

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 IPR2025-00193
Patent 11,761,030

DECLARATION OF CHARLES R. CANTOR

REFERENCES CITED

Exhibit Number	Description
EX1005	U.S. Patent No. 11,761,030 issued to Chee
EX1009	U.S. Provisional Application No. 61/321,124
EX1018	Victor Trevino et al., DNA Microarrays: a Powerful Genomic Tool for Biomedical and Clinical Research, <i>Mol Med</i> 13 (9-10) 527-541, 2007
EX1022	Michael Armani <i>et al.</i> , <i>2D-PCR: a method of mapping DNA in tissue sections</i> , <i>Lab Chip</i> , 2009, 9(24), 3526-3534; NIH Public Access Author Manuscript
EX1031	WO 2009/091934 A1 Cantor
EX1032	U.S. Patent No. 6,620,584 issued to Chee
EX1033	WO 2008/075086 A1 Southern <i>et al.</i>
EX1036	Norman P. Gerry, et al., Universal DNA Microarray Method for Multiplex Detection of Low Abundance Point Mutations, <i>J. Mol. Biol.</i> (1999) 292, 251-262
EX1038	U.S. No. 2007/0231824 A1 to Chee
EX1048	US6,133,436 issued to Köster
EX1103	Declaration of Joseph D. Puglisi, Ph.D.
EX2004	Web Capture of https://www.10xgenomics.com/products/spatial-gene-expression (December 18, 2024)
EX2005	Khaliq et al., “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 et al., “Pathogenic Human Viruses in Coastal Waters,” <i>Clin. Microbiol. Rev.</i> 16(1):129–143 (2003)

EX2008	Deak et al., “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)
EX2012	Gunderson et al., “Decoding Randomly Ordered DNA Arrays,” <i>Genome Research</i> 14:870–77 (2004)

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. 11,761,030 (“the ’030 patent”) (EX1005). The Petitioner in this case is Curio Bioscience, Inc. (“Petitioner” or “Curio”).

2. The ’030 patent is titled “Spatially Encoded Biological Assays.” (’030 Patent, EX1005 at cover page.) It was filed as U.S. Patent Application No. 18/074,992 on December 5, 2022 (“the ’992 application”) and issued as Patent No. 11,761,030 on September 19, 2023. (*Id.*) It claims priority to Provisional Application No. 61/321,124 (“the ’124 provisional,” EX1009), filed on April 5, 2010. (*Id.*) The ’030 patent has 30 claims. (EX1005, 33:16–34:60.) Of those claims, claims 1, 3, 6, 10, 11, 16, 18, 21, 24–26, and 30 are challenged (the “Challenged Claims”). (Petition, 10.)

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 (Retrotape) 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.

II. 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

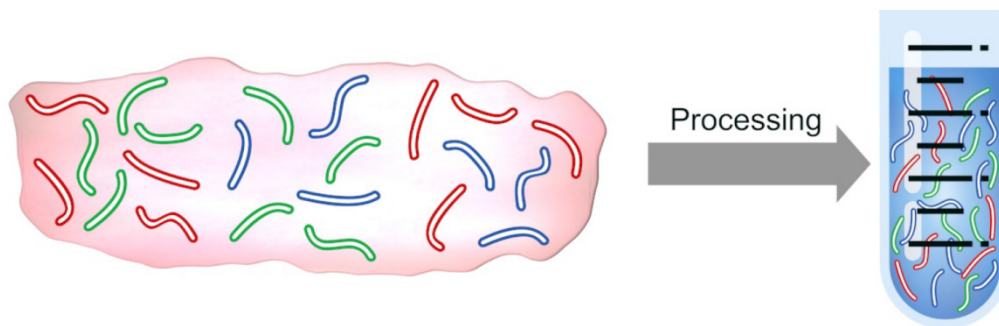
(“Petition”) of the ’030 patent in forming my opinions. A list of References Cited is set forth at the beginning of this Declaration.

III. THE ’030 PATENT AND SPATIAL ANALYSIS

15. The ’030 patent relates to spatial analysis assays for analyzing the spatial location of biological molecules in a tissue sample.¹ (EX1005, 1:38–41; 2:16–21, 20:60-21:14.) Before the earliest filing date for the ’030 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.” (EX1005, 2:16–21.) Thus, there was “a need for reproducible, high-resolution spatial maps of biological molecules in tissues.” (*Id.*)

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 IV.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.

¹ The ’030 patent refers to a “tissue sample” and a “tissue section.” Unless otherwise noted, I use these terms interchangeably throughout.



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 arrays and methods such as those described and claimed in the '030 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 '030 patent, however, answers both questions.

21. Indeed, the inventions described in the '030 patent addressed the “need for reproducible, high-resolution spatial maps of biological molecules in tissues.” (EX1005, 2:16–21.) The '030 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 positional information of such molecules in a biological sample, such as a tissue sample. (EX1005, 20:60–21:14.) 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.*)

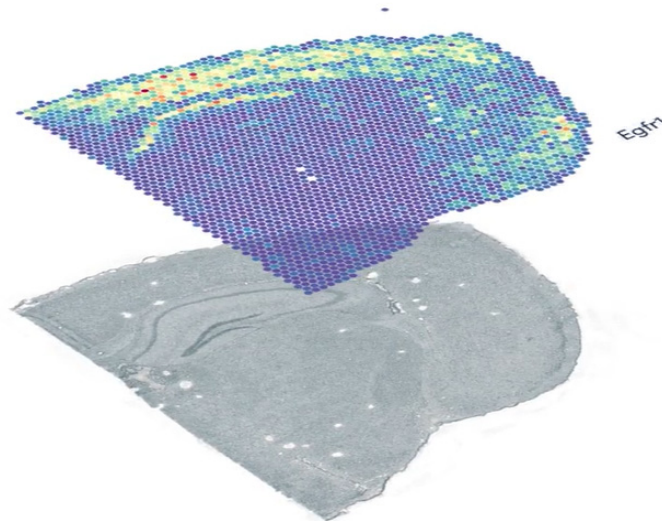
22. The spatial analysis technology claimed in the '030 patent has been commercialized by Patent Owner in the Visium® line of products for spatial analysis of tissue samples. (EX2004.) The Visium® technology allows researchers to assay gene expression patterns across an entire tissue sample for mRNA (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

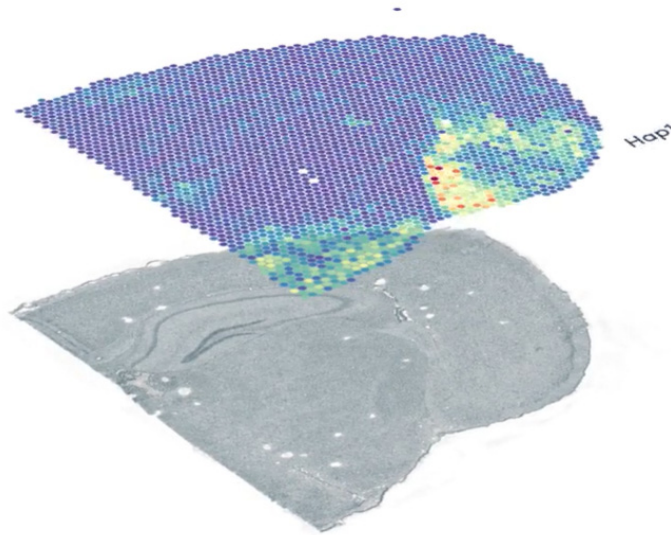
“microenvironment”), which, in turn, can help them develop new therapeutics.

(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.

IV. 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, 10.) 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 VII.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 ’030 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 III. 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.)

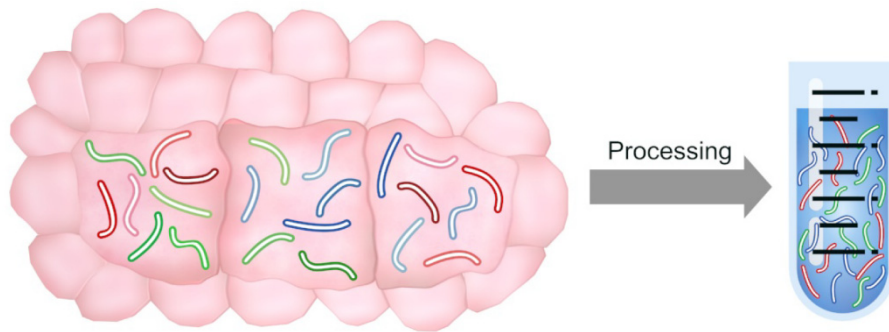
34. The sample nucleic acid that is tested could come from many different types of samples or sources—from environmental samples to human tissues. But whatever the source, the sample nucleic acid is derived (prepared) from the sample source and then mixed with the solid phase nucleic acid(s). If the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

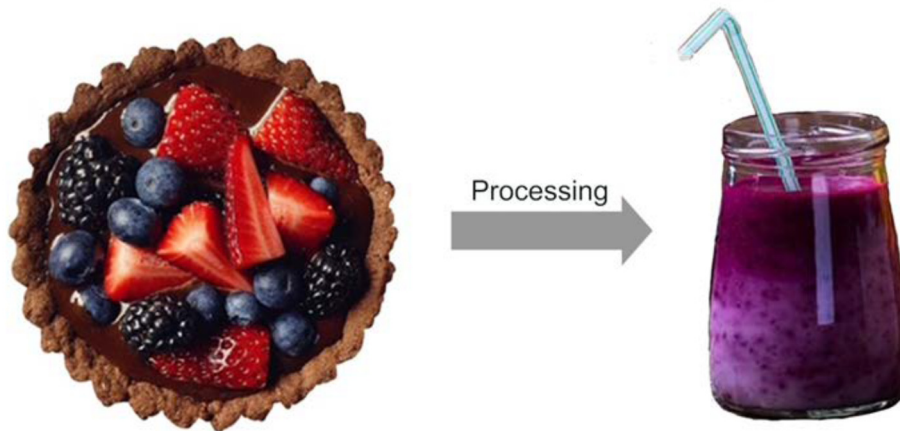
35. A POSA would have understood from my application that the first step in carrying out the disclosed methods is to derive (prepare) the sample nucleic acid from a sample or source (e.g., from a tissue sample). Specifically, as described in my application, “sample nucleic acid” is “derived from one 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 explains that [sample nucleic acid] may be ‘a solid material *such as a tissue ...or a biopsy.*’” (Puglisi Declaration, EX1103, ¶161, emphasis in original; *see also* ¶¶162, 175, 193, 335, 336, 343, 361) suggests that my disclosed solid supports and 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 (EX1103, ¶161; *see also* ¶¶162, 175, 193, 335, 336, 343, 361) 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,

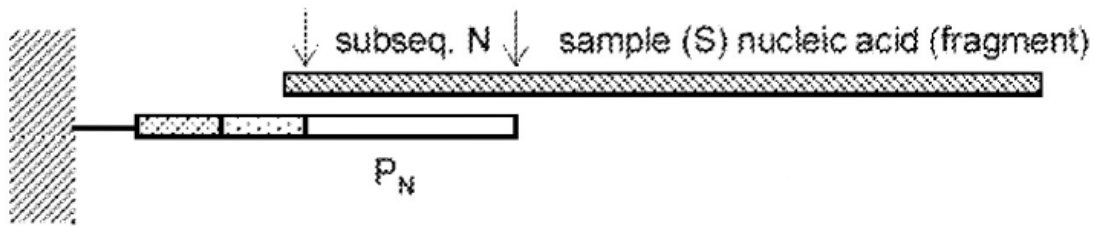
13, 14, 15, 16, 17, 18, 19 or 20 samples). A sample nucleic acid sample can contain host and non-host sample nucleic acid, and in some embodiments, a sample may contain two or more different species of sample nucleic acid (e.g., mutant vs. wild-type, transplants, forensics, mother vs. fetus). [emphasis 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 optional 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 (P_1) 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 (P_1) 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.

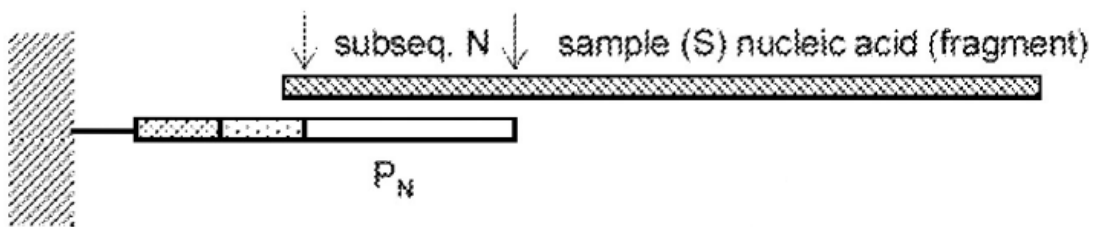


Figure 2 excerpt

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”).

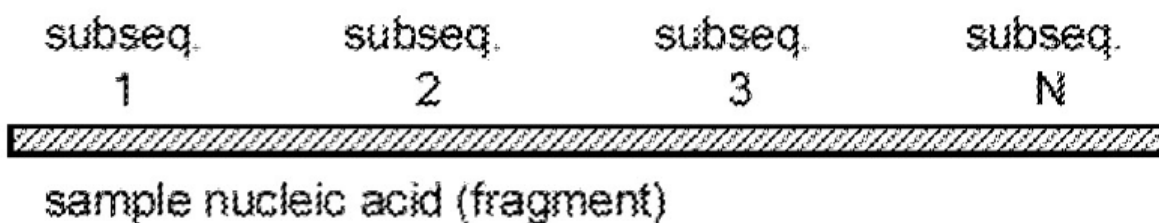


Figure 1A excerpt

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.

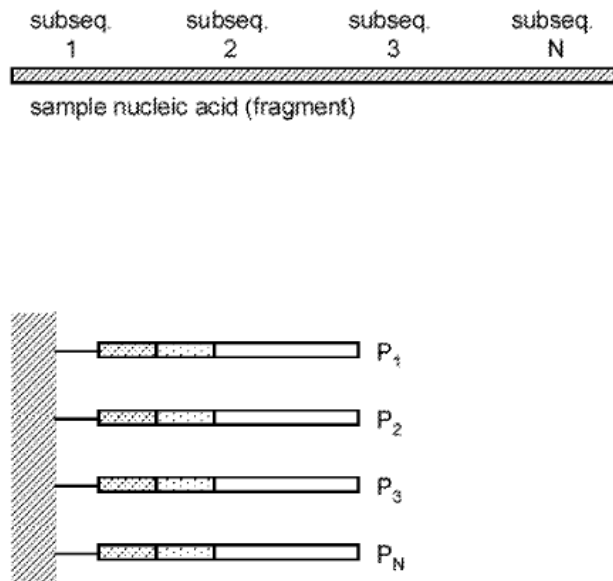


Figure 1A excerpt

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.

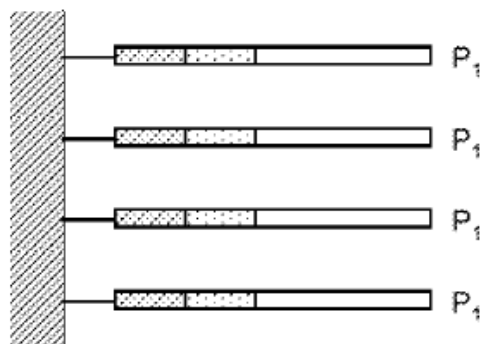
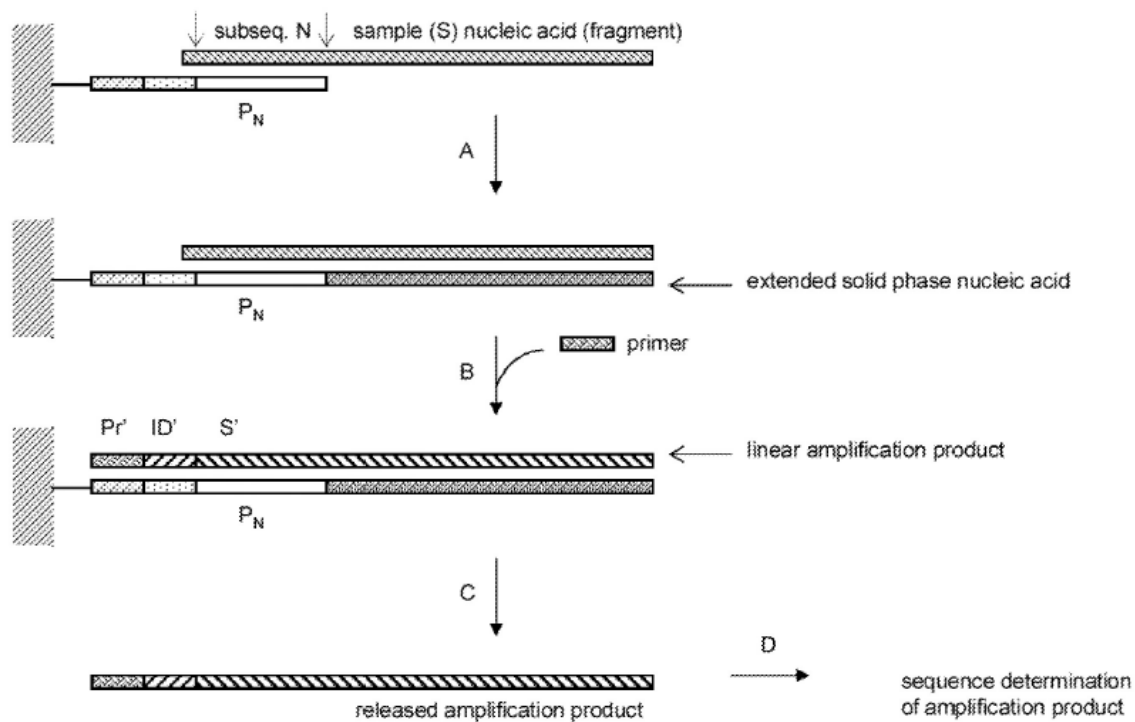


Figure 1C excerpt

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 IV.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.)

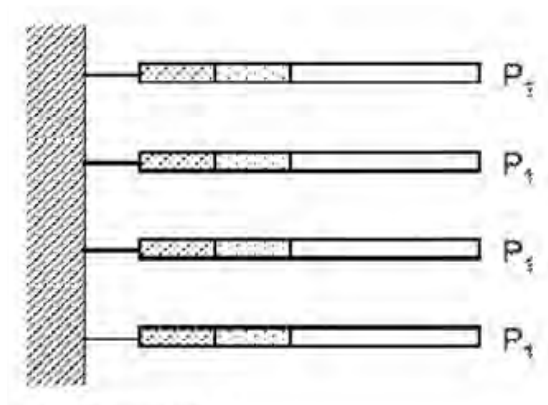


Figure 1C

59. 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 IV.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.)

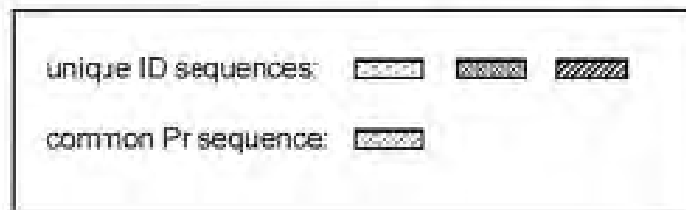
60. Likewise, if an identification sequence is present, each single-stranded nucleic acid on a single solid support species has the same identification sequence.

(EX1031, 15.)

5. Collections of solid supports

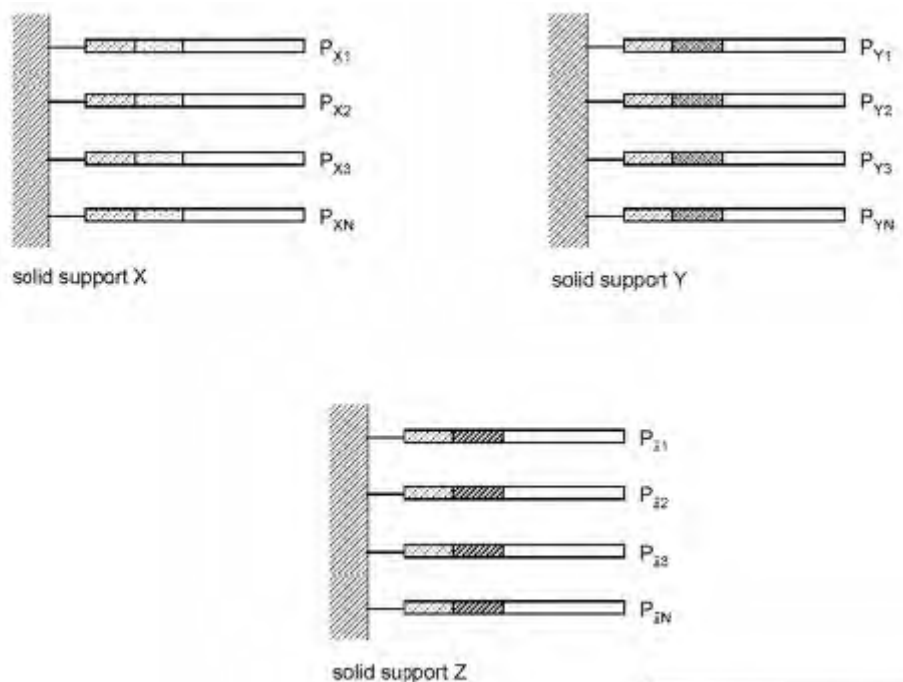
61. A “collection” of solid supports is one that contains multiple solid support species. (EX1031, 11, third paragraph.) My application discusses two different types of collections, depicted in Figure 1B and Figure 1C of my application. Each type of collection contains multiple different solid support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

62. 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.



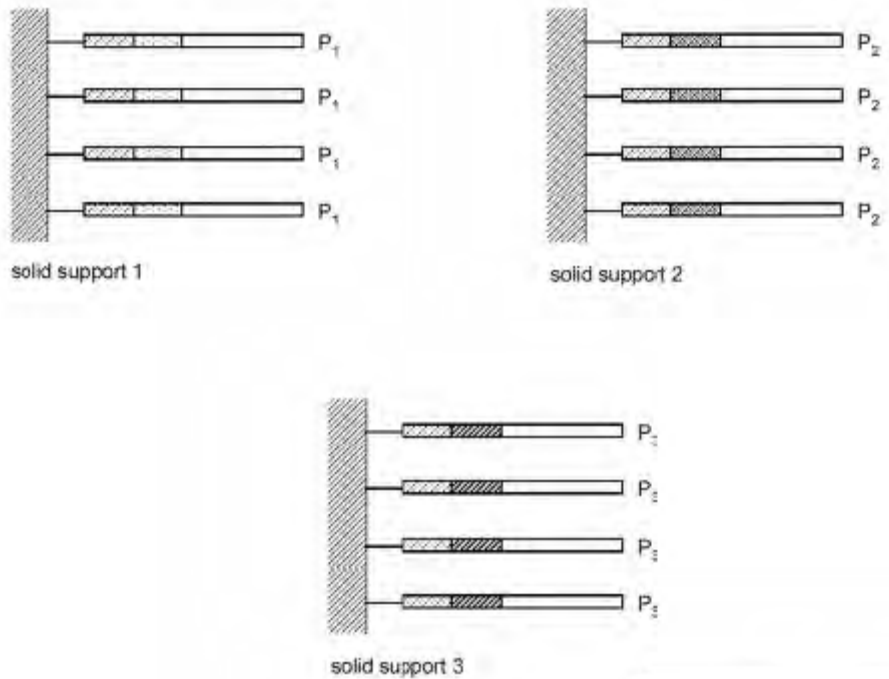
63. In other words, the “collection” contains three solid support species and three unique identification sequences, each identifying the particular solid support species (e.g., bead or particle) to which they are attached. As described below, however, in both types of collections no probe is the same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

64. 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:



65. 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.)

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



67. Each solid support species (1, 2, and 3) is depicted as having a single probe sequence (P_1 , P_2 , or P_3), which differs between the species. That is, P_1 for solid support 1; P_2 for solid support 2; and P_3 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

68. 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 IV.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.

69. 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 Cantor relate to nucleic acid analysis that could provide spatial information.

7. Sequence analysis and manufacture processes

70. 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.

71. 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.

72. *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.

73. *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.)

74. *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.)

75. *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.)

76. *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.)

77. *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.)

78. *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.*)

79. *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.)

80. *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).

81. *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.)

82. *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.)

83. *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).

84. *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.

85. *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.

86. *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.

87. *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.

88. *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.

89. *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.

90. 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).

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

B. Armani (EX1022)

92. 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 ’030 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. [EX1005, 2:9–15]

93. 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:

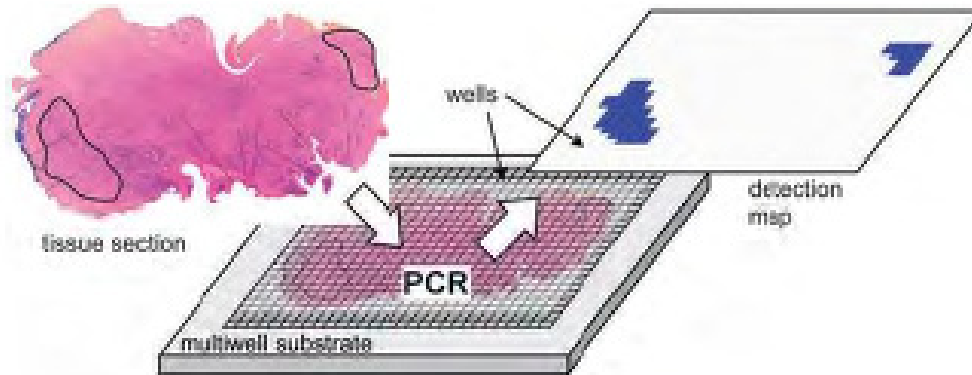
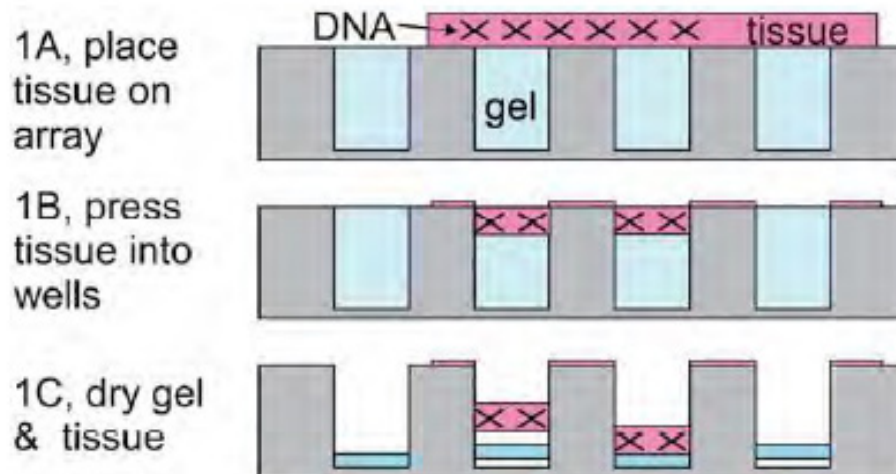


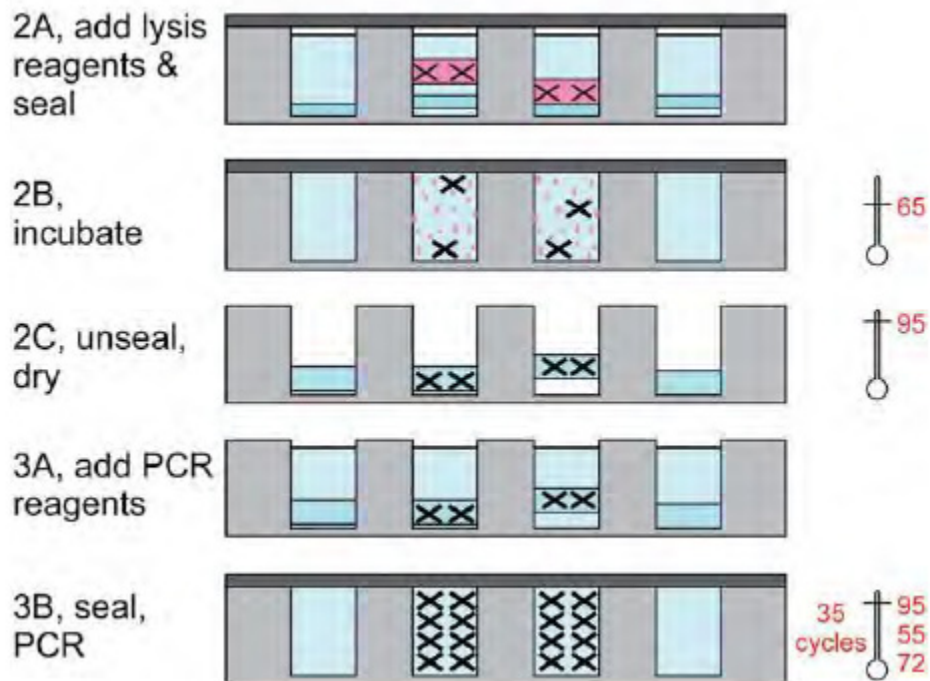
Fig. 1 2D-PCR is achieved by transferring a tissue section vertically into a multi-well array device, isolating tissue subregions. The DNA is extracted and then amplified by the polymerase chain reaction. A detection map is created by imaging the amplified DNA, for example using a DNA-intercalating dye.

94. 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.*):



95. 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.

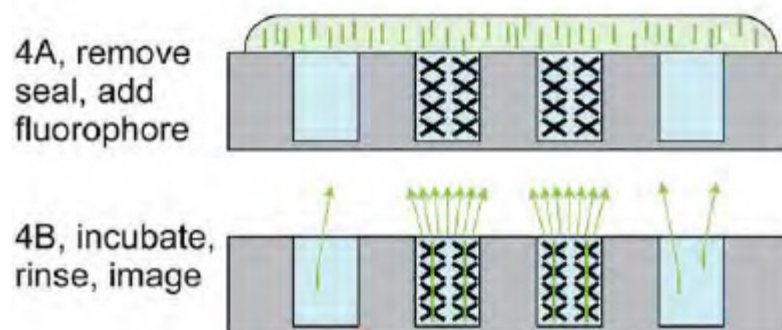
96. From that point on, each individual well is treated as a separate reaction. (*Id.*) Genomic DNA extraction and PCR amplification of the genomic DNA is carried out within each individual well. (*Id.*)



97. 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

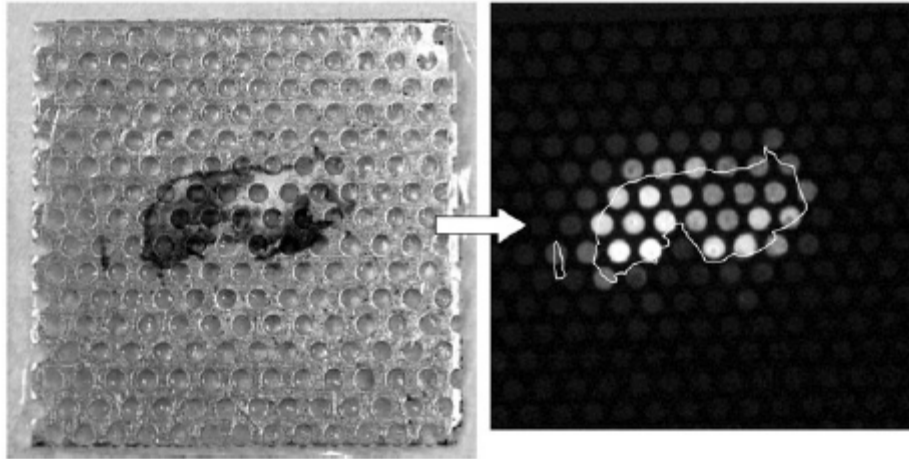
DNA with “x”s. (*Id.*) The solution is dried in step 2C, which removes the liquid in each well and allows for the next steps of PCR amplification. (*Id.*) In steps 3A-3B, PCR reagents are added to each well to amplify the genomic DNA, which are now represented by multiple “x”s in each well. (*Id.*)

98. 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.)



99. 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.*)



100. 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.)

101. Armani does not disclose or contemplate the use of beads or capture probes, contacting capture probes with a tissue section, or using capture probes comprising a nucleic acid sequence that identifies a unique location on the substrate.

V. PERSON OF ORDINARY SKILL IN THE ART

102. 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 '030 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, 13.)

103. 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.

VI. LEGAL STANDARDS

104. 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

105. 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.

106. 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

107. 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.

108. 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.

VII. DISCUSSION OF PATENTABILITY

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

A. Priority and Written Description

110. I understand from Patent Owner’s counsel that Petitioner identifies “two key elements of independent claims 1 and 16” that allegedly lack written-description support. (Petition, 52–60.) As I describe below, however, the ’124 provisional application provides written description of these “two key elements.” I address each in turn below.

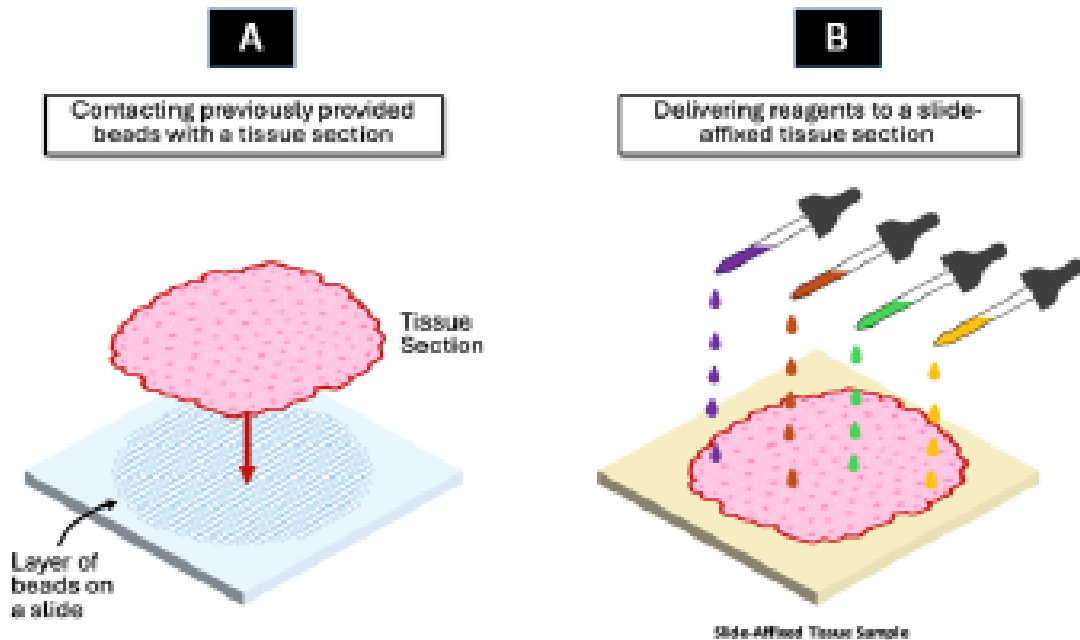
1. The ’124 provisional discloses a plurality of beads/capture probes attached to a substrate

111. Petitioner’s first argument is that a POSA would have understood the disclosure 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 “arrays that included a substrate with a plurality of probe-functionalized beads attached thereto.” (Petition, 55.)

112. As I describe below, however, the ’124 provisional² quite clearly teaches generating an array with a plurality of capture probes attached to it either directly or indirectly (e.g., via beads) before use in the disclosed methods (e.g., before disposing a tissue section on top of the array).

² 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.

113. Petitioner offers an illustration of an alleged dichotomy between what is taught and what is claimed (reproduced below), arguing that the '661 application is limited to “Figure B” (“[d]elivering reagents to a slide-affixed tissue 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 '030 patent. I presume that Petitioner created them.



114. The '124 provisional, in paragraphs 15–18, for example, discusses aspects of delivering binding agents (which bind to a molecule of interest) on the surface of a substrate. (EX1009, ¶¶7-8, 15-18.) 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 paragraphs 15–19 of the '124 provisional, 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]; EX1005, 22:15–19, 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]; EX1005, 22:20–28, 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]; EX1005, 22:29–37, 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]; EX1005, 22:38–49].

115. These passages from the '124 provisional clearly teach immobilizing binding agents (including nucleic acid binding agents) directly or indirectly onto a **substrate**. The specification states that the location of the nucleic acid sequences of the binding agents is known or determined prior to use of the substrate surface in the assay system. For example, the specification teaches that in some embodiments, the binding agents are provided in or on features of the substrate surface, such as wells or channels. It would not make sense at all for the substrate having wells or channels 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, and thus a “substrate” with wells or channels is **not** a tissue section, but instead is a structural support. Indeed, I describe as much in my own application.

(See EX1031, Page 18, describing manufactured substrates with wells and channels.)

116. Moreover, additional passages in the '124 provisional discuss the use of pre-generated arrays—with binding agents (e.g., capture probes) immobilized directly or indirectly onto a substrate surface. One passage does so in the context of peptide binding agents. (EX1009, ¶ [00021]; EX1005, 23:1–11). Another does so in the context of chemical binding agents. (EX1009, ¶ [00022]; EX1005, 23:12–22.)

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

118. Another of Petitioner’s (and Dr. Puglisi’s) arguments as to why the '030 disclosure is limited to their “Figure B” is that a “washing” step discussed in one section of the application would “not make sense” in the context of probes covalently bound to a substrate such as a slide or beads. (Petition, 54; EX1103, ¶ 202.) But here they cite to a completely separate portion of the '030 patent—Figures 1 and 2 as well as paragraph [0028].³ (*Id.*) These figures and passages

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 lines 10:8–32 of the '030 patent.

elaborate on additional types of assays also discussed in the '030 patent specification, which are in addition to the pre-generated array assays discussed above.

119. Petitioner also describes the '030 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 ['030 patent] discloses only the delivery of reagents, including probes, to a sample affixed to a solid support.”⁴ (Petition, 55–57.) But again, that additional passages may elaborate on additional types of assays in addition to the pre-generated 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.

120. 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 one or more agents provided in defined spatial patterns *on a substrate* surface . . .”

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 '030 patent: 2:35–45, 3:61–4:12, 8:61–9:25, 12:7–14, 16:28–53, 18:4–21, Figure 1, and Figure 2.

(EX1009, ¶ [0007]; EX1005, 20:60–21:14, emphasis added) and how in some cases “the encoding scheme employs limited reagent delivery to the spatial patterns *on the substrate* surface . . .” (EX1009, ¶ [0008]; EX1005, 21:27–29, 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]; EX1005 22:15–19, emphasis added.)

121. 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]; EX1005, 24:20–43]

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]; EX1005, 24:44–53]

122. Thus, it is my opinion that the '124 provisional supports “disposing a tissue on top of” an “an array of beads attached to a substrate, where each bead comprises a capture probe that binds a target nucleic acid.” (Petition, 53, referencing Petitioner’s “Figure A.”)

2. The '124 provisional discloses beads that (i) have a capture probe with a nucleic acid sequence that identifies a unique location on the substrate and (ii) are randomly distributed on the substrate

123. As to Petitioner's second "key element," Petitioner's first argument is that the '124 provisional does not disclose "beads that (i) have a capture probe with a nucleic acid sequence that identifies a unique location on the substrate." (Petition, 52.) Petitioner's logic is that "there would be no purpose for such an identifier because the beads to be [*sic*] 'provided in known locations on the substrate surface.'" (Petition, 60; *see also* EX1103, ¶¶227–228.) That is not the case. Even if the beads were provided in known locations, it would not, as Dr. Puglisi suggests, obviate the need for the use of coding identifiers. (EX1103, ¶226.)

124. First of all, the '124 provisional describes an assay system 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]; EX1005, 20:60–21:14.) This "unique encoding scheme" allows "multiple molecules and their respective multiple locations to be identified in a single system." (*Id.*)

125. For example, determining 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. (EX1009, ¶¶ [0007], [00018].)

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

127. Moreover, there is no reason a POSA would have understood that the ’124 provisional “does not disclose to a POSA a bead comprising a nucleic acid binding agent *plus* a nucleic acid sequence that identifies a unique location on the substrate,” as Petitioner suggests. (Petition, 60; EX1003, ¶¶227–228.) 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]; EX1005, 22:38–49.)

128. 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-

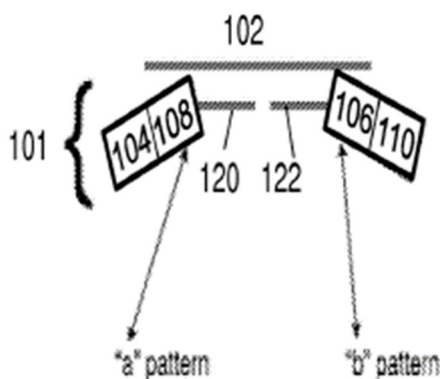
generation sequencing, is used to decode the spatial encoding scheme in the assay system of the invention. [EX1009, ¶ [0019]; EX1005, 22:50–57].

129. As explained above the “encoding scheme” uses both “molecule-specific binding agents and coding identifiers” (here, nucleic acid capture probes comprising “nucleic acid sequence[s] that identif[y] a unique location on the substrate” as recited in claim 1) to provide “specific positional 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.

130. If, as Petitioner suggests, a nucleic acid binding agent was attached to a bead at a known location on the substrate, without using any coding identifier, a POSA would have understood that it would be impossible to obtain “specific positional 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. In the absence of any coding identifier, once the target

nucleic acid is released from the substrate surface any location information is lost. Therefore, a POSA would understand that here the binding agent needs to include a coding identifier (e.g., the nucleic acid sequence that identifies a unique location on the substrate) in order for sequencing to be used to provide “specific positional information.”

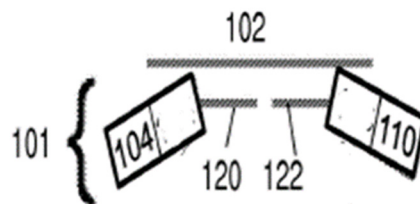
131. That the '124 provisional teaches “beads that (i) have a capture probe with a nucleic acid sequence that identifies a unique location on the substrate” is further demonstrated by the '124 provisional's Figure 1, which includes an illustration where the coding identifier is part of the same molecule as the binding agent. (See Fig. 1 of the '124 provisional, which is Fig. 4 of the '030 patent, partially excerpted below.) The coding identifiers (106 and 108) in this example are associated with the binding agents (120 and 122) which can capture the target nucleic acid (102).



132. 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]; EX1005, 24:1–19.) 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, ¶¶ [00018]–[00019]; EX1005, 22:38–57.)

133. To further illustrate why a POSA would have understood that the coding identifier (e.g., the nucleic acid sequence that identifies a unique location on the substrate) 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.



134. But because no coding identifier is used in this example at all, 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.

135. 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.

136. The second of Petitioner's arguments is that the '124 provisional "fails to disclose beads randomly distributed on an array that comprise nucleic acid

capture probes having a nucleic acid sequence that identifies a unique location on the substrate.” (Petition, 52, 59.)

137. As discussed above, the ’124 provisional teaches pre-generated arrays (e.g., bead arrays) in which binding agents and coding identifiers (e.g., capture probes) are deposited onto substrates at “discrete, independent measureable” locations. (EX1009, ¶ [00047]; EX1005, 24:20–43.) It also teaches that their location “is known or determined prior to use of the substrate surface in the assay system.” (EX1009, ¶¶ [00017]–[00018]; EX1005, 22:29–49.) That is, they are placed onto “discrete, independent measurable” locations and their location is either known (e.g., placed on the array knowing where each particular bead would bind to the array) or determined prior to use (e.g., placed on the array randomly, without prior knowledge of where each particular bead would bind to the array and later “decoded.”)

138. Petitioner acknowledges that the ’124 provisional discloses providing beads on a substrate at locations that are either *known or determined prior to use*. (Petition, 59; EX1009, ¶¶ [0017]–[0018].) Petitioner argues, however, that neither of these two alternatives refers to beads that are randomly distributed. (*Id.*) That is not the case.

139. Elsewhere in the Petition, Petitioner states that there are only two alternatives for placement of beads comprising identification sequences onto a

spatial analysis array: “selective loading,” and 2) “randomly loading” and “decoding”:

[A] POSA would have known that there are two options to correlate the identification sequence on the nucleic acid attached to a given bead and the beads location on the array: (i) selective loading of particular beads to particular locations on the array and/or (ii) ***randomly loading the beads*** and then decoding the array prior to use. [Petition, 33 (emphasis added).]

140. These two options originate from DNA microarray technology. As described in Gunderson et al., “Decoding Randomly Ordered DNA Arrays,” *Genome Research* 14:870–77 (2004) (EX2012), “[c]onventional microarrays are manufactured by spotting or synthesizing probes at known locations on a two-dimensional substrate.” (EX2012, 870.) This is equivalent to what Petitioner refers to as “selective loading,” which is when beads/probes are provided on a substrate at locations that are ***known***. That is, each bead/probe is physically addressed to a particular location on an array at the time of deposition on the array. But, as Gunderson explains, this physical addressing has drawbacks. An alternative approach, described in Gunderson, is to use “randomly assembled arrays in which the location of the probe is initially unknown.” (*Id.*)

141. Petitioner acknowledges, a POSA was also familiar with random loading methods, such as those described in Gunderson, in which functionalized beads are loaded on an array without prior knowledge as to which particular

location on the array each would land. In that case, the particular location of each bead/probe is *determined prior to use*. Petitioner refers to this as “randomly loading” followed by “decoding.”

142. A POSA would have understood providing beads on a substrate at locations that are either *known or determined prior to use*, in the context of spatial analysis arrays and assays as described in the ’124 provisional, refers to these two alternative options. Though, as noted in Section VII.B, a POSA would not have used either of these options for constructing arrays for use in Cantor’s bulk-analysis methods.

143. Given all of the above, it is my opinion that the ’124 provisional supports beads that (i) have a capture probe with a nucleic acid sequence that identifies a unique location on the substrate and (ii) are randomly distributed on the substrate.

B. Obviousness (Grounds 1 and 2)

144. 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 section” with my solid supports, as required by Claim 17 and its dependent claims, as Petitioner asserts. Second, Cantor fails to teach an identification sequence that “identifies a unique location on the substrate,” as is required by all the Challenged Claims.

1. Cantor does not teach contacting a “tissue section” with its solid supports

145. As I explained above in Section IV.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.

146. As further explained in Section IV.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. (EX1103, ¶¶164-165.) But Cantor teaches, and a POSA would have understood, that the cell lysis procedures derive the sample nucleic acid from the sample or source.

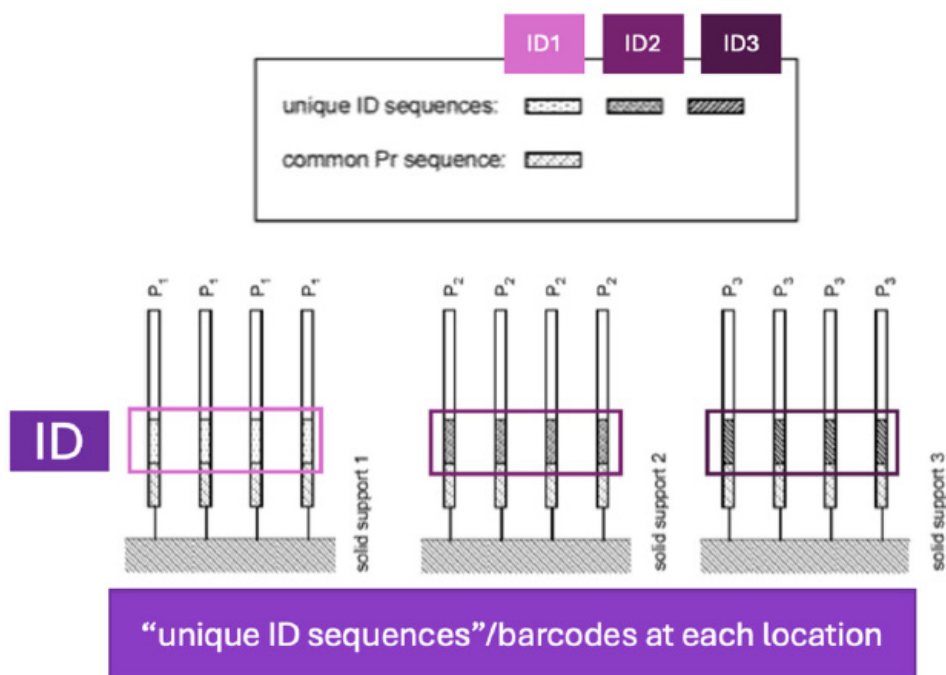
147. After derivation of the sample nucleic acid, a POSA would understand that the “sample nucleic acid” exists in the form of a homogenized (blended) mixture that does not retain the original spatial distribution of cells or nucleic acids

that an intact tissue would have. As I explain above in Sections III and IV.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).

148. 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 IV.A.2, my application explains the differences between “samples,” “biological samples,” and “sample nucleic acid.”

149. Furthermore, even if a POSA had constructed Petitioner’s array, a POSA would have had no reason “to perform array-based nucleic acid analysis on a tissue sample” as Petitioner alleges (Petition, 38) because the presence of different probes on each bead destroys any ability to obtain spatial information from a tissue section.

150. 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 “separate solid supports, such as beads, each hav[e] ‘unique ID sequences’ that are correlated to the specific solid supports.” (Petition, 29–30.)



151. As an initial matter, Petitioner’s schematic and description of Cantor’s array (Petition, 29–30) 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 IV.A, if they are used at all), 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., P₁, P₂, and P₃) to a particular nucleic acid target.

152. As described above in Section IV.A, 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.

153. 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—if they are used at all—are used for the assembly of large nucleotide sequences from smaller sequence “reads” by identifying overlapping sequences, as described in Section IV.A.3, above, not for determining a spatial location.

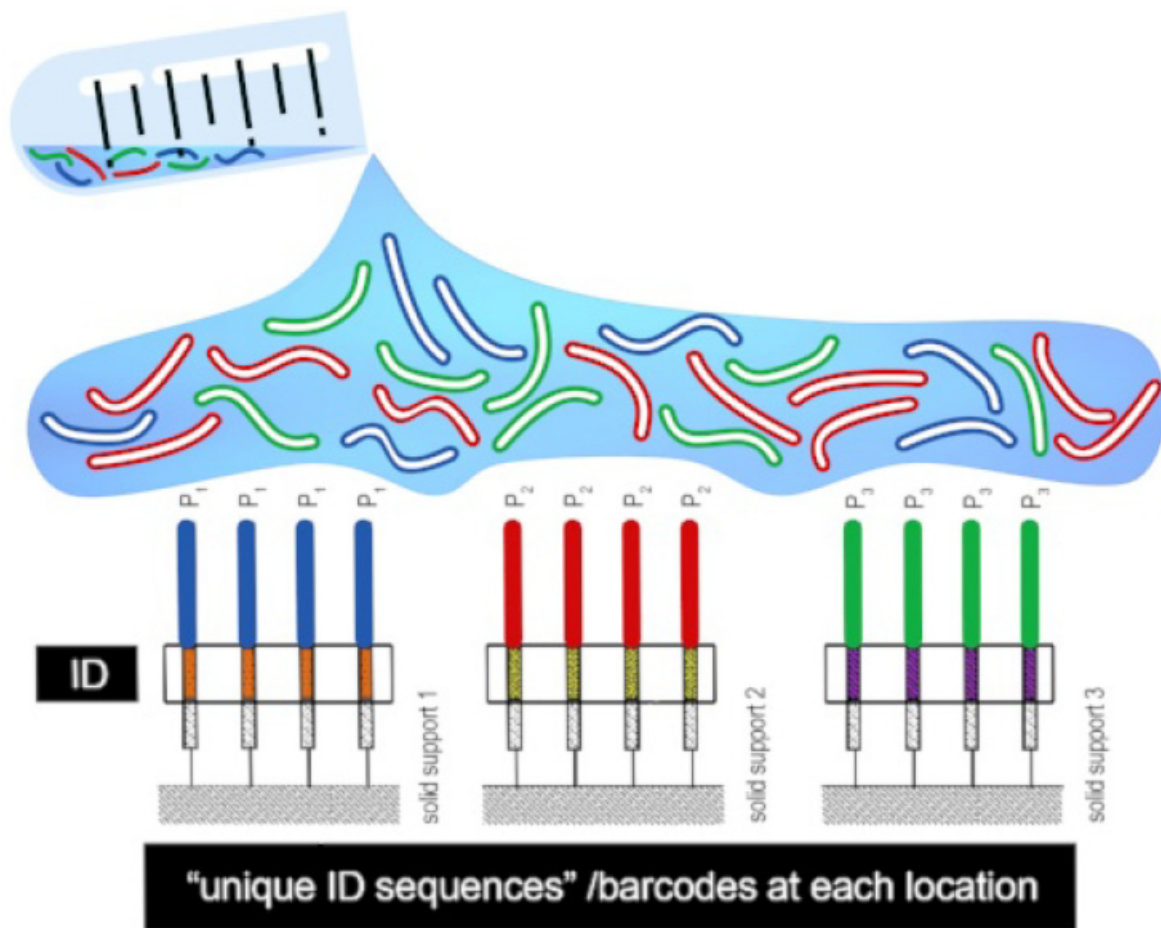
154. I also note that, as described above in Section IV.A.3, the copy of the captured nucleic acid (and identifier sequence, if used) is released from the solid supports before further sequencing and analysis (steps C and D of Figure 2). Once released and the physical connection between the bead and 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).

155. 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 sequencing methods, a POSA would only be able to determine where on the array a target nucleic acid was captured, 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.

156. 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.

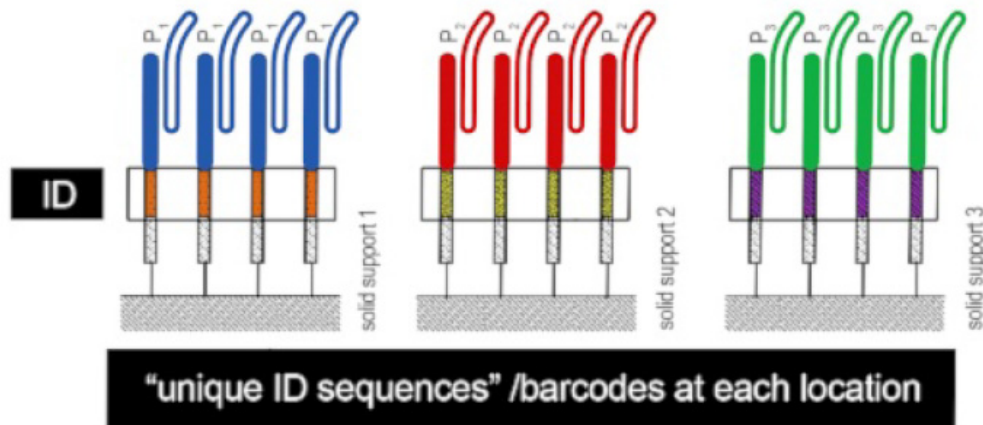
157. I note that Petitioner's proposed array has different identifiers on each bead and different *probe sequences* on each bead as well. As discussed in Sections IV.A.4 and IV.A.5, above, Cantor describes two different types of collections of solid support species, depicted in Figures 1A, 1B, and 1C. 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 IV.A.6 and IV.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).

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



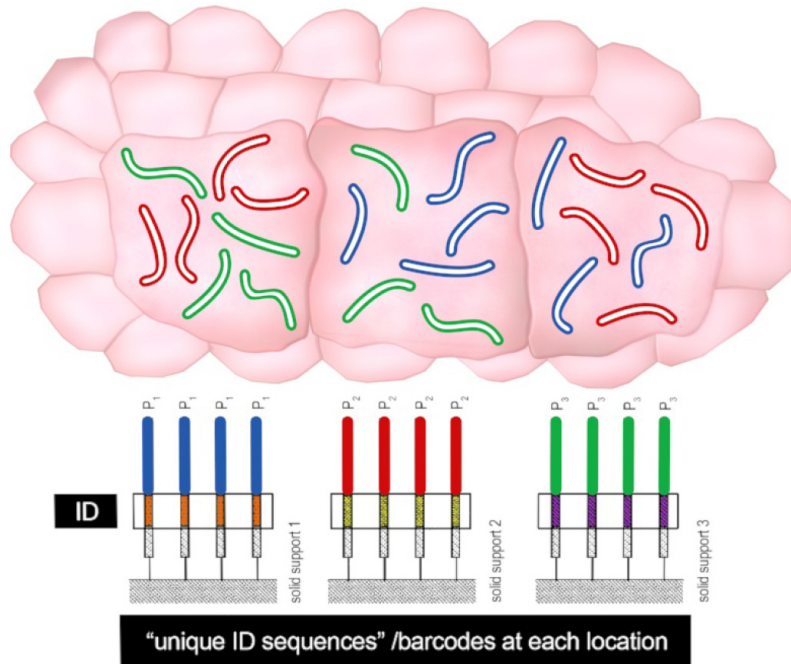
159. In this illustration, the probes of bead 1 are **solid blue**, the probes of bead 2 are **solid red**, and the probes of bead 3 are **solid green**. The **solid blue** probes are designed to hybridize to the **blue-outlined** nucleic acids in the sample nucleic acid; the **solid red** probes to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



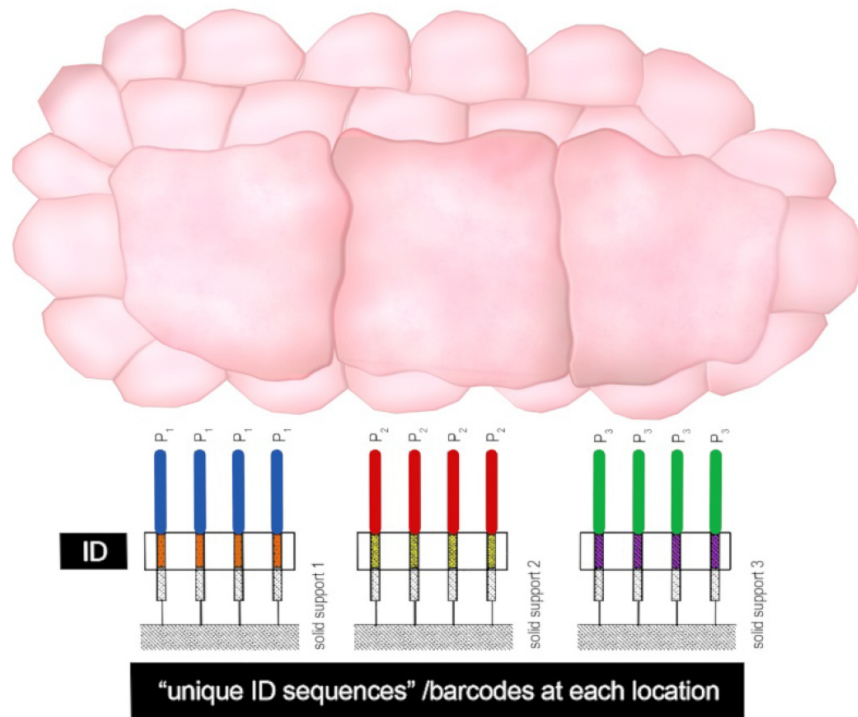
160. Each different bead on the array has a different probe sequence that will (or will not) hybridize to a particular target nucleic acid. This detects presence or absence. But the spatial distribution of the nucleic acids within the tissue sample would already have been lost, as all of the nucleic 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.

161. 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).



162. 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

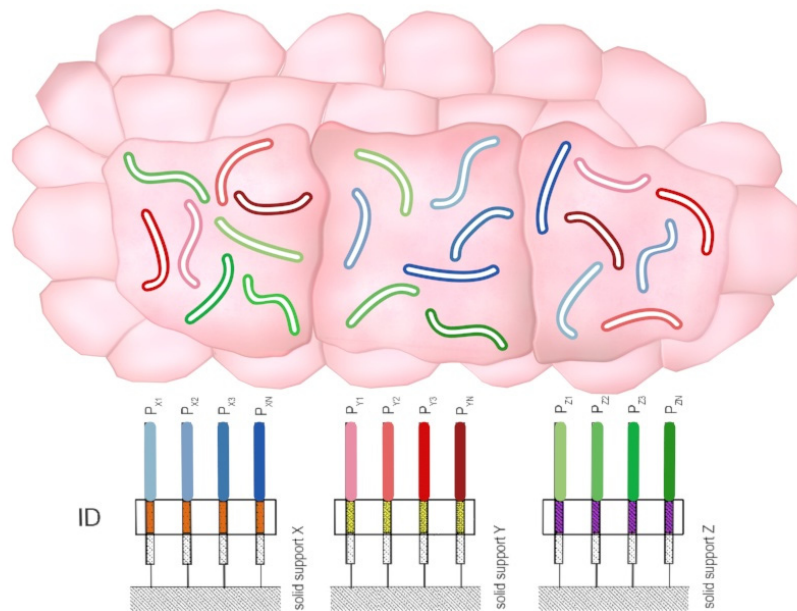
happens to be present in a spatial location of the tissue that overlays its corresponding probe. This is because when nucleic acids are released from a tissue sample onto the array, they can only hybridize to (be captured by) probes that are in proximity to (underneath) the location where the nucleic acid was released from the tissue. This is depicted in the next illustration:

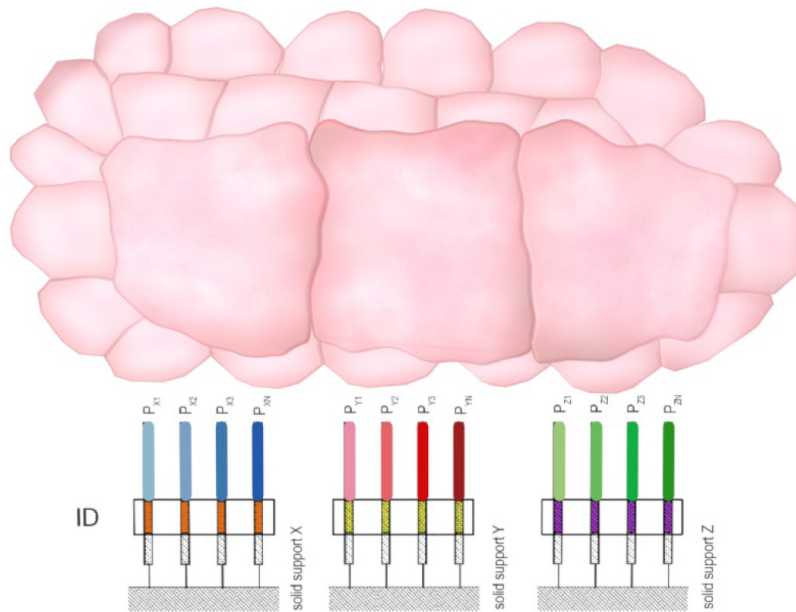


163. As this illustration shows, even though **blue-outlined**, **red-outlined**, and **green-outlined** target nucleic acids were *present* in the tissue (as shown in the first illustration), the results of the assay would nevertheless show *no* spatial information for these nucleic acids (as shown 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.

164. 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.





165. 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).

166. 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. At most, hybridization may result from a given set of circumstances. For example, if a tissue section was, by chance, overlaid in exactly the right location on Petitioner's array. But a POSA would not have

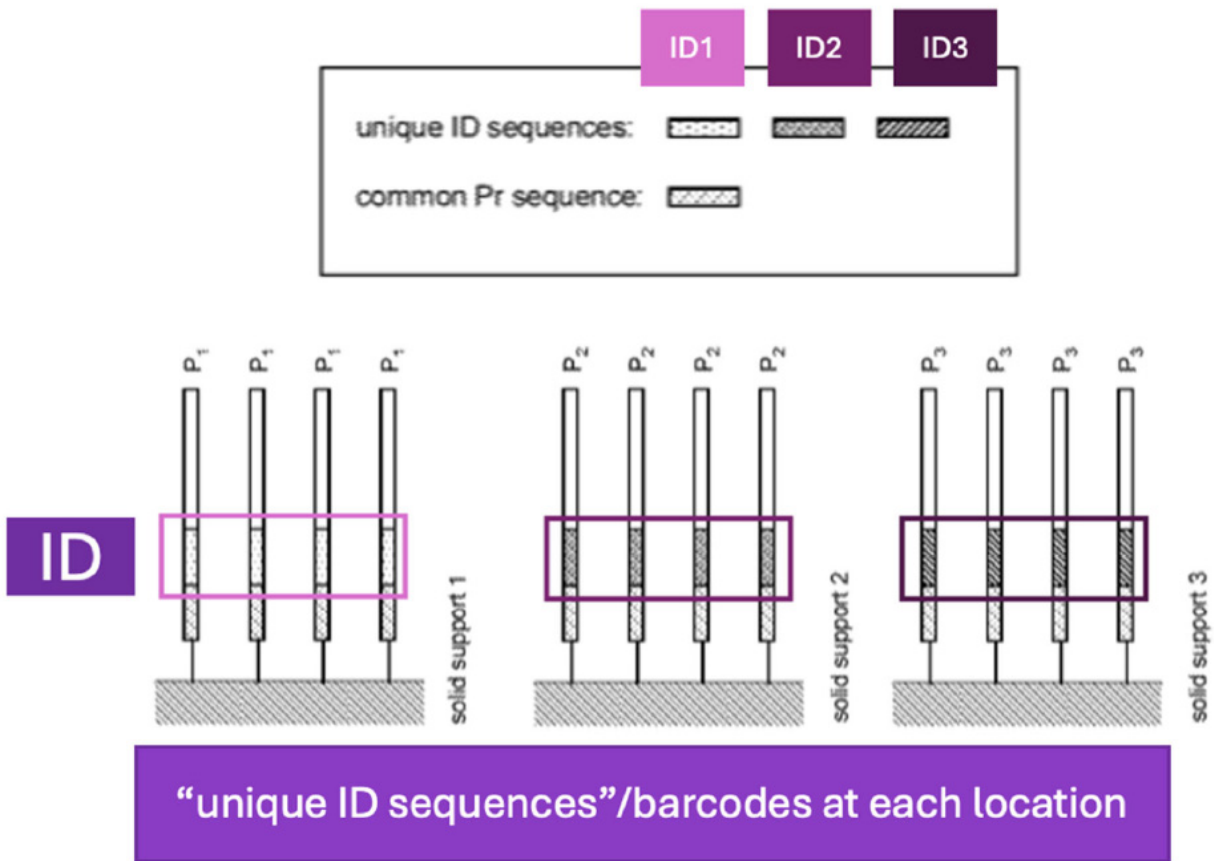
disposed a tissue section onto Petitioner's array at all because Cantor does not and cannot teach a POSA to use a tissue section with its beads and bulk-analysis methods. Simply put, spatial analysis does not matter to Cantor at all. Thus, a POSA would not have been motivated to "perform array-based nucleic acid analysis on a tissue sample" as Petitioner suggests (Petition, 38).

2. Cantor fails to teach an identification sequence that "identifies a unique location on the substrate"

167. As described in Section IV.A, Cantor's solid supports and methods are for sequence-based bulk analysis. They answer the question of whether a biological molecule of interest is present in a sample nucleic acid, not where. And Cantor's substrates are used to ease handling of its beads (solid supports) for use in those methods.

168. Again, as described above, Cantor never teaches or suggests that the beads with their corresponding identification sequences (used for downstream sequencing analysis, as described in Section IV.A, if they are used at all), can or should be traced back to a unique location on the substrate. For example, by placing them at known locations or placing them randomly and determining their locations prior to use.

169. Petitioner, however, assumes that they are, which is reflected in the modifications and rearrangements to Cantor's Figure 1C:



170. Petitioner states that this modified figure shows that Cantor’s identification sequences “necessarily correspond to, and thus identifies, the unique location (such as an (X,Y) coordinate) of the bead on the array.” (Petition, 29.)

171. But Petitioner mistakenly assumes that because Cantor’s beads can be arranged on a substrate, a POSA would have necessarily correlated the identifier sequence with a bead’s location and constructed the array in only one of two ways: (1) with beads placed at a known location (“selective loading”) or (2) random placement of beads with subsequent “decoding.” (Petition, 30.) This is not correct, and Cantor does not teach or suggest as much.

172. Cantor discusses how to physically attach beads to arrays. (EX1031, 12; *see also* EX1048.) But, again, as described in Section IV.A, the location of Cantor's beads on an array is not known, nor does it even need to be known. And for Cantor's bulk-analysis sequencing methods, it would only have made sense for a POSA to have done so *without* either "selective loading" or random loading and "decoding." This is true regardless of whether Cantor's optional identification sequence was present or not.

173. As described in Section IV.A, Cantor's substrates (e.g., arrays) are used for ease in handling the solid supports used in its bulk-analysis methods. In this context, it would not have made sense for a POSA to either selectively load or randomly load and decode the beads prior to use, as the location of a bead on the substrate is completely irrelevant to that use. Cantor says nothing about "correlation," and nothing about either "selective loading" or "decoding." At no point does Cantor express any interest in knowing or determining the location of any bead. Likewise, the location of beads on an array is completely irrelevant to Cantor's optional use of identification sequences for assembly of nucleotide sequences. A POSA would have arranged the beads without regard to or knowledge of where they were on the substrate, so as not to create additional unnecessary, expensive, and time-consuming steps.

174. Petitioner’s assumption, that a POSA would have necessarily correlated the identifier sequence with a bead’s location and chosen between selective loading and random loading with decoding, is based on Dr. Puglisi’s assertion in paragraph 321 of EX1103. But Dr. Puglisi provides no reasoning as to why this would be true in the context of Cantor. It wouldn’t. The only evidence that Dr. Puglisi cites is his own discussion in Section V.D.2 of EX1103 of “nucleic acid arrays” such as DNA microarrays. (EX1103, ¶¶ 63–71.) In this section, Dr. Puglisi describes the “selective loading” and random loading with “decoding” procedures, but does not explain why a POSA would have chosen to use either one with Cantor’s arrays. As described above, a POSA would not have. A POSA would not have needed or wanted to know the location of one of Cantor’s beads for Cantor’s sequencing-based bulk-analysis methods.

175. The types of nucleic acid arrays that Dr. Puglisi describes in Section V.D.2 of EX1103 are array sensors such as DNA microarrays. These are distinct from Cantor’s sequence analysis processes and compositions in that they generate a signal (such as fluorescence) directly on the array itself (i.e., *in situ*) when a target nucleic acid molecule hybridizes to a probe on the array. In that context, the identity of the probe at each location on the array is known or determined prior to use. This is so the signal generated *in situ* can provide the identity of the target nucleic acid molecule that hybridizes.

176. DNA microarrays are used to detect the presence and/or amount of specific target nucleic acid sequences in a bulk nucleic acid sample (e.g., a bulk nucleic acid sample as described in Section IV.A.2; like Cantor's arrays, they cannot be used for spatial analysis). This is achieved by placing nucleic acid probes onto a surface (e.g., slide) in spots, labeling a bulk nucleic acid sample with detectable labels (e.g., fluorescent labels), adding the labeled bulk nucleic acid sample to the slide so that it hybridizes to the probes, and then detecting hybridization on the array (e.g., by imaging). This process is depicted, for example, in Figure 1 of EX1018 (Victor Trevino et al., DNA Microarrays: a Powerful Genomic Tool for Biomedical and Clinical Research, *Mol Med* 13 (9-10) 527-541, 2007):

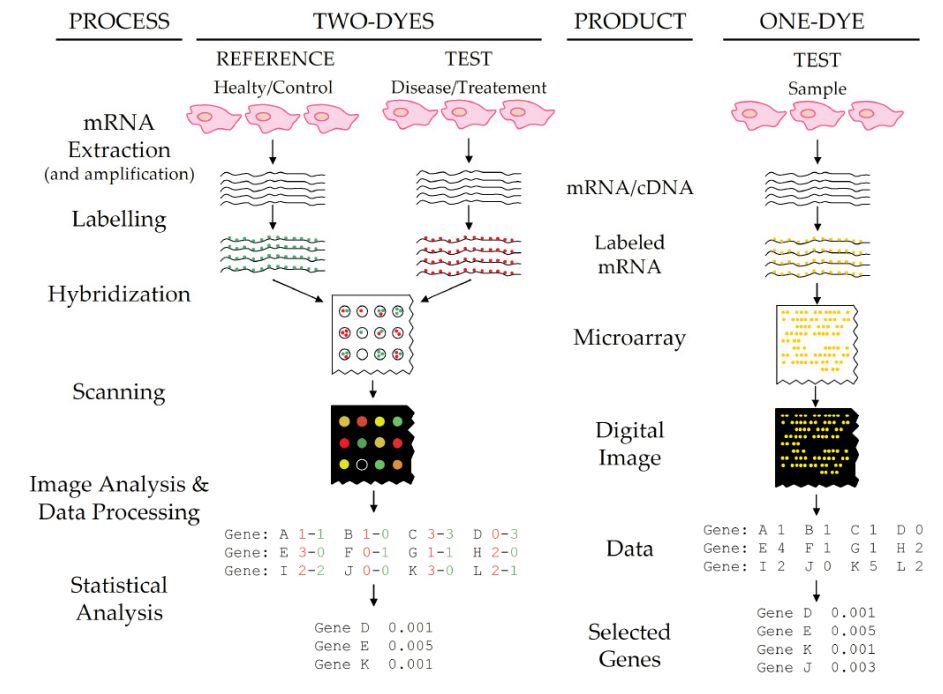
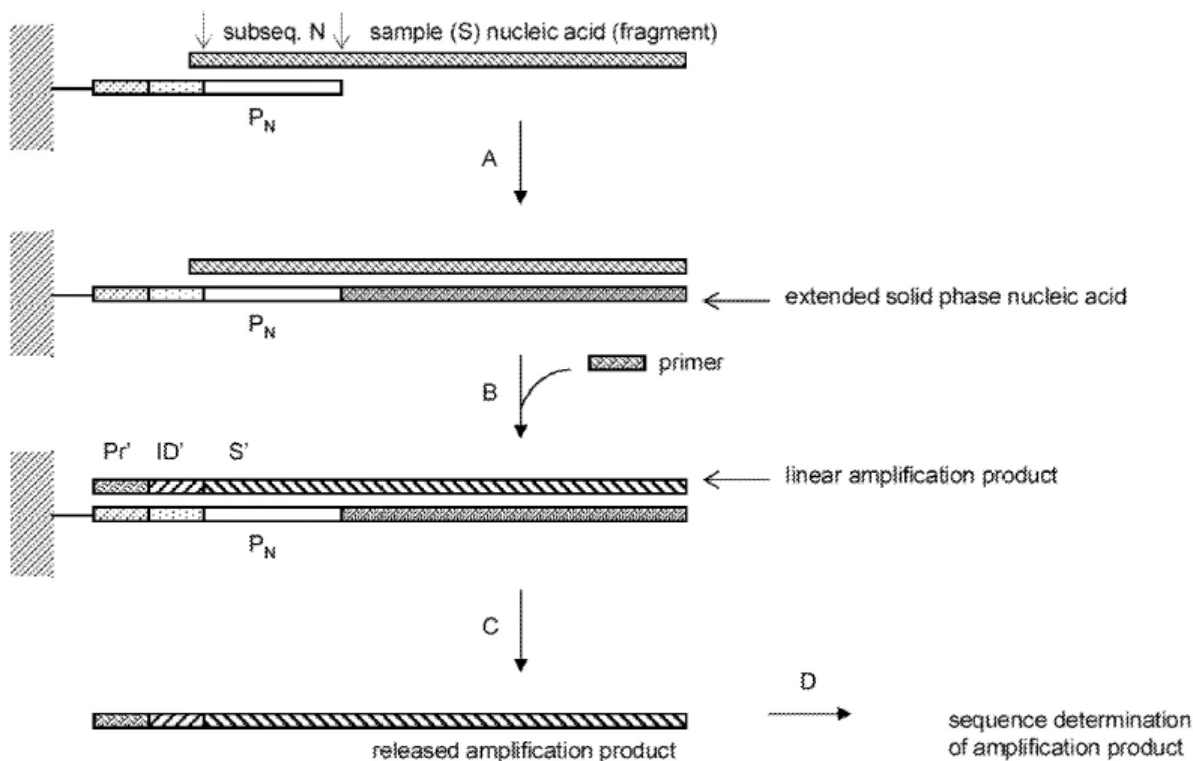


Figure 1. Schematic Representation of a Gene Expression Microarray Assay. Arrows represent process (left column) and pictures or text represent the product. Differences in the protocol in one- and two-dye technologies are specific to the technology rather than to the samples or question. For CGH, the process is similar, replacing mRNA by DNA.

177. The presence and/or amount of a target nucleic acid—as well as its identity—is determined by imaging the array to detect fluorescence at the physical location at which the DNA probe with the corresponding complementary sequence is located. Because the spots on a DNA microarray are designed to hybridize to many different target nucleic acids, the only way to determine presence and/or amount of any particular nucleic acid target is to know which spots on the microarray have a probe that binds to it. Without that knowledge, a POSA could not use the image to determine presence and/or amount of any particular nucleic acid target. Thus, with this type of array, a POSA would have used one of Petitioner’s two alternatives—either selective loading or random loading and

decoding. *See also* EX2012 (Gunderson et al., “Decoding Randomly Ordered DNA Arrays,” *Genome Research* 14:870–77 (2004), describing the use of these alternatives in the context of DNA microarrays). However, the selective loading or random loading and decoding would not have been done to correlate an identification sequence with a location on the array, but rather to determine the physical location of the probe on the DNA microarray for *in situ* imaging.

178. But Cantor is not a DNA microarray, and it does not use *in situ* detection of hybridization in this manner. A POSA would not have been constrained with placing Cantor’s beads in manners suitable only for *in situ* detection methods such as DNA microarrays, as Petitioner suggests. This becomes clear when considering Cantor’s Figure 2:



179. In contrast to DNA microarrays, as described in Section IV.A, Cantor's beads are not imaged to determine presence and/or amount of a target nucleic acid. Rather, in steps C and D, the linear amplification product is released from the solid support for downstream sequencing and analysis. This all happens off the array, not *in situ*. Again, the location of the bead, binding agent and (if used), coding identification sequence on the substrate is completely irrelevant to Cantor's sequence analysis methods.

3. A POSA would not have been motivated to generate Petitioner's proposed array

180. Furthermore, because of all these technical defects in Petitioner's analysis, a POSA would not have been motivated to construct Petitioner's array and would have recognized that doing so would have been futile.

181. As described above, a POSA would have had no reason to have correlated the identifier sequence with the bead's location and would not have been constrained to Petitioner's two options for arrangement of Cantor's beads. Indeed, a POSA would have chosen neither. As also described above, a POSA would not have disposed a "tissue section" on Petitioner's array. The resulting "by chance" hybridization is cross purposes to bulk-analysis and negates any reason why a POSA would have been motivated to do so. Indeed, as I explained above, out of the several dozen uses that my application *does* identify for the disclosed solid supports and methods, none are for spatial analysis.

182. Petitioner's obviousness case can only have been manufactured with the benefit of hindsight, starting from the '030 patent. Therefore, the Challenged Claims would not have been obvious over Cantor.

4. None of Petitioner’s “Background References” (Ground 1) or Armani (Ground 2) compensate for the deficiencies of Petitioner’s obviousness case

183. As discussed in Sections VII.B.2 and VII.B.3, Petitioner asserts that a POSA would have correlated Cantor’s identifier sequence with a bead’s location and chosen between “selective loading” or random loading with “decoding.” Petitioner cites to Chee-584 (EX1032), Chee-824 (EX1038), and Barany 1999 (EX1036) as describing these procedures. But of these two options, only random loading and decoding could even possibly be relevant to the Challenged Claims, as “selective loading” is incompatible with being “immobilized on the substrate in a random pattern,” as claimed. The only reference that Petitioner refers to regarding random loading and decoding is Chee-824, which relates to an array sensor that uses *in situ* on-substrate imaging. As discussed in Sections VII.B.2 and VII.B.3, a POSA would not have been constrained to Petitioner’s two options for arranging **Cantor’s** beads. Rather, a POSA would have chosen neither.

184. Petitioner also discusses Southern (EX1033) in its discussion of obviousness over Cantor with respect to claim 17. (Petition, 39–40.) 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. (EX1103, ¶¶ 45–51.)

185. 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 “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.)

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

186. 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.

187. 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.

188. I understand from Patent Owner’s counsel that Shirai, which Petitioner references on page 41 of the petition, is a post filing date reference and not relevant to a POSA’s background knowledge, as Petitioner suggests.

189. Petitioner’s Ground 2 combines Armani (EX1022) with Cantor. Armani is discussed above in Section IV.B. But Petitioner’s obviousness ground suffers from the same fatal flaws described above.

VIII. CONCLUSION

190. 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: February 25, 2025

A handwritten signature in black ink, appearing to read 'Charles R. Cantor', is written over a light gray grid background. The signature is fluid and cursive, starting with a large 'C' and ending with a long horizontal stroke.

CHARLES R. CANTOR