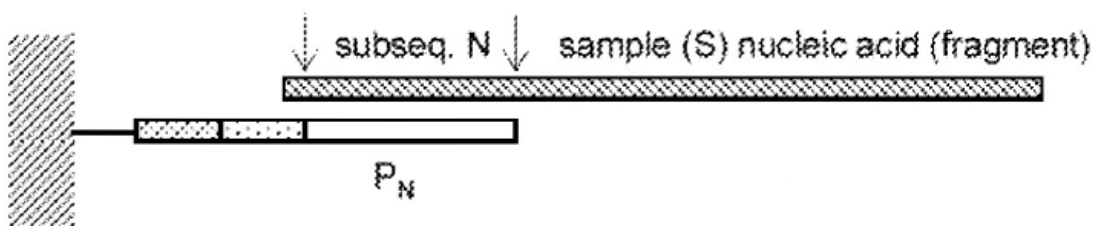
without limitation a solid material such as a tissue, cells, a cell pellet, a cell extract, or a biopsy, or a biological fluid such as urine, blood, saliva, amniotic fluid, exudate from a region of infection or inflammation, or a mouth wash containing buccal cells, urine, cerebral spinal fluid and synovial fluid and organs. A sample also may be isolated at a different time point as compared to another sample, where each of the samples are from the same or a different source. A sample nucleic acid may be from a nucleic acid library, such as a cDNA or RNA library, for example. A sample nucleic acid may be a result of nucleic acid purification or isolation and/or amplification of nucleic acid molecules from the sample. Sample nucleic acid provided for sequence analysis processes described herein may contain nucleic acid from one sample or from two or more samples (e.g., from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 samples). A sample nucleic acid sample can contain host and non-host sample nucleic acid, and in some embodiments, a sample may contain two or more different species of sample nucleic acid (e.g., mutant vs. wild-type, transplants, forensics, mother vs. fetus). [emphasis added]

43. This paragraph makes clear that the “tissue” is not the “sample nucleic acid” that is tested in my disclosed methods. Instead, the “tissue” is simply the *source* of the “sample nucleic acid” which is tested. A POSA would understand from this paragraph that if, for example, a human being was a “sample” organism, then the “solid material such as a tissue” would be the “biological sample.” A

POSA would further understand that the “sample nucleic acid” is derived (prepared) from the solid tissue sample as noted above.

3. Capturing and copying a nucleic acid molecule of interest

44. A POSA would have understood from my application that the next step in carrying out the disclosed methods is to capture the nucleic acid molecule of interest from the sample nucleic acid for analysis (e.g., copying for later sequencing). Target nucleic acids are captured using an assembly comprising single-stranded nucleic acid(s) attached to a solid support (e.g., bead or particle). I refer to single-stranded nucleic acid(s) attached to a solid support as “solid phase nucleic acid.” (See page 11, second paragraph; see also page 1, under “Summary” (“the solid support comprises single-stranded solid phase nucleic acid”)). The capture occurs between the solid phase nucleic acid and the target nucleotide sequence of a sample nucleic acid, which is depicted in the top panel of Figure 2 of my application:



45. A solid phase nucleic acid has two or three components: (1) a probe sequence, (2) a primer sequence, and (3) an optional identification sequence. (EX1031, 13–17.) The probe component is used to hybridize to specific target nucleotide sequences in the sample nucleic acid. (EX1031, 13–15.) The primer component is used in the copying process (EX1031, 15–16), and the identification sequence component, when present, and can be used to “construct[] partial sequence reads into larger sequence constructions in certain embodiments” and “may be of any length suitable for analyzing the nucleotide sequence or partial nucleotide sequence of the sample nucleic acid.” (EX1031, 15.) When the identification sequence is present, each solid support contains a unique identification sequence. (*Id.*) The identification sequences allow a POSA to assemble and analyze large nucleotide sequences from smaller sequence “reads” by identifying overlapping sequences. (EX1031, 25; 53–55 (Example 1).)

46. A single solid support (e.g., a single bead or particle) can have one or more single-stranded nucleic acids attached. Whether there are multiple nucleic acids attached to a single solid support or just one, I still refer to the whole as a “solid phase nucleic acid.” (*See* page 11, second paragraph, “[t]he term ‘solid phase nucleic acid’ as used herein generally refers to one or more different nucleic acid species in association with a solid support.”). Again, the solid phase nucleic

acid is mixed together with the sample nucleic acid to capture a nucleic acid molecule of interest. Examples of a solid phase nucleic acids are depicted in Figure 1C.

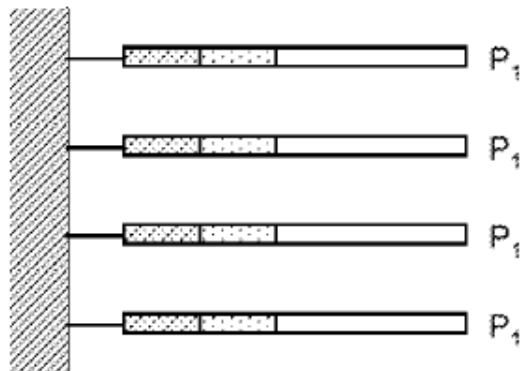


Figure 1C excerpt

47. The shading in Figure 1C's box identifies (in order from closest to the solid support to the furthest): the primer sequence (Pr), the optional identification sequence (ID), and the probe sequence (P₁) of the solid phase nucleic acid. In this figure, all four of the single-stranded nucleic acids attached to the solid support share a single probe sequence (P₁) as well as a single identification sequence.

48. Referring again to the top panel of Figure 2 of my application, reproduced below, the probe sequence (P_N) hybridizes to a specific target nucleotide sequence of a sample nucleic acid fragment. I refer to the target nucleotide sequence as "subseq. N" in Figure 2. The probe P_N has a sequence that is complementary to the sequence of the target nucleotide sequence. (See the

paragraph spanning pages 13–14 and first full paragraph on page 14 of my application.) The probe P_N is also “of a length sufficient to specifically hybridize to a sample nucleic acid sequence.” (See page 14, first full paragraph.) That is, the probe, by virtue of its sequence and length, is designed to hybridize to a target nucleotide sequence and thereby capture a sample nucleic acid fragment.

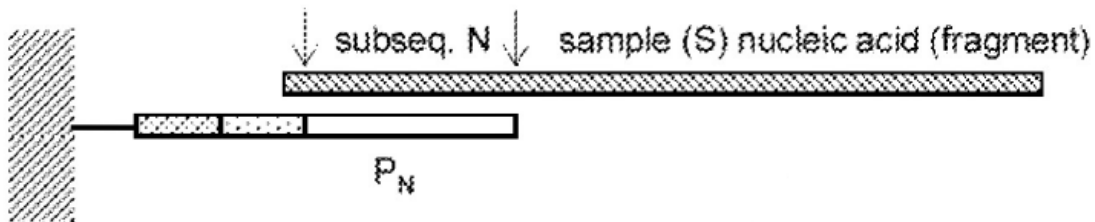


Figure 2 excerpt

49. A single nucleic acid molecule within a sample nucleic acid has multiple different subsequences along the length of the fragment that can be targeted for hybridization with a probe. These subsequences are depicted at the top of Figure 1A of my application, excerpted below (“subseq. 1,” “subseq. 2,” “subseq. 3” . . . “subseq N”).

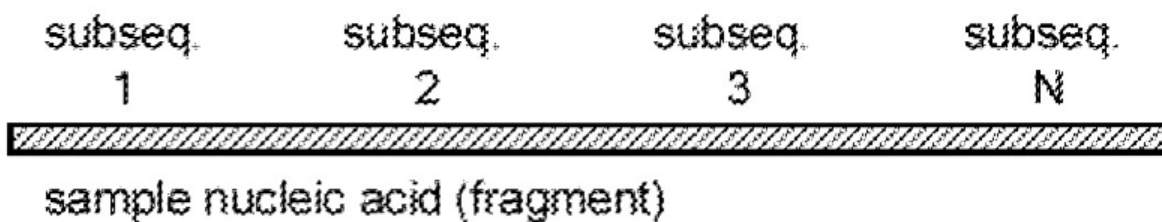


Figure 1A excerpt

50. The probe portions of the solid phase nucleic acid (P_1 , P_2 , P_3 , and P_N), are designed to hybridize with the various subsequences (or target nucleotide sequences) of a specific sample nucleic acid fragment. Thus, in this case, the solid phase nucleic acid contains, e.g., 4 different probe sequences, while the primer sequence and the identification sequence (if present) have the same sequences, as shown in the depiction of a solid phase nucleic acid Figure 1A of my application, excerpted below (along with the depiction of the subsequences in the target nucleic acid molecule in the same figure). A POSA would have understood that, in this case, the solid phase nucleic acid can capture different fragments of a target nucleic acid.

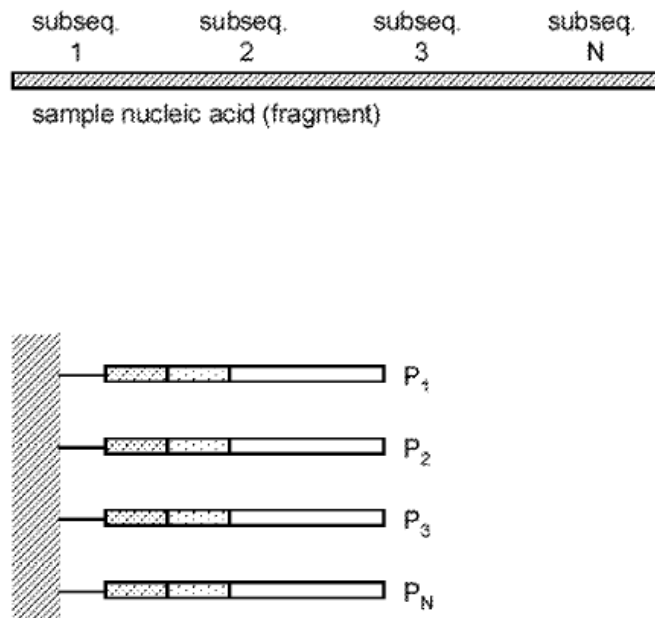


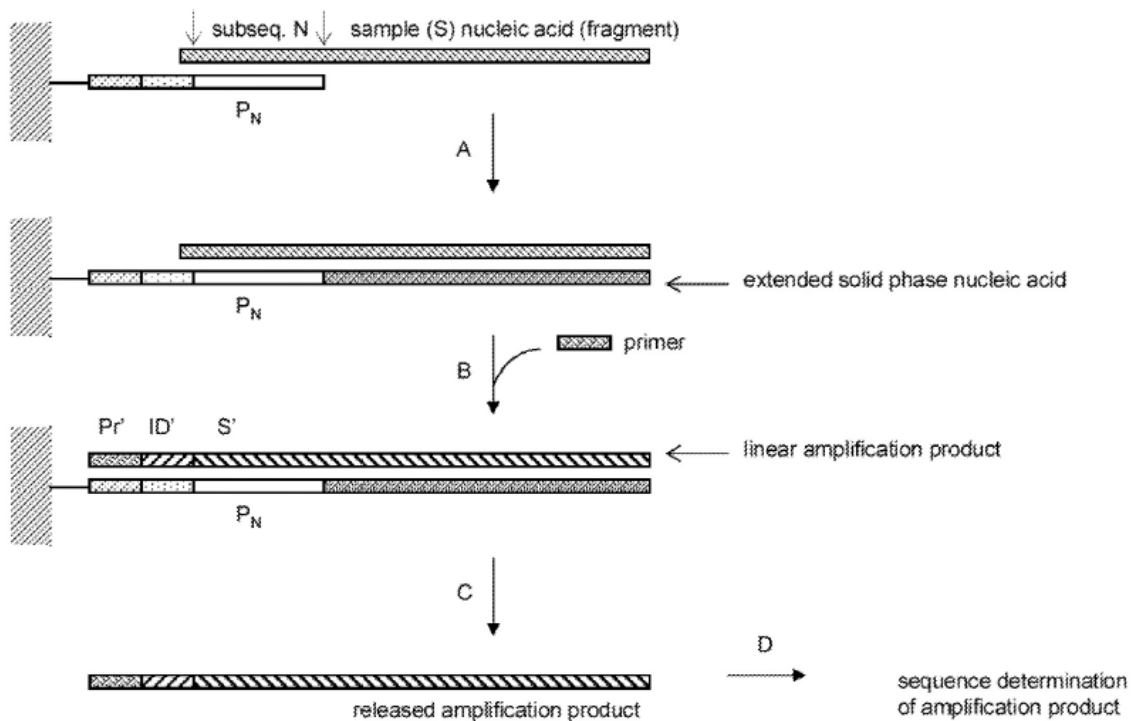
Figure 1A excerpt

51. In other cases, however, the solid phase nucleic acid contains four identical probe sequences, along with the same primer sequences and identification sequences, as shown in “solid support 1” of Figure 1C of my application, below. A POSA would have understood that, in this case, the solid phase nucleic acid captures just one subsequence of a target nucleic acid.



Figure 1C excerpt

52. Once hybridization occurs and the target nucleic acid fragment is captured, it can be copied. (See the paragraph spanning pages 24-25 of my application). A simplified explanation of this process is depicted in Figure 2 of my application:



53. In step A, the solid phase nucleic acid is extended by copying the captured sample nucleic acid fragment, which generates a molecule that is complementary to the captured nucleic acid (which I refer to as the “extended solid phase nucleic acid”). (See the paragraph spanning pages 24–25 of my application.)

54. In step B, the originally captured sample nucleic acid fragment is released, and then the extended solid phase nucleic acid is amplified to generate a “linear amplification product.” (*Id.*) In this step, a primer hybridizes to the primer sequence of the extended solid phase nucleic acid and is then extended using the extended solid phase nucleic acid as a template. So, a POSA would understand

from my application that the sequence of the resulting product contains: (1) a sequence complementary to the primer sequence of the solid phase nucleic acid (Pr' in Figure 2); (2) if an identification sequence is used, a sequence complementary to the identification sequence of the solid phase nucleic acid (ID' in Figure 2); and (3) a copy of the originally captured target nucleic acid fragment (S' in Figure 2). The product is then released from the solid support (step C) and the sequence is determined (step D). (*Id.*)

4. Solid support species

55. My application explains that a solid support (e.g., bead or particle) may be part of a “collection” having two or more “solid support species.” (EX1031, 11.)

56. As introduced in Section III.A.3, above, my application discusses two configurations of solid support “species,” the first of which has multiple different probes ($P_1, P_2, P_3, \dots P_N$), each configured to hybridize with different subsequences of a target nucleic acid, on a single solid support, and the second of which has a single probe (P_1) on a single solid support. (Figures 1A, 1B, and 1C; page 4, last paragraph; paragraph spanning pages 13–14; page 15, first full paragraph; paragraph spanning pages 16–17.) This is also described in claim 26 of Cantor, which recites “[a] solid support comprising single-stranded solid phase

nucleic acid having an identifier sequence and a probe sequence, wherein . . . the solid phase nucleic acid shares a common probe sequence [as in Figure 1C] or the solid phase nucleic acid does not share a common probe sequence [as in Figures 1A and 1B].” In both cases, the solid phase nucleic acid “shares a common identifier sequence,” as described in claim 26.

57. In the configuration shown in Figures 1A (excerpted below) and also Figure 1B of my application, the solid support species has multiple different probes on the same solid support (e.g., bead or particle), each of which hybridizes to different subsequences of a target nucleic acid fragment. This is the configuration that is described in claim 32 of Cantor, which depends on claim 26 and recites that “the solid phase nucleic acid of each solid support do not share a common probe sequence.” Thus, the released amplification products (step C in Figure 2 of my application) from this solid support would contain copies of different subsequences from across the target nucleic acid. (See *also* paragraph spanning pages 16–17.)



Figure 1A

58. In another configuration (depicted in Figure 1C of my application, below), the solid support species has just one probe sequence on the solid support (e.g., bead or particle). This is the configuration that is described in claim 31 of Cantor, which depends on claim 26 and recites that “the solid phase nucleic acid of each support share a common probe sequence.” Using this configuration, the released amplification products (step C in Figure 2 of my application) from this solid support would contain the same sequence. (*See also* paragraph spanning pages 16–17.)

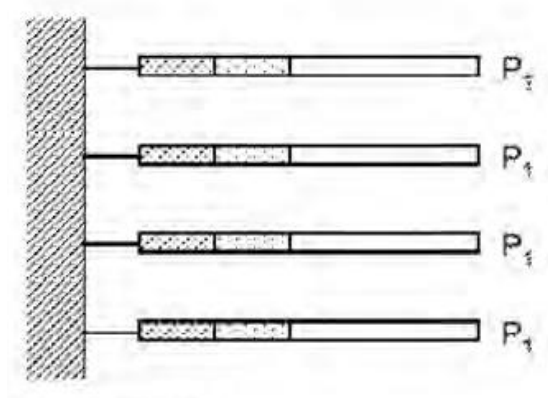


Figure 1C

59. Petitioner asserts, citing claims 31 and 32 that “Cantor discloses both arrays in which the probe sequence is the same at each position, and arrays in which the probe sequence is not the same at each position.” (Petition, 29.) But that is not the case. As discussed above, Claims 31 and 32 refer to the arrangement of the probes on a solid support (e.g., bead) rather than across a collection or an array, and depend only from claim 26, which is directed to a “solid support.”

60. While each single-stranded nucleic acid on a single solid support species may have different probes targeting different subsequences of a target nucleic acid, each has the same primer sequence, as depicted in Figures 1A, 1B, and 1C of my application. This is to allow a single primer sequence to be used during the copying phase (described generally in Section III.A.3, above):

In a collection of solid supports, each solid phase nucleic acid on each solid support species may have a common primer sequence (e.g., all solid support species have the same primer sequence species), in which case the primer sequence is referred to as a “universal” or “common” primer sequence.

(EX1031, 16, first full paragraph.)

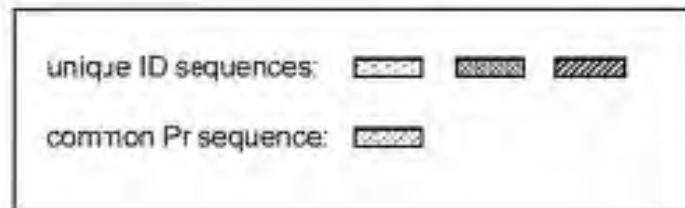
61. Likewise, if an identification sequence is present, each single-stranded nucleic acid on a single solid support species has the same identification sequence.

(EX1031, 15.)

5. Collections of solid supports

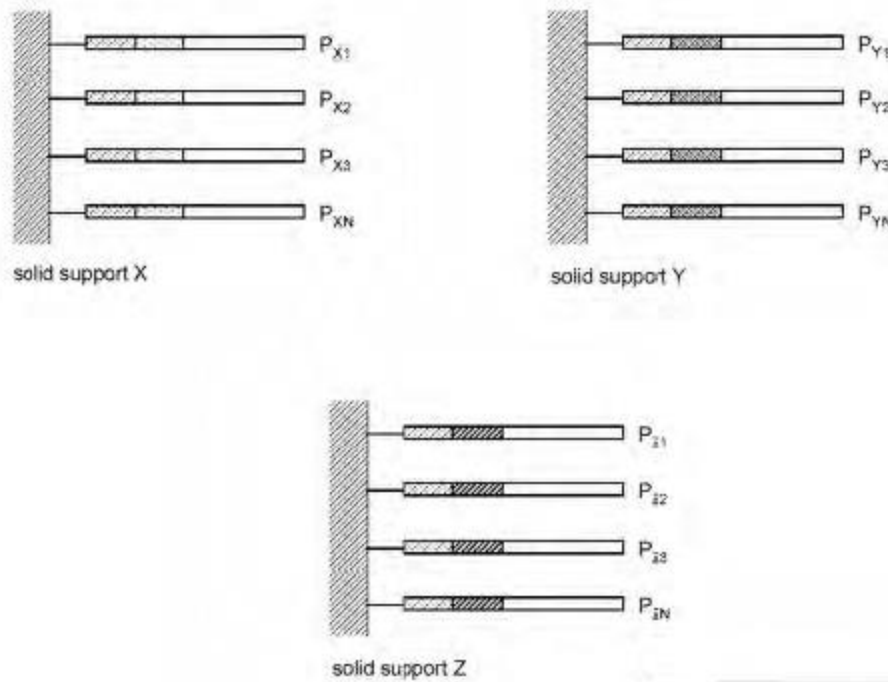
62. A “collection” of solid supports is one that contains multiple solid support species. (EX1031, 11, third paragraph.) My application discusses two different types of collections, depicted in Figure 1B and Figure 1C of my application. Each type of collection contains multiple different solid support species, with three solid support species depicted in each of Figures 1B and 1C.

63. In both Figures 1B and 1C, each solid support species in the collection has the same (common) primer sequence. But each of the solid support species has a unique identification sequence. So, as shown in the legend box in both Figures, there are as many unique identification sequences as there are solid support species in the collection.



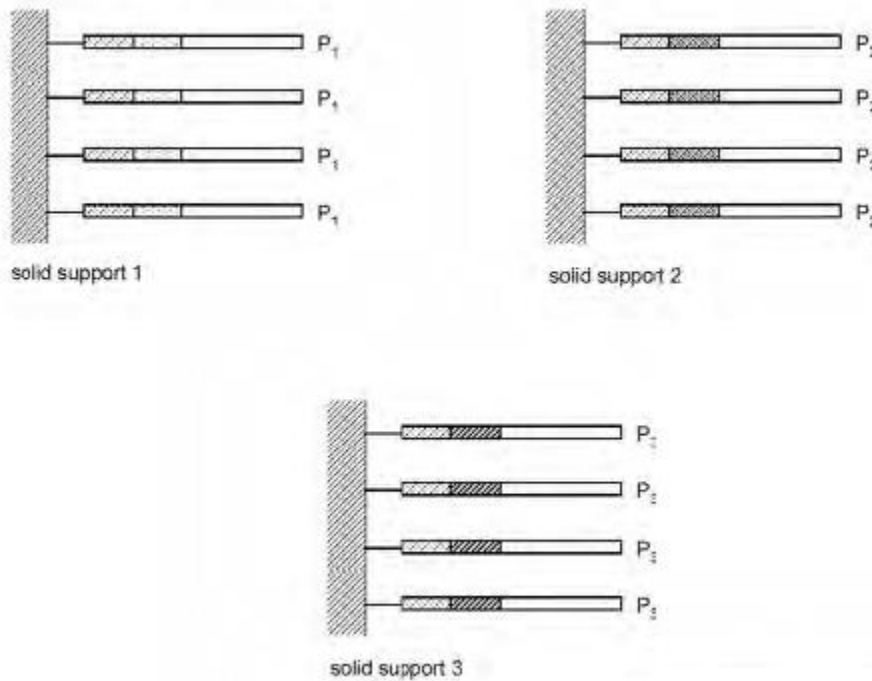
64. In other words, the “collection” contains three solid support species and three unique identification sequences, each identifying the particular solid support species (e.g., bead or particle) to which they are attached. As described below, however, in both types of collections no probe is the same between the beads.

65. The first type of collection, depicted in Figure 1B of my application, has solid support species with probes that target different subsequences of a target nucleic acid:



66. Each solid support species (X, Y, and Z) is depicted as having four different probe subsequences: X_1 , X_2 , X_3 , X_N for solid support species X; Y_1 , Y_2 , Y_3 , Y_N for solid support species Y; and Z_1 , Z_2 , Z_3 , Z_N for solid support species Z. A POSA would understand that this collection of solid support species, as depicted, has probes directed to three different target nucleic acids (X, Y, and Z). Each solid support has four probes, each of which targets a subsequence of the respective target nucleic acid. (See *also* paragraph spanning pages 16–17.)

67. The second type of collection, depicted in Figure 1C of my application, has solid support species with a single probe per species:



68. Each solid support species (1, 2, and 3) is depicted as having a single probe sequence (P₁, P₂, or P₃), which differs between the species. That is, P₁ for solid support 1; P₂ for solid support 2; and P₃ for solid support 3. A POSA would understand that this collection of solid support species, as depicted, has probes directed to three different target nucleic acids (1, 2, and 3). Each solid support species has four copies of the same probe. (See also paragraph spanning pages 16–17.)

6. Arrays

69. My application states that “[s]olid supports may be provided in an array in certain embodiments,” and “in part provides a substrate comprising a

collection of beads oriented in an array.” (EX1031, 4; 17, second full paragraph.)

The array “may contain one solid support species or multiple solid support species from a collection.” (EX1031, 17.) Collections of solid support species are shown in Figures 1B and 1C and described above in Section III.A.5. An array may be of any convenient shape (e.g., circular, oval, square, rectangular). (*Id.*) My application states that “[t]he array can be arranged on any substrate suitable for sequence analysis or manufacture processes described herein.” (EX1031, paragraph spanning pages 17–18.) Among the substrates are “flat substrates, filter substrates, wafer substrates, etched substrates, substrates having multiple wells or pits . . . substrates having one or more channels, substrates having one or more electrodes, and the like, and combinations thereof.” (*Id.*) The use of arrays such as bead arrays on a solid substrate facilitates the ease of handling of the beads in the described methods.

70. A POSA would have understood that my application does not describe or suggest arrays (e.g., of beads on a solid support) for determining a spatial distribution of a nucleic acid molecule of interest within a tissue sample. Indeed, none of the sequence analysis or manufacture processes described in the application relate to spatial distribution of a target nucleic acid in a tissue sample.

7. Sequence analysis and manufacture processes

71. None of the sequence analysis and manufacture processes described in the application relate to the spatial distribution of a nucleic acid molecule of interest in a tissue sample. Instead, as discussed above, my application describes bulk analysis methods that provide improvements for generating copies of nucleic acid molecules of interest. Those copies can then be used for later sequencing and analysis.

72. My application contains descriptions of eighteen different numbered applications for my solid phase nucleic acids. (EX1031, 30–52.) Each of these applications (briefly discussed below) relates to whether a particular sequence is present or absent in a sample nucleic acid (i.e., bulk analysis). This is not spatial distribution analysis.

73. *Microbial Identification* (EX1031, 31–32). In this application, my solid phase nucleic acids are used to “identify[] the presence or absence of [a] microbial nucleotide sequence in [a] sample nucleic acid.” (Page 32, first full paragraph.) For this and all other applications described below, the “sample nucleic acid” is mixed with the solid support-nucleic acid assembly under hybridization conditions (*id.* steps (a)–(b)) so that specific nucleic acid molecules

of interest out of the mixture are captured. Steps (c)–(e) are for copying the captured nucleic acid as I have described above.

74. *Detection of Sequence Variations* (EX1031,32–34). In this application, my solid phase nucleic acids are used to “identify[] the presence or absence of [a] disease marker nucleotide sequence in [a] sample nucleic acid.” (EX1031,34, first paragraph.)

75. *Detecting the Presence of Microbial Nucleic Acid Sequences Indicative of an Infection* (EX1031,34–36). In this application, my solid support-nucleic acid assemblies are used to “identify[] the presence or absence of [an] infectious microbial nucleotide sequence in [a] sample nucleic acid.” (EX1031,36, first paragraph.)

76. *Detecting the Presence of Specific Viral Nucleic Acid Sequences in a Viral Mixture or Mixed Viral Population* (EX1031,36–38). In this application, my solid phase nucleic acids are used to “identify[] the presence or absence of [a] viral nucleotide sequence in [a] sample nucleic acid.” (EX1031,38, first full paragraph.)

77. *Antibiotic Profiling* (EX1031,38–39). In this application, my solid phase nucleic acids are used to “detect[] the presence or absence of a target nucleic acid sequence indicative of drug resistance.” (EX1031,39, first full paragraph.)

78. *Identifying Disease Markers* (EX1031,39–40). In this application, my solid phase nucleic acids are used to “identify[] the presence or absence of [a] disease marker nucleotide sequence in [a] sample nucleic acid.” (EX1031,40, first full paragraph.)

79. *Haplotyping* (EX1031,40–41). In this application, my solid phase nucleic acids are used to “determin[e] the haplotype in [a] sample nucleic acid from one chromosome of a diploid organism.” (EX1031,41, first paragraph). That is, by identifying the presence of a nucleic acid molecule of interest in a sample nucleic acid. In this case, the “sample nucleic acid” is “from one chromosome of a diploid organism.” (*Id.*)

80. *Microsatellites* (EX1031,41–42). In this application, my solid phase nucleic acids are used to “determin[e] whether [a] microsatellite sequence is present in [a] sample nucleic acid.” (EX1031,42, first paragraph.)

81. *Short Tandem Repeats* (EX1031,42–43). In this application, my solid phase nucleic acids are used to “determin[e] whether [a] short tandem repeat sequence is present in [a] sample nucleic acid.” (EX1031,43, second full paragraph).

82. *Organism Identification* (EX1031,43–44). In this application, my solid phase nucleic acids are used to “determin[e] whether [a] target nucleotide sequence is present [in a nucleic acid sample].” (EX1031,44, first full paragraph.)

83. *Detecting Allelic Variation* (EX1031,44–45). In this application, my solid phase nucleic acids are used to “determin[e] whether a sequence variation in the target nucleotide sequence is present.” (EX1031,45, second full paragraph.)

84. *Determining Allelic Frequency* (EX1031,45–46). In this application, my solid phase nucleic acids are used to “identify[] the presence or absence of [a] target nucleotide sequence” in sample nucleic acid separately for multiple different individuals. (EX1031,46, first full paragraph).

85. *Epigenetics* (EX1031,46–48). In this application, my solid phase nucleic acids are used to “compar[e] the methylation pattern of [a] target nucleic acid to the methylation pattern of a reference nucleic acid.” (EX1031,47, second full paragraph.) In this case, the sample nucleic acid has been treated to convert methylated (or non-methylated) nucleotides to another nucleotide moiety. (*Id.*) So, comparing the sequence of the captured nucleic acid to a reference sequence identifies the presence or absence of methylation in the captured nucleic acid.

86. *Methylation Patterns* (EX1031,48–49). In this application, my solid phase nucleic acids are used to “determine[e] the methylation pattern [of a target

nucleic acid].” (EX1031,49, first full paragraph.) In this case, as for epigenetic analysis, the sample nucleic acid has been treated to convert methylated (or non-methylated) nucleotides to another nucleotide moiety. (*Id.*) So, determining the methylation pattern means determining the presence or absence of methylation in the captured nucleic acid.

87. *Resequencing* (EX1031,49–50). In this application, my solid phase nucleic acids are used to “compar[e] a sequence [of a target nucleic acid] to a reference nucleic acid sequence.” (EX1031,50, first full paragraph.) That is, determining the presence or absence of a specific sequence in the target nucleic acid sequence.

88. *Multiplexing* (EX1031,50–51). In this application, my solid phase nucleic acids are used to “identify[] [multiple] sequences in [a] sample nucleic acid.” (EX1031,51, first full paragraph.) That is, by identifying the presence or absence of two or more sequences within a sample nucleic acid.

89. *Disease Outbreak Monitoring* (EX1031,51–52). In this application, my solid phase nucleic acids are used to “compar[e] a sequence [of a target nucleic acid] to a reference sequence” to monitor disease outbreak. (EX1031, 52, first full paragraph.) That is, by identifying the presence of a nucleic acid molecule of interest in a sample nucleic acid.

90. *Vaccine Quality Control and Production Clone Quality Control*

(EX1031,52–53). In this application, my solid phase nucleic acids are used to “compar[e] a sequence [of a target nucleic acid] to a clone or vaccine reference sequence.” (EX1031,53, first paragraph.) That is, by identifying the presence of a target nucleotide sequence in a sample nucleic acid.

91. Numerous additional uses, none of which include spatial analysis, are also identified on page 5:

- (a) rapidly determine whether a particular target sequence is present in a sample;
- (b) perform mixture analysis, e.g., identify a mixture and/or its composition or determine the frequency of a target sequence in a mixture (e.g., mixed communities, quasispecies);
- (c) detect sequence variations (e.g., mutations, single nucleotide polymorphisms) in a sample;
- (d) perform haplotyping determinations;
- (e) perform microorganism (e.g., pathogen) typing;
- (f) detect the presence or absence of a microorganism target sequence in a sample;
- (g) identify disease markers;
- (h) detect microsatellites;
- (i) identify short tandem repeats;
- (j) identify an organism or organisms;
- (k) detect allelic variations;
- (l) determine allelic frequency;
- (m) determine methylation patterns;
- (n) perform epigenetic determinations;
- (o) re-sequence a region of a biomolecule;

- (p) human clinical research and medicine (e.g., cancer marker detection, sequence variation detection; detection of sequence signatures favorable or unfavorable for a particular drug administration),
- (q) HLA typing;
- (r) forensics;
- (s) vaccine quality control;
- (t) treatment monitoring;
- (u) vector identity;
- (v) perform vaccine or production strain quality control;
- (w) detect test strain identity;
- (x) identify a specific viral nucleic acid sequence or sequences in a viral mixture or population (e.g., hepatitis mixtures, HIV mixtures, mixed viral populations as might be found in an immuno-deficient, or immuno-compromised organism).

92. None of these applications relates to spatial analysis of nucleic acid molecules of interest within a tissue.

B. Armani (EX1022)

93. Armani is a 2009 article published in the journal *Lab on a Chip* that describes a method termed “2D-PCR.” (EX1022, 3526.) It is the same article referenced in the ’022 patent:

Certain PCR assays in a 2D format preserve spatial information (see Armani, et al., *Lab on a Chip*, 9(24): 3526-34 (2009)), but these methods have low spatial resolution because they rely on physical transference of tissue into wells, which also prevents random access to

tissue samples and high levels of multiplexing. [EX1001, 1:50–56]

94. In the 2D-PCR method, “tissue is sectioned and transferred into a multi-well array device.” (EX1022, 3527.) This is illustrated in Armani’s Fig. 1:

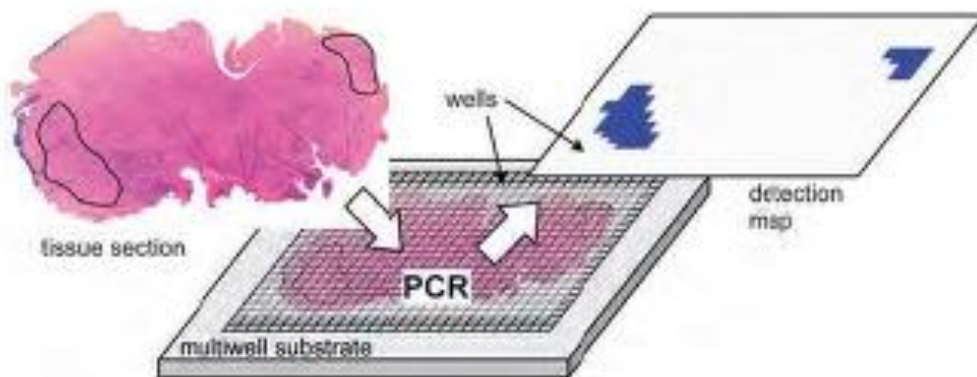
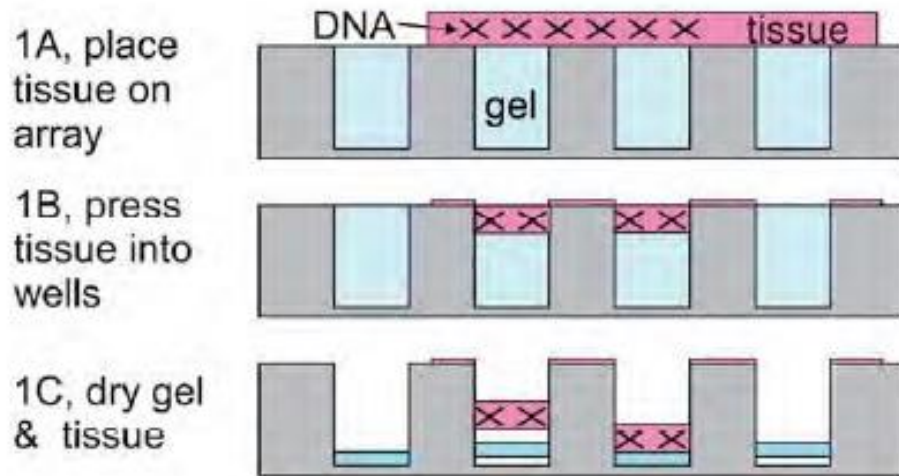


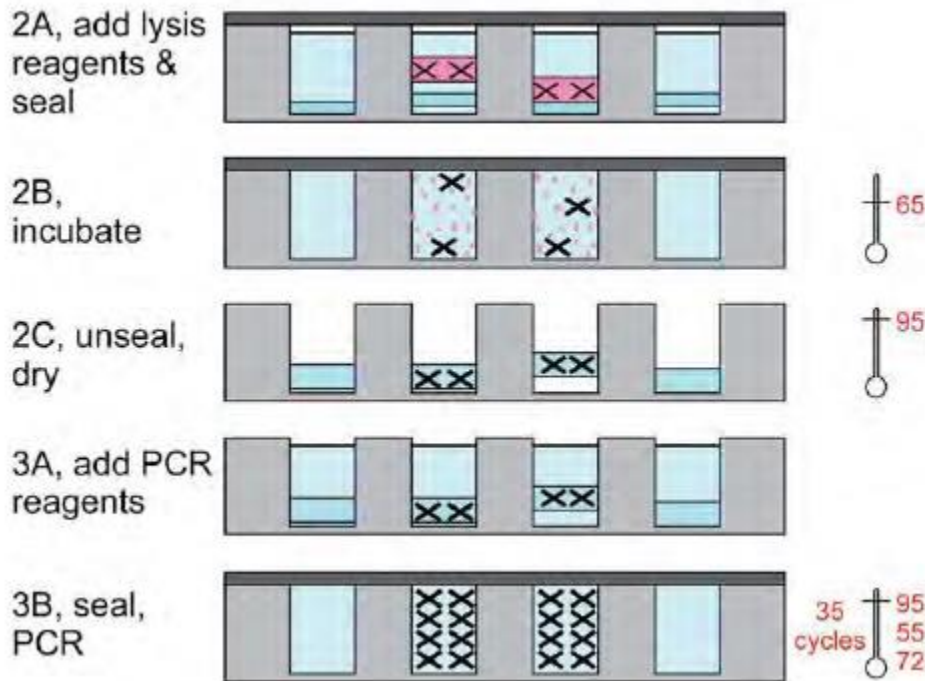
Fig. 1 2D-PCR is achieved by transferring a tissue section vertically into a multi-well array device, isolating tissue subregions. The DNA is extracted and then amplified by the polymerase chain reaction. A detection map is created by imaging the amplified DNA, for example using a DNA-intercalating dye.

95. As depicted in Armani’s Figure 2 (excerpted below), the tissue is effectively “punched” (i.e., pressed by force) into the wells containing agarose gel and dried, so that the wells contain sub-portions of the tissue sample immobilized in the agarose gel. (*Id.*):



96. By virtue of being punched into the wells, some portions of the tissue remain on the surface of the multi-well array device. These tissue portions are shown in Figure 2, above, as flat pink rectangles atop the pillars between the wells. A POSA would understand that the tissue portions that do not physically end up in a well are not analyzed in Armani's 2D-PCR method. The genomic DNA from those tissue portions is lost.

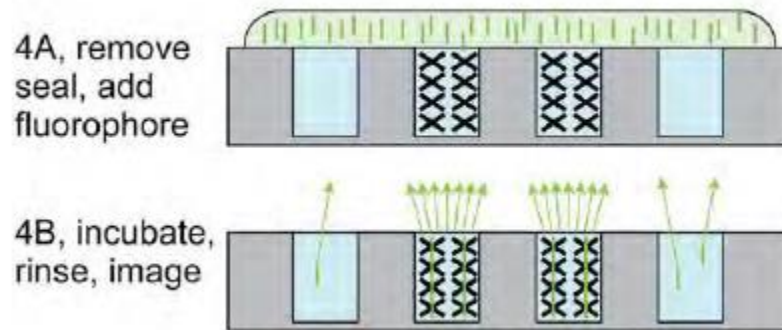
97. From that point on, each individual well is treated as a separate reaction. (*Id.*) Genomic DNA extraction and PCR amplification of the genomic DNA is carried out within each individual well. (*Id.*)



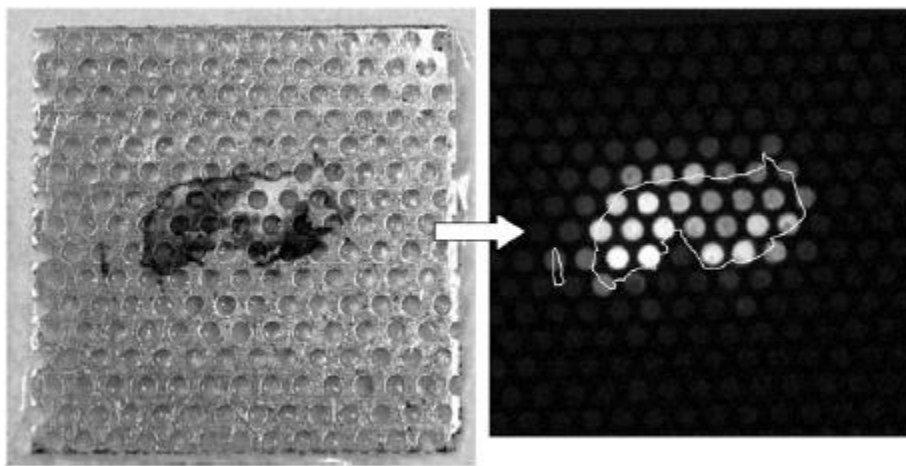
98. In steps 2A-2B, lysis reagents are added to each well to lyse the tissue section and release genomic DNA. (*Id.* at 3527.) Armani represents the genomic DNA with “x”s. (*Id.*) The solution is dried in step 2C, which removes the liquid in each well and allows for the next steps of PCR amplification. (*Id.*) In steps 3A-3B, PCR reagents are added to each well to amplify the genomic DNA, which are now represented by multiple “x”s in each well. (*Id.*)

99. For PCR amplification of the genomic DNA, Armani used primers that selectively amplify a GAPDH 167 bp genomic DNA target. (*Id.* at 3529.) Then, a “DNA-intercalating dye was used to visualize the PCR amplification products.” (*Id.*) Specifically, an intercalating fluorophore is added to the well. (*Id.* at 3527.) An intercalating fluorophore dye is a small molecule that can non-

specifically bind between adjacent basepairs of double-stranded DNA and fluoresce under light. The fluorescence coming from the array is thereafter imaged. (*Id.*, 3527, 3529.)



100. Armani shows a fluorescent image of the device in Figure 3, reproduced below. (*Id.* at 3530.) The image generally shows the presence or absence of the specifically amplified GAPDH genomic DNA target. The wells “completely under the tissue fluoresced the most” whereas wells not under the tissue “had only a low fluorescent background signal.” (*Id.*)



101. Because Armani's 2D-PCR method uses an intercalating dye for detection of genomic DNA, the only information the fluorescent signal provides is whether double-stranded DNA is present in the well following PCR amplification. To verify that the fluorescent signals actually correlated with the GAPDH genomic DNA target, Armani ran the contents of each well on an electrophoresis gel. (*Id.* at 3530.)

102. Armani does not disclose or contemplate the use of beads, using beads with a tissue section, or using binding agents configured to interact with a biological molecule of a tissue sample comprising a coding identifier having a nucleic acid sequence, or using a coding identifier for spatial analysis.

IV. PERSON OF ORDINARY SKILL IN THE ART

103. I understand from counsel that Patent Owner does not dispute Petitioner's definitions of the qualifications of a POSA at this time. I further understand that Patent Owner had reserved its right to challenge Petitioner's definition of a POSA and provide its own, should the Board decide to institute further proceedings. I understand Petitioner defined a POSA in the field of the '022 patent as of February 2010 to:

have had a doctorate degree (or equivalent) in
bioengineering, genetics, genomics, chemistry,
biochemistry, biophysics, molecular imaging or a related

discipline with at least 2-4 years of academic, research, or industry experience in genomic technology. In the course of their training and experience, the POSA would have acquired a working knowledge of nucleic acid arrays, combinatorial chemistry techniques for making and using such arrays, nucleic acid sequencing and sample preparation techniques, such as barcoding, imaging methods for signal detection, as well as histopathology techniques for cell permeabilization and tissue handling.

(Petition, 10.)

104. While I have based my analysis below on this description of a POSA and her level of skill, I have not been asked to offer an opinion as to whether I agree with Petitioner's definition nor have I been asked to offer my own description of the level of skill in the art at the relevant time. I reserve the right to offer my own description of the POSA and the requisite level of skill in the art if this proceeding is instituted.

V. LEGAL STANDARDS

105. My understanding of the legal requirements for priority, written description, and obviousness, as explained to me by Patent Owner's counsel, is set forth below.

A. Priority and Written Description

106. My understanding of the legal standard for priority, as described to me by Patent Owner's counsel, is that the priority document must provide "written description" of the claimed invention. I have been instructed by Patent Owner's counsel to consider written description support of the '124 provisional priority document.

107. I understand from counsel that to satisfy the written description requirement, the specification must describe the invention sufficiently to convey to a POSA that the patentee had "possession" of the claimed invention at the time of the application. I also understand from counsel that it is not necessary that the application describe the claim limitations exactly, but only so clearly that a POSA would recognize from the disclosure that the inventors possessed the invention. Furthermore, it is not necessary for a patent to teach what is well known in the art.

B. Obviousness

108. I understand the factual inquiries in determining obviousness include 1) the "scope and content of the prior art;" 2) the "differences between the prior art and the claims at issue;" and 3) "the level of ordinary skill in the pertinent art." A claimed invention is obvious if the prior art made obvious the invention as a whole, from the perspective of a POSA at the effective filing date (here, April 5, 2010). I

further understand a finding of obviousness requires consideration of the claims in their entirety and that the obviousness analysis must be carried out without the benefit of hindsight.

109. Also, a finding of obviousness requires a showing that a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so. I also understand for a single prior art reference to invalidate a patent claim, the prior art must show a suggestion or motivation to modify the teachings of the prior art reference to obtain the claimed invention, again, with a reasonable expectation of success.

VI. DISCUSSION OF PATENTABILITY

110. Below I set forth my opinions on patentability with respect to Petitioner's Grounds 1–3.

A. Priority and Written Description

111. I understand from Patent Owner's counsel that Petitioner identifies "three key elements of independent claim 1" that allegedly are not entitled to the April 5, 2010, priority date of the '124 provisional for lack of written description. (Petition, 55.) As I describe below, however, the '124 provisional application

provides written description of these “three key elements of independent claim 1.”

I address each in turn below.

1. The '124 provisional supports delivering a tissue sample to a previously generated array of bead-affixed probes

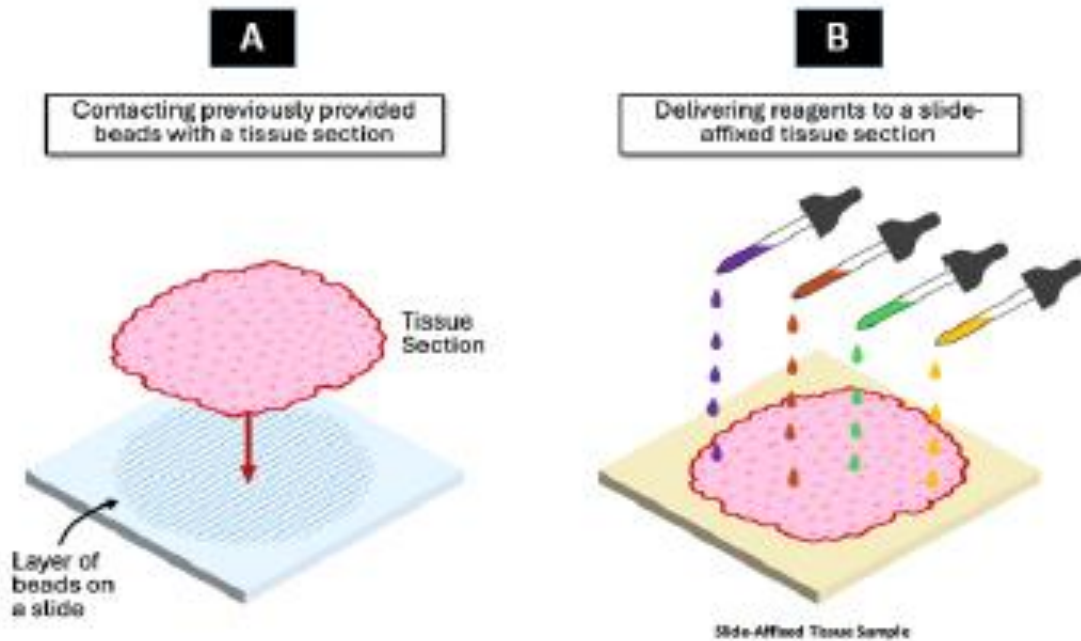
112. Petitioner argues that “a POSA would have understood the disclosure of the '124 Provisional to have been limited to the delivery of reagents, including binding agents and encoded probes, to a tissue sample already affixed to the solid support” as opposed to “delivering a tissue sample to a previously generated array of bead-affixed probes.” (Petition, 56–58.)

113. I disagree. As I describe below, the '124 provisional¹ quite clearly teaches generating an array of bead-affixed probes, in which binding agents are attached to beads, which are, in turn, attached to a substrate surface (e.g., a slide) before use in the disclosed methods.

114. Petitioner offers an illustration of an alleged dichotomy between what is taught and what is claimed (reproduced below), arguing that the '661 application² is limited to “Figure B” (“[d]elivering reagents to a slide-affixed tissue

¹ I understand that Petitioner analyzes the '661 application. I have been instructed by Patent Owner’s counsel to analyze the support in the '124 provisional.

section”), while the Challenged Claims are limited to “Figure A” (“[c]ontacting previously provided beads with a tissue section”). I note that these figures are not from the ’022 patent. I presume that Petitioner created them.



115. The ’124 provisional does discuss bead arrays, which both Petitioner and Dr. Puglisi acknowledge, pointing us to paragraph [00018]. (Petition, 60; EX1100, ¶ 268.) Petitioner states that this passage “give[s] no indication that they refer to providing beads in an array as claimed, as opposed to depositing beads comprising a binding agent to a tissue sample that was previously affixed to a solid support using the disclosed ‘reagent delivery systems.’” (*Id.*)

116. But Petitioner and Dr. Puglisi must misunderstand what this paragraph says. These passages certainly describe an array as depicted in Petitioner's "Figure A" that, once constructed, would also have to be contacted with a tissue sample as depicted in "Figure A" to carry out the types of spatial analyses described in the '124 provisional. I have excerpted the paragraph Petitioner cites, as well as the three preceding paragraphs of the 124 provisional for full context, below.

Numerous reagent delivery systems can be used with the assay system of the invention. The primary criteria of such reagent delivery systems is the ability to ***direct delivery of specific agents based on spatial patterns on the substrate surface.*** [EX1009, ¶ [00015]; EX1001, 21:51–55, emphasis added]

In one preferred aspect, the encoding scheme utilizes a reagent delivery system based on printing and informatics technologies to implement the spatial patterns used for identification and localization of the biological materials. For example, the patterns found in the encoding scheme may be created using ink jet printing technology to provide reagents at specific locations ***on one or more substrate surfaces.*** The desired patterns are set out in specific coding patterns ***on the substrate surface.*** [EX 1009, ¶ [00016]; EX1001, 21:56–54, emphasis added]

In certain aspects of the invention, the binding agents are immobilized directly to the substrate surface, and the location of the binding agents is known or determined prior to use of the substrate surface in the assay system. ***In another aspect, the binding agents are immobilized***

onto beads or other separate structural elements that are then provided in known locations on the substrate surface. In yet another aspect, the binding agents may be provided in or on features of the substrate surface, *e.g.*, provided in wells or channels. [EX1009, ¶ [00017]; EX1001, 21:65–22:6, emphasis added]

In specific aspects of the invention, the binding agents are nucleic acids immobilized directly or indirectly to the substrate surface, *e.g.*, directly through the use of amino groups on the substrate surface or indirectly through the use of a linker. ***The location of the nucleic acid sequences is known or determined prior to use of the substrate surface in the assay system. In another specific aspect, the nucleic acids may be immobilized directly or indirectly onto beads that are then provided in known locations on the substrate surface.*** In yet another aspect, the nucleic acids may be provided in or on features of the substrate surface, *e.g.*, provided in wells. [EX1009, ¶ [00018]; EX1001, 22:7–18].

117. These passages from the '124 provisional clearly teach immobilizing binding agents (including nucleic acid binding agents) directly or indirectly onto a ***substrate surface***. A POSA would understand that “substrate surface” is ***not*** a tissue sample, but instead is a structural support. The specification states that the binding agents are immobilized onto beads which are ***then*** provided in known locations on the substrate surface, such as wells or channels. It would not make sense at all for the substrate surface being referenced in these paragraphs to be a

tissue sample. A POSA would understand that a tissue sample does not have features such as wells or channels. Rather, a POSA would understand that wells or channels are features of manufactured solid supports. Indeed, I describe as much in my own application. (See EX1031, Page 18, describing manufactured substrates with wells and channels.)

118. Moreover, additional passages in the '124 provisional discuss the use of these types of pre-generated bead arrays, with beads immobilized directly or indirectly onto a substrate surface. One passage does so in the context of peptide binding agents. (EX1009, ¶ [00021]; EX1001, 22:37–47). Another does so in the context of chemical binding agents (EX1009, ¶ [00022]; EX1001, 22:48–57).

119. Again, once such an array is constructed, it would have to be contacted with a tissue sample as depicted in “Figure A” to carry out the types of spatial distribution analyses described in the '124 provisional.

120. Aside from the misreading of the bead-related disclosures in the '124 provisional and ignoring other pre-generated array teachings, another of Petitioner’s (and Dr. Puglisi’s) argument is that a “washing” step discussed in one section of the application would “not make sense” in the context of probes covalently bound to beads. (Petition, 57; EX1100, ¶ 252.) But here they cite to a completely separate portion of the '022 patent—Figures 1 and 2 as well as

paragraph [0028].³ (*Id.*) These figures and passages elaborate on additional types of assays also discussed in the '022 patent specification, which are in addition to the pre-generated bead array assays discussed above.

121. Petitioner also describes the '022 patent as being “replete with statements confirming that its ‘invention’ requires (i) providing a sample affixed to a support before (ii) delivering probes to the sample” or as “[f]urther confirming that the ['022 patent] discloses only the delivery of reagents, including probes, to a sample affixed to a solid support.”⁴ (Petition, 58.) But again, that additional passages may elaborate on additional types of assays in addition to the pre-generated bead array assays discussed above does not negate that the '124 provisional teaches a pre-generated array (as depicted in Petitioner’s “Figure A”), as described above.

122. And those are not the only passages in the '124 provisional that teach a pre-generated array. Passages directly preceding the paragraphs quoted above discuss how the '124 provisional provides assays and assay systems “comprising

³ While Petitioner cites to the '661 application, I understand from Patent Owner’s counsel that the cited portions correspond to Figures 1 and 2 as well as paragraph [0028] of the '022 patent.

⁴ While Petitioner cites to the '661 application, I understand from Patent Owner’s counsel that the cited portions all correspond to passages in the '022 patent: 2:9–19, 3:36–54, 8:39–9:3, 11:50–57, 16:3–27, 17:44–61, Figure 1, and Figure 2.

one or more agents provided in defined spatial patterns ***on a substrate surface . . .***” (EX1009, ¶ [0007]; EX1001, 20:29–33, emphasis added) and how in some cases “the encoding scheme employs limited reagent delivery to the spatial patterns ***on the substrate surface . . .***” (EX1009, ¶ [0008]; EX1001, 20:63–65, emphasis added). As the ’124 provisional discusses, “[n]umerous reagent delivery systems can be used with the assay system of the invention,” and “[t]he primary criteria of such reagent delivery systems is the ability to direct delivery of specific agents based on spatial patterns ***on the substrate surface.***” (EX1009, ¶ [00015]; EX1001 21:51–55, emphasis added.)

123. Even more passages from the ’124 provisional confirm that Petitioner’s “Figure A” embodiment is described in the ’124 provisional:

The assay systems of the invention are particularly advantageous in that they are compatible with numerous samples [sic] types, such as fresh samples, such as primary tissue sections, and preserved samples including but not limited to frozen samples and paraformalin-fixed paraffin-embedded (FFPE) samples. ***An important aspect of the assay systems of the invention is that the binding agents are immobilized on a substrate surface in discrete, independent measureable areas. These discrete areas can be formed by spatially selective deposition of the binding agents on the substrate surface.*** Numerous methods can be used for the deposition of the agent and the coding identifiers associates [sic] with the agent. For example, the coding identifiers can be delivered together

or separately from the agent. If delivered together they can be attached (*e.g.*, synthesized as a single molecule or attached through ligation or a chemical coupling mechanism) or simply mixed together to be attached after delivery to the substrate. In a preferred aspect, the agent and the coding identifier are made separately, mixed together for attachment, and delivered either attached or as a mixture to be attached on the surface. In a specific aspect the binding agents are delivered generally over the substrate surface and the coding identifiers are delivered in a pattern-specific manner. [EX1009, ¶ [00047]; EX1001, 23:52–24:8]

Examples of methods that can be used for deposition of agents and/or coding identifiers onto the substrate surface include, but are not limited to, ink jet spotting, mechanical spotting by means of pin, pen or capillary, micro contact printing, fluidically contacting the measurement areas with the biological or biochemical or synthetic recognition elements upon their supply in parallel or crossed micro channels, upon exposure to pressure differences or to electric or electro magnetic potentials, and photochemical or photolithographic immobilization methods. [EX1009, ¶ [00048]; EX1001, 24:9–18]

124. Given all of the above, it is my opinion that the '124 provisional supports “delivering a tissue sample to a previously generated array of bead-affixed probes,” as depicted in Petitioner’s “Figure A.”

2. The '124 provisional supports contacting a plurality of beads with a tissue section “such that the binding agent interacts with the biological molecule”

125. Petitioner’s second argument is that “the ’661 Application contain[s] no teaching on how to prepare the tissue section ‘such that the binding agent interacts with the biological molecule’ when a tissue section is contacted with a plurality of beads.” (Petition, 62; EX1100, ¶ 271.)

126. I understand from Patent Owner’s counsel that techniques that are conventional or well known to a POSA need not be disclosed in detail within a patent application’s specification. Techniques for preparing tissues to interact with biological molecules were well known to a POSA and would not have needed to be explained in the specification. For example, *in situ* hybridization (referenced in the ’124 provisional at paragraph [0003]) had been around since the 1970s. These techniques would not need to have been explained to a POSA circa 2010.

127. Petitioner and Dr. Puglisi themselves confirm that is the case, acknowledging that “techniques for local release of nucleic acids or other biological molecules from tissue samples for capture on probes in bead or other arrays were known” to a POSA at the time that the ’124 provisional was filed (Petition, 62–63; EX1100, ¶ 273.)

128. Furthermore, the '124 provisional does point a POSA to “standard laboratory manuals” describing “conventional techniques and descriptions” of “organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology which are within the skill of those who practice in the art.” (EX1009, ¶ [00037].) For example, PCR PRIMER: A LABORATORY MANUAL (Carl W. Dieffenbach and Gabriela S. Dveksler, eds. (2003)). (*Id.*) The Dieffenbach manual contains an entire section on “Sample Preparation” that includes detailed protocols for liberating nucleic acids (both DNA and RNA) from a variety of different samples, including tissue. (EX2009.) It also includes a chapter by Gerard J. Nuovo describing *in situ* methods for “Reverse Transcriptase In Situ PCR” that detail techniques for local release of nucleic acids from tissue samples (EX2010.)

129. As Nuovo’s *in situ* chapter correctly notes, since the 1970s, “direct cellular localization of a DNA or RNA target [had] been achieved routinely using *in situ* hybridization.” (*Id.*, 251.) By 2003, accessing nucleic acids from tissue samples *in situ* was routine and there were many different protocols available to a POSA to use, depending on the particular sample and type of analysis desired. (*See id.*) The Nuovo chapter describes a protocol for *in situ* PCR, but the basic steps for liberating nucleic acids from a tissue sample (tissue preparation and

protease digestion) apply to other types of *in situ* analysis, including *in situ* hybridization. (*Id.*, 255–257, 261.) *In situ* tissue preparation protocols vary depending on whether the tissue sample was, for example, fresh frozen or formalin fixed and paraffin embedded. (*Id.*, 255–256.) But, generally, protease digestion is used to permeabilize the cells (open cell membranes) for local release of nucleic acids. (*Id.*, 256–257.) A number of different proteases could be used; Nuovo specifically mentions proteinase K, pepsin, trypsin, trypsinogen, and pronase. (*Id.*, 257.)

130. Petitioner also points to the discussion of the Armani reference (EX1022) within the '124 provisional. (Petition 62.) While the '124 provisional and, as noted above in Section III.B, the '022 patent, both contain the same discussion of the Armani references, a POSA would not have read the discussion as “expressly criticiz[ing] and disclaim[ing]” a “technique for local release of nucleic acids or other biological molecules from tissue samples for capture on probes in beads or other arrays,” as Dr. Puglisi suggests. (EX1100, ¶ 271–274; *see also* Petition, 62–63.) Rather, a POSA would have understood that passage (excerpted again below) to be a criticism of the low spatial resolution that the Armani 2D-PCR method provides, which results from physically pressing the tissue into the individual wells of the array (as depicted in Fig. 2 of Armani,

excerpted below). This criticism of Armani has nothing to do with the POSA's knowledge of laboratory techniques.

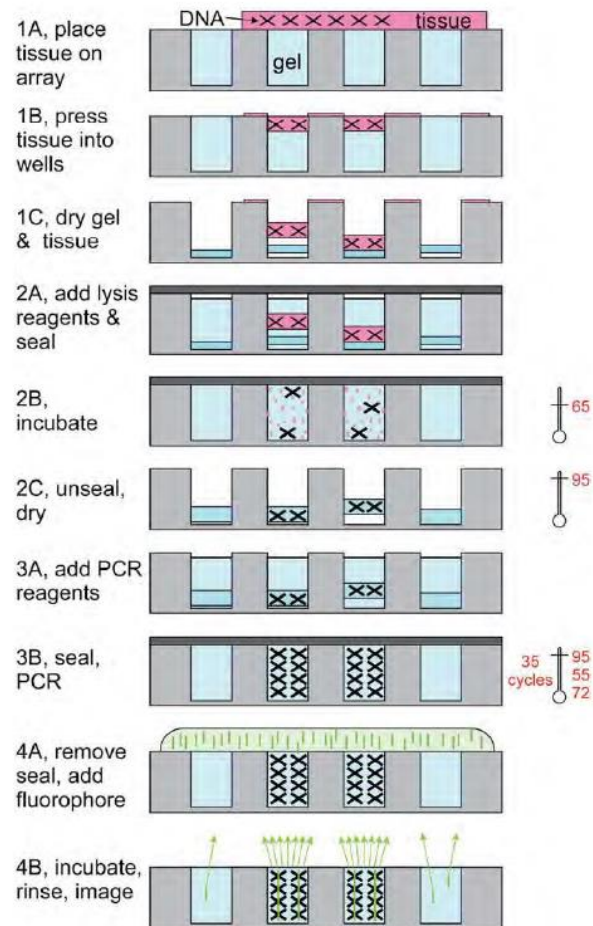


Fig. 2 Experimental protocol used to demonstrate the 2D-PCR concept. Tissue is transferred into wells, isolated, and lysed to extract the genomic DNA (represented by “x”s). PCR is performed to amplify the target DNA, and the targets are visualized with fluorescent dye (indicated by vertical lines “|”).

131. The methods described in the '124 provisional do not require individually partitioning portions of a tissue sample into discrete wells, and performing individual well-by-well PCR reactions followed by fluorophore

detection and, therefore, do not suffer from the same low resolution as Armani (as the '124 provisional notes):

Certain PCR assays in a 2D format preserve spatial information (Armani, M et al (2009) *Lab on a Chip*, 9(24): 3526-34 (2009)), but these methods have low spatial resolution because they rely on physical transference of tissues into wells, which also prevents random access to tissue samples and high levels of multiplexing. [EX1009, ¶ [0004]]

132. Because methods for contacting a plurality of beads with a tissue section “such that the binding agent interacts with the biological molecule” were well known and conventional, the '124 provisional meets the requirements for priority.

3. The '124 provisional supports “providing a plurality of beads, wherein the plurality of beads . . . (i) comprises a coding identifier having a nucleic acid sequence”

133. Finally, Petitioner alleges that the '124 provisional “fails to disclose beads comprising nucleic acid binding agents having a nucleic acid coding identifier.” (Petition, 63.) In particular, Petitioner alleges that, “[b]ecause the '124 Provisional’s disclosures pertaining to beads involve providing them at known locations, a POSA would have understood these disclosures to have obviated the need for the use of coding identifiers as claimed in the '022 Patent.” (Petition, 64;

EX1100, ¶278.) That is not the case. Even if the beads were provided in known locations, it would not, as Petitioner suggests, “obviate[] the need for the use of coding identifiers.”

134. First of all, the ’124 provisional describes an assay based on an encoding scheme that uses “**both molecule-specific binding agents and coding identifiers** to provide a practical and cost-effective determination of information on multiple biological molecules, including specific positional information of such molecules in a biological sample, *e.g.*, a tissue section.” (EX1009, ¶ [0007]; EX1001, 20:37-46., emphasis added.) This “unique encoding scheme” allows “multiple molecules **and their respective multiple locations** to be identified in a single system.” (*Id.*)

135. And the presence or absence and specific positional information of the biological molecules is done based on detection of both the binding agent, the coding identifier, and the knowledge of the location of the binding agents (and thus coding identifier) on the substrate surface. (EX1009, ¶¶ [0007], [00018].)

136. Thus, the ’124 provisional clearly describes the invention as using both binding agents and coding identifiers, directly refuting Petitioner’s allegation that a POSA would have believed coding identifiers were superfluous.

137. Moreover, there is no reason a POSA would have understood that the '124 provisional “fails to disclose beads comprising nucleic acid binding agents having a nucleic acid coding identifier,” as Petitioner suggests. (Petition, 63.) The '124 provisional specifically discusses an aspect in which its “binding agents are nucleic acids” and that the nucleic acids can be attached to a bead. (EX1009, ¶ [0018]; EX1001, 22:7–18.)

138. The '124 provisional explains that when the binding agent is a nucleic acid, nucleic acid sequencing is preferably used to “decode the spatial encoding scheme”:

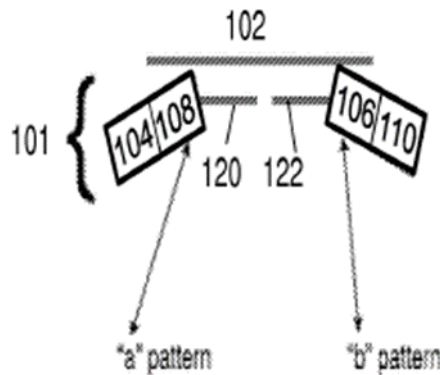
In these aspects involving nucleic acid [binding] agents, any methods of sequence determination can be used, *e.g.*, sequencing, hybridization and the like. ***In a preferred aspect, nucleic acid sequencing, and preferably next-generation sequencing, is used to decode the spatial encoding scheme in the assay system of the invention.*** [EX1009, ¶ [0019]; EX1001, 22:19-23].

139. As explained above the “encoding scheme” uses both “molecule-specific binding agents and coding identifiers” to provide “specific position information.” When nucleic acid sequencing is used to decode the spatial encoding scheme and thus determine the spatial location of the biological molecule captured by the binding agent, the coding identifier must also be a nucleic acid. If it were not, it could not be sequenced using nucleic acid sequencing.

140. If, as Petitioner suggests, a nucleic acid binding agent was attached to a bead at a known location on the substrate surface but without a coding identifier, a POSA would have understood that it would be impossible to obtain “specific position information” for captured target nucleic acids by sequencing, including next generation sequencing, as contemplated by the ’124 provisional. The captured target nucleic acid would be at a known location on the substrate surface when it remained bound to the binding agent on the bead. But in order for next-generation sequencing to be used to identify the captured target nucleic acid, it would have to be released from the substrate surface so that it could be processed to undergo sequencing. Once released from the substrate surface, without a coding identifier, any location information is lost. Therefore, a POSA would understand that the binding agent needs to include a coding identifier in order for sequencing to be used to provide “specific position information.”

141. That the ’124 provisional teaches “providing a plurality of beads, wherein the plurality of beads . . . (i) comprises a coding identifier having a nucleic acid sequence” is further demonstrated by the ’124 provisional’s Figure 1, which includes an illustration where the coding identifier is part of the same molecule as the binding agent. (See Fig. 1 of the ’124 provisional, which is Fig. 4 of the ’022 patent, partially excerpted below.) The coding identifiers (106 and 108) are

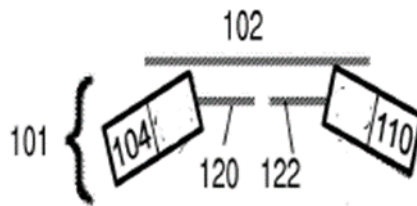
associated with the binding agents (120 and 122) which can capture the target nucleic acid (102).



142. In this depiction the coding identifiers are also associated with universal priming sites (104 and 110). The universal priming sites can be used to facilitate sequence-based decoding by making copies of the binding agent, captured target nucleic acid and linked coding identifier which can be sequenced. (EX1009, ¶ [00046]; EX1001, 22:34–51). It is the sequence of the coding identifier that, after sequencing, provides the specific position on the substrate surface where the target nucleic acid hybridized to the binding agent, provided that the location of the binding agents (and therefore coding identifiers) is known or determined prior to use of the substrate surface in the assay system. (EX1009, ¶ [0018]-[0019]; EX1001, 23:7–24.)

143. To further illustrate why a POSA would have understood that the coding identifier is not superfluous in this case, as Petitioner suggests, consider the

case where this system does not include a coding identifier at all. I've shown this by removing the coding identifiers 106 and 108 from the binding agents 120 and 122 in Figure 1. In the revised schematic depicted below, the binding agents (120, 122) would bind to and capture the target nucleic acid molecule (102). Then, as I describe above, a new nucleic acid molecule would be generated using the universal priming sites. It would contain a copy of the binding agent and the captured target nucleic acid.



144. But because no coding identifier was used, the new nucleic acid molecule would **not** contain a copy of any coding identifier. As a result, if the copies were sequenced, the sequence readout would likewise lack any coding identifier sequence and a POSA would not be able to derive any spatial location information. And this would be true even if the binding agents were originally at known locations on the array. As a POSA would readily understand, sequencing requires release of the captured target nucleic acid from the substrate surface so that it can undergo sequencing.

145. The copy can be released from the probe without the probe being released from the solid surface (e.g., by heating so that the hydrogen bonds weaken and break). Alternatively, the entire double-stranded complex could be cleaved from the solid support (e.g., by breaking the bond between the probe and the solid support). In either case, the released nucleic acids will be pooled together in solution and will no longer be associated with the solid surface or any particular location on a solid surface.

146. Given all of the above, the '124 provisional supports “providing a plurality of beads, wherein the plurality of beads . . . (i) comprises a coding identifier having a nucleic acid sequence.”

B. Obviousness (Grounds 1 and 2)

147. Petitioner’s Ground 1 is obviousness over my application (Cantor, EX1031). But Petitioner’s arguments are faulty for several reasons. First, my application does not teach contacting a “tissue sample” with my solid supports, as Petitioner asserts. My application repeatedly explains that the disclosed methods relate to preparing a “sample nucleic acid” and contacting that sample nucleic acid to the solid supports (e.g., beads) for later sequencing and analysis. In every description of “the invention,” Cantor refers to “sample nucleic acid” and nowhere mentions contacting a tissue sample with the solid supports.

148. As I explained above in Section III.A.2, a POSA would have understood that to carry out the method steps of my application, the POSA would first have to derive the sample nucleic acid from a sample or source. A POSA could not have “prepar[ed] a mixture comprising sample nucleic acid and a solid support” (EX1031, 1) as a next step unless the sample nucleic acid was first derived (prepared) from a sample or source.

149. As further explained in Section III.A.2, above, a POSA would have understood that the “sample nucleic acid” is derived from a sample or source “by any means commonly known in the art, including but not limited to cell lysis procedures using chemical, physical or electrolytic lysis methods.” (EX1031, 54.) I understand that Dr. Puglisi states that a POSA would have been motivated to apply such cell lysis techniques to the tissue or biopsy samples disclosed by Cantor. (EX1100, ¶149.) But Cantor teaches, and a POSA would have understood, that the cell lysis procedures derive the sample nucleic acid from the sample or source.

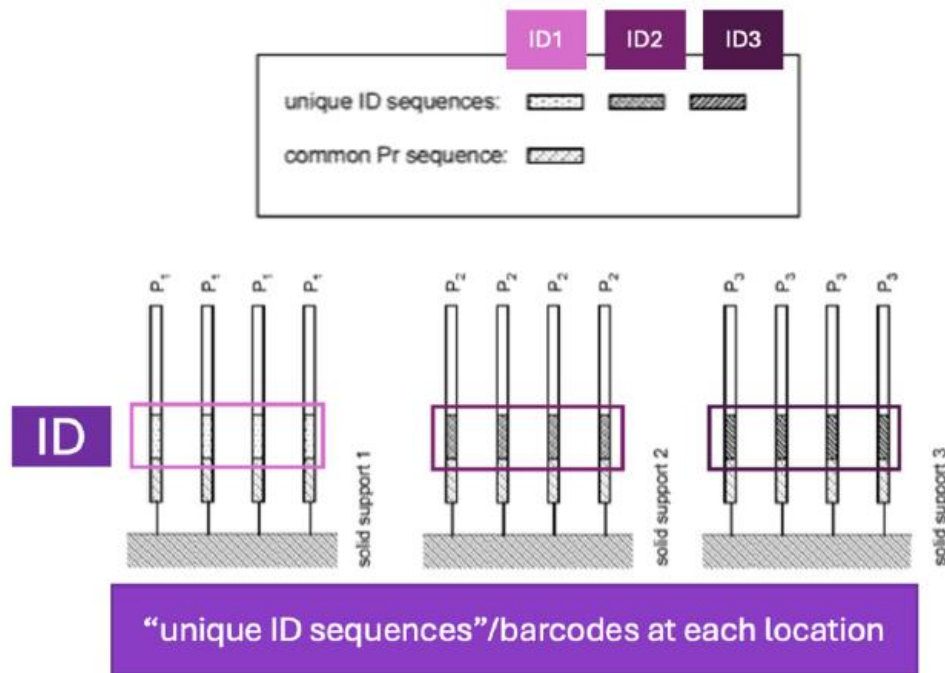
150. After derivation of the sample nucleic acid, a POSA would understand that the “sample nucleic acid” exists in the form of a homogenized (blended) mixture that does not retain the original spatial distribution of cells or nucleic acids that an intact tissue would have. As I explain above in Sections II and III.A.2, the

homogenized (blended) mixture is analogous to a fruit smoothie made from the fruit of a fruit tart. A POSA could obtain information about whether a particular fruit (target nucleic acid) is present in the smoothie (sample nucleic acid) but could not obtain information about the spatial distribution of those fruits relative to one another within the original fruit tart (tissue sample).

151. A “sample nucleic acid” and a “sample” as used and defined in my application are neither synonymous nor equivalent. Again, as described above in Section III.A.2, my application explains the differences between “samples,” “biological samples,” and “sample nucleic acid.”

152. Second, I understand that Petitioner argues that using my application’s “identification sequences” would necessarily provide the spatial location of a biological molecule of a tissue sample. This is not correct for the reasons explained below.

153. I understand that Petitioner creates a schematic that places solid supports 1, 2, and 3 of Figure 1C side-by-side, which is reproduced below. Petitioner states that “each binding agent on support/bead 1 has an ID/bead barcode that is different than the ID/bead barcode present on each of the other supports/beads.” (Petition, 23.)



154. As an initial matter, Petitioner’s schematic and description of “Cantor’s bead arrays” (Petition, 24) assumes that the identification sequence of the beads can be traced back to a unique location on the substrate. But while Cantor teaches that a collection of beads can be disposed on an array, such as an “X by Y array,” (EX1031, 11, 17), it never teaches or suggests that the beads with their corresponding identification sequences (used for downstream sequencing analysis, as described in Section III.A), can or should be traced back to a unique location on the substrate. And Petitioner ignores that on its proposed array each bead has different probes (i.e., P_1 , P_2 , and P_3) to a particular nucleic acid target.

155. As described above in Section III.A.3, A POSA would have understood that my application does not describe or suggest arrays (e.g., of beads on a solid support) for determining a spatial distribution of a nucleic acid molecule of interest within a tissue sample. Rather, the use of arrays such as bead arrays on a solid substrate facilitates the ease of handling of the beads for use in the described bulk-analysis methods. As my application states, “[t]he array can be arranged on any substrate suitable for sequence analysis or manufacture processes described herein.” (EX1031, paragraph spanning pages 17–18.) The sequence and analysis steps of the bulk-analysis methods described in Cantor are more efficient and sample handling is easier when the solid supports (e.g., beads) are arranged on a substrate surface. Rather than having to manipulate numerous individual solid supports throughout each of the steps of a multi-step preparation and sequencing process, a POSA could simply manipulate a single substrate that holds numerous individual solid supports.

156. In Cantor’s methods, if a POSA were using beads with unique identifier sequences, a POSA would have known that the bead was present somewhere on the substrate surface. However, a POSA would not have known the location of that bead on the substrate, nor would a POSA have had a reason to determine its location. That information would have been completely irrelevant to

Cantor's bulk-analysis methods, and negate the efficiencies gained by Cantor's methods. Indeed, in Cantor the identification sequences are used for the assembly of large nucleotide sequences from smaller sequence "reads" by identifying overlapping sequences, as described in Section III.A.3, above, not for determining a spatial location.

157. I also note that, as described above in Section III.A.3, the copy of the captured nucleic acid (and identifier sequence, if used) is released from the solid supports before further sequencing and analysis (steps C and D of Figure 2). Once released and the physical connection between the bead and the copy is broken, any information about where the bead was located on the array is lost. All that is left is the copy, in solution (with a copy of the identifier sequence, if used).

158. Even if a POSA were to have traced one of Cantor's identifier sequences back to a location on an array, because Cantor's methods are bulk-analysis methods, a POSA would only be able to determine where on the array a target nucleic acid was captured, but not where in the original tissue sample the nucleic acid came from. Again, all information about the spatial location of a target nucleic acid in a tissue sample is lost when the nucleic acid sample is prepared as Cantor teaches.

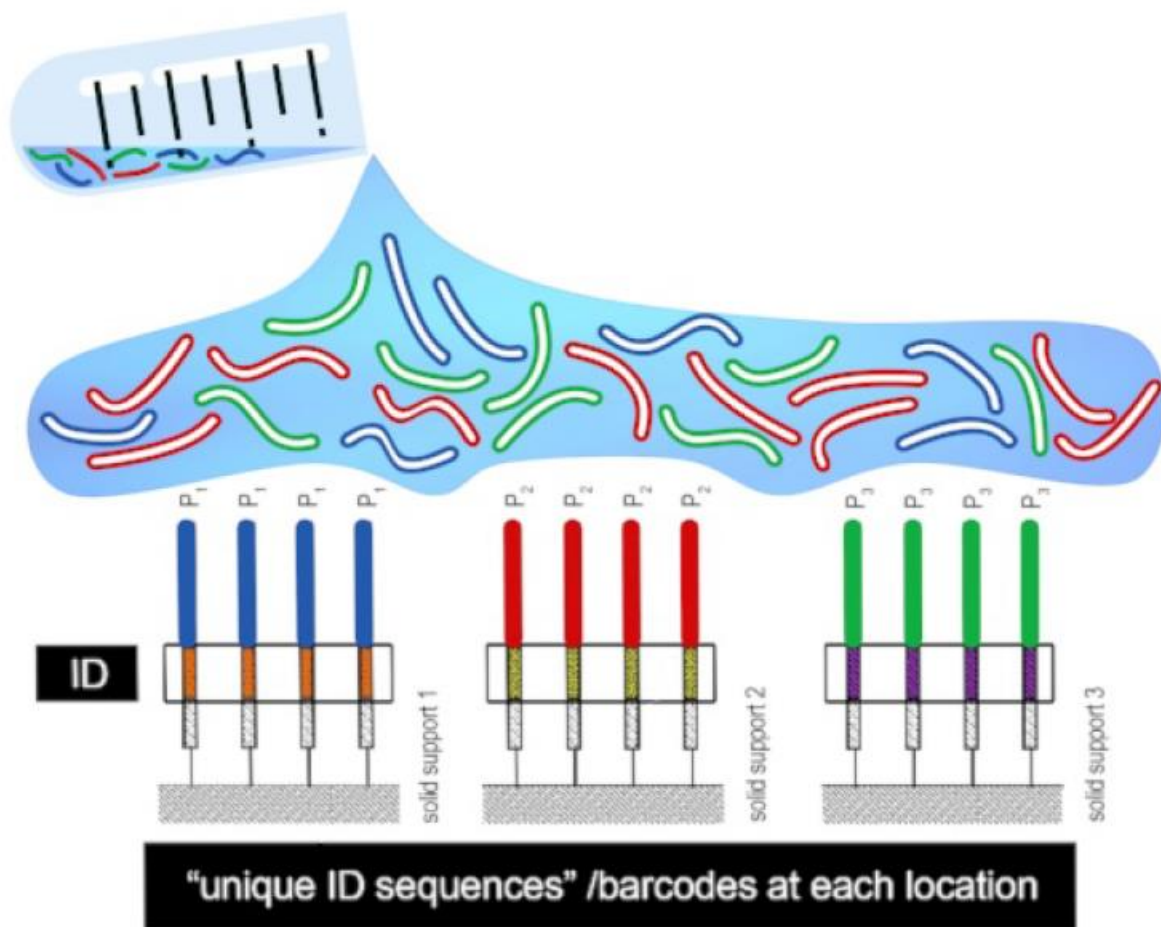
159. Given all of the above, Cantor simply does not teach or suggest contacting its solid supports (e.g. beads) with a tissue sample and certainly not with its beads at known locations to obtain spatial information.

160. I note that Petitioner's proposed array has different identifiers on each bead and different **probe sequences** on each bead as well. As discussed in Sections III.A.4 and III.A.5, above, Cantor describes two different types of collections of solid support species, depicted in Figures 1A, 1B, and 1C. Putting either of these collections onto an array results in each bead in the array having different probe sequence that are not shared between beads. This is consistent with Cantor's bulk-analysis methods (as opposed to spatial analysis). (See Sections III.A.6 and III.A.7.) Having different probes on each bead would destroy the ability to obtain any spatial information from a tissue sample (which, as described above, a POSA would not have been used with Cantor's arrays anyhow).

161. Petitioner states in a footnote that "Cantor discloses both arrays in which the probe sequence is the same at each position, and arrays in which the probe sequence is not the same at each position." Pet. 29, footnote 2. That is not the case. As explained above in Section III.A.4, a POSA would understand that claims 31 and 32 refer to the arrangement of the probes on a solid support (e.g.,

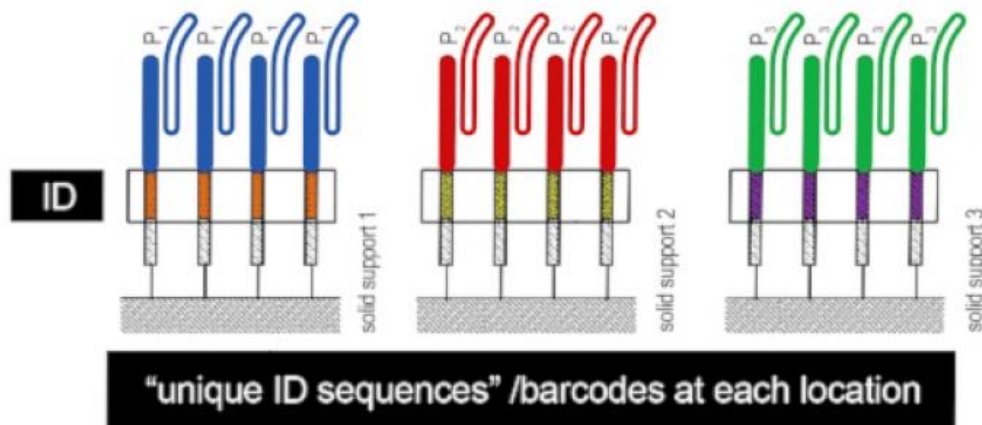
bead) rather than across a collection or an array, and depend only from claims directed to a “solid support.”

162. The illustration below depicts what would happen if a POSA contacted Petitioner’s proposed array with a **sample nucleic acid**:



163. In this illustration, the probes of bead 1 are **solid blue**, the probes of bead 2 are **solid red**, and the probes of bead 3 are **solid green**. The **solid blue** probes are designed to hybridize to the **blue-outlined** nucleic acids in the sample

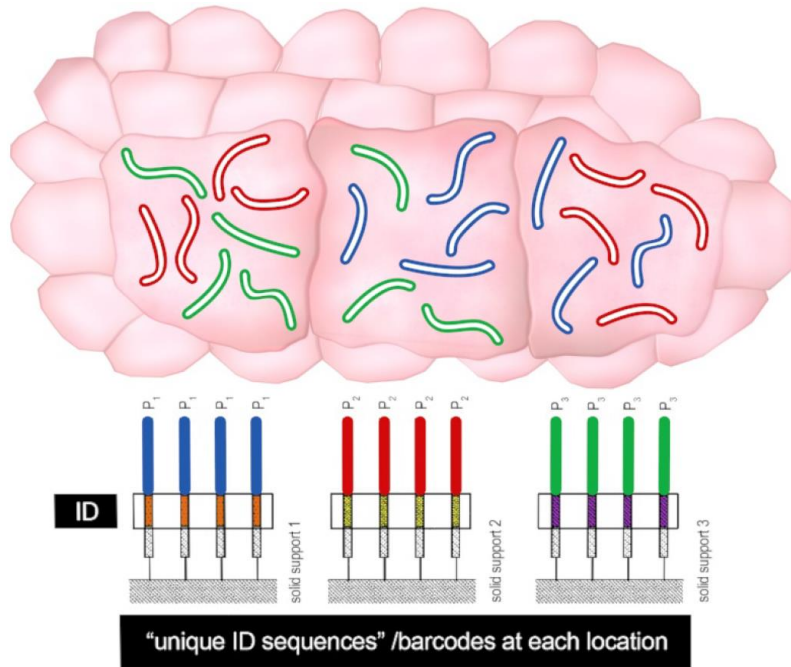
nucleic acid; the **solid red** probes to the **red-outlined** nucleic acids; and the **solid green** probes to the **green outlined** nucleic acids. Because the nucleic acids are free-flowing in solution and thus able to flow across the entire array, they can hybridize to (be captured by) their corresponding probes on the array regardless of where the nucleic acid was located in the original tissue sample. Hybridization between the probes and the target nucleic acids, when contacted with a **sample nucleic acid**, is depicted in the following illustration:



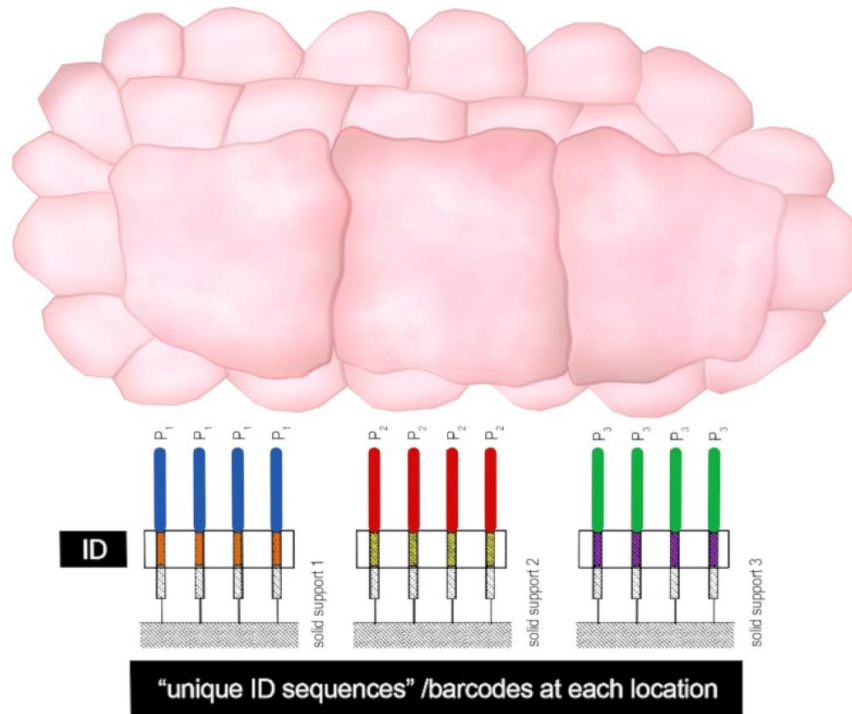
164. Each different bead on the array has a different probe sequence that will (or will not) hybridize to a particular target nucleic acid. This detects presence or absence. But the spatial distribution of the nucleic acids within the tissue sample would already have been lost, as all of the nucleic acids would be blended together (like a fruit smoothie). So, as this depiction highlights, while Petitioner's array can reveal that the **blue-outlined** nucleic acids hybridized to the **solid blue**

probes of bead 1 (and thus was **present** in the sample), it cannot reveal **where** in a tissue itself that **blue-outlined** nucleic acid was spatially located (like in the fruit tart). Accordingly, any binding (or lack thereof) would indicate only the *presence or absence* of a target nucleic acid in the originating tissue sample, but not the *location* in the tissue sample. Therefore, a POSA could not have used Petitioner's arrays to determine a spatial location of the biological molecule of the tissue sample, as required by the Challenged Claims.

165. The illustrations below depict what would happen if a POSA contacted Petitioner's proposed array with a **tissue sample**. In the first illustration, just as above, the probes of bead 1 are **solid blue** (designed to hybridize to the **blue-outlined** nucleic acids in the tissue), the probes of bead 2 are **solid red** (designed to hybridize to the **red-outlined** nucleic acids in the tissue), and the probes of bead 3 are **solid green** (designed to hybridize to the **green-outlined** nucleic acids in the tissue).

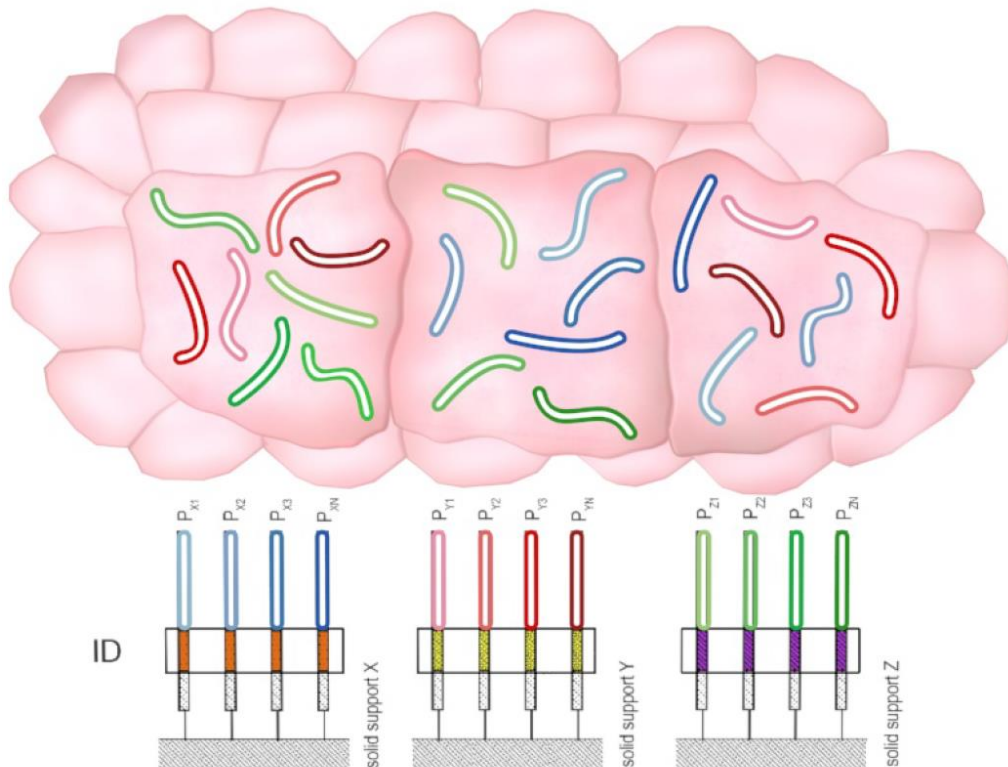


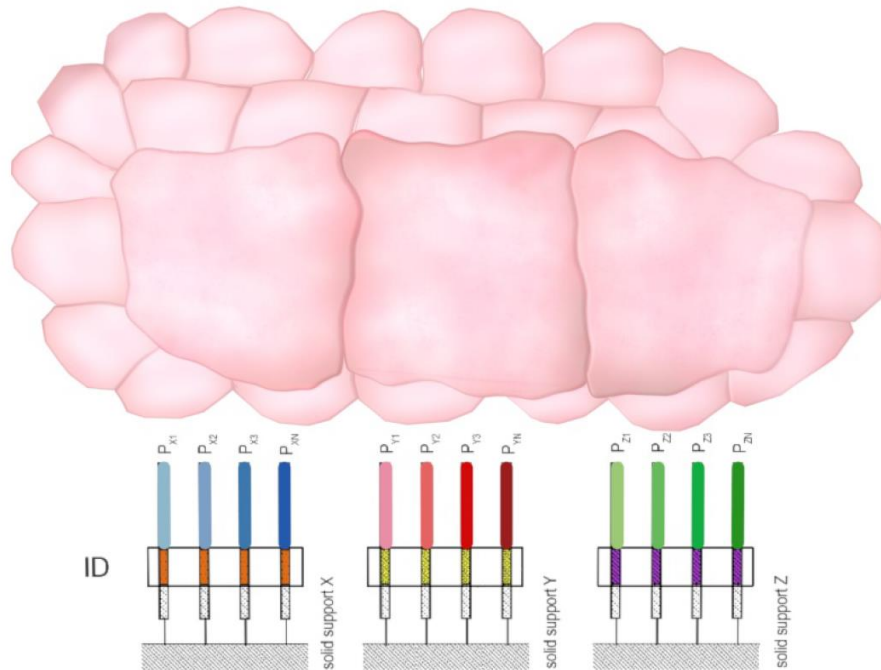
166. The target nucleic acid would bind to the probe of the same color on the array if they were put in contact. But because the target nucleic acids are heterogeneously (non-uniformly) distributed within the tissue sample **and** the probes are not repeated across the array, if a tissue sample were contacted with the array, binding (hybridization) would only happen if that target nucleic acid just happens to be present in a spatial location of the tissue that overlays its corresponding probe. This is because when nucleic acids are released from a tissue sample onto the array, they can only hybridize to (be captured by) probes that are in proximity to (underneath) the location where the nucleic acid was released from the tissue. This is depicted in the next illustration:



167. As this illustration shows, even though **blue-outlined**, **red-outlined**, and **green-outlined** target nucleic acids were **present** in the tissue (as shown in the first illustration), the results of the assay would nevertheless show **no** spatial information for these nucleic acids (as shown by the lack of any binding between the probes and any nucleic acids in the second illustration). This is because the spatial location of these target nucleic acids in the tissue **just happened** not to overlay the beads containing each target's corresponding **solid blue**, **solid red**, and **solid green** probes. That is, the target nucleic acids were not in a spatial location where they could hybridize to their corresponding probes and no spatial location of the biological molecules is necessarily obtained from the array.

168. This would be true whether all of the probes on a given solid support were identical (as depicted above and in Cantor's Figure 1C) or if they the array had multiple different subsequence probes per bead (as depicted in Cantor's Figure 1B). In the illustration below, even though the target nucleic acids were in the tissue sample, a POSA would conclude that the target nucleic acids are not present because they are not in the proper location to hybridize with the probes.





169. Again, even though various shades of **blue-outlined**, **red-outlined**, and **green-outlined** target nucleic acids were **present** in the tissue (as shown in the first illustration), the results of the assay would nevertheless show **no** spatial information for these nucleic acids (as shown by the lack of any binding between the probes and any nucleic acids in the second illustration).

170. All of this goes to show that constructing an array and using it as Petitioner suggests would not necessarily result in hybridization between a probe and a biological molecule in a tissue sample and, thus, would not necessarily provide a spatial location.

171. Furthermore, because of all these technical defects in Petitioner's analysis, a POSA would not have been motivated to use Cantor's arrays (again,

used for facilitating downstream sequencing and analysis steps for bulk-analysis methods) to determine a spatial location of a biological molecule of a tissue sample. A POSA would have recognized that doing so would have been futile.

172. A POSA would not be motivated to carry out an assay that could detect the spatial location of a biological molecule of a tissue by chance or not at all to determine a spatial location of a biological molecule of a tissue sample. Such “by chance” hybridization would not only be cross purposes to bulk-analysis, as described above, but negates any reason why a POSA could have expected to be able to determine the spatial location of a biological molecule of the tissue sample. Indeed, as I explained above, out of the several dozen uses that my application *does* identify for the disclosed methods, none are for spatial analysis.

173. In sum, Petitioner’s obviousness case fails. It requires a POSA to have modified Cantor’s methods to use a tissue sample (a POSA would not have) and presumes that a POSA would further modify Cantor to trace back its coding identifiers to locations on the substrate surface by creating a reference map either by placing the beads in known locations or determining their location before using an array (a POSA would not have). But even if a POSA would have done exactly as Petitioner suggests, for the reasons discussed above, it would not result in determining the spatial location of a biological molecule of the tissue sample.

Petitioner's obviousness case can only have been manufactured with the benefit of hindsight, starting from the '022 patent. Therefore, the Challenged Claims would not have been obvious over Cantor.

174. Petitioner also discusses Southern (EX1033) in its discussion of obviousness over Cantor. (Petition, 32.) In this discussion, the Petition mischaracterizes the teachings of Southern's "tissue samples" in the same way as it did for Cantor. Southern does not teach contacting a solid support with a tissue. Southern's methods use cells, not tissues. (EX1033, 2:17–27.)⁵ A POSA would understand that cells are not tissues and do not maintain the spatial relationship of the biological molecules of a tissue. Dr. Puglisi explains the difference between cells and tissues in his declaration, as well. (EX1100, ¶¶ 45–50)

175. Southern mentions tissue samples only in the way that Cantor does—in the context of processing it to derive a sample that can be used in its methods. In Cantor that is sample nucleic acid. In Southern that is cells. (EX1033, 16:26–17:10.) Southern teaches that it is "[a]fter separation of cells of interest from other components in the starting samples" that "the cells of interest can be analysed using the devices of the invention." (EX1033, 17:6–10.) When Southern discusses

⁵ Alternatively, Southern teaches that the released material from cells may be overlayed on the solid support. (EX1033, 17.)

“spatial arrangement,” it is in respect to the sample itself (the cells) on the support, not the spatial arrangement of target biological molecules of a tissue. For example, Southern describes an array in which individual samples are placed on a surface at known locations, creating an arrangement of individual samples. (EX1033, 22:20–31.) The array can be used to simultaneously analyze multiple samples (i.e., cells of interest separated from the other components in the tissue) by a variety of methods. (EX1033, 26:19–32.)

176. The way that Southern uses samples is similar to how a POSA might arrange test tubes in a particular order in a rack. Each test tube contains a single sample and the arrangement of test tubes in the rack is maintained throughout subsequent testing. As Southern puts it, “[t]he spatial arrangement of the samples is maintained” so that the location of “results of the analysis [may] be match[ed] to individual samples”—i.e., the location of that test tube in the rack. EX1033, 17:6–10. But the spatial location of a biological molecule of a tissue is not maintained.

177. Southern also uses the word “tissue” in the Examples section of the application (*see, e.g.*, EX1033, 40:19), but a POSA would readily understand that in this context a “tissue” is a paper tissue used for wetting membranes or blotting excess liquid.

178. I understand from Patent Owner's counsel that Shirai, which Petitioner references on page 32 of the petition, is a post filing date reference and not relevant to a POSA's background knowledge, as Petitioner suggests.

179. Petitioner's Ground 2 combines Armani (EX1022) with Cantor. Armani is discussed above in Section III.B. But Petitioner's obviousness ground suffers from the same fatal flaws described above even if a POSA were to have constructed one of Petitioner's arrays, though, this time with "the plurality of beads disposed in wells" of a multi-well plate rather than on a different kind of solid surface (e.g., a flat surface), in other words, placing one of the beads of Petitioner's array in each of Armani's wells.

VII. CONCLUSION

180. 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 United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: December 24, 2024

A handwritten signature in black ink, appearing to read 'Charles R. Cantor', written over a light gray rectangular background.

CHARLES R. CANTOR