

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.

IPR2025-00192 & IPR2025-00193
U.S. Patent Nos. 11,549,138 & 11,761,030

**DECLARATION OF JOSEPH D. PUGLISI, PH.D. IN SUPPORT OF
PETITION FOR *INTER PARTES* REVIEW**

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I. Introduction

1. My name is Joseph Daniel Puglisi. I am currently the Jauch Professor and Professor of Structural Biology at the Stanford University School of Medicine. I have 40 years of experience in the investigation of nucleic acid and protein chemistry, biophysics, and biology as a professor and as a student.

2. I have been retained by Petitioner Curio Bioscience to provide an independent expert opinion regarding the technology, patents, and relevant prior art at issue in this proceeding. I am being compensated for my time spent consulting on this matter at my normal hourly rate. My compensation does not depend on the outcome of this proceeding.

3. I am an expert in biophysics, biochemistry, molecular biology, cell diagnostics, molecular interactions, DNA and RNA sequencing, genetic testing, nucleic acid preservation, and related fields. My qualifications and credentials are set forth in my *curriculum vitae*, attached as Exhibit 1101. Briefly, I received a B.A. in Chemistry in 1984 from The Johns Hopkins University and a Ph.D. in Biophysical Chemistry in 1989 from the University of California, Berkeley. While pursuing my Ph.D., I received the Bruce Mahan Memorial Teaching Award from the University of California Berkeley's Department of Chemistry.

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4. From 2004 through 2014, I was the Chair of Stanford's Department of Structural Biology. I have been the Director of the Stanford Magnetic Resonance Laboratory since 1997. I have also been the Director of the International School of Biological Magnetic Resonance since 2003. From 1993 through 1997, I was part of the faculty at the University of California, Santa Cruz in the university's Molecular, Cellular, & Developmental Biology Department. From 1991 to 1993, I was a postdoctoral fellow at the Massachusetts Institute of Technology in the institute's Department of Chemistry. From 1989 to 1991, I was a European Molecular Biology Organization (EMBO) postdoctoral fellow at the Institute of Molecular and Cellular Biology at the French National Centre for Scientific Research.

5. I have authored or co-authored over 175 publications in the fields of biophysics, chemistry, molecular and cell biology, including at least five book chapters. I have co-authored a textbook on Physical Chemistry. I have been invited to present on topics in the fields of molecular and cell biology on at least 300 occasions, and presented numerous named Lectureships, including the Linus Pauling Lecture (Stanford), Tom Steitz Lecture (Yale), Ferry Lecture (University of Wisconsin), O'Keefe Lecture (Arizona State University).

6. I am a member of numerous technical societies, including the US National Academy of Sciences since 2014. I have been a member of the Advisory

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Editorial Board for the EMBO since 2011. I was a council member of the Biophysical Society from 2014–2017. I have been the lead organizer of the past 12 NATO Advanced Study Institute courses on the fields of structural biology and molecular biophysics. In 2007, I was the co-chair for both the Nucleic Acids Gordon Conference hosted by the Salve Regina University, and the American Chemical Society's RNA Biophysics Symposium.

7. I have been appointed as an editor of a number of scientific publications. I have been a member of the Editorial Board for the Proceedings of the National Academies of Science since 2017. I have been an Editor of the *Biophysics Journal* since 2014. I have been a member of the Advisory Editorial Board for EMBO reports since 2011. I have been an editor of the *Structure* division of the prestigious Cell Press since 2007, and previously served as the Senior Editor from 2003-2007.

8. I received the Merit Award from the National Institute of Health in 2011. I also received the National Institute of Health Director's Transformative R01 Program Award in 2011. I was an Alfred P. Sloan Research Fellow for the Alfred P. Sloan Research Foundation in 1997. I was also the David and Lucille Packard Fellow for the David and Lucille Packard Fellowship in Science and Engineering from 1994–1999. I thrice received the University of California at Berkeley's Bruce

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Mahan Memorial Teaching Award in the university's Department of Chemistry. In 1989, I was inducted into the Sigma Xi Honor Society.

9. My research for the past four decades involved the study of RNA structure and function. In particular, my research includes the investigation of RNA function by studying molecular structure and dynamics using a variety of biophysical and biological tools, such as nuclear magnetic resonance (NMR) spectroscopy, x-ray crystallography, cryogenic electron microscopy, and single-molecule fluorescence methods. I have developed and characterized methods to synthesize, purify, characterize, and stabilize highly pure and structurally stable RNA molecules for these applications, and applied/developed DNA/RNA analysis tools, including sequencing methods. I have also helped develop commercial DNA sequencing and analysis tools, in particular as a longtime advisor to Pacific Biosciences.

II. Legal Standards

10. I have been informed by counsel of general principles concerning patent law and the analysis of whether a patent claim is valid. I have followed these principles in arriving at my conclusions in this declaration. The sections below set forth my understanding of the legal principles that are relevant to my opinions in this declaration.

A. Claim Construction

11. I understand that the claims of a patent define the scope of the inventions that are protected by the patent. I understand that the language of the claims is construed as it would be understood by a hypothetical person having ordinary skill in the art (a “POSA”) at the time of the invention. Accordingly, where I refer below to a POSA—for example, where I discuss how such a person would have understood the specification or the claims, or what a POSA might have known, among other things—I am referring to such a person working at the time of the invention, even if for the sake of brevity I omit that specific timeframe from my statements.

12. I understand words in a claim will be given their ordinary or customary meaning unless it appears that the inventor used them differently.

13. I also understand that, in construing patent claims, courts seek to determine what a person skilled in the art would have understood the claims to mean primarily in light of the intrinsic evidence of record, which includes the language of the claim itself and other issued claims, the specification, the art cited during prosecution, and the prosecution history.

B. Anticipation and Obviousness

14. I understand that, for an invention claimed in a patent to be found patentable, or to be valid, it must be, among other things, novel and not obvious in light of what came before it. I understand that patents and publications that were filed and/or published before the filing of a challenged patent are generally referred to as “prior art.”

15. I understand that a patent claim is unpatentable or may be found invalid as anticipated if all claim elements are found in a single prior art reference as arranged in the claim.

16. I understand that a patent claim is unpatentable or may be found invalid as obvious if, at the time when the invention was made, the subject matter of the claim, considered as a whole, would have been obvious to a person having ordinary skill in the field of the technology (the “art”) to which the claimed subject matter belongs.

17. I understand that a claim in a patent is obvious when the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains.

18. I understand that obviousness may be shown by considering more than one item of prior art, and that the following factors should be considered in analyzing obviousness: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims; and (3) the level of ordinary skill in the pertinent art.

19. I understand that a person of ordinary skill in the art is assumed to have knowledge of all relevant prior art. I understand that one skilled in the art can combine the teachings of various prior art references based on the teachings of the individual prior art references, the general knowledge present in the art, or common sense. I understand that a teaching, motivation or suggestion appearing in the prior art may provide a basis for combining two (or more) prior art references or modifying a given prior art reference. A motivation to combine references may also be implicit in the prior art, and there is no requirement that there be an actual or explicit teaching to combine two references. Thus, one may take into account the inferences and creative steps that a person of ordinary skill in the art would employ to combine the known steps in the prior art in the manner claimed by the patent at issue.

20. I understand that the combination of familiar steps according to known methods is likely to be obvious when it does no more than yield predictable results. I understand that when a work is available in one field of endeavor, design incentives

and other market forces can prompt variations of that work, either in the same field or a different one. Likewise, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using that technique to improve the other device in a predictable manner would normally be obvious.

21. I understand that a person of ordinary skill is also a person of ordinary creativity in the field. A person of ordinary skill attempting to solve a problem will not be restricted only to those steps of prior art designed to solve the same problem. Rather, in exercising common sense and ordinary creativity, a person of ordinary skill can draw upon prior art directed to any need or problem known in the field of endeavor at the time of the invention. In other words, the prior art does not need to be directed towards solving the same problem that is addressed in the patent. Further, the individual prior art references themselves need not all be directed towards solving the same problem.

22. I further understand that in many fields, it may be that there is little discussion of obvious techniques or combination, and it often may be the case that market demand, rather than scientific literature or knowledge, will drive design trends. When there is such a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary

skill has good reason to pursue the known options within their technical grasp. If this leads to the anticipated success, it is likely the result not of innovation but of ordinary skill and common sense. In that instance, the fact that a combination was obvious to try might show that it was obvious. I understand that the fact that a particular combination of prior art steps was “obvious to try” may indicate that the combination was obvious even if no one had attempted the combination at the time of filing. Likewise, duplicating a known feature from the prior art multiple times in order to take advantage of proven technology typically does not require a leap of inventiveness.

C. Priority and Written Description

23. I understand that during patent prosecution, the applicant may amend the originally filed claims, or submit new claims, between the time an original application is filed and the time at which the patent is issued. I understand that such changes may be made in a continuation application, which is an application that stems from a parent application.

24. I understand that, for a continuation application to be entitled to the filing date of its parent application or parent applications, the claims must find written description support in the earlier application bearing the filing date on which the applicant seeks to rely. If the earlier application on which the applicant seeks to

rely is separated from the application for which the benefit is sought by one or more intervening applications, the claims also must find written description support in each intervening application.

25. To evaluate whether claims are supported by a disclosure, such as the patent specification or the specification of a parent filing, I understand that one must consider the written description (including the figures/drawings, text, and original claims) from the perspective of a person of ordinary skill in the art at the time the application was filed. The written description requirement is satisfied if such a person would have recognized that it describes the full scope of the claimed invention as it is finally claimed in the issued patent and that the inventor actually possessed that full scope by the filing date of the original application.

26. I further understand that, while the full scope of a claim or any particular requirement in a claim need not be expressly disclosed in the original patent application, or described in the exact same words used in the claim, a written description that merely renders the claimed subject matter obvious does not satisfy the written description requirement.

III. Overview of the '138 and 030 Patents

27. I understand that in co-pending litigation, EX1006 (District Court Complaint), Patent Owner and its exclusive licensee, 10x Genomics, have accused

Petitioner of infringing five related patents, including U.S. Patent Nos. 11,549,138 (EX1004) and 11,761,030 (EX1005), addressed here, as well as, U.S. Patent Nos. 10,480,022 (EX1001), 10,662,468 (EX1002), and 11,001,879 (EX1003).

28. The '138 and '030 Patents are titled “Spatially Encoded Biological Assays,” and names as their inventor Mark S. Chee. EX1004, EX1005. According to their faces, the '138 and '030 Patents issued, respectively, from Application No. 17/825,719, which was filed on May 26, 2022, and Application No. 18/074,992, which was filed on December 5, 2022. EX1004 & EX1005, Code (22). The '138 and '030 Patents claim priority to “Provisional application No. 61/321,124, filed on Apr. 5, 2010.” EX1004 & EX1005, Code (60).

29. The **ABSTRACT** of the '138 and '030 Patents state that “[t]he present invention provides assays and assay systems for use in spatially encoded biological assays,” and that the assay system of the invention is “capable of high levels of multiplexing where reagents are provided to a biological sample in defined spatial patterns; instrumentation capable of controlled delivery of reagents according to the spatial patterns; and a decoding scheme providing a

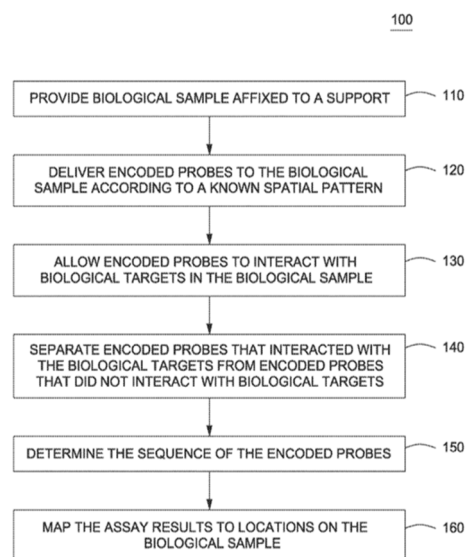


FIG. 1

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readout that is digital in nature.” EX1004 & EX1005, Abstract. The approach of the patent is illustrated in the flow-diagram provided in Figure 1. EX 1004 & EX1005, FIG. 1.

IV. Level of Ordinary Skill in the Art

30. I have been advised that a “person of ordinary skill in the art,” or “POSA,” is a hypothetical person and that I should consider the following factors in determining the qualifications of a POSA: (1) the educational and experience level of active workers in the field; (2) the sophistication of the technology; (3) the rapidity with which innovations are made; (4) the types of problems encountered in the art; (5) the prior art solutions to those problems. I understand that not all such factors may be present in every case, and one or more of them may predominate.

31. The relevant technology field for the '138 and '030 Patents is nucleic acid analysis and detection. By virtue of my education, experience, and training, I am familiar with the level of skill in the art of the '138 and '030 patent before the April 5, 2010 filing date of the '124 Provisional Application.

32. In my opinion, a POSA in the field of the '030 and '138 Patent as of the April 2010 filing date of the '124 Provisional Application would 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

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years of academic, research, or industry experience in genomic technology.¹ 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 preservation and handling. This is confirmed by the '124 Provisional:

The practice of the techniques described herein may employ, unless otherwise indicated ***conventional techniques*** and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology, ***which are within the skill of those who practice in the art.*** Such conventional techniques include polymer array synthesis, hybridization and ligation of polynucleotides, and detection of hybridization using a label.

EX1009, [00037]; *see also id.*, [0003] (“Conventional methods such as *in situ* hybridization and multiplexed detection of multiple different transcripts have revealed spatial patterns of gene expression and have helped to shed light on the molecular basis of development and disease. Other technologies that have enabled the quantitative analysis of many RNA sequences per sample include microarrays,

¹ I understand that the POSA is a fictional person. In practice, an individual may have obtained equivalent skill through a combination of less education and greater research and/or industry experience, for example.

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serial analysis of gene expression (SAGE), and high-throughput implementations of qPCR.”) (citations omitted); *id.*, [00083] (“Conventional sequencing is used as a readout....”); *id.*, [00085] (using “conventional sequencing to determine the structure of the assay products.”).

33. By April 5, 2010, I would have had at least the foregoing level of education and experience.

34. In the grounds below, where a priority date of February 14, 2019 applies, a POSA would have at least the foregoing level of skill and education, plus the additional knowledge acquired and documented in the literature in the intervening years.

V. Overview of a Person of Ordinary Skill in the Art’s Knowledge

35. In this section, I provide an overview of the various concepts and techniques that a POSA would have known and understood by April 5, 2010.

A. Background Molecular Biology

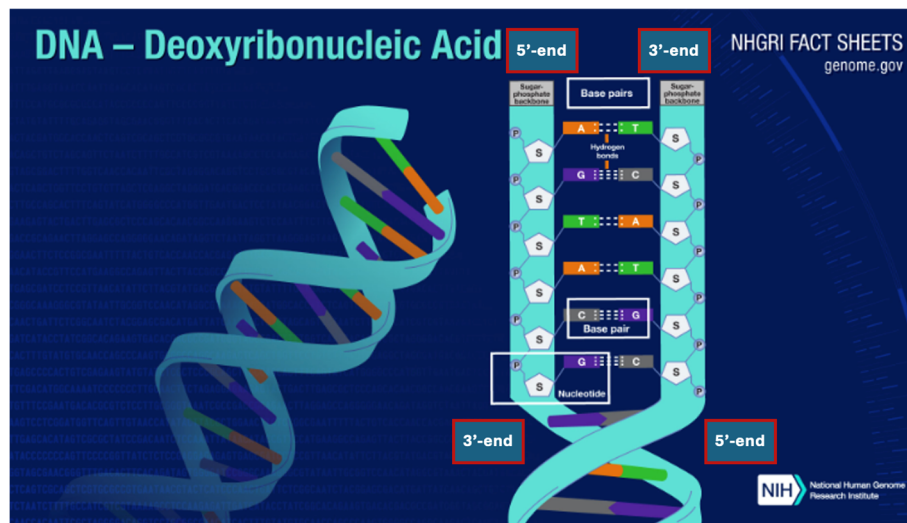
36. The common specification of the ’030 and ’138 Patents refer to the analysis and detection of “biological molecules.” These “biological molecules” may be members of several different classes of biological molecules, such as nucleic acids, proteins, lipids, carbohydrates, small-molecule metabolites, or ions, EX1005, 20:66-21:1, but in this declaration, I focus primarily on nucleic acids.

37. The two main types of nucleic acids relevant to this declaration are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the genetic material found in all living organisms. A fraction of the DNA in an organism encodes genes.

38. DNA is made up of two strands that wind around each other in a shape called a “double helix,” which biologists often depict in schematics that look like a twisting ladder. Each DNA strand is composed of subcomponents called nucleotides consisting of nitrogenous aromatic bases; the nucleotides are linked together to form a continuous strand through phosphodiester linkages. DNA is typically composed of sequences of four such bases, T (thymine), A (adenine), C (cytosine), and G (guanine), which make up the genetic code.

39. Each base on one strand of DNA pairs with a base on the other strand through predictable hydrogen bonding interactions, referred to as “base-pairing.” C preferentially pairs with G and A preferentially pairs with T. This consistent pairing of bases allows DNA to accurately replicate itself, and to be accurately transcribed into a copy of RNA (see below). As discussed in detail below, many methods of analyzing nucleic acids leverage this predictable complementarity to capture, amplify, and detect specific nucleic acids present in a biological system.

40. Each strand is asymmetric and thus has a “directionality,” which scientists refer to by noting the “5’-end” and “3’-end” of a given DNA (or other nucleic acid) strand. In double-stranded DNA, one strand runs from 5’ to 3’, while the other runs from 3’ to 5’. I have annotated this concept on the figure below.²



41. The bases are in different orders on different parts of the DNA strand. DNA is “read” by the order of the bases, that is by the order of the As, Cs, Gs, and Ts. The order of these bases is what is known as the DNA sequence.

42. In gene expression, DNA genes are converted into an intermediate called messenger RNA (mRNA) through a process called transcription. During

² Deoxyribonucleic Acid (DNA) Fact Sheet, Nat’l Hum. Genome Rsch. Inst. (Aug. 24, 2020), <https://www.genome.gov/about-genomics/fact-sheets/Deoxyribonucleic-Acid-Fact-Sheet>

transcription, one strand of DNA holds the information that codes for various genes; this strand is often called the template strand or antisense strand and is copied by a protein machine called RNA polymerase. The other, and complementary, strand is called the coding strand or sense strand (containing codons). Since mRNA is made by copying the template strand, it has the same information as the coding strand. The code for the mRNA will be identical to the coding strand, except in RNA the Thymine (T) bases are replaced with Uridine (U). The illustration below shows the sequence of two complementary strands of DNA, along with the mRNA that would be transcribed from the template strand.

	Directionality	Sequence
DNA Coding Strand	(5'→3')	ACGTTGCATTCCGG
DNA Template Strand	(3'→5')	TGCAACGTAAGGCC
mRNA Transcribed from Template Strand	(5'→3')	ACGUUGCAUCCGG

43. The transcribed mRNA can then be converted into protein through a process called translation. During translation the sequence information within the RNA, which originally derives from DNA, is “translated” to a sequence of amino acids using 3 base code words (called codons). The flow of genetic information from a gene encoded by DNA, into a transcribed mRNA, which in turn is translated to

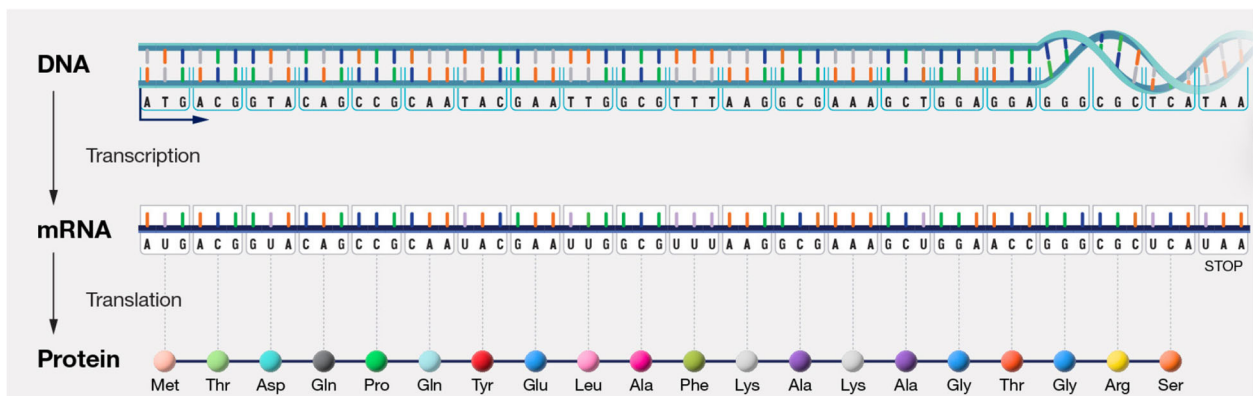
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protein is referred to as the central dogma of molecular biology.³ If there is a change to the DNA sequence of a gene (a mutation), that would change the mRNA sequence and could lead to the incorporation of a different amino acid into a protein, possibly disrupting its function. An essential aspect of modern biological research is to understand how this process occurs properly during life and is aberrant in disease.



44. The '030 and '138 Patent's **"BACKGROUND OF THE INVENTION"** explains that prior art "gene expression analysis and protein analysis have been useful tools in understanding mechanisms of biology," and that "[c]onventional methods such as in situ hybridization and other multiplexed detection of different transcripts have revealed spatial patterns of gene expression and have helped shed light on the molecular basis of development and disease." EX1005, 1:52-61. The concept of "gene expression" refers to the process by which a gene encoded by DNA

³ Central Dogma, Nat'l Hum. Genome Rsch. Inst. (Nov. 23, 2024), <https://www.genome.gov/genetics-glossary/Central-Dogma>

gets turned on in a cell to make RNA and proteins. Gene expression reflects the amounts of mRNA or protein, or both, and is analyzed accordingly.

B. Cells and Tissues

45. Cells are the basic building blocks of living things, and can be sorted into two groups: prokaryotes and eukaryotes. Human cells (and other mammalian cells) are eukaryotic cells; such cells are more complex than prokaryotic cells, which comprise many unicellular organisms such as bacteria. Prokaryotic cells lack an enclosed nucleus or other organelles found in eukaryotic cells.

46. Eukaryotic cells are highly organized and contain a number of organelles, which are specialized internal components that serve particular functions. These include the cell membrane, which is an outer casing comprised of a double layer of lipid (i.e., fatty acid) molecules, in which a variety of proteins are embedded to serve myriad cellular functions. The nucleus of the cell, also enclosed by a lipid membrane, is where the chromosomes are located, as well as the nucleolus (a membraneless structure), which produces the ribosomes that initiate the processes that produce proteins from the cell using the information encoded by the chromosome.

47. A POSA would have been familiar with a number of well-known techniques for analyzing cells and their components. Light, fluorescence and

electron microscopy methods were used traditionally over the prior several decades to visualize cellular architectures and components. Many methods were developed to characterize cells based on proteins expressed on their surfaces. For example, fluorescence activated cell sorting (FACS) uses fluorescent antibodies for cell surface markers to allow individual cells of a given type to be purified. DNA and RNA sequencing methods allow the analysis of the nucleic acid components, and by 2009, could be performed at a single-cell level.

48. Tissues are groups of cells that function together as a unit. For example, tissues can be from different organs (e.g., liver, kidney, heart, muscle, brain, etc.), and often carry cells that are specific to that organ. Tissues can contain multiple cell types, such as muscle and fat cells in skeletal muscle tissues, and neurons and glial cells in brain tissue. Important to many aspects of biological research, tissues can be in a normal, healthy state, or in a disease state (e.g., a cancerous tumor, or degenerated brain tissue).

49. To facilitate research, many techniques for isolating and preparing tissue samples for further investigation have been developed. Once a tissue sample is isolated from a patient or an animal (for example from a surgical resection or biopsy), it must be preserved for further analysis. These techniques are designed “to

prepare tissues for study so that they closely resemble their natural, living state.”

EX1044, 1. Steps that may be involved in such processes include:

- **Fixation:** the treatment of tissues with chemical agents to preserve the tissue architecture, such as through dehydration (typically using methanol) or cross-linking methods (typically using formalin, a relative of formaldehyde). This creates a meshwork that maintains the location of biological macromolecules as well as the overall structure of the cell.
- **Freezing:** tissues can be preserved using deep freezing, such as cryogenic preservation in liquid nitrogen.
- **Embedding:** a technique in which tissues are embedded in a material, often paraffin, which hardens to form a block around the tissue, facilitating the slicing of the sample into thin sections.
- **Sectioning:** a technique that involves slicing a tissue into thin slices using a tool called a microtome, which can be performed on embedded or frozen specimens.

See EX1044, 1-2.

50. In the art, there were only a few common techniques for preserving tissues samples, these included (1) freezing the tissues samples and (2) fixing the tissue samples in formalin followed by embedding them in paraffin. Formalin fixation and paraffin embedding (FFPE) was the most common fixation method practiced in the art in 2010 and was known as the “gold standard” for tissue fixation. Ex. 1046, 1229. However, FFPE samples were known to be the inferior choice for nucleic acid analysis because they yielded poor quality RNA in comparison to frozen or fresh tissue samples. *Id.*; Ex. 1047, 1933. During the tissue fixation process, RNA

molecules in particular can be degraded or chemically modified, hindering their detection.

51. Once preserved, the tissues can be analyzed using methods to characterize cellular and tissue architectures. Tissues can be stained for traditional microscopy or imaged using fluorescent antibody probes. To explore the interiors of cells, biologists also possess techniques to “permeabilize” the cells to allow large molecules to transit out of the cell’s lipid membrane, for example using alcohols or detergents.

C. Spatial Analysis of Nucleic Acids

52. Biologists can find important information not only in the identity and quantitation of the genes expressed in a biological system, but also in the location at which particular genes are expressed (or not expressed). Not all genes are transcribed at all times. Instead, transcription of each gene in a biological system is highly regulated. Typically, only the genes whose products (i.e., mRNA and protein) are required by a given system at a given time are transcribed.⁴ Not only do these levels of transcription differ depending on the requirements of a biological system at a

⁴ There are many instances in which transcription is not properly regulated, which results in the over- or under-expression of certain genes. Such issues are implicated in a number of diseases, and are also an important area of investigation.

given time, they also may differ depending on the location of given cells in a biological system. For example, levels of transcription of a particular gene may differ in an eye cell as compared to a liver cell. And at an even more localized level, gene expression may differ in different cell types that make up the same tissue. *See, e.g.,* EX1028, 869 (“To explore the relationships between brain regions, we first examined the correlations between each of voxels⁵ in terms of gene expression levels.”). Finally different transcripts of a gene may have different locations within a cell. For example, a transcript may be localized in the nucleus, in the cytoplasm or targeted to membranes or organelles. Thus, understanding the spatial aspects of gene expression is central to delineating biological functions and responses.

53. Accordingly, by at least several years before the priority date, scientists had been devising ways to analyze the spatial relationships of biological molecules:

Spatial organization is perhaps the most conspicuous quality of cells and organisms, and also the most elusive. The dissection of life into its molecular constituents—the genes, enzymes, and lipid bilayers—

⁵ Voxels are the three-dimensional equivalent of pixels. In this study, the voxels analyzed were segments of mouse brain tissue. EX1028, 868 (“Here, a method called voxelation is used to investigate the gene expression changes that occur in the mouse brain as a result of a pharmacological model of Parkinson’s disease (PD). The name of the method is derived from the term voxel, which is a cubic 3D image element. Conceptually, voxelation is simple, and uses the direct creation of voxels (cubes) *spatially registered with the brain. RNA extracted from the voxels is analyzed using high-throughput techniques, allowing 3D gene expression patterns to be deduced.*”).

necessarily starts with the destruction of spatial order. Yet we know that most complex functions, such as motility and division, depend on having the right molecules in their proper places. Architecture is what ultimately distinguishes a living cell from a soup of the chemicals of which it is composed. How cells generate, maintain, and reproduce their spatial organization is central to any understanding of the living state.

EX1027, 544 (“Over the past 15 years [before 2005], spatial organization has moved to the forefront of research in cell biology.”). Accordingly, numerous tools and techniques have evolved to answer questions such as “whether there is...a spatial relationship between the position of genes and the location of their products in cell space.” *Id.*, 552.

54. Since “many genes need to be properly regulated in space for the system to function,” many techniques have emerged “[t]o study the spatial patterns of gene expression” by “produc[ing] spatially localized quantification of messenger RNA (mRNA) transcripts as proxies for gene expression.” EX1023 (Pachter), 534. These methods may be referred to as “spatial transcriptomics methods.” *Id.*

55. Pachter’s review article, entitled “Museum of spatial transcriptomics” deeply surveys the literature in this field and “presents a database of literature dating back to 1987 comprehensively documenting the historical evolution and current development in data collection and analysis in spatial transcriptomics.” *Id.* “[I]mportant technologies enabling spatial transcriptomics date back to the 1970s,”

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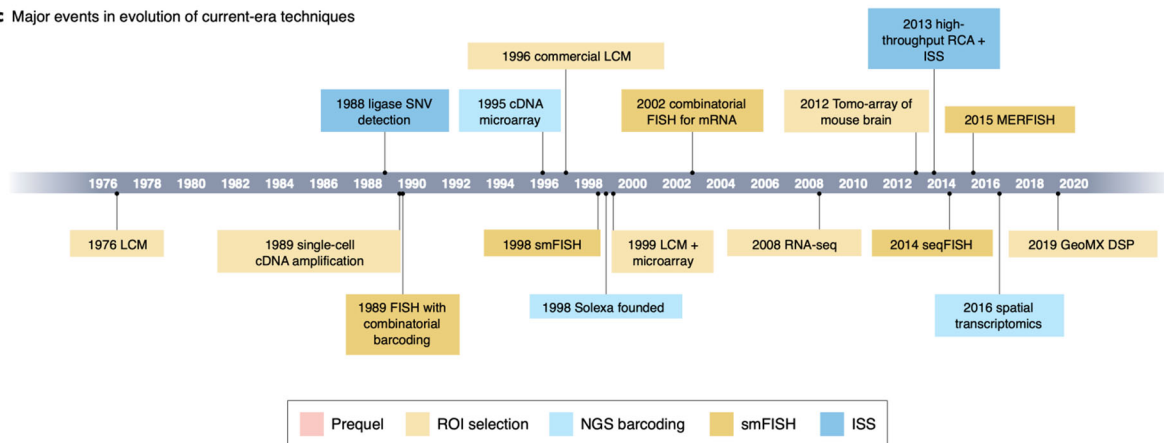
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and “[t]he foundation for many current-era technologies was built in the decades between the 1970s and 2000s (Fig. 1c).” *Id.*

c Major events in evolution of current-era techniques



56. Pachter continues:

For example, ultraviolet (UV) laser was first used to cut tissue in 1976. Popular infrared (IR) and UV LCM systems were first reported in 1996 and were soon commercialized. Some highly multiplexed smFISH technologies, such as sequential FISH (seqFISH), rely on combinatorial barcoding, that is encoding each gene with a combination of colors so transcripts of more genes with easily discernible colors (up to 5) can be quantified simultaneously. Combinatorial barcoding was first reported in immunological DNA FISH in 1989 and was first used for transcripts in 2002. The first unequivocal demonstration of smFISH showing each mRNA molecule as a spot was reported in 1998. Highly multiplexed smFISH would not have been possible without the development of these technologies.

EX1023, 534-536.

57. This historical context documents the existing motivation a POSA would have had as of April 2010 to analyze the spatial distribution and quantification of biological molecules (e.g., nucleic acids, proteins) in biological samples, such as

tissues. It also documents the examples of the relevant technologies and background literature that a POSA would have been familiar with at that time.

D. Background Techniques for Spatial Tissue Analysis

58. In this section, I discuss some of the background techniques and technologies with which a POSA would have been familiar, and which are used in the specific implementations of spatial analysis technologies discussed in the following section.

1. Detection of Nucleic Acids

59. Many nucleic acid technologies historically used fluorescence and other labelling and imaging techniques to detect the presence or absence of a target nucleic acid sequence. Fluorescence is a type of emission spectroscopy, in which a molecule absorbs light of a given wavelength, and then releases some of the absorbed energy by re-emitting light of a longer wavelength. For this purpose, many fluorescent dye molecules that absorb and emit light efficiently have been developed. Such molecules can be excited by exposure to light (often using a laser), and because emission occurs at a different wavelength than the exciting light, the exciting light can be effectively filtered out, leading to a high sensitivity of detection of the fluorescent molecule. This high sensitivity is a power of fluorescence-based detection methods.

60. Fluorescence has been particularly useful for the analysis of molecular interactions. For example, nucleic acids are readily labeled with fluorescent dyes to form labelled probes, and when such probes are hybridized with a target, complementary sequence using the principles of base pairing described above, will cause the target-probe complex to be fluorescent upon exposure to an appropriate light source. Thus, the signal is readily detectable with simple imaging or spectroscopic methods. For example, such techniques were frequently used to detect nucleic acid binding events in microarrays, as discussed further below.

61. Sequencing generally refers to techniques used to determine the order of nucleotide bases present in the DNA or RNA of a biological sample. Around the priority date, next-generation sequencing (NGS) technologies were becoming available. “NGS platforms have dramatically increased throughput and substantially lowered expenditure” as compared to first generation Sanger sequencing, EX1029, 44, “offer[ing] the ability to produce an enormous volume of data cheaply.” *Id.*, 31.

62. Some applications of these techniques included the use of “custom-designed oligonucleotide microarrays...for targeting regions of interest.” *Id.*, 41. Such techniques include “oligonucleotide microarrays [that] have been designed to provide complementary sequences for solid-phase hybridization to enrich” for sequences of interest, before subjecting the captured nucleic acids to NGS. *Id.*, 41

(explaining that “[t]he concept of targeting specific regions of the genome is well established, with PCR being the most widely used method,” and that this may be accomplished “us[ing] NGS platforms to target specific regions of interest.”).

2. Nucleic Acid Arrays

63. Nucleic acid arrays are a class of technologies in which DNA or other nucleic acid sequences are provided on a solid support. Typical solid supports include glass microscope slides and beads. Because “[i]t is well known that complementary single-stranded sequences of nucleic acids form double stranded hybrids,” nucleic acid arrays leverage this property by presenting “thousands of single-stranded sequences that are complementary to target sequences.” EX1018, 528. “[T]he targeted nucleic acid specie” can then be “detected by designing several complementary oligonucleotides spanning each entire target sequence” and hybridization events can be read out by a variety of methods, including fluorescence, dyes, and sequencing. *See id.*

64. The illustration below, provided by the National Institutes of Health, provides a general example of one type of implementation of a nucleic acid array:⁶

⁶ DNA Microarray Technology Fact Sheet, Nat’l Hum. Genome Rsch. Inst. (Aug. 15, 2020), <https://www.genome.gov/about-genomics/fact-sheets/DNA-Microarray-Technology>

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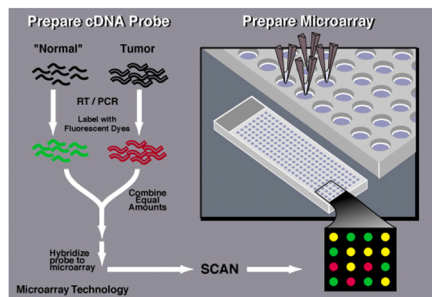
How does a DNA microarray work?

To determine whether an individual possesses a mutation for a particular disease, a scientist first obtains a sample of DNA from the patient's blood as well as a control sample - one that does not contain a mutation in the gene of interest.

The researcher then denatures the DNA in the samples - a process that separates the two complementary strands of DNA into single-stranded molecules. The next step is to cut the long strands of DNA into smaller, more manageable fragments and then to label each fragment by attaching a fluorescent dye (there are other ways to do this, but this is one common method). The individual's DNA is labeled with green dye and the control - or normal - DNA is labeled with red dye. Both sets of labeled DNA are then inserted into the chip and allowed to hybridize - or bind - to the synthetic DNA on the chip.

If the individual does not have a mutation for the gene, both the red and green samples will bind to the sequences on the chip that represent the sequence without the mutation (the "normal" sequence).

If the individual does possess a mutation, the individual's DNA will not bind properly to the DNA sequences on the chip that represent the "normal" sequence but instead will bind to the sequence on the chip that represents the mutated DNA.



65. Around the priority date, there were two main classes of solid supports on which the hybridization probes, sometimes called capture probes, were typically affixed. In one class, “thousands of single-stranded sequences that are complementary to target sequences are bound, synthesized, or spotted to a glass support whose size is similar to a typical microscope slide.” EX1018, 528. A “different technology...uses designed oligonucleotide probes attached to beads that are deposited randomly in a support. The position of each bead and hence the sequence it carries is determined by a complex pseudo-sequencing process.” *Id.*

66. Well before 2010, a POSA would have been familiar with a variety of techniques for “decoding” bead arrays to determine the identity and location of each

bead in an array. For example, in a 2007 publication, the named inventor, Dr. Chee explained:

U.S. Ser. Nos. 08/818,199 and 09/151,877 describe array compositions that utilize microspheres or beads on a surface of a substrate, for example on a terminal end of a fiber optic bundle, with each individual fiber comprising a bead containing an optical signature. Since the beads go down randomly, a unique optical signature is needed to ‘decode’ the array; i.e., ***after the array is made, a correlation of the location of an individual site in the array with the bead or bioactive agent at that particular site can be made.*** This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the in situ synthesis or spotting techniques of the prior art. Once the array is loaded with beads, the array can be decoded, or can be used, with full or partial decoding occurring after testing, as is more fully outlined below.

EX1038 (Chee-824), [0013]. Other known techniques for spatially addressing bead arrays so that the identity of the probes on a given bead were known included “positional decoding” methods, such as “targeting the placement of the beads” to specified locations, or “selective loading of the sites” of particular locations on an array. *Id.*, [0043].

67. Another decoding method disclosed in Chee-824 (EX1038) involves analyzing “identification sequences” that are also affixed to the beads. EX1038, [0042] (“The present invention is generally based on previous work comprising a bead-based analytic chemistry system in which beads, also termed microspheres, carrying different chemical functionalities are distributed on a substrate comprising

a patterned surface of discrete sites that can bind the individual microspheres.”). In a common method of making bead arrays, “the beads may be randomly distributed on the array,” and “can later be ‘decoded’, i.e. after the array is made,” by making “a correlation of the location of an individual site on the array with the bead or candidate agent at that particular site.” *Id.*; *see also id.*, [0043] (“[T]he placement of the bioactive agents is generally random, and thus a coding/decoding system is required to identify the bioactive agent at each location in the array.”). “Once the identity of the bioactive agent and its location in the array has been fixed, the array is exposed to samples containing target analytes.” *Id.*, [0044].

68. Chee-824 further discloses decoding that “does not rely on the use of optical signatures, but rather on the use of decoding binding ligands that are added during a decoding step.” *Id.*, [0045]. “The decoding binding ligands will bind either to a distinct identifier binding ligand partner that is placed on the beads, or to the bioactive agent itself, for example when the beads comprise single-stranded nucleic acids as the bioactive agents.” *Id.* “The decoding binding ligands are either directly or indirectly labeled, and thus decoding occurs by detecting the presence of the label.” *Id.* Chee-824 discloses that “[p]referably at least 5000 bead types are simultaneously decoded or decoded in a single decoding experiment.” *Id.*, [0046].

69. Chee-824 discloses methods in which “the bioactive agent is synthesized with encoding sequences that allows decoding to be accomplished independent of the useable portion of the bioactive agent, i.e., nucleic acid sequence.” *Id.*, [0141] (“By ‘useable’ portion is meant the adapter sequence or target sequence.”). For example, “FIG. 12 depicts decoding by sequencing” in which “dye-labeled chain-terminating nucleotides are used to sequence the encoding region of a DNA strand present on a bead.” *Id.*, [0037].

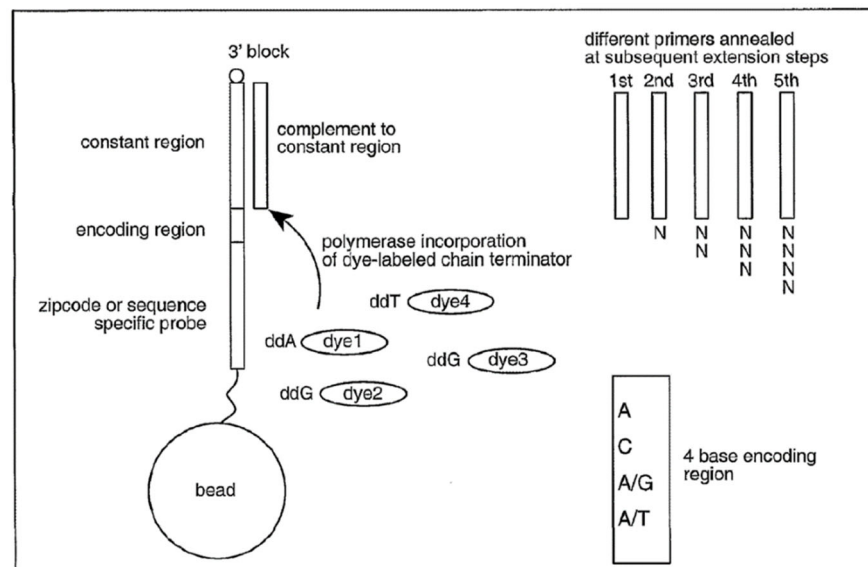
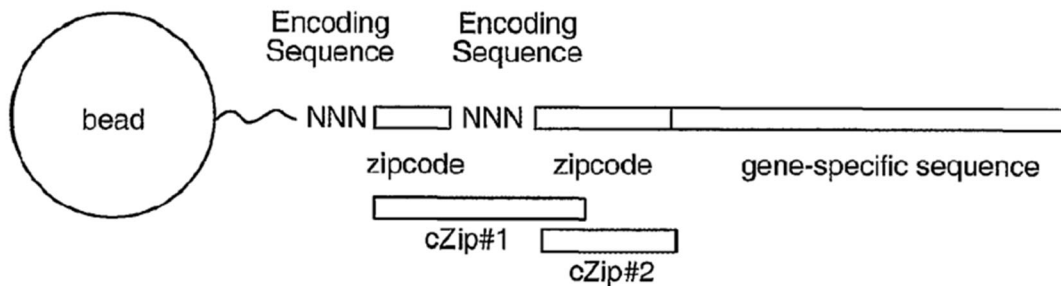


FIG. 12

70. Figure 13 further shows “construction of probes on [a] bead containing encoding sequences, zipcodes, and a gene-specific sequence.” *Id.*, [0038].

**FIG. 13**

71. After the encoding sequences are decoded using labels, such as fluorescent labels, the location of the bead with a particular encoding sequence may be recorded, and the decoding reagents may be removed or destroyed so that the array can be used analytically. For example, “following a decoding step where the fluorescent signal of the dyes are detected, the fluorophore is destroyed by an appropriate chemical solution that degrades the fluorescent molecule sufficient[ly] leaving no trace of fluorescence.” *Id.*, [0146]. “In this way, beads are tagged with fluorescent labels, i.e., barcodes of sorts, their location or identity deciphered and recorded.” *Id.* “The beads are then exposed to the ‘fluorescence destroying solution’ and the fluorescence is eliminated, allowing an analytical assay to be conducted.” *Id.*

3. Sequence Barcoding

72. Multiplexing, which allows higher throughput analysis of many samples in parallel, was a key development for nucleic acid sequence analysis before

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the priority date. As such, a POSA would have been familiar with the concept of barcoding, including using nucleic acid sequences as barcodes to identify and differentiate between different nucleic acid molecules or sets of molecules. *See, e.g.*, EX1037 (Fodor 2009) 3:55-64 (“Methods are disclosed herein for digital counting of individual molecules of one or more targets. In preferred embodiments the targets are nucleic acids.... Targets are labeled so that individual occurrences of the same target are marked by attachment of a different label to different occurrences. The attachment of the label confers a separate, determinable identity to each occurrence of targets that may otherwise be indistinguishable. Preferably the labels are different sequences that tag or mark each target occurrence uniquely.”). *See also* EX1030 (“Multiplexed high-throughput pyrosequencing is currently limited in complexity (number of samples sequenced in parallel), and in capacity (number of sequences obtained per sample).... To overcome these limitations, we have devised a novel barcoding approach to allow for pooling and sequencing of DNA from independent samples, and to facilitate subsequent segregation of sequencing capacity. Forty-eight forward-reverse barcode pairs are described: each forward and each reverse barcode unique with respect to at least 4 nt positions.”).

73. Consistent with this knowledge, I note that in a prior proceeding involving the Fodor patent (EX1037 discussed above), 10x Genomics (Patent

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Owner's exclusive licensee and co-plaintiff in this matter) asserted the following with respect to nucleic acid barcoding:

The claims of the Fodor patents are directed to the abstract idea of labeling different objects with different labels. ***This concept is as old as the scientific method and is familiar to every student in any introductory science class*** – when performing an experiment, one needs a control and an experiment and will label them accordingly (whether that means written labels of “control” and “experiment” or “test 1” and “test 2” or “sample A” and “sample B” or some other form of labeling such as placing one in a green cup and one in a blue cup). Even outside the context of scientific experiments, using different labels to differentiate and distinguish between different instances of similar objects is commonplace. For example, different instances of the same model of television set are given different serial numbers for warrantee or recall purposes; people attending a convention are given different name tags; and different books in a library are given different classification numbers (under the Dewey Decimal System) and are assigned different International Standard Book Numbers (ISBNs) (by the Library of Congress). ***The Fodor patents did not invent this idea they simply take this abstract idea and attempt to limit the use of the idea to the particular technological environment of DNA (and other nucleic acids) by adding “attaching”, “amplifying”, and “detecting” steps and other similarly generic techniques that were well-known and conventional before 2009*** (the earliest provisional application).

EX1035, 4-5.

74. Examples of nucleic acid barcoding approaches specifically for correlating particular spatial locations in arrays (such as X,Y coordinates) with particular nucleic acid sequences (barcodes) were disclosed in numerous references before the priority date. For example, Barany 1999 (EX1036) describes a barcoding scheme for microarrays, which is used to direct target nucleic acids “to specific zip-

code addresses attached covalently to a three-dimensional gel-matrix array.”

EX1036, 251. This barcoding system, which the authors refer to as “zip-codes” defines particular spatial coordinate locations in an array based on unique nucleic acid sequences. For example, by using tetramer (4 nucleotide) bar codes, an array may be designed that uniquely barcodes up to 256 (4^4) separate locations according to, for example, a grid pattern.⁷

	TT	TC	TG	TA	CT	CC	CG	CA	GT	GC	GG	GA	AT	AC	AG	AA
TT																
TC																
TG																
TA																
CT																
CC																
CG																
CA																
GT																
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GG																
GA																
AT																
AC																
AG																
AA																

Figure 2. Design of tetramers for use in zip-code arrays. The checkerboard pattern shows all 256 possible tetramers. A given square represents the two bases on the left followed by the two bases on the top of the checkerboard. To be included, each tetramer must differ from all others by at least two bases, and be non-complementary. The chosen tetramers are shown in the white boxes, while their complements are listed as (number)'. Thus, as an example, the complementary sequences GACC (20) and GTGC (20') are mutually exclusive in this scheme. In addition, tetramers that are palindromic, e.g. TCGA (off-diagonal hatched boxes) or repetitive, e.g. CACA (hatched boxes on diagonal from upper left to lower right) have been eliminated. All other sequences which differ from the 36 tetramers by only one base are shaded in light gray. Four potential tetramers were not chosen as they are either all A·T or G·C bases (open boxes).

⁷ In Barany’s method, certain potential barcodes were excluded, such as those having tetramer repeats (e.g., AAAA or TTTT).

75. Each additional nucleotide added to a barcode exponentially increases the number of unique available barcodes. For example, using 5-mers as opposed to Barany's tetramers would yield 1,024 (4^5) potential barcodes, and using 10-mers would yield 1,048,576 (4^{10}) potential barcodes. *See also* EX1032, 16:10-17 ("Thus, for an array size of 1000, the decoding sequences must be 5 nucleotides long ($4^5=1024$) to ensure a unique decoding sequence for each candidate agent. In a preferred embodiment the decoding sequence contains from 1 to 1000 nucleotides....").

76. To take another example, Chee-584 (EX1032) describes barcoded bead arrays in which "a spatial or positional coding system is done." EX1032, 28:6-7. These systems allowed for further re-use of barcodes by positioning them on a given array in a manner similar to telephone area codes:

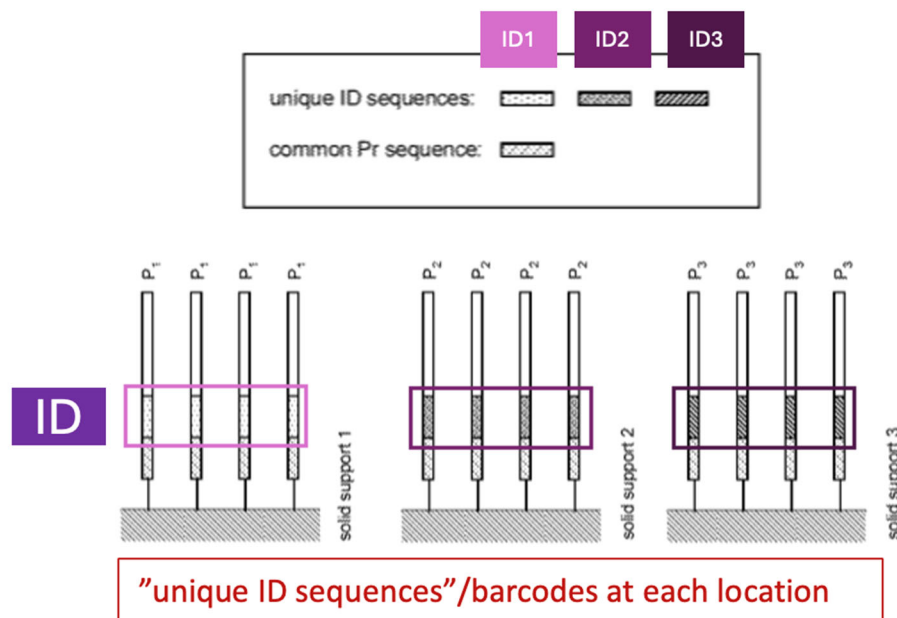
[S]ub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an "area code" that can have the same tags (i.e. telephone numbers) of other subarrays, that are separated by virtue of the location of the subarray. Thus, for example, the same unique tags can be reused from bundle to bundle. Thus, the use of 50 unique tags in combination with 100 different subarrays can form an array of 5000 different bioactive agents. In this embodiment, it becomes important to be able to identify one bundle from another; in general, this is done either manually or through the use of marker beads, i.e. beads containing unique tags for each subarray.

EX1032, 28:6-19; *see also* EX1038 (Chee-824), [0146] (“In this way, beads are tagged with fluorescent labels, i.e., barcodes of sorts, their location or identity deciphered and recorded.”).

77. Cantor, which I address further in Grounds 1 through 2 below, disclosed bead arrays in which each bead was labelled or barcoded with a unique “identification sequence” that differed from one bead to another, thereby distinguishing between different beads at different locations in its arrays. A specific example of Cantor’s disclosure “provides a substrate comprising a collection of beads oriented in an array.” EX1031, 4. Each bead “is in association with single-stranded nucleic acid.” *Id.* The bead-associated nucleic acids, which Cantor at times refers to as “the solid phase nucleic acid,” include “a probe sequence,” which “hybridizes to [a] sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid.” *Id.* The bead-associated nucleic acids also include “an identifier sequence.” *Id.* Cantor discloses that in its bead arrays, “each of the solid supports,” i.e., beads, “in the collection share a unique identifier sequence different than the identifier sequence of solid phase nucleic acid of the other solid supports,” i.e., beads. *Id.* In other words, each bead is identified with unique “identifier sequences.” Since each bead necessarily occupies a distinct physical location, and because each bead possesses an “identifier sequence” unique

to that bead alone, Cantor's identifier sequences, or barcodes, necessarily correspond with the spatial location of that bead in the array.

78. Cantor's Figure 1 is reproduced below, with annotations showing its use of unique "identification sequences" to identify each unique solid support (i.e., bead) in its arrays, differentiating solid supports at different locations from one another.



E. Overview of Relevant Spatial Analysis Technologies

79. By 2010, a POSA would have been familiar with several methods for analyzing biological molecules in tissue sections, including their spatial arrangement.

1. In Situ Hybridization

80. In situ hybridization (ISH) including fluorescence in situ hybridization (FISH) was a well-established technique by the priority date. The method works by hybridizing a probe molecule that contains a fluorescent tag to nucleic acid targets in fixed, permeabilized cells or tissues. The probe is bound to the target DNA or RNA molecule by targeting and hybridizing to target nucleic acid sequences. Free, unbound probes are then washed away to prevent background signal (e.g., fluorescence from probes that did not bind their target). Originally, this approach used radioactive labeling (simple in situ hybridization). By the late 1980s, the approach had moved to using fluorescently-labeled probes for improved performance by using fluorescence microscopy methods to localize the hybridized probe. By the early 2000s, researchers were using bright probes with multiple fluorescent dyes for single-molecule detection in cells, and combinations of different fluorophores to multiplex detection.

2. 2D-PCR

81. By 2009, a POSA would have been familiar with 2D-PCR techniques in which “[a] 2d map of [a gene] was created from a tissue section and shown to correlate with the spatial area of the sample.” EX1022 (Armani), 3526 (Abstract). These techniques were anticipated to “be easily adapted to assess the status of

multiple genes within tissue sections, yielding a molecular map that directly correlates with the histology of the sample,” thus “provid[ing] investigators with a new tool to interrogate the molecular heterogeneity of tissue specimens.” *Id.* This approach merges the power of spatial observation with the nucleic acid analysis methods described above.

82. Armani explained that “[v]ariations in DNA status (mutation, epigenetic modification, insertions, deletions) by location within a tissue section are of interest for elucidating their roles in biology and as clinical markers for diseases.” *Id.*, 3526. Thus, Armani noted a desire and motivation in the art to study nucleic acids in a manner that maintained the spatial organization of the tissue, in contrast to prior methods that “identify genes of interest from tissue specimens by examining cell homogenates (all the cells in the sample mixed together).” *Id.* The use of such cell homogenates “masks a tremendous amount of information” because the intact “tissue comprises a complex milieu of diverse cell populations that define the local microenvironments.” *Id.* Thus, Armani observed, techniques were necessary to correlate nucleic acid variability within a sample with “the physical location [of nucleic acids] within the tissue section.” *Id.* Armani successfully demonstrated its “method of mapping DNA as a step towards the more challenging task of mapping mRNA.” *Id.*, 3527. Armani subsequently demonstrated the feasibility of its approach

with mRNA. EX1021, 69-73 (Example 23) (“This experiment has been performed several times, and shows that it is possible to transfer a tissue vertically into an array of wells, simultaneous[ly] isolating and preserving (without cross-contamination between wells) the positions of the mRNA for subsequent analysis.”).

83. Armani’s approach, which the authors called “2D-PCR, makes use of a miniature array of wells together with protocols that allow tissue lysis, DNA amplification, and target detection within the same well.” EX1022, 3527. The authors demonstrated this technique “via the creation of a 2D detection map, showing the presence/absence of the target DNA.” *Id.* Such methodologies therefore “advance[] studies of gene expression as a function of the type of cell and its position within a tissue.” *Id.*

84. In Armani’s 2D-PCR method, “[a] tissue is sectioned and transferred into a multi-well array device.” *Id.* “The DNA is then extracted from the tissue and subsequently amplified by PCR in the same wells.” *Id.* “The PCR products can be detected either within the wells or after products are transferred onto one or more detection membranes.” *Id.* This is depicted in Figure 1.

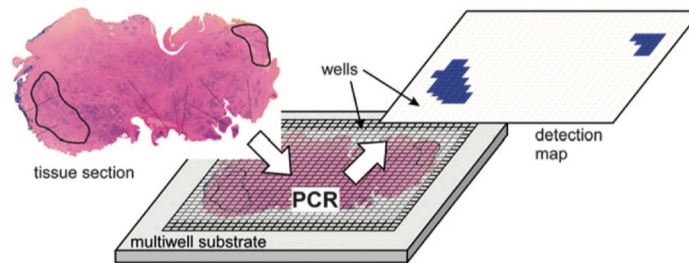


Fig. 1 2D-PCR is achieved by transferring a tissue section vertically into a multi-well array device, isolating tissue subregions. The DNA is extracted and then amplified by the polymerase chain reaction. A detection map is created by imaging the amplified DNA, for example using a DNA-intercalating dye.

85. I note that the Challenged Patents state with respect to Armani that, “[c]ertain PCR assays in a 2D format preserve spatial information [citing EX1022], 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. The Challenged Patents provide no support for these conclusory assertions, nor do they explain how to solve these purported problems with Armani’s methods. Indeed, the only examples set forth in the Challenged Patents are prophetic examples with no supporting data or results. *See* EX1005, 30:33-33:18. Moreover, the Challenged Claims do not preclude methods in which tissue is physically transferred into wells, nor do they recite a particular level of spatial resolution or multiplexing. *See generally* EX1004-1005, claims.

3. Array-Based In-Situ Reverse Transcription

86. A common feature among mature mRNAs that are ultimately translated to protein, especially in eukaryotes, is the presence of a poly(A) tail at the 3' end. EX1013, 501. The poly(A) tail is a series of "A" bases added to the end of an mRNA after it is transcribed. The poly(A) tail is sometimes referred to as polyadenylation. Thus, regardless of the identity of the gene, most mRNAs end with a poly(A) tail, which is typically ~250 "A" nucleotides in mammalian cells. EX1014, 22803.

87. Reverse transcription refers to a biological process by which mRNA is copied into DNA by enzymes called reverse transcriptases. The resulting DNA is referred to as complementary DNA, or cDNA. By the early 1990s, a POSA would have been aware of techniques for "performing cDNA synthesis *in situ*," for example on a tissue sample, "incorporating radioactivity into the cDNA, and examining the autoradiographic distribution of the resultant radiolabeled cDNA." EX1015, 81. This procedure was sometimes referred to as *in situ* transcription (IST), and "was originally used as an mRNA localization technique." *Id.*

88. In short, the IST process involved (1) "annealing a specific oligonucleotide primer to the mRNA in the tissue section" (2) "washing away the unhybridized primer"; and (3) "initiating cDNA synthesis by addition of reverse transcriptase and deoxynucleotide triphosphates." *Id.* This "allows radiolabeled

deoxynucleotides to be incorporated into the growing cDNA molecule in an anatomically defined region,” and subsequently visualized by autoradiographic exposure. *Id.* Those skilled in the art applied this technique for “several applications, including cellular localization of specific mRNAs,...expression profiling of mRNA populations from anatomically defined areas,...and analysis of gene expression in live cells.” *Id.*

89. Early methods of IST were “initiated by in situ hybridization of an oligonucleotide complementary to a specific mRNA, providing a primer that is required for the polymerase activity of reverse transcriptase.” EX1016, 1661. “This priming step allows one to select the individual mRNA to be transcribed,” and “[t]he enzyme then extends the primer with the mRNA acting as a template.” *Id.* This is shown in the figure reproduced below, which shows hybridization of “[a]n oligonucleotide 36 nucleotides (nt) in length, complementary to the sequence encoding amino acids 100 to 111 of rat POMC,” a particular protein encoded by a particular sequence, “as the primer.” *Id.*

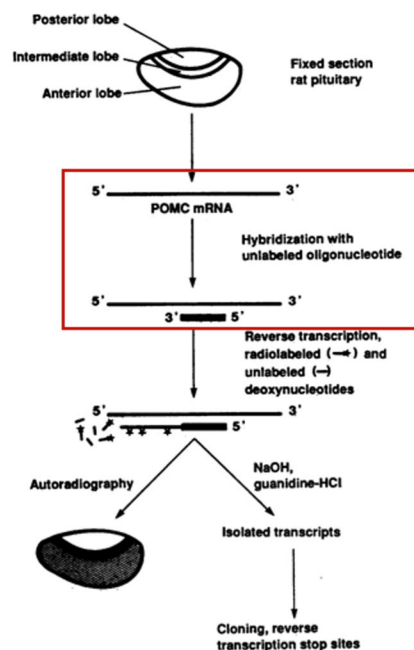


Fig. 1. Schematic outline of the in situ transcription (IST) procedure for the detection of POMC mRNA. An unlabeled oligonucleotide is hybridized to POMC mRNA, followed by reverse transcription within the tissue section in the presence of radiolabeled deoxynucleotides. Autoradiography is then performed for the localization of radiolabeled POMC cDNA. After autoradiography, the transcripts may be denatured and eluted by NaOH or guanidine-HCl treatments, and the transcripts may be either cloned or separated by electrophoresis for subsequent analysis.

90. Subsequently, techniques were developed in which poly-T sequences were used as primer sequences to initiate reverse transcription by hybridizing to the poly-A sequences on any given mRNA. In such techniques, the identity of the gene could then be determined by sequencing the cDNA strand generated by reverse transcription. *See* EX1017, 3-4 (detecting “polyA RNA sequences in permeabilized HeLa cells” using primers consisting of “oligo dT15,” i.e., TTTTTTTTTTTTTTTT, as well as oligo dT20 and oligo dT25). The use of oligo dT as a generic primer allows the capture and analysis of a generic class of molecules, namely polyadenylated mRNAs. EX1017, 1 (“In this study, we have developed an approach based on a

reverse transcription enabling highly specific, effective and fast detection of polyA RNA sequences *in situ*.”).

91. Below I discuss several exemplary references that discuss the use of *in situ* reverse transcription to capture, identify, and analyze the location of nucleic acids in tissue samples. Following reverse transcription of target nucleic acids, these techniques describe detecting and localizing the targets by different techniques, including visualization techniques (e.g., fluorescence) and sequencing techniques.

(a) *Detection of Reverse-Transcription Products on Spatial Arrays with Fluorescence*

92. Another related set of technologies involved the spatial analysis of gene expression using arrays of capture agents, including poly-T capture agents and gene-specific capture agents, to immobilize mRNA in two-dimensional space and detect its presence using fluorescent or other labeled probes. Representative references demonstrating such techniques include Shirai (EX1024), Yasuda (EX1025), and Southern (EX1032).

93. Shirai is a United States Patent that was filed on November 29, 2010. EX1024, (63). I understand that even if the challenged claims are determined to be entitled to a priority date of April 5, 2010 (as set forth in Sections VIII.C.1 & IX.C.1, below, they should not), Shirai is sufficiently contemporaneous to constitute appropriate evidence of what was known for indicating the level of ordinary skill in

the art, the motivations of a POSA, and how a POSA would have understood other prior art disclosures.

94. Shirai discloses methods of creating cDNA libraries from tissue sections using bead arrays that capture target nucleic acids while maintaining information about the spatial locations of those target nucleic acids in the tissue slice. For example, Shirai explains that it “provides a method and/or means for collecting and analyzing an individual cell in a tissue, and at the same time, quantitatively monitoring the expression levels of various genes *while keeping two-dimensional information in the tissue.*” EX1024, Abstract. Moreover, Shirai “provides a method comprising preparing a cDNA library from mRNA while keeping two-dimensional cellular distribution information and obtaining the gene expression levels at any site or all sites at a level of single cell.” *Id.*

95. In Example 4, Shirai describes providing a plurality of beads: “beads smaller than cells are arranged on a surface.” EX1024, 27:5-6. These beads comprise a plurality of binding agents: “[o]n the beads surface, poly-T probes are immobilized.” *Id.*, 27:6-7. The poly-T probes immobilized on the beads’ surface are configured to interact with at least one mRNA present in a tissue sample: “On the resultant structure,” that is, an array of poly-T coated beads, “a tissue slice is mounted” and the cells are permeabilized or lysed to release their mRNA, which is

then reverse transcribed into complementary cDNA molecules. *Id.*, 27:5-19 (“Cells were lysed in the same manner as above and mRNA molecules were electrophoretically trapped on the surface of beads and reverse-transcribed to obtain a cDNA library sheet.”). Thus, Shirai discloses a plurality of beads comprising binding agents that are configured to interact with nucleic acids (mRNA) present in the tissue slice.

96. The interaction between the poly-T functionalized beads (1901) arrayed on substrate (1903) and mRNA in the tissue (1101) slice is depicted in Figure 19. EX1024, Figure 19; 5:38-39 (“FIG. 19 shows an example of a method for preparing a cDNA library on beads.”); 27:5-32.

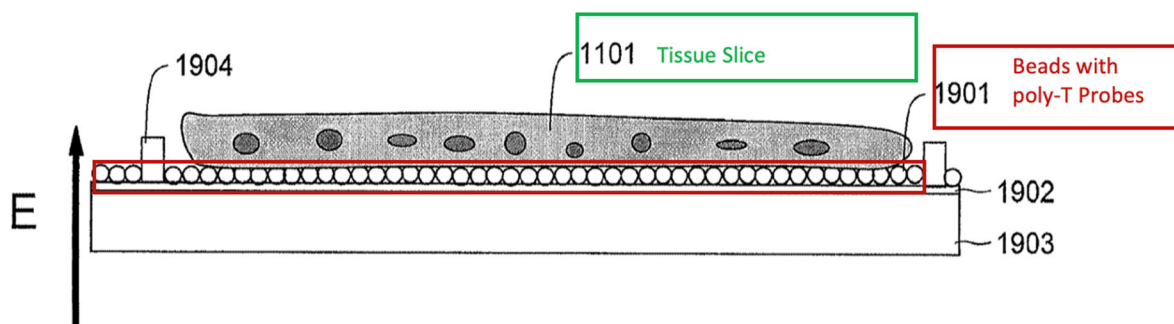


FIG. 19

97. Shirai further discloses a variation of the above method, in which the functionalized beads are arrayed in spatially defined microwells or chambers, each of which corresponds with a different spatial location of an overlaid tissue sample.

EX1024, 27:33-40. Specifically, Figure 20 shows an implementation in which “a number of beads are spread in the bottom of reaction cells in which cells are housed, and the same treatment,” i.e., the mRNA release and capture discussed in connection with Figure 19, “may be performed.”

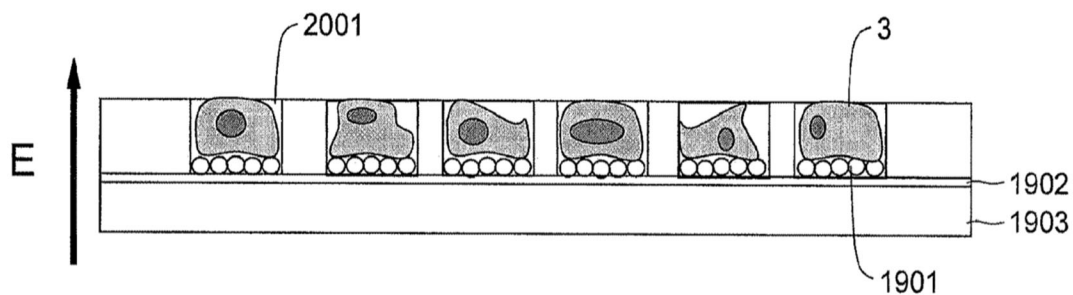


FIG. 20

98. One method that Shirai discloses for preserving spatial/location information regarding the presence or abundance of mRNAs in the tissue sample is by transferring the cDNA library (which contains nucleic acid complements to the mRNA from the tissue) to a sheet or membrane. EX1024, 27:15-25 (“Cells were lysed in the same manner as above and mRNA molecules were electrophoretically trapped on the surface of beads and reverse-transcribed to obtain a cDNA library sheet.”). Gene expression may then be detected based on, for example the use of fluorescent probes, and a spatial map may be generated. *See, e.g., id.*, 10:46-55 (“In the present invention, the results from the detection of gene expression in the two-

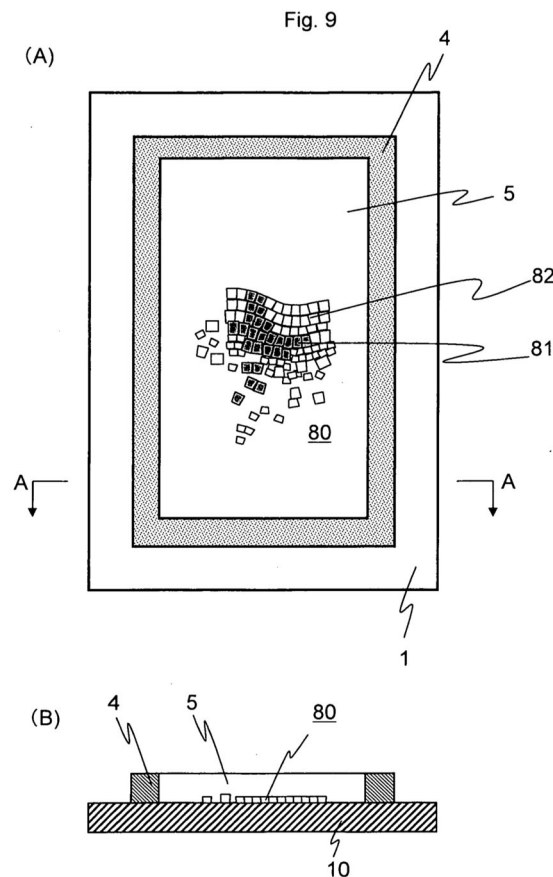
dimensional cDNA library obtained as mentioned above can be compared to two-dimensional positional information of a sample to obtain correlation data between the specific position in the sample and gene expression. Examples of such two-dimensional positional information of a sample include a microscopic image of a cell sample or a tissue section sample, and a fluorescent image or a chemiluminescent image obtained by other labeling methods.”); Figs. 24-26.

99. The disclosures of Yasuda are similar. Yasuda was published on December 9, 2009, and generally describes “a method, a chip device, and a system for quantitatively measuring intercellularly and intracellularly-localized mRNA and protein *without loss of local space information.*” EX1025, Abstract. Yasuda explains that “cell content is trapped atop a substrate or an electrode in the form of a two-dimensional projection of a cell,” and the cellular contents are released such that “mRNA is immobilized to a two-dimensional surface,” where it can be “identified with a labeled probe which hybridizes to the mRNA.”

100. Yasuda explains that by 2009, POSAs were motivated to identify and detect mRNA and proteins in tissue samples while maintaining the spatial orientation of such molecules. For example, Yasuda notes that “methods for the identification and detection of mRNA and proteins, which are the respective products of transcription and translation performed using a DNA probe chip or

protein chip” had been “thoroughly investigated,” and that such methods typically were “performed if a sample in the form of a solution can be prepared.” *Id.*, [0007]. For example, “[i]n mRNA testing, the RNA component is extracted in advance...reverse transcriptase is used to synthesize cDNA by means of a reverse transcription reaction, and amplification is performed by means of PCR amplification and cRNA synthesis.” *Id.* Yasuda observed that in the case of cell and tissue samples, “there are instances in which it is problematic to convert the cell component being measured into a solution state,” because the process of doing so would “cause[] localized information for each cell to be lost.” *Id.*, [0008]. Since “even within individual cells, mRNA, which is an expressed gene, is localized,” and “change[s] in accordance with a cell’s state of activity,” Yasuda noted the desirability of detecting such molecules while maintaining information about their spatial context. Thus, Yasuda described and established a POSA’s motivation to perform methods in which “mRNA and proteins localized between or inside cells” can “be quantitatively detected in a state in which local space information is retained,” enabling the generation of “information useful for medically-directed testing drug discovery, and to more accurately describe biological functions.” *Id.*, [0008].

101. For example, Yasuda's Figure 9 shows plan and cross-sectional views of "the cell component sorting chip of Example 3," which describes "an instance in which a tissue-like specimen of aggregated cells, for example, a histological section is immobilized to a two-dimensional surface while position information of an intracellular substance is retained." EX1025, [0072].



102. "[A] tissue-like specimen of aggregated cells is the target," and this specimen is applied to an array of "indexing probes in which 10 nm gold particles

are bonded to probes for an EpCAM^[8] mRNA sequence.” *Id.* These probes “function as a bonding means for trapping a biological substance,” and when “the biological substance is mRNA,” as in Example 3, such substances are those which “have[] a complementary sequence that can hybridize” to the target mRNA. *Id.*, [0014]. “[T]wo-dimensional position information and quantity can be measured at the cellular level of each biological substance by arranging multiple probes to the spots in each section as with a conventional DNA microarray.” *Id.*, [0031]. Thus, “cell position is maintained, and labeling probes are provided with appropriate identifiers that selectively bond to intracellular substances that are, in turn, bonded with affinity to a plurality of probes arranged in cell immobilization sections.” *Id.*

103. Similarly, Southern discloses the detection of target nucleic acids on arrays so that “[t]he spatial arrangement of the samples is maintained” and the location of “results of the analysis to be matched to individual samples.” EX1033, Abstract. “One analytical procedure that can be used with” Southern’s arrays “involves capture of mRNA by hybridisation to an immobilised capture DNA, followed by reverse transcription of the mRNA using the immobilised DNA as a primer.” *Id.*, 9:17-25. In this procedure, Southern discloses creating a

⁸ This refers to the gene for “Epithelial Cellular Adhesion Molecule.”

complementary cDNA by extending the probe, using the captured mRNA as a template, using in situ reverse transcription. Southern discloses using optical means, such as fluorescence, as the method for detecting the spatial location of an interaction between its binding agent and a target nucleic acid:

For RNA analytes, detection can be achieved by incorporating fluorescent nucleotides into a complementary strand using an enzyme such as reverse transcriptase (e.g. the avian myeloblastosis virus (AMV) reverse transcriptase). ***For example, cDNA may be made in situ by hybridising mRNA to oligonucleotide probes on a support, and using the immobilised probe as a primer. The reverse transcription reaction preferably incorporates labelled nucleotides into the cDNA in order to facilitate detection of the hybridisation*** [10]. This can be achieved by the use of dNTPs with suitable fluorophores attached. Unlike a sequencing reaction, it is not necessary to use different coloured fluorophores for different nucleotides, because individual nucleotides do not need to be distinguished. Similarly, there is no need to label every nucleotide, and so 1, 2, 3 or 4 of dATP, dCTP, dGTP and dTTP may be labelled, and a mixture of labelled and unlabelled dNTPs can be used. Incorporation of a large number of fluorophores into the cDNA (e.g. in at least 5% of incorporated dNTPs, such as $\geq 10\%$, $> 20\%$, $\geq 30\%$, $\geq 40\%$, $\geq 50\%$, $\geq 75\%$, or more) means that ***the cDNA can readily be detected by any of the familiar means of fluorescence detection, thus revealing a positive signal even for a single hybridisation event. Thus even low-abundance mRNAs can be detected.***

EX1030, 27:12-28. In addition to detection by fluorescence, Southern continues by noting that “[a]fter in situ reverse transcription has been performed, there is initially a RNA/DNA hybrid, wherein the DNA will typically include a label for detection.”

Id., 28:8-9. Southern observes that detection can include an “amplification reaction,”

Id., 28:19-22, and that “[a]mplification in situ of captured target analytes should

enable a wide range of useful applications” including “single molecule sequencing.”

Id., 39:25-28.

104. As explained further below, others expressly reported using sequencing as a readout for analyzing nucleic acids on spatially addressable arrays.

(b) Detection of Reverse-Transcription Products on Spatial Arrays with Sequencing

105. Other techniques utilize nucleic acid capture arrays and reverse transcription (or analogous techniques when the targeted nucleic acid is DNA) to analyze nucleic acid samples and use sequencing as the readout. Such techniques include those disclosed by Cantor (EX1031), which is discussed in more detail in the grounds of unpatentability presented below, and Frisen (EX1012).

106. Approaching the priority date, a POSA would have found using sequencing-based techniques as a readout for nucleic acid assays as an alternative for fluorescence-based detection methods to have been an obvious and natural substitution. First, sequencing methods provide a direct readout of the nucleic acid identity, without the use of probe hybridization, imaging, or microscopy to indirectly confirm the identity of a target sequence. Second, using the analogy presented by 10x Genomics regarding the Fodor patent, labelling target nucleic acids with fluorescence would have been akin to a “form of labeling such as placing one in a green cup and one in a blue cup,” whereas nucleic acid barcodes simply apply the

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concept of “serial numbers” to nucleic acids. EX1035, 4-5. The use of nucleotide “serial numbers” or “barcodes” allows the creation of an exponentially greater number of labels as compared to using colors like green or blue. As noted above, the number of unique barcodes that can be generated by barcodes consisting of only A/C/G/T is 4^n , where n is the number of nucleotides in each barcode. This allows the creation of large sets of unique barcodes or tags, thereby enabling higher degrees of multiplexing and throughput using the short read-length sequencing technologies available before April 2010. *See e.g.*, EX1030. As Chee-824 explains, “[d]ecoding by sequencing has the advantage that encoding a vast number of distinct codes” may be accomplished “by using only a small number of nucleotides.” EX1038, [0142].

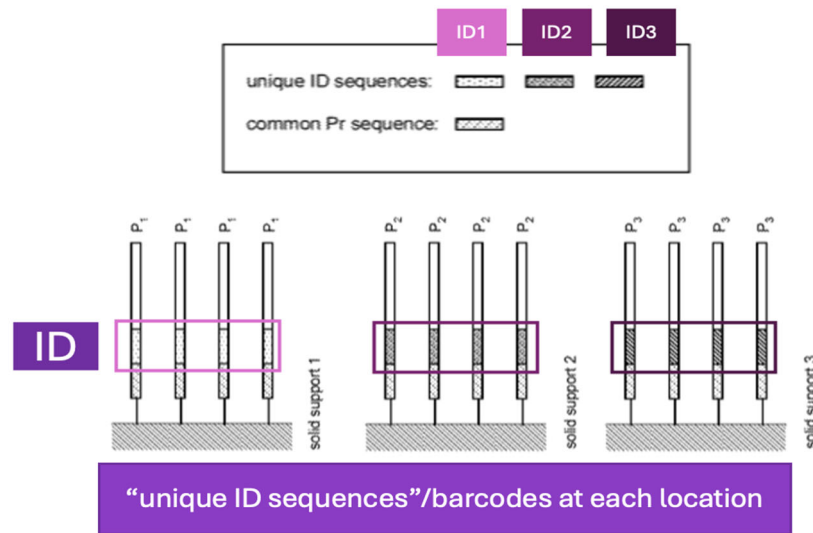
VI. Prior Art References of the Grounds

A. Cantor (Grounds 1-2)

107. Cantor, Patent Cooperation Treaty App. No. WO 2009/091934A1, titled “Single Molecule Nucleic Acid Sequence Analysis Processes and Compositions,” is an international patent application publication that was published on July 23, 2009. EX1031. Accordingly, Cantor is prior art to the ’030 and ’138 Patents.

108. As explained above, Cantor discloses bead arrays in which each bead is labelled or barcoded with a unique “identification sequence” that differs from

one solid support (e.g., bead) to another, thereby differentiating different beads at different locations in its arrays. This is depicted in the annotated version of Fig. 1:



109. A POSA would have recognized Cantor’s “identification sequences” as a type of sample barcoding, as Cantor disclosed associating each solid support, which necessarily occupies a distinct physical location on its arrays, with a unique “identification sequence” that therefore corresponds to a given support’s identity and location.

B. Armani (Ground 2)

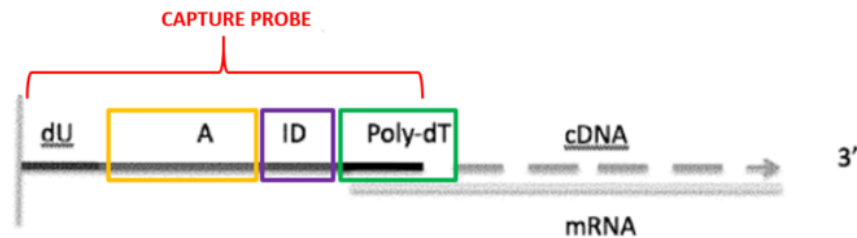
110. Armani (EX1022) is an article that was published in the journal *Lab on a Chip* on October 12, 2009. EX1022, 3526. Armani is therefore prior art to the ’030 and ’138 Patents. Armani discloses the 2D-PCR approach discussed in Section V.E.2, above. As discussed in detail below, Armani provides additional evidence of

a POSA's motivations to determine the spatial locations of nucleic acids in a tissue sample, and provides tissue preparation workflows that a POSA would have found obvious to combine with the nucleic acid analysis arrays disclosed by Cantor.

C. Frisen (Ground 3)

111. Frisen, Patent Cooperation Treaty App. No. WO 2014/060483 A1 is an international patent application publication that was filed in 2013 and published in 2014. EX1045, Fields (22) and (43). As explained below, the priority applications leading to the '138 and '030 Patents do not support the issued claims, so they are not entitled to a priority date before February 14, 2019. Therefore, Frisen is prior art to the '138 and '030 Patents.

112. Frisen describes “methods and products for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof.” *Id.*, Field (57). Frisen's products include an array of **capture probes** attached to a substrate, wherein each capture probe comprises “**a positional domain** that corresponds to the position of the capture probe on the array,” and “**a capture domain**” that binds to a nucleic acid. *Id.*, 10-11. The **capture probes** can also contain an **amplification domain** that has “a distinct sequence to which an amplification primer may hybridize.” *Id.*, 32. A capture probe with each of these domains is depicted below in Figure 6.



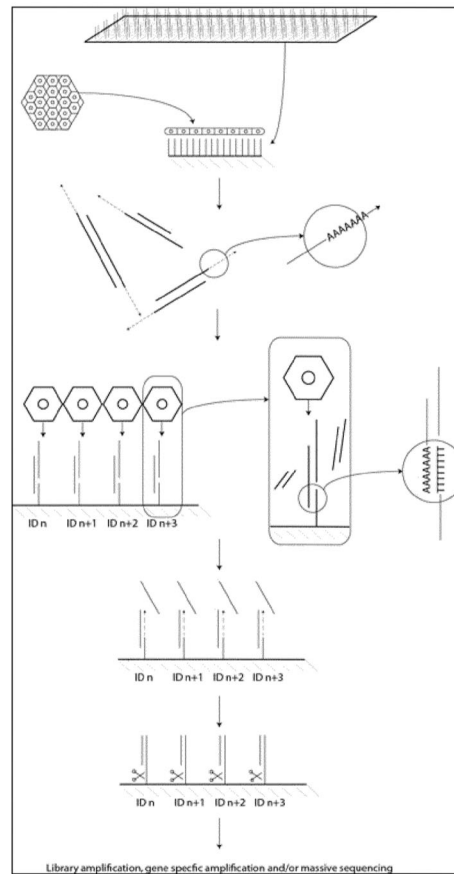
113. Frisen discloses contacting said array “with a tissue sample and allowing RNA of the tissue sample to hybridise to the capture probes,” and then using various techniques to analyze the identity and location of the captured nucleic acids. *Id.*, Abstract. This is depicted in Figure 8, which “shows a schematic illustration” of the “use of microarrays with immobilized DNA oligos (capture probes) carrying spatial labeling tag sequences (positional domains).” *Id.* Each position has “1) a unique labeling tag (positional domain) and 2) a capture sequence (capture domain).” *Id.*, 78-79; Figure 8 (depicting different ID for each spot on the array).

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114. I note that in a different case involving 10x Genomics (but not involving Petitioner or the Chee patents), 10x submitted a declaration from Dr. Paul T. Spellman which stated:

As early as 2011, Stahl *et al.* developed a pioneering spatially barcoded array paradigm for determining the locations of target mRNAs in a biological sample. The scientists disclosed this foundational technology in a patent application filed in 2011 (WO 2012/140224; Ex. 1017 [EX1020 in this proceeding]) and subsequently published it in a 2016 article in Stahl *et al.*, “Visualization and analysis of gene expression in tissue selections [sic: sections] by spatial transcriptomics,” *Science* 353(6294):78-82 (July 1, 2016), and associated supplementary material (“Stahl 2016”; Ex. 1016 [EX1019 in this proceeding]). This technology

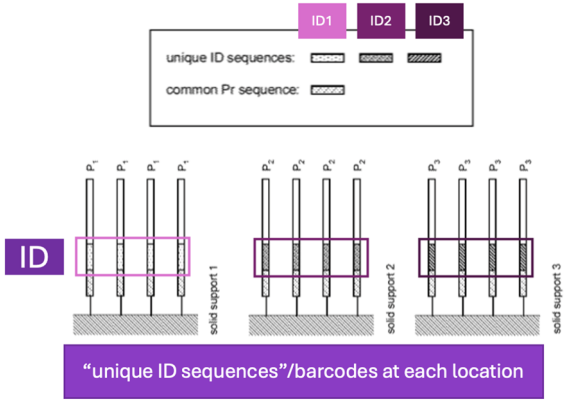
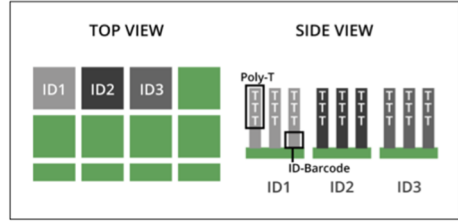
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was also applied in the spatial detection products by Spatial Transcriptomics, Inc. (ST).

EX1034, ¶82. I understand that EX1020 is another international patent application by Frisen that has similar disclosures to WO 2014/060483 A1 (EX1045)—the basis for Ground 3 in this proceeding.

115. I note that 10x’s characterization of the EX1020 as “pioneering” and “foundational” in proceedings not involving the Chee Patents is consistent with my analysis in Ground 3, which shows that the applications leading to the ’138 and ’030 patents do not provide written description support for the issued claims of the ’138 and ’030 Patents.

116. Moreover, despite 10x’s characterization of the Stahl/Frisen array designs in EX1020 as “pioneering” and “foundational” in other proceedings, it is also important to note that the design of the nucleic acid constructs attached to Cantor’s solid supports—which include an identification sequence and a probe sequence—is essentially identical to the ST “array paradigm” described in EX1020. The chart below compares the barcoded arrays disclosed by Cantor in 2009 with those disclosed by Stahl/Frisen in 2011 and 2016.

Cantor 2009 (EX1031) Fig. 1C (Annotated)	“ST Array” (Spellman Declaration, EX1034), ¶¶84-85
 “The solid supports in the collection share a unique identifier sequence different than the identifier sequence of the solid phase nucleic acid of the other solid supports.” EX1031, 2. “A probe [P_N] nucleotide sequence is of a length sufficient to hybridize to a sample nucleic acid nucleotide sequence.” EX1031, 13.	 EX1018, 3. “As depicted above, each spot (identified by a different “ID”) on the solid surface contains a cluster of array probes (vertical rectangular bars in the ST diagram[.]) comprising a unique ‘spatial barcode’ (‘ID’) having a nucleic acid sequence specific to that spot. All the array probes in one spot on the array share the same spatial barcode sequence, which is different from the spatial barcode sequence in the array probes for the other spots.” EX1034, ¶85 (citations omitted). The “Ts” boxed and annotated by Dr. Spellman and captioned “Poly-T” represent poly-T probes that are designed to hybridize to the poly-A tails present on any given mRNA in a sample.

VII. Claim Construction

117. In my analysis throughout this declaration, I have applied the plain and ordinary meaning of the terms of the '138 and '030 Patents, consistent with the way Patent Owner has read these terms in asserting infringement by Petitioner's products

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in co-pending district court litigation. I understand that, according to Patent Owner, no claim terms require construction.

118. I understand that in the litigation, the parties dispute whether a poly-T sequence on a capture probe constitutes a “target-nucleic acid binding domain” or “target-binding domain.” I understand that Patent Owner/Plaintiffs contend, and Petitioner disputes, that any mRNA molecule can be a “target nucleic acid,” and thus, a poly-T tail on a capture probe constitutes a “target-nucleic acid binding domain”/“target-binding domain” because it binds to the poly-A tail on mRNA. Because the prior art identified in each ground discloses gene-specific probes, in addition to probes that satisfy the broader implicit interpretation advanced by PO, the claims are obvious and/or anticipated regardless of which interpretation is applied.

VIII. Prior Art Anticipates and/or Renders Obvious the Challenged Claims of the '030 Patent

119. I have determined that the Challenged Claims are unpatentable on each of the grounds tabulated below:

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Ground	Basis	Priority Date Applied
1	Obviousness over Cantor in view of the knowledge of a POSA	April 5, 2010
2	Obviousness over Cantor in view of Armani and the knowledge of a POSA	April 5, 2010
3	Anticipation by Frisen	February 14, 2019

A. Ground 1: Obvious over Cantor in view of the Knowledge of a POSA

1. Claim 1

120. Claim 1 recites:

A composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes, wherein:

the plurality of beads are attached to the substrate;

each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain;

the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain; and

the plurality of beads was previously positioned on the substrate in a random pattern.

121. As described below, Cantor discloses and/or renders obvious in view of the knowledge of a POSA each limitation of Claim 1.

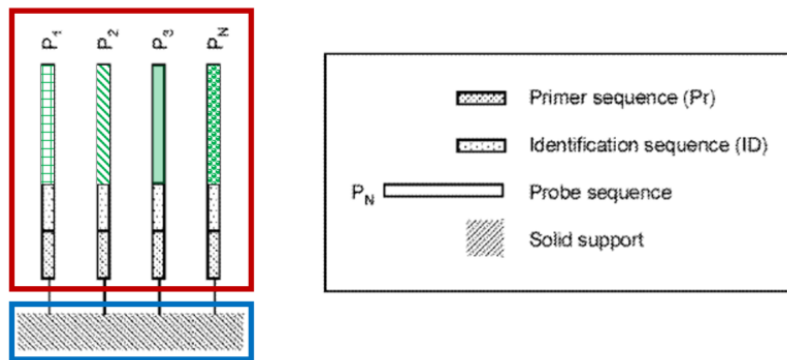
(a) ***“A composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes”***

122. Cantor discloses “[a] composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes.” Thus, to extent the preamble is limiting, it is disclosed by Cantor.

123. In short, Cantor discloses arrays comprising a substrate with a plurality of beads (*i.e.*, solid supports) that each include a capture probe. In detail, Cantor discloses “[i]mproved solid supports and methods for analyzing target nucleotide sequences.” EX1031, 1. A specific example of Cantor’s disclosure “provides ***a substrate*** comprising ***a collection of beads*** oriented in ***an array***.” *Id.*, 4. Each bead “is in association with [one or more] single-stranded nucleic acid.” *Id.* The bead-associated nucleic acids, which constitute the claimed capture probes, include “a probe sequence,” which “hybridizes to” (*i.e.*, captures) “a sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid.” *Id.*

124. Figure 1 below depicts examples of the single stranded nucleic acids (*i.e.*, the claimed “**capture probes**”). Each of the capture probes include a probe

sequence, which is a **target nucleic acid binding domain** that is configured to interact with a target nucleic acid. Figure 1 also shows a **solid support**, which may be a **bead**. EX1031, 10 (“Examples of solid supports for use with processes described herein include, without limitation, beads...”); *see also id.* at 4. I have annotated this figure to show the **capture probes**, **target nucleic acid binding domain**, and the **solid support (i.e., bead)**.



(b) “the plurality of beads are attached to the substrate”

125. Cantor discloses that “the plurality of beads are attached to the substrate” in the disclosed arrays. In particular, Cantor describes that solid supports (e.g., beads) can be associated with a substrate to form an array. As just one example, Cantor explains that “the invention in part provides a substrate comprising a collection of beads oriented in an array.” EX1031, 4. The “array may contain one solid support species or multiple solid support species from a collection. ***The array can be arranged on any substrate*** suitable for sequence analysis or manufacture

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processes described herein. Examples of substrates include without limitation flat substrates, filter substrates, wafer substrates, etched substrates, substrates having multiple wells or pits.” EX1031, 17-18. An exemplary embodiment is described in Cantor’s published claim 48:

48. A substrate comprising a collection of beads oriented in an array, wherein:
- each bead is in association with single-stranded nucleic acid;
 - the solid phase nucleic acid comprises an identifier sequence and a probe sequence;
 - the probe sequence hybridizes to sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid;
 - at least one nucleic acid of the solid phase nucleic acid of each of the solid supports in the collection has a unique probe sequence different than a probe sequence of the solid phase nucleic acid of the other solid supports; and
 - the solid phase nucleic acid of each of the solid supports in the collection share a unique identifier sequence different than the identifier sequence of solid phase nucleic acid of the other solid supports.

126. Cantor discloses the claimed “attaching” by disclosing orienting the beads on, and in contact with, the substrate. For example, the beads may be oriented in wells or pits on the substrate. EX1031, 18 (“Wells or pits of multiple well and pit substrates may contain one or more solid support units (e.g., each unit being a single bead or particle).”). In addition to physical attachment, a POSA would also immediately understand, including from references incorporated by reference by Cantor, that Cantor discloses chemically attaching its beads to the substrate through various methods known in the art. Indeed, Cantor incorporates by reference U.S. Patent No. 6,133,436, which is titled “Beads bound to a solid support and to nucleic

acids” and discloses methods to attach beads to a substrate. *See, e.g.*, EX1048, 2:56-57 (“FIG. 1 is a schematic showing the covalent attachment of a bead to a solid support and DNA to the bead.”); EX1031, 12 (“Methods for linking solution phase nucleic acid to a solid support also are known (e.g., U.S. Patent No. 6,133,436, naming Koster et al. and entitled ‘Beads bound to a solid support and to nucleic acids’ and WO 91/08307, naming Van Ness and entitled ‘Enhanced capture of target nucleic acid by the use of oligonucleotides covalently attached to polymers’). Examples include, without limitation, thioether linkages (e.g., thiolated nucleic acid); disulfide linkages (e.g., thiol beads, thiolated nucleic acid); amide linkages (e.g., Wang resin, amino-linked nucleic acid); acid labile linkages (e.g., glass beads, tritylated nucleic acid) and the like.”).

- (c) ***“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain”***

127. Cantor discloses each of the capture probes of the plurality of capture probes comprising a nucleic acid sequence comprising from the bead surface: (i) a

primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain.

128. For example, Cantor discloses that the “[p]robe, identification and primer hybridization sequences in solid phase nucleic acid can be arranged in any suitable orientation with respect to one another for performing the methods described herein,” and “[i]n certain embodiments, the sequences are contiguous and in the following orientation: 5' - (primer sequence) - (identification sequence) - (probe sequence) - 3'.” EX1031, 17. Furthermore, Cantor discloses that the solid-phase nucleic acid (*i.e.*, capture probe) can be attached to the solid support (*i.e.*, bead) at the 5' or 3' end. Ex. 1031, 12 (“Methods for sequentially synthesizing nucleic acid directly on a solid support are known. For example, the 3' end of nucleic acid can be linked to the solid support (e.g., phosphoramidite method described in Caruthers, Science 230: 281-286 (1985)) or the 5' end of the nucleic acid can be linked to the solid support[.]”). Thus, Cantor discloses capture probes with a nucleic acid sequences comprising from the bead surface a primer binding site, a nucleic

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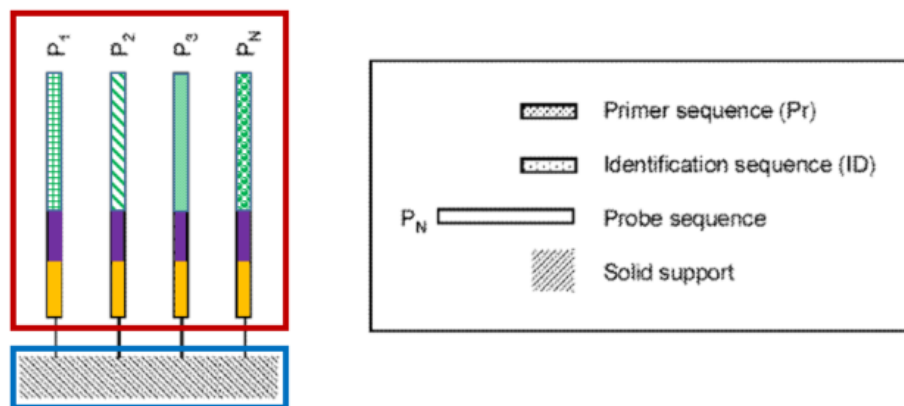
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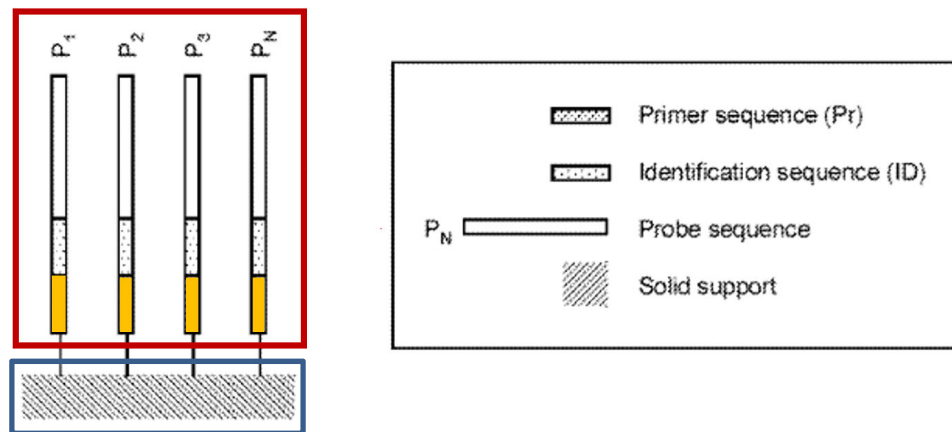
acid sequence that identifies a unique location on the substrate, and a target-binding domain.

129. Indeed, Cantor depicts this very structure in Figure 1. As explained in greater detail in the following sections, in Figure 1 (annotated below), each single-stranded nucleic acid (*i.e.*, **capture probe**) in Cantor's arrays comprise from the **solid support (*i.e.*, bead surface)**: (1) **a primer binding site**, (2) **a nucleic acid sequence that identifies a unique location on the substrate** and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (3) **a target nucleic acid binding domain**, wherein the target nucleic acid-binding domain comprises a sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain.



- (i) *“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site”*

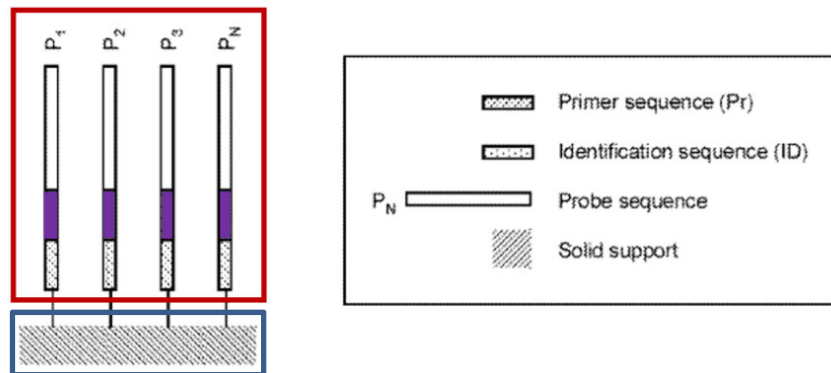
130. Cantor discloses that the single-stranded nucleic acid can “include[] a primer sequence (Pr), which is also referred to herein as a ‘primer hybridization sequence.’ *Primer sequence (Pr) can hybridize [i.e., bind] to a complementary nucleotide sequence in a primer nucleic acid* that can be utilized to amplify extended solid phase nucleic acid previously hybridized to a sample nucleic acid. As used herein, the term ‘primer nucleic acid’ refers to a nucleic acid (e.g., naturally occurring or synthetic) that has a nucleic acid sequence complimentary to a primer hybridization sequence, and can hybridize to the primer hybridization sequence under hybridization conditions and can be extended in an amplification process (e.g., primer extension, PCR amplification, and the like).” EX1031, 15. Exemplary **primer sequences** (i.e., **primer binding sites**) on **capture probes** are shown in Figure 1 below. As can be seen, the **primer binding site** is the first sequence from the **solid support (i.e., bead) surface**.



(ii) *“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface:... (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain”*

131. Each of the capture probes in Cantor’s arrays (e.g., the single-stranded nucleic acids depicted in Fig. 1) also includes a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain. In particular, Cantor discloses that in its arrays, “each of the solid supports,” *i.e.*, beads, “in the collection share a unique identifier sequence different than the identifier sequence of [the] solid phase nucleic acid of the other solid supports,” *i.e.*, beads. *Id.*, 2. In other words, each bead is coded with a unique nucleic acid “identifier sequence” that differs from the “identifier sequence” coding every other bead. Figure 1 below shows that for each **capture probe**, the **identifier sequence** is the second sequence from the **solid**

support, i.e., bead, on the **capture probes**. Since Figure 1 depicts a single bead, each capture probe on a given bead shares a common identifier sequence (*i.e.*, each identifier sequence is the same).

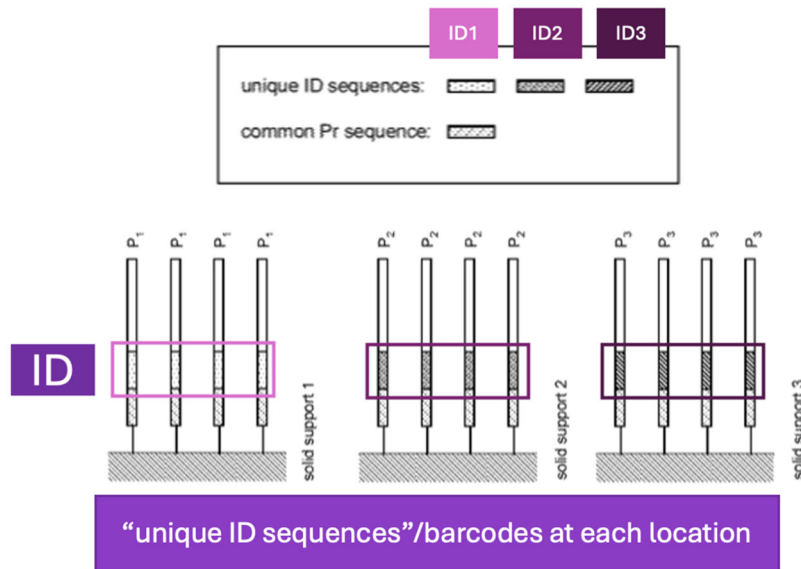


132. Additionally, Cantor explains that the disclosed “[s]olid supports may be provided in an array” including for example “an ‘X by Y array’ for square or rectangular arrays, where the array includes X number of sample locations or solid supports,” such as beads, “in one dimension and Y number of sample locations or solid supports,” such as beads, “in a perpendicular dimension.” EX1031, 17.

133. Thus, Cantor discloses an array of beads at which each bead is necessarily deposited at a particular (X, Y) coordinate location, and thus, each identification sequence necessarily corresponds to, and thus identifies, the unique location (such as an (X,Y) coordinate) of the bead on the array.

134. Indeed, this is shown in Cantor’s Figure 1C, which depicts separate solid supports, such as beads, each having “unique ID sequences” that are correlated

to the specific solid supports “solid support [e.g., bead] 1,” “solid support [e.g., bead] 2,” and “solid support [e.g. bead] 3.”



135. Thus, a POSA would understand that the “identification sequence” described in Cantor is a “nucleic acid sequence that identifies a unique location on the substrate.” For example, in the figure annotated above, ID1 identifies the unique location “solid support [e.g., bead] 1,” ID2 identifies the unique location “solid support [e.g., bead] 2,” and ID3 identifies the unique location “solid support [e.g., bead] 3.”

136. Cantor also discloses that the “nucleic acid sequence that identifies a unique location on the substrate” and “is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain.” Rather, as shown in

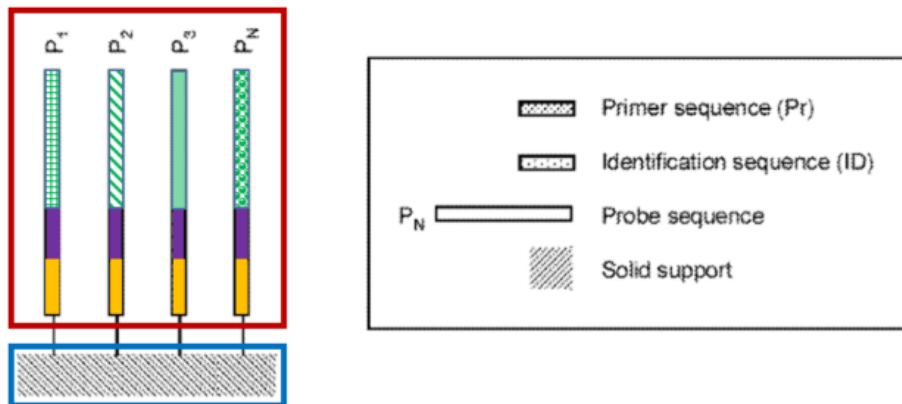
Figure 2, only the target nucleic acid binding domain is configured to bind to the target nucleic acid. See Section VIII.A.1(c)(iii) below.

(iii) “each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface:... (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain”

137. Cantor also discloses that each of the capture probes has a target nucleic acid-binding domain comprising a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain. As mentioned previously, Cantor discloses that the application is directed to “[i]mproved solid supports and methods for analyzing target nucleotide sequences.... Certain improvements are directed to 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.” EX1031, 1. In particular, Cantor discloses that the capture probes (*i.e.*, single-stranded nucleic acids) contain a sequence that is “complementary to or substantially complementary to a sample nucleic acid nucleotide subsequence and allows [the] solid phase nucleic acid to hybridize to [the] sample nucleic acid. Such

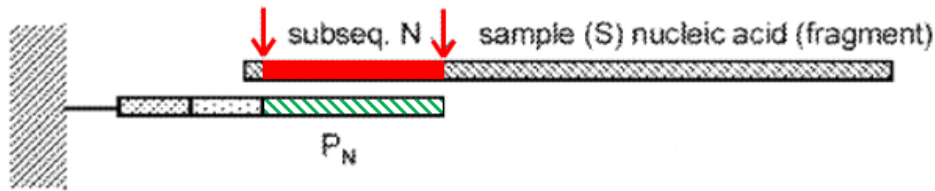
a subsequence (e.g., illustrated in Figures 1A-1C) is referred to herein as a ‘probe’ sequence.” *Id.*, 13.

138. Cantor’s “**probe sequence**” (*i.e.*, **target nucleic acid binding domain**) is shown below in Figure 1A. This sequence is the third sequence of the **capture probe** from the bead surface. As depicted below, starting from the **solid support** (*i.e.*, **bead**) surface, the three claimed subsequences of the capture probe are ordered (i) **primer binding site**; (ii) **identification sequence (ID)** (*i.e.*, the nucleic acid sequence that identifies a unique location on the substrate); (iii) **probe sequence**, exactly as claimed.



139. Cantor discloses that the target nucleic acid-binding domain is “a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain.” For example, Cantor’s “probe sequences” bind to target nucleic acids (including those from a sample such

as a tissue section) because they are “complementary to subsequences in the sample nucleic acid (e.g., probe sequence species P_1 , P_2 , P_3 ,... P_n are complementary to sample nucleic acid subsequences 1, 2, 3,... N , respectively).” EX1031, 16. Cantor explains: “*A probe nucleotide sequence is of a length sufficient to specifically hybridize to a sample nucleic acid nucleotide sequence.* In certain embodiments a probe sequence is about 5 to about 100 nucleotides in length, and sometimes is about 5 to about 40 nucleotides in length.” *Id.*, 14; *see also id.* (“Generally, a shorter probe sequence is selected for applications where the target nucleotide sequence is known or partially known and longer probe sequence are selected for applications in which the target nucleotide sequence or portions thereof are not known. In some embodiments, a probe sequence is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000 or 5000 nucleotides in length.”). This is further confirmed in Figure 2, which depicts the target nucleic acid binding domain hybridized to the target nucleic acid. Cantor explains that in Figure 2 “[s]ample nucleic acid molecule (S) may include a **nucleotide sequence (i.e., subseq.N)** that hybridizes to a **complementary probe sequence (P_N)** of the solid phase nucleic acid.” EX1031, 24. Cantor does not describe any other part of the single-stranded nucleic acid hybridizing (*i.e.*, binding) to the target nucleic acid.



(d) “the plurality of beads was previously positioned on the substrate in a random pattern”

140. Cantor in view of the knowledge of POSA renders obvious “the plurality of beads was positioned on the substrate in a random pattern.” Cantor discloses that “[t]he array can be arranged on any substrate suitable for sequence analysis or manufacture processes described herein,” thereby disclosing that its beads are necessarily positioned on the substrate. EX1031, 17-18.

141. Moreover, as described above, Cantor discloses each of the beads having an identification sequence that, for example, correlates the bead to a specific (X,Y) coordinate on the array. In view of this disclosure, 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 bead’s 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. *See* Section V.D.2.

142. Cantor discloses that its “identifier sequence is about 5[-]50 nucleotides in length.” EX1031, 15. In a selective loading procedure, a POSA would have found it obvious to provide beads having, for example, 5 nucleotide identifier sequences

that correspond to particular locations in Cantor's (X, Y) array. A POSA would have reasonably expected success in correlating such sequences with particular (X, Y) coordinates given the disclosure of success in using such systems in the prior work of the named inventor and others. *See* EX1032 (Chee-584), 7:30-33 (beads are spatially arranged in "a regular pattern of sites such that the sites may be addressed in the X-Y coordinate plane."); EX1038 (Chee-824), [0044] ("Once the identity of the bioactive agent and its location in the array has been fixed, the array is exposed to samples containing target analytes."); EX1036, 251 (Barany 1999) (describing 4 nucleotide "zip-code" sequences to define spatial locations on an array in a "checkerboard" (X, Y) array pattern).

143. For example, the below illustration shows an example of a 16 bead array with unique 5 nucleotide identifier sequences at each (X,Y) location. X coordinates are numbered 1-4, and Y coordinates are lettered A-D. In a selective loading technique, to correlate position A1 with the identification sequence AGTGA, a POSA would have found it obvious to deposit a bead (or beads) that was (or were) manufactured to include such sequences according to known methods at the A1 coordinate in the array. *See* EX1038, [0043] (describing "positional decoding" by "targeting the placement of the beads" to predetermined locations on

the array). A POSA would have found it obvious to target the placement of the other barcoded beads to the other locations according to similar methods.

	1	2	3	4
A	AGTGA	TGTGC	TCAAA	AGCTA
B	GTACG	TATCC	GCTTG	CTCGA
C	CCTTC	TTCGT	CTACC	TGGGC
D	TGAAT	CCTTA	CATTG	GATTA

144. While the illustration above uses 16 total positions, the use of 5 nucleotide barcodes allows uniquely addressing 1,024 unique positions, and each additional nucleotide added to a barcode would have been known to exponentially increase the number of unique available barcodes and thus the number of beads that can be uniquely addressed and loaded on an array.

145. Selectively loading the beads in a defined pattern becomes more difficult as more beads are added to the array. Thus, alternatively, “the beads may be randomly distributed on the array,” and “can later be ‘decoded’, i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or candidate agent at that particular site can be made.” EX1038, [0042]. A POSA would have been motivated to use such a random loading procedure to simplify and

expedite the array generation and fabrication process, and would have found it obvious to decode the array by using any of a number of known decoding procedures. For example, a POSA would have found it obvious to use the decoding by sequencing procedure disclosed in Chee-824, which allows simultaneous decoding of many bead types in a single experiment. *Id.*, [0046]. For example, Chee-824 discloses “decoding by sequencing” of bead arrays comprising probes structured similarly to those used in Cantor’s bead array, comprising an encoding sequence used to identify the particular bead as well as a gene-specific sequence that may be used to assay target nucleic acids. *Id.*, [0037]-[0038], Figs. 12-13. Moreover, the procedures disclosed in Chee-824 allow the array to be used in analytical assays without issue following the decoding procedure. *Id.*, [0146].

146. Thus, assuming that barcoded beads were randomly deposited on a substrate and landed in the positions illustrated in the table above, decoding by sequencing, such as the methods disclosed in Chee-824, would have been an obvious method for obtaining knowledge of the identification sequence at each location before use in subsequent assays. That is, using such methods, a POSA would have been motivated to decode the array to learn that position A1 had the identification sequence AGTGA, position B1 had the identification sequence GTACG, and so on. A POSA would have reasonably expected success in performing such decoding, as

doing so would have involved the mere application of well-known techniques, including those described in Chee-824.

147. Accordingly, Cantor in view of the knowledge of a POSA renders obvious this limitation.

(e) Conclusion on Claim 1

148. Accordingly, Cantor in view of the knowledge of a POSA renders obvious Claim 1.

2. Claim 3⁹

149. Claim 3 recites “[t]he composition of claim 1, wherein the plurality of capture probes are attached to the plurality of beads by their 5’ ends.”

150. Cantor discloses that the “[p]robe, identification and primer hybridization sequences in solid phase nucleic acid can be arranged in any suitable orientation with respect to one another for performing the methods described herein,” and “[i]n certain embodiments, the sequences are contiguous and in the following orientation: 5' - (primer sequence)- (identification sequence)- (probe sequence)- 3'.” EX1031, 17. Furthermore, Cantor discloses that the solid-phase nucleic acid (i.e., capture probe) can be attached to the solid support (i.e., bead) at

⁹ My analysis of each dependent claim throughout this declaration incorporates by reference my analysis of each parent claim.

the 5' or 3' end. EX1031, 12 (“Methods for sequentially synthesizing nucleic acid directly on a solid support are known. For example, the 3' end of nucleic acid can be linked to the solid support (e.g., phosphoramidite method described in Caruthers, Science 230: 281-286 (1985)) or the 5' end of the nucleic acid can be linked to the solid support[.]”).

151. Thus, Cantor discloses the following arrangement: 5' – (solid support, i.e., bead) – (primer binding site) – (nucleic acid sequence that identifies a unique location on the substrate) – (target nucleic acid binding domain) – 3'.

3. Claim 6

152. Claim 6 recites “[t]he composition of Claim 1, wherein each of the plurality of beads comprises at least 1,000 capture probes.”

153. Cantor discloses each of the plurality of beads (*i.e.*, solid supports) containing at least 1,000 capture probes (*i.e.*, nucleic acid molecules). For example, Cantor discloses that the “solid phase nucleic acid density per solid support unit [e.g., capture probes per bead] is about 5 nucleic acids to about 10,000 nucleic acids per solid support. The density of the solid phase nucleic acid per unit solid support in some embodiments is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 or 10000 nucleic acids per solid support.” EX1031,

13. Thus, Cantor discloses beads with “at least 1000 capture probes,” including up to at least 10,000 capture probes per bead.

4. Claim 10

154. Claim 10 recites “[t]he composition of claim 1, wherein the target nucleic acid is RNA.”

155. Cantor discloses the target nucleic acid being RNA. For example, Cantor discloses that the “[s]ample [i.e, target] nucleic acid may comprise or consist essentially of any type of nucleic acid suitable for use with processes of the invention, such as sample nucleic acid that can hybridize to solid phase nucleic acid (described hereafter), for example. A sample nucleic in certain embodiments can comprise or consist essentially of DNA (e.g., complementary DNA (cDNA), genomic DNA (gDNA) and the like), *RNA (e.g., message RNA (mRNA), short inhibitory RNA (siRNA), ribosomal RNA (rRNA), tRNA and the like), and/or DNA or RNA analogs* (e.g., containing base analogs, sugar analogs and/or a non-native backbone and the like).” EX1031, 6; *see also id.*, cl. 10 (“A method according to any one of claims 1-7, wherein the sample nucleic acid comprises RNA.”); *see also id.*, 5 (“Sample nucleic acid may be derived from one or more samples or sources. As used herein, ‘nucleic acid’ refers to polynucleotides such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).”).

5. Claim 11

156. Claim 11 recites “[t]he composition of claim 10, wherein the RNA is mRNA.”

157. As described above in relation to Claim 10, which is herein incorporated by reference, Cantor discloses the RNA being mRNA. For example, Cantor discloses that the “[s]ample [i.e, target] nucleic acid may comprise or consist essentially of any type of nucleic acid suitable for use with processes of the invention, such as sample nucleic acid that can hybridize to solid phase nucleic acid (described hereafter), for example. A sample nucleic in certain embodiments can comprise or consist essentially of DNA (e.g., complementary DNA (cDNA), genomic DNA (gDNA) and the like), *RNA (e.g., message RNA (mRNA)*, short inhibitory RNA (siRNA), ribosomal RNA (rRNA), tRNA and the like), and/or DNA or RNA analogs (e.g., containing base analogs, sugar analogs and/or a non-native backbone and the like).” EX1031, 6.

6. Claim 16

158. Claim 16 is reproduced below:

A composition comprising (i) an array comprising a substrate, a plurality of beads, and a plurality of capture probes, and (ii) a tissue section, wherein:

the tissue section is disposed on the array;

the plurality of beads are attached to the substrate;

each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain;

the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain; and

the plurality of beads was previously positioned on the substrate in a random pattern.

159. As described below, Cantor discloses each limitation of Claim 16.

(a) “A composition comprising (i) an array comprising a substrate, a plurality of beads, and a plurality of capture probes, and (ii) a tissue section”

160. As described above in Section VIII.A.1(a), Cantor discloses “[a] composition comprising (i) any array comprising a substrate, a plurality of beads, and a plurality of capture probes.” As described below, Cantor also discloses a tissue section.

161. In particular, Cantor explains that its capture probes interact with a “sample nucleic acid” by hybridization. EX1031, 2. (“[T]he solid support and sample nucleic acid are contacted in the mixture under conditions that allow hybridization of the solid phase nucleic acid,” i.e., the probe, “to the sample nucleic

acid.”). Cantor explains that this sample may be “a solid material *such as a tissue...or a biopsy*.” *Id.*, 6. A POSA would have immediately appreciated that a biopsy is a type of tissue section. *See, e.g.*, EX1005, 12:20-22 (“Exemplary biological samples include *tissue sections (e.g., including* whole animal sectioning and *tissue biopsies*), cell populations on slides or culture dishes, and the like.”). Thus, Cantor discloses a tissue section.

(b) “the tissue section is disposed on the array”

162. Cantor in view of the knowledge of a POSA renders obvious “the tissue section is disposed on the array.” Cantor explains that its sample may be “a solid material such as a tissue...or a biopsy,” EX1031, 6. The literature evidenced multiple reasons why a POSA would have been motivated to perform array-based nucleic acid analysis on a tissue sample (such as an intact tissue section or a biopsy sample), including because the analysis of such samples would provide information, such as spatial information, that could not be obtained through the analysis of, for example, a tissue homogenate:

Variations in DNA status (mutation, epigenetic modification, insertions, deletions) by location within a tissue section are of interest for elucidating their roles in biology and as clinical markers for diseases. However, to study the molecular basis of normal and pathological conditions, many researchers identify genes of interest from tissue specimens by examining cell homogenates (all the cells in the sample mixed together). Since tissue comprises a complex milieu of diverse cell populations that define the local microenvironments,

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Patent No. 11,761,030

combining the multiple cell populations into one measurement masks a tremendous amount of information. Therefore, to achieve a more complete view, DNA variability within a tissue sample needs to be correlated with the type of cell from which it originates (for example, stroma vs. epithelium), the physical location within the tissue section, and/or histopathological features (for example, a specific disease lesion).

EX1022 (Armani), 3526.

When handling cells, which constitute a large percentage of specimens, there are instances in which it is problematic to convert the cell component being measured to a solution state. Since balance of the whole is achieved through mutual interaction between a cell and adjacent cell groups in multi-cellular organisms, pulverizing cell groups will result in a uniform state, causing localized information for each cell to be lost. Further, even within individual cells, mRNA, which is an expressed gene, is localized. Proteins, which are the final product, are also localized within the cell in accordance with their function. These local mRNA and proteins change in accordance with a cell's state of activity, in other words the level of communication between a cell and the adjacent cell group, and the environment in which the cell is placed. Therefore, if an attempt is made to obtain information useful for medically-directed drug discovery, and to describe biological functions more accurately, the mRNA and proteins localized between or inside cells must be quantitatively detected in a state in which local space information is retained.

EX1026 (Okano), [0008]. Thus, a POSA would have been motivated to contact Cantor's array with a tissue section, yielding the claimed combination.

163. Additionally, Cantor explains that its capture probes interact with the target nucleic acid by contacting the nucleic acids with the array to allow for hybridization between the capture probes and the nucleic acids. EX1031, 2. (“[T]he

solid support and sample nucleic acid are contacted in the mixture under conditions that allow hybridization of the solid phase nucleic acid,” i.e., the probe, “to the sample nucleic acid.”). Thus, Cantor teaches that the tissue section would need to be contacted with (i.e., disposed on) the array.

164. Cantor further explains in its Example 1, “Sequence analysis methodology,” that “[s]ample nucleic acid may be prepared by any means commonly known in the art, including but not limited to cell lysis procedures using chemical, physical or electrolytic lysis methods.” *Id.*, 54. A POSA would have been familiar with, and would have been motivated to apply such techniques to intact tissue or biopsy samples such that the tissue or biopsy sample disposed on the array is then treated such that the capture probes can hybridize to the nucleic acids in the tissue sample.

165. Indeed, these techniques were frequently applied in the art. For example, Southern similarly discloses the spatial analysis of nucleic acid molecules by contacting tissue-derived nucleic acid molecules with a binding agent affixed to a solid support. EX1033, 16:28-30; 7:3-7. Southern discloses that, “[i]n some methods, *samples are applied directly to the support to generate a spatial arrangement of samples*, as illustrated in Fig. 1. Thus, *when the samples are biological samples comprising cells, the step a) of applying the samples to a*

support may comprise: (i) applying cells to the support; then (ii) releasing material from the cells.” Id., 2:17-27. A POSA would recognize that “releasing material from the cells” is synonymous with lysing the cells, as disclosed by Cantor. A POSA would similarly have been familiar with methods for releasing and capturing locally target nucleic acid from cells in a tissue sample disposed on an array, including a bead array, from the background references discussed in Section V.E, above. *See* EX1021-1022 (Armani) (physical pressing and chemical lysis of tissue samples to release target nucleic acid); EX1024 (Shirai) (use of electrophoretic force to lyse cells in tissue sample to locally release target nucleic acid for capture on probes attached to beads); EX1025 (Yasuda) (same); EX1026 (Okano) (same). Thus, a POSA would have been further motivated to dispose the tissue section of the array disclosed in Cantor and lyse the cells such that the probes can interact with the target nucleic acids within the tissue sample.

(c) “the plurality of beads are attached to the substrate”

166. Cantor discloses “the plurality of beads are attached to the substrate.” For example, see Section VIII.A.1(b) above, which is herein incorporated by reference.

- (d) ***“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain”***

167. Cantor discloses “each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain.” For example, see Section VIII.A.1(c), which is herein incorporated by reference.

(e) *the plurality of beads was previously positioned on the substrate in a random pattern*

168. Cantor in view of the knowledge of a POSA renders obvious “the plurality of beads was previously positioned on the substrate in a random pattern.”

See Section VIII.A.1(d), which is herein incorporated by reference.

(f) *Conclusion on Claim 16*

169. As outlined above, Claim 16 is rendered obvious by Cantor in view of the knowledge of a POSA.

7. Claim 18

170. Claim 18 recites “[t]he composition of claim 16, wherein the plurality of capture probes are attached to the plurality of beads by their 5’ ends.”

171. Cantor discloses “wherein the plurality of capture probes are attached to the plurality of beads by their 5’ end.” For example, see Section VIII.A.2 above, which is herein incorporated by reference.

8. Claim 21

172. Claim 21 recites “[t]he composition of claim 16, wherein each of the plurality of beads comprises at least 1,000 capture probes.”

173. Cantor discloses “where each of the plurality of beads comprises at least 1,000 capture probes.” For example, see Section VIII.A.3 above, which is herein incorporated by reference.

9. Claim 24

174. Claim 24 recites “[t]he composition of claim 16, wherein the tissue is a fresh frozen tissue section.” Cantor in view of the knowledge of a POSA renders obvious “wherein the tissue is a fresh frozen tissue section.”

175. As mentioned previously, Cantor explains that its target/sample nucleic acid may be “a solid material *such as a tissue...or a biopsy*.” EX1031, 6. Furthermore, Cantor explains in its Example 1, “Sequence analysis methodology,” that the “[s]ample nucleic acid may be prepared by any means commonly known in the art.” *Id.*, 53-54. As described in Section V.B., there were only a handful of methods of preparing a tissue sample known in the art prior to 2010, including freezing a fresh tissue section to create a fresh frozen tissue sample. Given the limited number of options, it would have been obvious to a POSA to try disposing a fresh-frozen tissue sample on the array disclosed in Cantor, particularly in light of Cantor’s disclosure to utilize a solid material such as a tissue or biopsy as a source of sample nucleic acid.

176. Moreover, a POSA would have been motivated to use a fresh-frozen tissue sample with the array disclosed in Cantor. In 2010, fresh-freezing tissue samples was a common method for preserving tissue samples in a laboratory and clinical setting. Given the prominence of fresh-frozen tissue samples in the field, a

POSA would have been motivated to try using a fresh frozen tissue sample with Cantor's array. Additionally, because fresh frozen tissue samples were known to have better RNA/nucleic acid quality than formalin-fixed paraffin embedded tissues, see EX1046,1229; EX1047, 1933, a POSA would have been motivated to use fresh-frozen samples because their high quality, highly intact nucleic acid content made them highly suitable for use with arrays designed to detect RNA/nucleic acids, like Cantor's.

10. Claim 25

177. Claim 25 recites "[t]he composition of claim 16, wherein the target nucleic acid is RNA."

178. Cantor discloses "wherein the target nucleic acid is RNA." For example, see Section VIII.A.4, which is herein incorporated by reference.

11. Claim 26

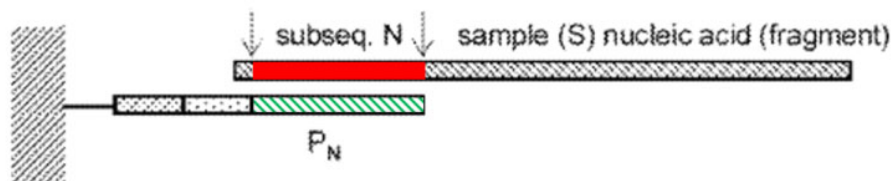
179. Claim 26 recites "[t]he composition of claim 25, wherein the RNA is mRNA."

180. Cantor discloses "wherein the RNA is mRNA." For example, see Section VIII.A.5 above, which is herein incorporated by reference.

12. Claim 30

181. Claim 30 recites “[t]he composition of claim 16, wherein the composition further comprises the target nucleic acid hybridized to a target nucleic-acid binding domain of at least one of the plurality of capture probes.”

182. Cantor discloses “wherein the composition further comprises the target nucleic acid hybridized to a target nucleic-acid binding domain of at least one of the plurality of capture probes.” For example, in Figure 2, Cantor depicts the target nucleic acid binding domain hybridized to the target nucleic acid. Cantor explains that in Figure 2 “[s]ample nucleic acid molecule (S) may include a **nucleotide sequence (i.e., subseq.N)** that hybridizes to a **complementary probe sequence (P_N)** [i.e., target nucleic acid binding domain] of the solid phase nucleic acid.” EX1031, 24.



B. Ground 2: Obviousness over Cantor in view of Armani and Knowledge of a POSA.

1. Claim 1 and Claim 16

183. I incorporate by reference my analysis in Ground 1 above, which outlines why the Challenged Claims are obvious over Cantor in view of the

knowledge of a POSA. Armani provides additional information rendering obvious disposing the tissue section on the array as recited by Claim 16 and the tissue being a fresh frozen tissue section as required by Claim 24. Nevertheless, all challenged claims are still rendered obvious for the reasons laid out in Ground 1 and said reasoning applies to this ground with equal force. Moreover, because Claim 16 recites the same features as claim 1 combined with “(ii) a tissue section, wherein: the tissue section is disposed on the array,” claim 1 is rendered obvious over Cantor in view of Armani and Knowledge of a POSA for the same reasons as claim 16. Thus, all challenged claims are rendered obvious under this ground as well.

184. Claim 16 is reproduced below.

A composition comprising (i) an array comprising a substrate, a plurality of beads, and a plurality of capture probes, and (ii) a tissue section, wherein:

the tissue section is disposed on the array;

the plurality of beads are attached to the substrate;

each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain;

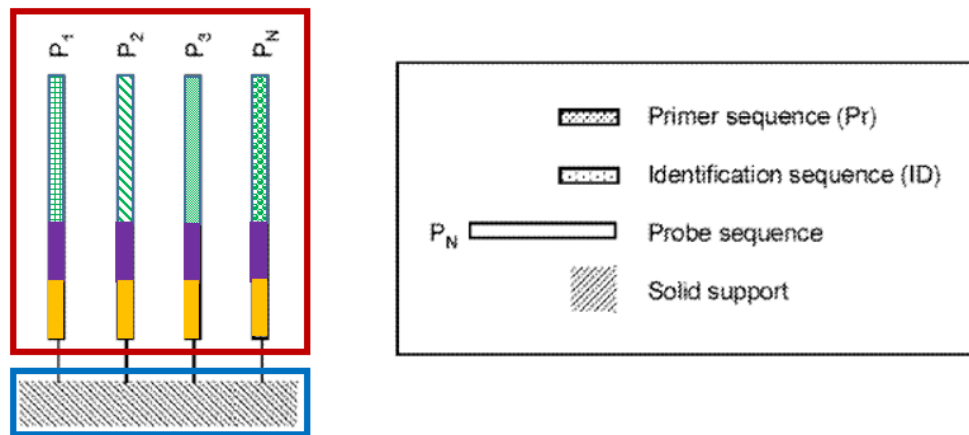
the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of

hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain; and

the plurality of beads was previously positioned on the substrate in a random pattern.

185. Cantor in view of Armani and the knowledge of a POSA further renders obvious the composition of claim 16 wherein “the tissue section is disposed on the array.”

186. As explained above, Cantor describes a device for detecting nucleic acids using nucleic acid arrays that capture, extend, amplify, and detect target nucleic acids with sequencing. In summary, Cantor discloses providing a substrate with a plurality of **beads (i.e., solid supports)** with the claimed “**capture probes**” in the form of nucleic acid constructs that include a “**probe sequence**” that is configured to hybridize with a target nucleic acid, as well as a “**coding identifier**” or “**identification sequence**” that may be used to identify a unique location on the substrate. Cantor’s Figure 1A shows an example of its **solid support, such as a bead surface**, with a plurality of “**capture probes**,” each having the same “**primer sequence**” and “**identification sequence**” and different “**probe sequences**.” I have annotated this figure to show the correspondence between Cantor’s Figure 1 and the requirements of the claimed “capture probes” below.



187. Cantor further explains that its “array can be arranged on any substrate suitable for sequence analysis or manufacture processes described herein,” including “substrates having multiple wells or pits,” with varying volumes ranging from the microliter to picoliter scale. EX1031, 17-18. Cantor explains that “[w]ells or pits of multiple well and pit substrates may contain one or more solid support units (e.g., each unit being a single bead or particle).” *Id.*, 18; *see also id.*, 19 (“In some embodiments, the microreactor volume is large enough to accommodate one solid support bead in the microreactor and small enough to exclude the presence of two or more beads in the microreactor.”). Thus, Cantor discloses providing a plurality of beads containing the claimed binding agents by arranging the beads in a multiple well plate. This arrangement of binding agents in wells also motivates combining the teachings of Cantor with the teachings of Armani, as discussed below.

188. Armani further discloses methods for the analysis of nucleic acids in a tissue section. In Armani's method, (1) "the tissue section is transferred onto the surface of the multi-well array (step 1A) and the pressed onto the surface (1B)," after which "[t]he contents of the well are dehydrated to enable the addition of lysis reagents (1C); (2) "the tissue is lysed to release genomic DNA into solution"; (3) the "DNA is amplified" using PCR; and (4) "the DNA is imaged" using fluorescent detection techniques. EX1022, 3527. This is depicted in Armani's Figure 2.

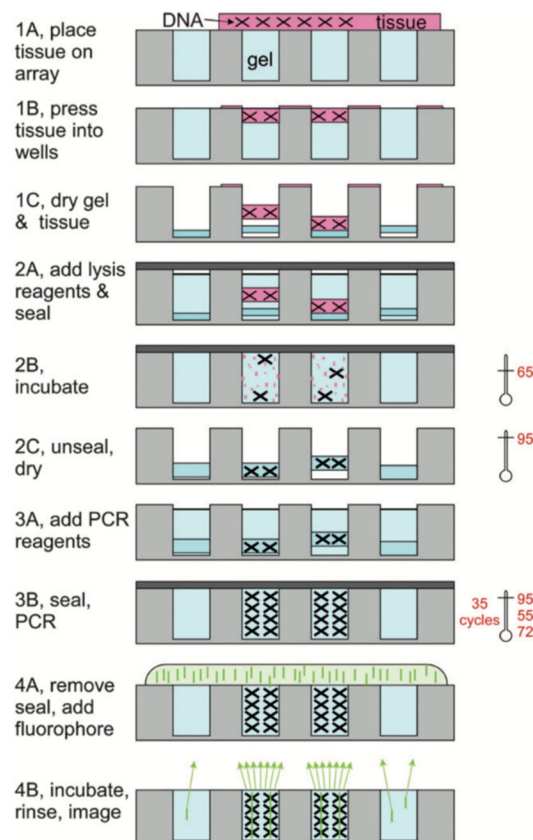


Fig. 2 Experimental protocol used to demonstrate the 2D-PCR concept. Tissue is transferred into wells, isolated, and lysed to extract the genomic DNA (represented by "x"s). PCR is performed to amplify the target DNA, and the targets are visualized with fluorescent dye (indicated by vertical lines "|").

189. A POSA would have found it obvious to combine the teachings of Cantor and Armani by providing Cantor's bead array with the plurality of beads disposed in wells (as noted above, an embodiment expressly contemplated by Cantor), contacting that array with a tissue slice as disclosed by Armani, and then pressing the tissue sample into the wells and lysing the tissue as described by Armani. EX1022, 3531 (describing "loading the tissue vertically, pressing it directly into the array of wells to isolate tissue subregions while preserving the overall 2D spatial relationship.... This tissue loading technique also has the advantage of beginning with traditional histological sectioning and transfer steps, which are familiar to investigators and clinicians who may wish to adopt this technique for research or diagnosis.").

190. A POSA reviewing Cantor and Armani would have been motivated to combine their teachings for several reasons. Cantor's protocols facilitate the capture, amplification, and detection of mRNA by sequencing, which Armani indicates was a desirable application for spatial analysis. By using sequencing as a detection technique, rather than direct visualization (e.g., fluorescence), the combination would allow the detection of even low abundance mRNAs. *See* EX1022, 3532 ("We are currently working on mapping RNA from tissue, since current visualization techniques for RNA cannot reliably detect below a few dozen copies per cell in

situ.”). Moreover, the use of Cantor’s methods to generate cDNA libraries of target nucleic acids coded with location information (via Cantors’ identification sequences) would simplify Armani’s detection procedure by eliminating at least the steps of unsealing the array, adding a fluorophore, incubating, rinsing, and imaging in Armani’s steps 4A-4B. Instead, using sequencing as a detection method would allow a POSA to simply pool the contents of all wells together in a multiplexed sequencing procedure, where the data can then be deconvoluted and spatially mapped based on the read out of location-correlated identification sequences.

191. A POSA would have reasonably expected success in combining the teachings of Cantor and Armani. Indeed, the combination would have involved using Cantor’s device and methods in the manner they are disclosed—to detect nucleic acids from a tissue sample, while specifically using the identification probes to identify the location at which beads were provided. Armani further provides additional specific implementations of how to prepare successfully and process effectively a nucleic acid tissue sample for localized detection, methods not claimed in the ’030 Patent, but the practice of which confirms the obviousness of the claimed arrangement.

2. Claim 24

192. Claim 24 recites “[t]he composition of claim 16, wherein the tissue is a fresh frozen tissue section.”

193. As mentioned previously, Cantor explains that its target/sample nucleic acid may be “a solid material *such as a tissue...or a biopsy.*” EX1031, 6. Furthermore, Cantor explains in its Example 1, “Sequence analysis methodology,” that “[s]ample nucleic acid may be prepared by any means commonly known in the art.” *Id.*, 53-54. A POSA would have been familiar with, and would have been motivated to apply such techniques to the tissue or biopsy samples disclosed by Cantor such that the tissue or biopsy sample could be disposed on the array and then treated such that the capture probes can hybridize to the nucleic acids in the tissue sample.

194. Armani describes an apparatus for the analysis of nucleic acids in a frozen tissue section. In particular, Armani discloses placing a fresh-frozen tissue section on a multi-well array as shown below to detect the presence of a target nucleic acid at various location throughout the tissue sample. EX1022, 3528 (“Two types of tissue were used in these studies: frozen tissue samples from a human prostate with benign hyperplasia and breast tissue with lobular carcinoma....Tissue specimens were snap frozen and embedded in O.C.T. compound embedding medium

(Sakura Fine-Tek, Torrance, CA).”); *id.* (“Tissue sections were transferred to the multi-well device immediately after sectioning while still frozen[.]”). After “the tissue section is transferred onto the surface of the multi-well array (step 1A),” (1) the tissue section is “pressed onto the surface (1B),” (2) “[t]he contents of the well are dehydrated to enable the addition of lysis reagents (1C); (3) “the tissue is lysed to release genomic DNA into solution”; (4) the “DNA is amplified” using PCR; and (5) “the DNA is imaged” using fluorescent detection techniques. *Id.*, 3527. This is depicted in Armani’s Figure 2.

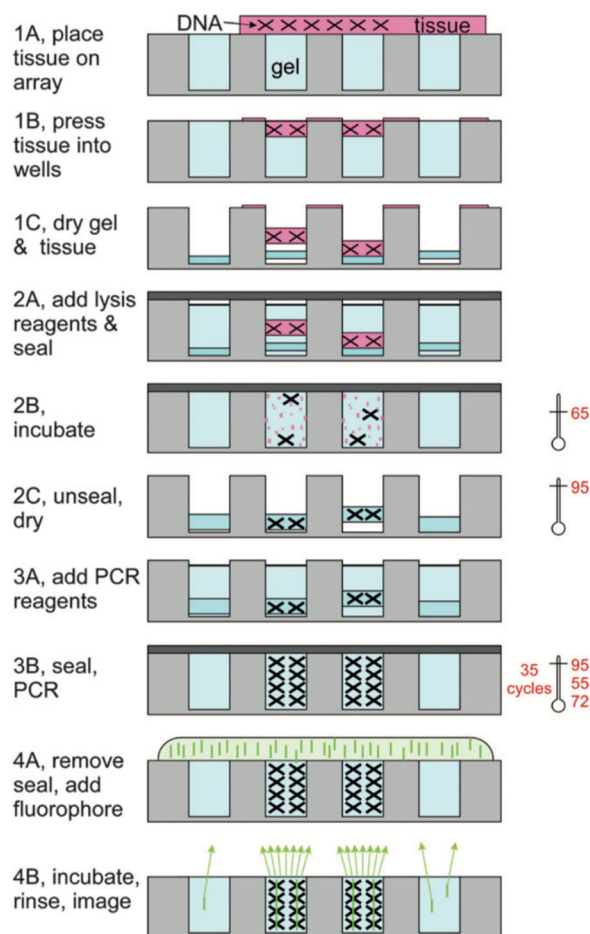


Fig. 2 Experimental protocol used to demonstrate the 2D-PCR concept. Tissue is transferred into wells, isolated, and lysed to extract the genomic DNA (represented by “x”s). PCR is performed to amplify the target DNA, and the targets are visualized with fluorescent dye (indicated by vertical lines “|”).

195. A POSA would have found it obvious to combine the teachings of Cantor and Armani by providing Cantor’s bead array with the plurality of beads disposed in wells (as noted above, an embodiment expressly contemplated by Cantor), contacting that array with a tissue slice as disclosed by both Cantor and Armani, and then pressing the tissue sample into the wells and lysing the tissue as described by Armani. EX1022, 3531 (describing “loading the tissue vertically,

pressing it directly into the array of wells to isolate tissue subregions while preserving the overall 2D spatial relationship.... This tissue loading technique also has the advantage of beginning with traditional histological sectioning and transfer steps, which are familiar to investigators and clinicians who may wish to adopt this technique for research or diagnosis.”).

196. A POSA reviewing Cantor and Armani would have been motivated to combine their teachings for several reasons. Cantor’s protocols facilitate the capture, amplification, and detection of mRNA by sequencing, which Armani indicates was a desirable application for spatial analysis. By using sequencing as a detection technique, rather than visualization (e.g., fluorescence), the combination would allow the detection of even low abundance mRNAs. *See* EX1022, 3532 (“We are currently working on mapping RNA from tissue, since current visualization techniques for RNA cannot reliably detect below a few dozen copies per cell in situ.”). Moreover, the use of Cantor’s methods to generate cDNA libraries of target nucleic acids coded with location information (via Cantors’ identification sequences) would simplify Armani’s detection procedure by eliminating at least the steps of unsealing the array, adding a fluorophore, incubating, rinsing, and imaging in Armani’s steps 4A-4B. Instead, using sequencing as a detection method would allow a POSA to simply pool the contents of all wells together in a multiplexed

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sequencing procedure, where the data can then be deconvoluted and spatially mapped based on the read out of location-correlated identification sequences.

197. A POSA would have reasonably expected success in combining the teachings of Cantor and Armani. Indeed, the combination would have involved using Cantor's device and methods in the manner they are disclosed—to detect nucleic acids from a tissue sample, while specifically using the identification probes to identify the location at which beads were provided. Armani further provides additional specific implementations of how to successfully prepare and process effectively a nucleic acid tissue sample for localized detection.

C. Ground 3: Anticipation by Frisen

1. Priority Date

198. The '030 Patent issued from Application No. 18/074,992 ("the '922 Application"), which was filed on December 5, 2022. I understand that the '030 Patent/'922 Application purports to claim priority to the following applications:

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Related U.S. Application Data

- (63) Continuation of application No. 17/850,538, filed on Jun. 27, 2022, now Pat. No. 11,519,022, which is a continuation of application No. 17/480,914, filed on Sep. 21, 2021, now Pat. No. 11,371,086, which is a continuation of application No. 17/321,090, filed on May 14, 2021, now Pat. No. 11,156,603, which is a continuation of application No. 17/145,210, filed on Jan. 8, 2021, now Pat. No. 11,067,567, which is a continuation of application No. 16/669,246, filed on Oct. 30, 2019, now Pat. No. 10,982,268, which is a continuation of application No. 16/437,637, filed on Jun. 11, 2019, now Pat. No. 10,494,667, which is a continuation of application No. 16/430,015, filed on Jun. 3, 2019, now abandoned, which is a continuation of application No. 16/414,213, filed on May 16, 2019, now Pat. No. 10,787,701, which is a continuation of application No. 16/402,098, filed on May 2, 2019, now Pat. No. 10,472,669, which is a continuation of application No. 16/276,235, filed on Feb. 14, 2019, now Pat. No. 10,480,022, which is a continuation of application No. 15/187,661, filed on Jun. 20, 2016, now Pat. No. 10,308,982, which is a continuation of application No. 13/080,616, filed on Apr. 5, 2011, now Pat. No. 9,371,598.
- (60) Provisional application No. 61/321,124, filed on Apr. 5, 2010.

EX1005, Codes (63), (60). These purported priority documents are tabulated below.

Because the '661 Application (filed on Jun. 20, 2016), '616 Application (filed on Apr. 5, 2011), and '124 Application (filed on Apr. 5, 2010) do not provide written description support for the challenged claims, the priority date for the '030 Patent is no earlier than the February 14, 2019 filing date of the '235 Application.¹⁰

¹⁰ The '235 Application fails to support the challenged claims for the same reasons discussed herein. However, I understand that for purposes of determining the priority date to which the Challenged Claims are entitled, Frisen is eligible as prior art based on the break in the chain of priority at the '661 Application. Because Frisen is eligible as prior art based on this analysis, I have not formed an opinion as to whether any subsequently filed application supports the challenged claims, and reserve the right to perform that analysis if raised by Patent Owner.

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Accordingly, Frisen, which was published on April 24, 2014, is prior art to the '030 Patent. EX1045, Code (43).

'030 Patent Priority Documents	Filing Date	Exhibit
17/850,538 (the "'538 Application")	June 27, 2022	EX1049
17/480,914 (the "'914 Application")	September 21, 2021	EX1050
17/321,090 (the "'090 Application")	May 14, 2021	EX1051
17/145,210 (the "'210 Application")	January 8, 2021	EX1052
16/669,246 (the "'246 Application")	October 30, 2019	EX1053
16/437,637 (the "'637 Application")	June 11, 2019	EX1054
16/430,015 (the "'015 Application")	June 3, 2019	EX1055
16/414,213 (the "'213 Application")	May 16, 2019	EX1056
16/402,098 (the "'098 Application")	May 2, 2019	EX1057
16/276,235 (the "'235 Application")	February 14, 2019	EX1010
15/187,661 (the "'661 Application")	June 20, 2016	EX1011
13/080,616 (the "'616 Application")	April 5, 2011	EX1012
61/321,124 (the "'124 Provisional")	April 5, 2010	EX1009

199. Based on my review, and as discussed in detail below, the challenged claims of the '030 Patent are not entitled to a priority date earlier than February 14,

2019, the filing date of the '235 Application. In my opinion, the challenged claims of the '030 patent are not supported by sufficient written description in the Specification of the '661 Application, the next priority application in the chain, such that a POSA would have understood the inventor to be in possession of the subject matter recited in the challenged claims. Accordingly, I understand that since the '661 Application does not support the challenged claims, they are not entitled to a priority date earlier than the February 14, 2019 filing date of the '235 Application.

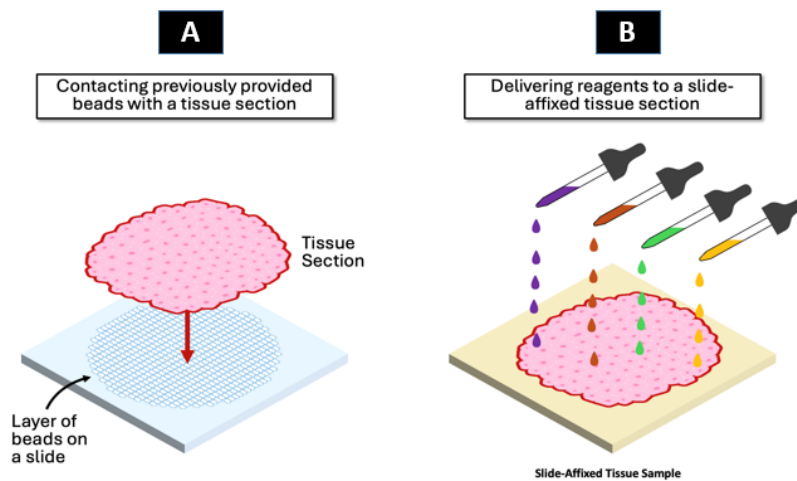
200. As I explain below, the '661 Application fails to disclose the subject matter recited in the claims of the '030 patent. The application preceding the '661 Application—'616 Application—shares the same specification as the '661 application, and thus fails to provide adequate description of the subject matter recited in the challenged claims for the same reasons. Finally, and as discussed further below, the passages of the '124 Provisional that were omitted from the '661 and '616 Applications also fail to describe the subject matter of the challenged claims.

(a) *The '661 Application discloses delivering probes to a fixed tissue sample; not delivering a tissue sample to a previously generated array of bead-affixed probes.*

201. Claims 1 and 16 of the '030 Patent recite “[a] composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture

probes” wherein “the plurality of beads are attached to the substrate.” Additionally, Claim 16 recites wherein “the tissue section is disposed on the array.” In my opinion, the ’661 Application (including the ’124 Provisional) fails to disclose these limitations.¹¹ The claims as drafted and as read by Patent Owner to assert infringement require an array of beads attached to a substrate, where each bead comprises a capture probe that binds a target nucleic acid, as shown in Figure A below. Claim 16 as read by Patent Owner to assert infringement then recites disposing a tissue on top of this bead array, as depicted in Figure A below. But the ’661 Application focuses exclusively on providing a tissue sample affixed to a solid support, and then delivering reagents, such as capture probes/binding agents, to the top of the support-affixed tissue sample according to a spatial pattern, as depicted in Figure B below.

¹¹ As discussed further below, the ’661 Application contains no disclosures relating to beads. Accordingly, I also discuss certain disclosures of the ’124 Provisional purportedly incorporated by reference into the ’661 Application.



In fact, the specification emphasizes that the novelty of the “invention” lies in the delivery instrumentation used in the system of Figure B. “The assay system further provides instrumentation with an ability to deliver reagents in a spatially-defined pattern. This instrumentation, together with software, reagents and protocols, provides a key component of the highly innovative assay system of the invention....” EX1005, 9:6-10. “In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology.” *Id.*, 28:40-42. The claims of the ’030 patent do not cover the allegedly “highly innovative assay system of the invention” as depicted in Figure B.

202. The differences in these methodologies are not trivial, and the implementation of each requires different sophisticated laboratory instrumentation, techniques, and surface chemistries. For example, in the methods described in the

'030 Patent and its priority applications, probes are delivered to the tissue in solution, as illustrated in Figure B. Such techniques require, as discussed below, precise liquid handling and dispensing techniques. And because the probes are provided in solution, the disclosed embodiments repeatedly include a step of “washing” or separating encoded probes that did not bind to a target away from the sample. EX1011, Figs. 1-2; [0028] (“Once encoded probes interact with the biological targets, the encoded probes that interacted with the biological targets must be separated from the encoded probes that did not interact with the biological targets at step 140. In the case where the biological targets are nucleic acids and the encoded probes are oligonucleotides, the separation can be accomplished by, e.g., washing the unhybridized encoded probes from the sample.”). The removal of unhybridized probes is important in the Figure B approach, because if such probes are not washed away, they contribute background “noise” to the readout signal, such as sequencing, making it difficult to detect the interactions of interest. By contrast, the claims as read by patent owner to assert infringement against Petitioner purport to encompass probes provided in a solid phase. Such techniques require fabrication technologies and chemistries to create beads or other surfaces with the nucleic acids (such as probes) affixed to the desired locations. A step of separating or washing encoded probes that did not bind to a target would not make sense in the context of these bead

chemistries, because each bead would typically consist of multiple probes, some of which would bind a sample nucleic acid and some would not. But because such probes are covalently bound to the beads, it would be extremely difficult and unfeasible to remove unhybridized probes from the array-sample combination without also disrupting the interactions between hybridized bead-affixed probes and the sample. Moreover, such a washing step could displace beads spatially with respect to the sample, impeding the array's function and the accuracy of its readouts.

203. Moreover, the '030 Patent criticizes Armani, which provides a variation of the Figure A approach, stating that its reliance on the transfer of tissue into wells, "prevents random access to tissue samples and prevents high levels of multiplexing." *See* EX1005, 2:9-15. To the extent that the disclosures of the '030 Patent (but not its claims) purport to address these alleged deficiencies, it is through the delivery of reagents from above the tissue sample. EX1005, 16:28-30 ("Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto* the biological sample.").

204. A POSA reviewing the '661 Application (including the '124 Provisional) would not have understood it to demonstrate that the inventor possessed methods of contacting a pre-existing array or arrangement of binding agents, such as capture probes, with a tissue sample, whether affixed to beads or otherwise.

Instead, a POSA would have understood the disclosure of the '124 Provisional to have been limited to the delivery of reagents, including binding agents and encoded probes, to a tissue sample already affixed to the solid support.

205. For example, the **SUMMARY OF THE INVENTION** explains that “[t]he assay system comprises an assay capable of high levels of multiplexing where encoded probes *are provided to a biological sample* in a defined spatial patterns....” EX1011 ('661 Application), [0007].¹²

206. The first “embodiment” disclosed is one in which “the assay system performs the following steps: *providing a sample affixed to a support; delivering encoded probes* for the multiple biological targets to the multiple sites....” *Id.*, [0008]. The next “embodiment” is one in which “there is provided an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample, where the assay system performs the following steps: *providing a sample affixed to a support; delivering oligonucleotide probes* for multiple nucleic acid targets *to the multiple sites in the sample* in a known spatial pattern....” *Id.* [00014]. “Other embodiments of the invention provide an assay system” that “performs the following steps: *providing a sample affixed to support;*

¹² I note that the '661 Application's paragraph numbering re-starts following the **SUMMARY OF THE INVENTION** section.

delivering encoded probes for the multiple protein targets to the multiple sites in the sample....” *Id.*, [00015]. The final “embodiment” described in the **SUMMARY OF THE INVENTION** section similarly involves “*providing a sample affixed to a support; delivering encoded probes* for the multiple biological targets at multiple sites in a sample in a known spatial pattern....” *Id.*, [00016].

207. Thus, a POSA reviewing the ’661 Application would understand from the inventor’s **SUMMARY OF THE INVENTION** that the alleged invention related to (i) providing a sample affixed to a support and (ii) delivering probes to such a sample. A POSA would not understand these disclosures to demonstrate that the inventor possessed methods of creating arrays of encoded probes on solid supports, such as beads, attaching said beads to a substrate, and subsequently disposing a tissue sample on top of such an array. Such an approach inverts the workflow of the methods disclosed in the ’661 Application’s **SUMMARY OF THE INVENTION**, requires a structure that is not disclosed, and is not mentioned in that section (or elsewhere in the Application).

208. Turning to the '661 Application's figures, "Figure 1 provides a simplified overview of *the assay system of the present invention*."

EX1011, [0001]. This overview is explicit that the "assay system of the present invention" involves first "provid[ing] biological sample affixed to a support" and then "deliver[ing] encoded probes to the biological sample according to a known spatial pattern."

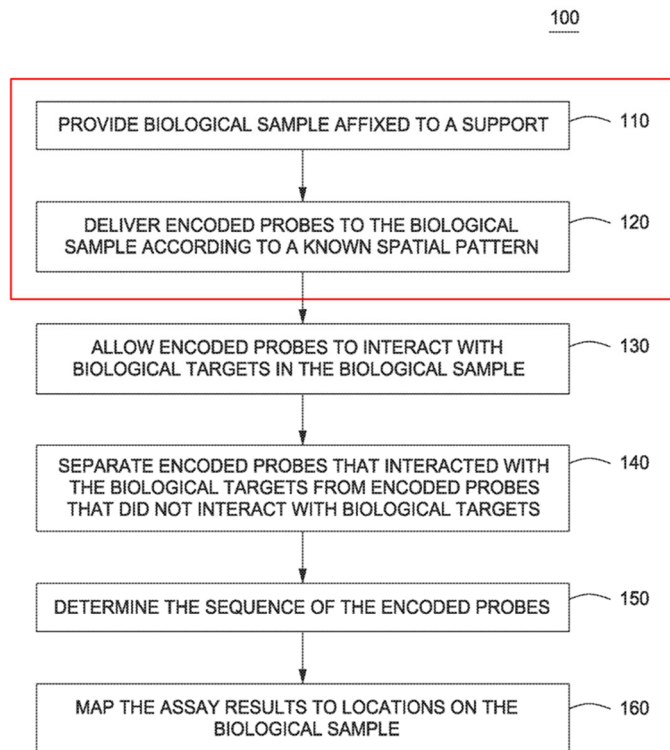


FIG. 1

EX1011, Fig. 1; *see also id.*, [00025] (describing Fig. 1).

209. Thus, a POSA reviewing Figure 1 and its caption, which provides an "overview of *the assay system of the present invention*" would understand the inventor considered the methods of the alleged invention to require fixing a sample to a support, followed by delivering encoded probes to the sample affixed to the support.

210. Similarly, Figure 2 "provides a simplified overview of one embodiment of *the assay of the present invention* for detecting nucleic acids." *Id.*, [0002]. This

disclosure again requires “provid[ing] biological sample affixed to a support” and then “deliver[ing] probes” and “deliver[ing] encoding oligonucleotides” to the biological sample.” EX1011, Fig. 2. *See also id.*, [00030], [00031] (describing Fig. 2).

211. A POSA would not have understood Figures 1-2 to describe providing an array of probes, and then delivering a tissue sample to that array.

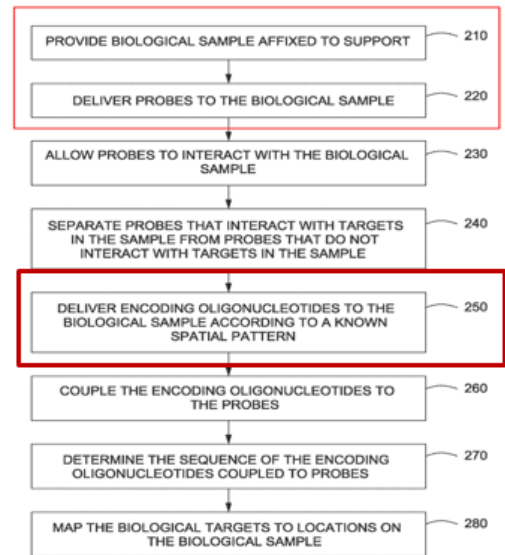


FIG. 2

212. The remainder of the '661 Application's Specification is replete with additional statements confirming that the “invention” requires (i) providing a sample affixed to a support before (ii) delivering probes to the sample.

213. For example, the **DETAILED DESCRIPTION OF THE INVENTION** section begins with a subsection headed, “**The Invention in General.**” EX1011, [00023].

This section begins:

[00023] *The assay systems of the invention provide* spatially-encoded, multiplexed assays comprising 1) an assay capable of high levels of multiplexing with an efficient spatial encoding scheme; 2) *instrumentation capable of delivering reagents according to a spatial pattern*; and 3) decoding determined by a readout that is digital in nature. The assay systems of the invention detect the presence or absence and relative amount of a biological target or biological activity

indicative of a biological target, as well as the location of the biological target or activity in a biological sample, *e.g., a tissue section or other biological structure disposed upon a support such as a microscope slide or culture dish.*

EX1011, [00023]; *see also id.*, [00024] (“The assay system further provides *instrumentation with an ability to deliver reagents* in a spatially-defined pattern. *This instrumentation*, together with software, reagents and protocols, *provides a key component* of the highly innovative assay system *of the invention....*”).

214. Confirming that the ’661 Application discloses only the delivery of reagents, including probes, to a sample affixed to a solid support, the Specification further notes that the above-referenced “instrumentation capable of delivering reagents according to a spatial pattern” “onto the biological sample” is “*integral to the assay system of the invention.*” EX1011, [00055] (“Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto the biological sample.*”); *see also, e.g., id.*, [00046] (“Coding tag a1, for example, *is deposited on the biological sample* in ten discrete areas or spots.... Coding tag a2 *is deposited on the biological sample* in ten spots on the second horizontal line.... Coding tag a3 *is deposited on the biological sample....*”); *id.*, [00048] (“A deposit or dispensing scheme is shown at 530. Like the grid exemplified in Figure 4, encoding oligonucleotides a1 through a4 *are deposited* in spots in a pattern.... The

grid though shown as a square with spots is actually *a deposition pattern on a biological sample* (not shown) such as tissue Section 416 shown in Figure 4.”); *id.*, [00049] (“The target-specific oligonucleotides *are delivered to the biological sample....*”).

215. Under the subsection headed, “**Assays**” the ’661 Application states that “[t]he assay portion of the assay systems of the present invention comprise the following general steps: *delivering probes and encoding agents* where the encoding agents (in some embodiments pre-coupled to the probes) are *delivered to the sample* according to a known spatial pattern....” EX1011, [00034]. Further confirming that the invention as disclosed by the priority documents requires delivering probes or reagents to a sample, this section goes on to state that “[t]he samples of the present invention include virtually any biological sample or samples *that can be affixed to a support* or provided essentially in a two-dimensional manner....” *Id.*, [00035].

216. Because the ’661 Application’s alleged inventions require the delivery of reagents to samples affixed to a support, the **DETAILED DESCRIPTION OF THE INVENTION** section contains a subsection titled, “**Reagent Delivery Systems.**” EX1011, [00054]. “The reagent delivery system *of the invention* includes instrumentation that allows the delivery of reagents to discrete portions of the biological sample.” *Id.* The ’661 Application goes on to note that “reagent delivery

may be to many different biological samples at one time,” although “[a] single tissue section has been exemplified herein.” *Id.* The Specification does not, however, contain any disclosure indicating that the system “of the invention” may operate by delivering a tissue section to an array of probes.

217. As noted, the ’661 Application provides that “[i]ntegral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto* the biological sample.” *Id.*, [00055]. Such techniques are disclosed to include, for example, “ink jet spotting; mechanical spotting...; micro contact printing; photochemical or photolithographic methods; and the like.” *Id.*, [00056]; *see also id.*, [00064] (“[T]he reagent delivery system controls the delivery of reagents to specific patterns *on a biological sample surface* using semiconductor techniques such as masking and spraying.”); *id.*, [00065] (“In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology.”).

218. A POSA would not have understood such disclosures, which necessarily involve delivering reagents on top of a substrate-affixed tissue sample, to demonstrate possession of methods in which a tissue sample is placed on top of an array of binding agents, or the devices required to perform such methods, such as an array of functionalized beads, as claimed.

219. Indeed, even where the '124 Provisional mentions beads, there is no indication that these disclosures refer to providing beads in an array as claimed, as opposed to depositing beads comprising a binding agent to a tissue sample that was previously affixed to a solid support. EX1009, [0018] (“[T]he nucleic acids may be immobilized directly or indirectly onto beads that are then provided in known locations on the substrate surface.”). As discussed further below, a POSA would not have read the '124 Provisional's limited disclosures regarding bead arrays and probes immobilized on solid supports—passages which do not mention tissue analysis—to have been disclosures related to the spatial analysis of tissue samples.

220. Further suggesting that the '124 Provisional's disclosures regarding immobilizing nucleic acids on beads refer to reagents that are delivered to a support-affixed tissue sample, and not to the creation of bead arrays, the only disclosure of a “bead array” in the application is to refer to genotyping using prior art techniques. EX1009, [0052] (“Genotyping may be performed using any technique known to those of skill in the art. Preferred techniques permit rapid, accurate determination of multiple variations with a minimum of sample handling. Some examples of suitable techniques involve but are not limited to direct DNA sequencing, capillary electrophoresis, hybridization, allele-specific probes or primers, single-stranded conformation polymorphism analysis, nucleic acid arrays, bead arrays, restriction

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length polymorphism analysis, random amplified polymorphic DNA, ligase detection reaction, heteroduplex or fragment analysis, differential sequencing with mass spectrometry, atomic force microscopy, pyrosequencing, FRET, and other related techniques. Several methods for DNA sequencing are well known and generally available in the art.”) (internal citations omitted). A POSA would not have understood this single reference to bead arrays (and nucleic acid arrays) as part of a laundry list of known and conventional nucleic acid detection techniques for genotyping to disclose that the inventor possessed methods of directly using bead arrays (or the other listed techniques) on tissue samples to perform the claimed spatial assays, *see, e.g.*, Figs. 1-2, or the claimed bead array compositions comprising beads affixed to a substrate, wherein the beads were deposited on the substrate in a random pattern. *See* EX1005, cls. 1, 16.

221. Accordingly, the '661 Application (including the '124 Provisional) fails to support the challenged claims of the '030 patent. The additional limitations of the challenged dependent claims do not narrow claim 1 or 16 in a manner that resolves these deficiencies, and thus all challenged claims are unsupported for the same reasons.

(b) *The '661 Application does not disclose “an array comprising a substrate, a plurality of beads, and a plurality of capture probes,” wherein “the plurality of beads are attached to the substrate” and “each of the capture probes of the plurality of capture probes comprises...a nucleic acid sequence that identifies a unique location on the substrate”*

222. First, I note that the '661 Application itself does not refer to or mention “beads.”

223. However, I understand that the '661 Application states: “This application claims benefit of United States Provisional Patent Application No. 61/321,124, filed April 5, 2010 and is incorporated herein by reference.” EX1011, [0001]. Thus, I have further evaluated the '124 Provisional to determine whether it provides support for the claimed “array comprising a substrate, a plurality of beads, and a plurality of capture probes,” wherein “the plurality of beads are attached to the substrate” and “each of the capture probes of the plurality of capture probes comprises...a nucleic acid sequence that identifies a unique location on the substrate.”

224. Even assuming that the full text of the '124 Provisional is incorporated by reference into the '661 Application, and thus effectively part of that disclosure, it is my opinion that the disclosures of the '124 Provisional fail to describe the subject

matter of the challenged claims, including beads comprising a binding agent having a nucleic acid coding identifier.

225. The '124 Provisional contains the following disclosures that reference beads comprising nucleic acid binding agents and coding identifiers:

[00017] 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.

[00018] 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.

[00021] In other specific aspects of the invention, the binding agents are peptides. In one aspect, *these peptides are associated directly or indirectly to known locations on a substrate surface*, e.g., using binding protein pairs or through oligonucleotide linkers complementary to oligonucleotides on the substrate surface. *In another aspect, the binding agents are peptides are [sic] immobilized directly or indirectly onto beads or other separate structural elements that are then provided in known locations on the substrate surface.* In yet another aspect, the peptides may be provided in or on features of the substrate surface, e.g., provided in wells.

[00051] In a particular aspect, the assay system is used to analyze nucleic acids, e.g genotyping, gene expression analysis, localization of particular transcripts within samples, and the like.

[00052] Genotyping may be performed using any technique known to those of skill in the art. Preferred techniques permit rapid, accurate determination of multiple variations with a minimum of sample handling. Some examples of suitable techniques involve but are not limited to direct DNA sequencing, capillary electrophoresis, hybridization, allele-specific probes or primers, single-strand conformation polymorphism analysis, nucleic acid arrays, *bead arrays*....

226. As an initial matter, as the foregoing passages demonstrate, the '124 Provisional's disclosures involve providing beads at *known locations* on the substrate—*i.e.*, at locations that are *known or determined prior to use*. In sharp contrast, the claims of the '030 Patent expressly recite “the plurality of beads was previously positioned on the substrate in a random pattern,” and the beads include a “nucleic acid sequence that identifies a unique location on the substrate” that identifies the location where the bead-bound capture probe interacted with the target nucleic acid. Because the '124 Provisional's disclosures pertaining to beads involve providing them at known locations, a POSA would have understood these disclosures to have obviated the need for the use of “nucleic acid sequence that identifies a unique location on the substrate” sequences as claimed in the '030 Patent. A closer examination of these disclosures makes this point even clearer.

227. Paragraph [00017] provides that in certain aspects, “the binding agents are immobilized onto beads or other separate structural elements that are then provided in known locations on the substrate surface.” It makes no reference to including coding identifiers in the beads, and a POSA would not have understood that a coding identifier would be included in the beads, as there would be no purpose for such a coding identifier because the binding agents are to be “provided in known locations on the substrate surface.”

228. Similarly, Paragraph [00018] provides that “[i]n specific aspects of the invention, the binding agents are nucleic acids immobilized directly or indirectly to the substrate surface....” EX1009, [00018]. It goes on to state that “[i]n 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.” *Id.* A POSA would have understood this disclosure to refer to providing beads where “the binding agents are nucleic acids,” but would not have understood this disclosure to encompass a bead comprising a nucleic acid binding agent *plus* a nucleic acid ID sequence, as again there would be no apparent purpose for a nucleic acid ID sequence in this embodiment.

229. The specification does not describe “binding agents” or capture probes as necessarily including “coding identifiers,” and in fact repeatedly considers them

to be separate structures. *See, e.g.*, EX1009, [0007] (“This encoding scheme uses both molecule-specific binding agents and coding identifiers....”); [0008] (“In a specific aspect, the encoding scheme employs limited reagent delivery to the spatial patterns on the substrate surface, and access of the coding identifiers and/or binding agents to portions of the sample is controlled through such limited delivery.”); [0012] (“This aspect includes systems with two or more substantially identical spatial patterns using different binding agents and/or coding identifiers....”). Because the disclosure of paragraph [0018] makes no reference to “coding identifiers,” a POSA would not have understood this passage to demonstrate the inventor’s possession of beads comprising a nucleic acid binding agent and a coding identifier.

230. Finally, as noted above, each of the passages describing the use of beads above describes providing beads at “known locations on the substrate surface.” *See, e.g.*, EX1009, [0018]. As claimed, the purpose of the coding identifier is to identify the location where the bead-bound capture probe interacted with the biological molecule. Because the ’124 Provisional’s disclosures pertaining to beads involve providing beads with surface-bound binding agents, but not coding tags, at known locations, a POSA would have understood these disclosures to have obviated the need for the use of coding tags as claimed in the ’030 Patent.

231. Accordingly, the '661 Application (including the '124 Provisional) fails to support the challenged claims of the '030 patent. The additional limitations of the challenged dependent claims do not narrow claims 1 or 16 in a manner that resolves these deficiencies, and thus all challenged claims are unsupported for the same reasons.

(c) Conclusion on Priority Date

232. For the reasons set forth above, the challenged claims are not supported by the '661 Application filed on June 20, 2016, and thus are entitled to a priority date no earlier than the February 14, 2019 filing date of the '235 Application. I reserve the right to respond to any arguments advanced by Patent Owner suggesting that the challenged claims are supported by one or more of the '030 Patent's priority applications.

2. Claim 1

233. Claim 1 recites:

A composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes, wherein:

the plurality of beads are attached to the substrate;

each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic

acid binding domain, and (iii) the target nucleic acid-binding domain;

the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain; and

the plurality of beads was previously positioned on the substrate in a random pattern.

234. As described below, Frisen discloses each limitation of Claim 1.

(a) “A composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes”

235. Frisen discloses “[a] composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes.” Thus, to extent the preamble is limiting, it is anticipated by Frisen.

236. Frisen discloses “methods and products for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof.” EX1045, Abstract. In particular, Frisen claims methods “for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof” by utilizing a composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes.

237. For example, Claims 1, 13 and 14 in Frisen recite:

1. A method for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof, comprising:

(a) providing *an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer;*

(b) contacting said substrate with a tissue sample and allowing RNA of the tissue sample to hybridise to the capture probes;

(c) generating cDNA molecules from the captured RNA molecules using said capture probes as RT primers;

(d) labelling the cDNA molecules generated in step (c), wherein said labelling step may be contemporaneous with, or subsequent to, said generating step;

(e) detecting a signal from the labelled cDNA molecules; and optionally

(f) imaging the tissue sample, wherein the tissue sample is imaged before or after step (c).

13. The method of any one of claims 1 to 13, wherein the capture probes further comprise a positional domain which is 5' relative to the capture domain, wherein said positional domain comprises a sequence that corresponds to the position of the capture probe on the object substrate, preferably wherein the positional domain of each species of capture probe comprises a unique barcode sequence.

14. The method of any one of claims 1 to 13, wherein the capture probes further comprise a universal domain which is 5' relative

to the capture domain or, if present the positional domain, wherein said universal domain comprises:

(i) an amplification domain, for amplifying the generated DNA molecules; and/or

(ii) a cleavage domain for releasing the generated DNA molecules from the surface of the object substrate.

238. Of particular relevance here is the limitation of Frisen's Claim 1¹³ requiring "an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer." Thus, Frisen discloses an array comprised of *a substrate* with *a plurality of capture probes* being immobilized directly or indirectly thereon. EX1045, 2 ("The invention also provides an object substrate, such as a chip or slide (e.g. an array).").

239. Frisen describes multiple methods for directly or indirectly immobilizing capture probes on a substrate, including the use of beads. In particular, Frisen discloses:

[T]he probes may be immobilized on beads, e.g. plastic microbeads, which can be used to immobilize the probes on the substrate. Suitable techniques for immobilizing the nucleic acid molecules on beads may be selected from the techniques discussed above or selected from

¹³ I have been informed by counsel that because Claims 13 and 14 depend from Claim 1, this limitation is also a limitation in Claims 13 and 14.

methods known in the art. The beads may be contacted with, and immobilized on, an object substrate, thereby indirectly immobilizing the probes on the surface of the substrate. For example, after contacting the beads with the substrate, the substrate may be treated [to] crosslink the beads to each other and/or the surface of the substrate, e.g. the substrate may [be] heated to partially melt the beads which are allowed to solidify, to generate an object substrate on which probes are indirectly immobilized.

EX1045, 20. Thus, a POSA would understand Frisen to disclose “[a] composition comprising an array comprising a substrate, a plurality of beads, and a plurality of capture probes.” *Id.* (describing “probes immobilized on beads...which can be used to immobilize the probes on the substrate”).

(b) “the plurality of beads are attached to the substrate”

240. Frisen discloses that “the plurality of beads are attached to the substrate” in the disclosed array.

241. As mentioned previously, Frisen discloses that

[T]he probes may be immobilized on beads, e.g. plastic microbeads, which can be used to immobilize the probes on the substrate.... The beads may be contacted with, and immobilized on, an object substrate, thereby indirectly immobilizing the probes on the surface of the substrate. For example, after contacting the beads with the substrate, the substrate may be treated [to] crosslink the beads to each other and/or the surface of the substrate, e.g. the substrate may be heated to partially melt the beads which are allowed to solidify, to generate an object substrate on which probes are indirectly immobilized.

EX1045, 20. A POSA would understand that by “immobilizing” beads on the surface of the substrate as described by Frisen, the beads are attached to the substrate.

Indeed, Frisen even discloses a method of attaching the beads to the surface of the substrate by treating the beads so that they are crosslinked to the substrate, for example, by melting the beads to the substrate. *Id.*

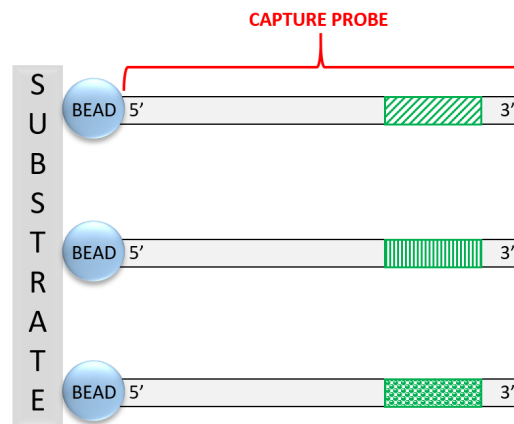
- (c) ***“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain”***

242. As described in the sections below, Frisen discloses “each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain”

- (i) *“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising...(iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain”*

243. As described previously, Claim 1 of Frisen requires “an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer.”

244. A POSA would recognize that Claim 1 is describing capture probes attached to a substrate by their 5' ends such that their 3' ends are free. Moreover, as described above, one method that Frisen discloses for attaching probes to the substrate is using beads. Thus, a POSA would understand Frisen to disclose a substrate with a plurality of **capture probes** with a **target-nucleic acid binding domain**, each attached to beads (and the substrate) by their 5' end, as shown below.



245. Additionally, Frisen explains that the disclosed “capture domain comprises a nucleotide sequence that is capable of hybridizing to nucleic acid, e.g. RNA (preferably mRNA) present in the cells of the tissue sample contacted with the array.” EX1045, 24-25. Moreover, “the capture domain may be selected or designed to bind (or put more generally may be capable of binding) selectively or specifically to the particular nucleic acid, e.g. RNA, it is desired to detect or analyse.” *Id.*, 25. For example, Frisen discloses that the capture domain may be designed for the “selective capture of mRNA,” as opposed to other classes of nucleic acids (e.g., DNA, rRNA, tRNA), using poly-T capture domains:

As is well known in the art, this may be on the basis of hybridisation to the poly-A tail of mRNA. Thus, in a preferred embodiment the capture domain comprises a poly-T DNA oligonucleotide, i.e. a series of consecutive deoxythymidine residues linked by phosphodiester bonds, which is capable of hybridizing to the poly-A tail of mRNA.

*Id.*¹⁴ As another example, Frisen discloses that “[t]he capture domain may be capable of binding selectively to a desired subtype or subset of nucleic acid, e.g. RNA, for example a particular type of RNA such mRNA or rRNA etc. as listed above, or to a particular subset of a given type of RNA, for example, a particular mRNA species e.g. corresponding to a particular gene or group of genes. Such a capture probe may be selected or designed based on sequence of the RNA it is desired to capture. Thus it may be a sequence-specific capture probe, specific for a particular RNA target or group of targets (target group etc). Thus, it may be based on a particular gene sequence or particular motif sequence or common/conserved sequence etc., according to principles well known in the art.” *Id.* at 26.

246. Thus, a POSA would understand that the capture domain (*i.e.*, target nucleic acid-binding domain) of each of the plurality of capture probes described in Claim 1 of Frisen comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain.

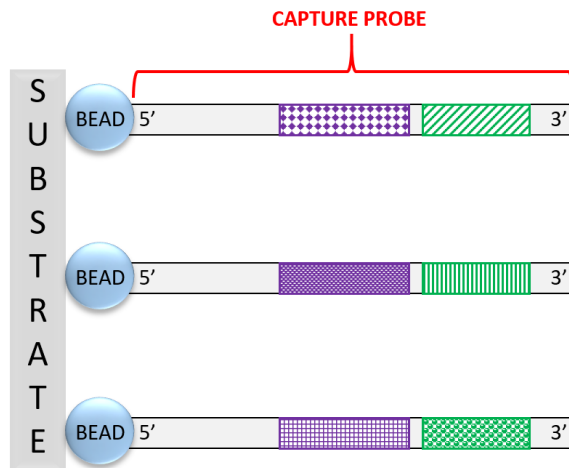
¹⁴ I understand that in the District Court litigation, 10X Genomics and Prognosys have taken the position that polyT domains are “target nucleic acid binding domains.” Thus, Frisen’s disclosure satisfies the claim for the same reasons.

- (ii) *“each of the capture probes of the plurality of capture probes comprises... (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain”*

247. Published Claim 13 of Frisen depends from Claim 1 and recites “wherein the capture probes further comprise a positional domain which is 5' relative to the capture domain, wherein said positional domain comprises a sequence that corresponds to the position of the capture probe on the object substrate, preferably wherein the positional domain of each species of capture probe comprises a unique barcode sequence.” *See also id.*, 26 (“The positional domain (feature identification domain or tag) of the capture probe, if present, is located directly or indirectly upstream, i.e. closer to the 5' end of the capture probe nucleic acid molecule, of the capture domain.”)

248. I have been informed by counsel that a dependent claim incorporates all of the limitations from the claim from which it depends. Thus, Claim 13 of Frisen discloses an embodiment in which the substrate and **capture probes** of Claim 1 are further defined to have capture probes with “a **positional domain** which is 5' relative to the **capture domain**, wherein said **positional domain** comprises a sequence that corresponds to the position of the **capture probe** on the object substrate, preferably

wherein the **positional domain** of each species of **capture probe** comprises a unique barcode sequence,” as shown below.



249. Frisen explains that “[t]he positional domain (or tag),” when present in the disclosed capture probes, “comprises the sequence which is unique to each feature or a group of directly or indirectly adjacent features, and acts as a positional or spatial marker (the identification tag). In this way, each region or domain of the tissue sample, e.g. each cell in the tissue, may be identified by spatial resolution across the array linking the nucleic acid, e.g. RNA (e.g. the transcripts) from a certain cell to a unique positional domain sequence in the capture probe. The positional domain of a capture probe in the array is one aspect of the methods of the invention that allows a specific transcript (or group of transcripts) to be correlated to a position in the tissue sample, for example it may be correlated to a cell in the sample. Thus, the positional domain of the capture domain may be seen as a nucleic acid tag

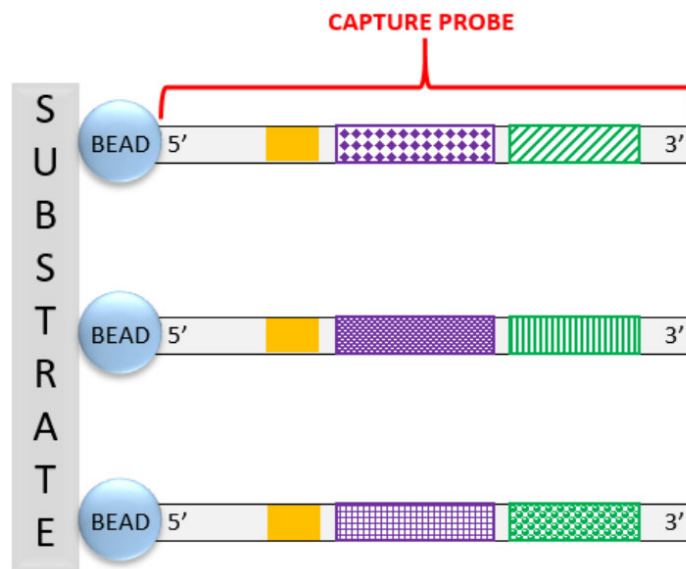
(identification tag) and enables the position of a transcript to be correlated to a position in the sample indirectly, e.g. by analysis of the sequence of the captured transcript.” *Id.*, 28.

250. Frisen also explains that while “[a]ny suitable sequence may be used as the positional domain in the capture probes of the invention,” “the positional domain should not interfere with (i.e. inhibit or distort) the interaction between the RNA of the tissue sample and the capture domain of the capture probe. For example, the positional domain should be designed such that nucleic acid molecules in the tissue sample do not hybridize specifically to the positional domain. Preferably, the nucleic acid sequence of the positional domain of the capture probes has less than 80% sequence identity to the nucleic acid sequences in the tissue sample. Preferably, the positional domain of the capture probe has less than 70%, 60%, 50% or less than 40% sequence identity across a substantial part of the nucleic acids molecules in the tissue sample. Sequence identity may be determined by any appropriate method known in the art, e.g. using the BLAST alignment algorithm.” *Id.*, 29-30.

251. Thus, Frisen discloses that “each of the capture probes of the plurality of capture probes comprises...(ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain.”

(iii) “each of the capture probes of the plurality of capture probes comprises... (i) a primer binding site”

252. Claim 14 of Frisen, which depends from Claim 13, further requires “wherein the **capture probes** further comprise a universal domain which is 5' relative to the **capture domain** or, if present the **positional domain**, wherein said universal domain comprises: (i) **an amplification domain**, for amplifying the generated DNA molecules,” as shown below:



253. When describing the amplification domain in detail, Frisen explains that “it may be advantageous to amplify the nucleic acid, e.g. cDNA molecules, for example after they have been released (e.g. removed or cleaved) from the substrate. It will be appreciated, however, that the initial cycle of amplification, or indeed any or all further cycles of amplification may also take place in situ on the substrate. *The*

amplification domain comprises a distinct sequence to which an amplification primer may hybridize. The amplification domain of the universal domain of the capture probe is preferably identical for each species of capture probe. Hence a single amplification reaction will be sufficient to amplify all of the nucleic acid, e.g. cDNA, molecules (which may or may not be released from the substrate prior to amplification).” *Id.*, 32.

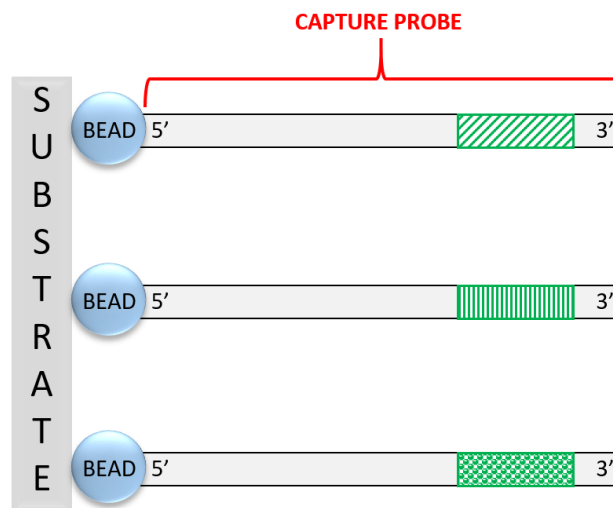
254. Thus, the “amplification domain” disclosed by Frisen constitutes the “primer binding site” required by Claim 1 of the ’030 Patent.

(iv) *“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate..., and (iii) the target nucleic acid-binding domain”*

255. As I described above, Frisen discloses that each capture probe comprise a primer binding site (i.e., an amplification domain), a nucleic acid sequence that identifies a unique location on a substrate (i.e., a positional domain), and a target binding domain (i.e., capture domain). Frisen also discloses the particular arrangement of these sequences required by claim 1.

256. For example, published Claim 1 of Frisen requires “an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3’

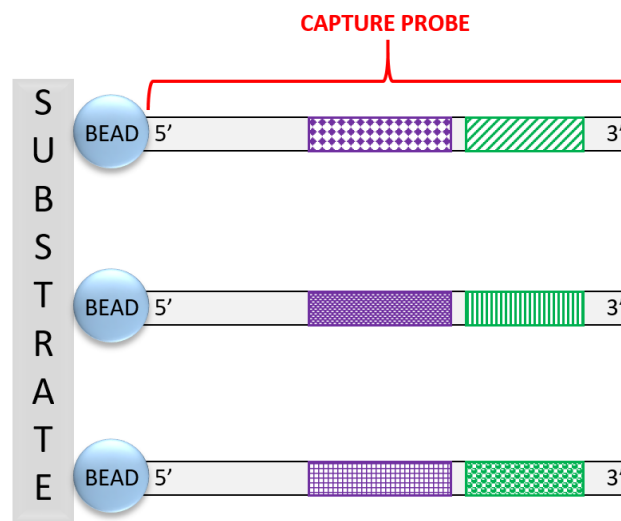
end to enable said probe to function as a reverse transcriptase (RT) primer.” A POSA would understand Frisen to disclose a substrate with a plurality of **capture probes** with a **capture domain** attached thereto by their 5' ends using a **bead**, as shown below.



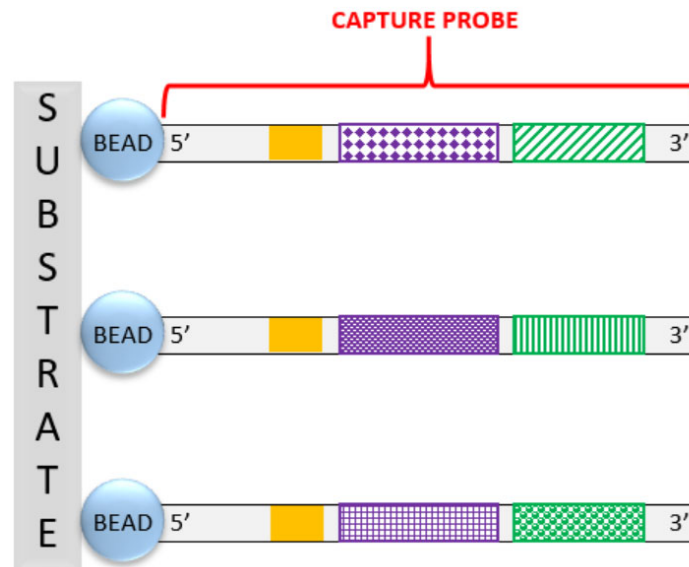
257. Published Claim 13 of Frisen depends from Claim 1 and recites “wherein the capture probes further comprise a positional domain which is 5' relative to the capture domain, wherein said positional domain comprises a sequence that corresponds to the position of the capture probe on the object substrate, preferably wherein the positional domain of each species of capture probe comprises a unique barcode sequence.”

258. I have been informed by counsel that a dependent claim incorporates all of the limitations from the claim from which it depends. Thus, Claim 13 of Frisen

requires the substrate and **capture probes** of Claim 1 in addition to the capture probes comprising “a **positional domain** which is 5' relative to the **capture domain**, wherein said **positional domain** comprises a sequence that corresponds to the position of the **capture probe** on the object substrate, preferably wherein the **positional domain** of each species of **capture probe** comprises a unique barcode sequence,” as shown below.

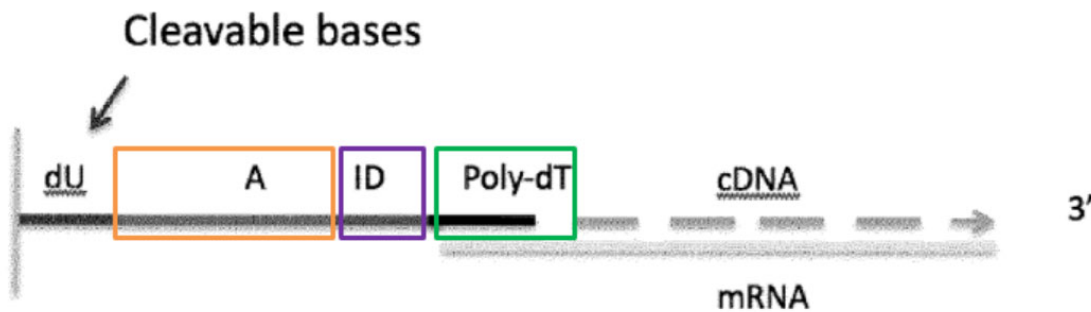


259. Published Claim 14 of Frisen, which depends from Claim 13, further requires “wherein the **capture probes** further comprise a universal domain which is 5' relative to the **capture domain** or, if present the **positional domain**, wherein said universal domain comprises: (i) **an amplification domain**, for amplifying the generated DNA molecules” as shown below.



260. Thus, Claim 14 of Frisen discloses that each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) **a primer binding site** (i.e., amplification domain), (ii) **a nucleic acid sequence that identifies a unique location on the substrate** (i.e., positional domain), and (iii) the **target nucleic acid-binding domain** (i.e., capture domain).

261. This is also shown in Frisen's Figure 6, which discloses from the substrate surface: (i) **a primer binding site** (i.e., amplification domain), (ii) **a nucleic acid sequence that identifies a unique location on the substrate** (i.e., positional domain), and (iii) the **target nucleic acid-binding domain** (i.e., capture domain).



(d) the plurality of beads was previously positioned on the substrate in a random pattern

262. Frisen discloses “the plurality of beads was previously positioned on the substrate in a random pattern.” For example, Frisen states that “the probes may be immobilized on beads, e.g. plastic microbeads, which can be used to immobilize the probes on the substrate. Suitable techniques for immobilizing the nucleic acid molecules on beads may be selected from the techniques discussed above or selected from methods known in the art. The beads may be contacted with, and immobilized on, an object substrate, thereby indirectly immobilizing the probes on the surface of the substrate.” EX1045, 20. A POSA would appreciate from this disclosure that, as known in the art, the beads could either be (1) linked randomly to the substrate, such as described in Chee-824,¹⁵ or (2) linked in a predetermined manner, such as

¹⁵ Ex. 1038, [0013] (“Since the beads go down randomly, a unique optical signature is needed to ‘decode’ the array; i.e., after the array is made, a correlation of the location of an individual site in the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly

described in Chee-584.¹⁶ See Section V.D.2. above. Thus, a POSA would immediately envisage the plurality of beads/capture probes being positioned on the substrate in a random pattern.

3. Claim 3

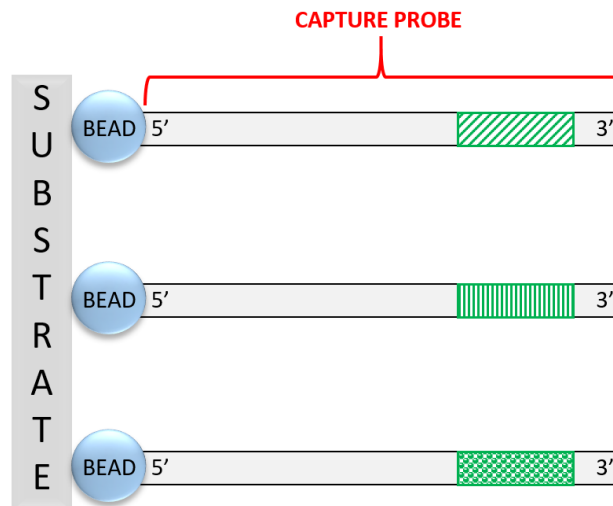
263. Claim 3 recites “[t]he composition of claim 1, wherein the plurality of capture probes are attached to the plurality of beads by their 5’ ends.”

264. As described previously, Claim 1 of Frisen requires “an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3’ end to enable said probe to function as a reverse transcriptase (RT) primer.”

distributed on the array, a fast and inexpensive process as compared to either the in situ synthesis or spotting techniques of the prior art. Once the array is loaded with beads, the array can be decoded, or can be used, with full or partial decoding occurring after testing, as is more fully outlined below.”).

¹⁶ Ex. 1032, 28:6-19 (“[S]ub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an “area code” that can have the same tags (i.e. telephone numbers) of other subarrays, that are separated by virtue of the location of the subarray. Thus, for example, the same unique tags can be reused from bundle to bundle. Thus, the use of 50 unique tags in combination with 100 different subarrays can form an array of 5000 different bioactive agents. In this embodiment, it becomes important to be able to identify one bundle from another; in general, this is done either manually or through the use of marker beads, i.e. beads containing unique tags for each subarray.”).

265. A POSA would recognize that Claim 1 is describing capture probes attached to a substrate by their 5' ends such that their 3' ends are free. Moreover, as described above, one method that Frisen discloses for attaching probes to the substrate is using beads. Thus, a POSA would understand Frisen to disclose a substrate with a plurality of **capture probes** attached thereto by their 5' ends using a plurality **beads**, as shown below.



4. Claim 6

266. Claim 6 recites “[t]he composition of claim 1, wherein each of the plurality of beads comprises at least 1,000 capture probes.”

267. Frisen discloses that “[t]he object substrate (often simply referred to as the ‘substrate’) of, and for use in the methods of, the invention may contain multiple spots, or features’. Accordingly, in some embodiments of the invention the object substrate may be an array, i.e. an object substrate, e.g. slide or chip, on which the

immobilized probes are arrayed on the surface of the substrate. A feature may be defined as an area or distinct position on the array substrate at which a single species of capture probe is immobilized. Hence each feature will comprise a multiplicity of probe molecules, of the same species.” EX1045, 36. Frisen also states that “[a]s used herein the term "multiple" means two or more, or at least two, e.g 3, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 400, 500, 1000, 2000, 5000, 10,000, or more etc.” *Id.*, 17.

268. A POSA would understand that a “bead” would constitute a “feature” on the arrays disclosed in Frisen. Moreover, because Frisen discloses that “each feature will comprise a multiplicity of” capture probes and “multiple” is defined to include 1,000 or more, a POSA would immediately envisage a bead with at least 1,000 capture probes.

5. Claim 10

269. Claim 10 recites “[t]he composition of claim 1, wherein the target nucleic acid is RNA.”

270. Frisen explains that the disclosed “capture domain comprises a nucleotide sequence that is capable of hybridizing to nucleic acid, e.g. RNA (preferably mRNA) present in the cells of the tissue sample contacted with the array.” EX1045, 24-25. Moreover, “the capture domain may be selected or designed

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Patent No. 11,761,030

to bind (or put more generally may be capable of binding) selectively or specifically to the particular nucleic acid, e.g. RNA, it is desired to detect or analyse.” *Id.*, 25. For example, Frisen discloses that the capture domain may be designed for the “selective capture of mRNA” using poly-T capture domains:

As is well known in the art, this may be on the basis of hybridisation to the poly-A tail of mRNA. Thus, in a preferred embodiment the capture domain comprises a poly-T DNA oligonucleotide, i.e. a series of consecutive deoxythymidine residues linked by phosphodiester bonds, which is capable of hybridizing to the poly-A tail of mRNA.

Id.

271. Thus, a POSA would understand that Frisen discloses the target nucleic acid being RNA.

6. Claim 11

272. Claim 11 recites “[t]he composition of claim 10, wherein the RNA is mRNA.”

273. Frisen explains that the disclosed “capture domain comprises a nucleotide sequence that is capable of hybridizing to nucleic acid, e.g. RNA (preferably mRNA) present in the cells of the tissue sample contacted with the array.” EX1045, 24-25. For example, Frisen discloses that the capture domain may be designed for the “selective capture of mRNA” using poly-T capture domains:

As is well known in the art, this may be on the basis of hybridisation to the poly-A tail of mRNA. Thus, in a preferred embodiment the capture

domain comprises a poly-T DNA oligonucleotide, i.e. a series of consecutive deoxythymidine residues linked by phosphodiester bonds, which is capable of hybridizing to the poly-A tail of mRNA.

Id., 25.

274. Thus, a POSA would understand that Frisen discloses the target nucleic acid being mRNA.

7. Claim 16

275. Claim 16 is reproduced below:

A composition comprising (i) an array comprising a substrate, a plurality of beads, and a plurality of capture probes, and (ii) a tissue section, wherein:

the tissue section is disposed on the array;

the plurality of beads are attached to the substrate;

each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain;

the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain; and

the plurality of beads was previously positioned on the substrate in a random pattern.

276. As described below, Frisen discloses each limitation of Claim 16.

- (a) ***“A composition comprising (i) an array comprising a substrate, a plurality of beads, and a plurality of capture probes, and (ii) a tissue section”***

277. As described above in Section VIII.C.2(a) and herein incorporated by reference, Frisen discloses “[a] composition comprising (i) any array comprising a substrate, a plurality of beads, and a plurality of capture probes.” Frisen also discloses a tissue section.

278. For example, Claim 1 in Frisen recites:

A method for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof, comprising:

- (a) providing an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer;
- (b) ***contacting said substrate with a tissue sample*** and allowing RNA of the tissue sample to hybridise to the capture probes;
- (c) generating cDNA molecules from the captured RNA molecules using said capture probes as RT primers;
- (d) labelling the cDNA molecules generated in step (c), wherein said labelling step may be contemporaneous with, or subsequent to, said generating step;
- (e) detecting a signal from the labelled cDNA molecules; and optionally
- (f) imaging the tissue sample, wherein the tissue sample is imaged before or after step (c).

279. A POSA would understand that a tissue sample is synonymous with a tissue section. *See also* EX1045, 6 (“In its simplest form, the invention may be illustrated by the following summary. The invention requires reverse transcription (RT) primers to be immobilised on an object substrate, e.g. a glass slide or chip. ***Thin tissue sections*** are placed onto the substrate and a reverse transcription reaction is performed in the tissue section on the substrate.”)

(b) “the tissue section is disposed on the array”

280. Frisen also discloses disposing the tissue section on the array. Indeed, Claim 1 above requires “contacting said substrate [i.e., array] with a tissue sample.”

281. Frisen also explains that “[i]n its simplest form, the invention may be illustrated by the following summary. The invention requires reverse transcription (RT) primers to be immobilised on an object substrate, e.g. a glass slide or chip. ***Thin tissue sections are placed onto the substrate*** and a reverse transcription reaction is performed in the tissue section on the substrate.” EX1045, 6.

(c) “the plurality of beads are attached to the substrate”

282. Cantor discloses “the plurality of beads are attached to the substrate.” For example, see Section VIII.C.2(b) above, which is herein incorporated by reference.

- (d) ***“each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain”***

283. Frisen discloses “each of the capture probes of the plurality of capture probes comprises a nucleic acid sequence comprising from the bead surface: (i) a primer binding site, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (iii) the target nucleic acid-binding domain; the target nucleic acid-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence capable of hybridizing to at least a portion of the target nucleic acid that binds to the target nucleic acid-binding domain.” For example, see Section VIII.C.2(c) above, which is herein incorporated by reference.

(e) “the plurality of beads was previously positioned on the substrate in a random pattern.”

284. Frisen discloses “the plurality of beads was previously positioned on the substrate in a random pattern” as described in Section VIII.C.2.(d) above, which is herein incorporated by reference.

8. Claim 18

285. Claim 18 recites “[t]he composition of claim 16, wherein the plurality of capture probes are attached to the plurality of beads by their 5’ ends.”

286. Frisen discloses “wherein the plurality of capture probes are attached to the plurality of beads by their 5’ end.” See analysis for Section VIII.C.3 above, which is herein incorporated by reference.

9. Claim 21

287. Claim 21 recites “[t]he composition of claim 16, wherein each of the plurality of beads comprises at least 1,000 capture probes.”

288. Frisen discloses “where each of the plurality of beads comprises at least 1,000 capture probes.” See analysis for Section VIII.C.4 above, which is herein incorporated by reference.

10. Claim 24

289. Claim 24 recites “[t]he composition of claim 16, wherein the tissue section is a fresh frozen tissue section.”

290. Frisen discloses the tissue sample being a fresh frozen tissue sample. In particular, Frisen discloses that “in one embodiment of the invention, the tissue sample, i.e. the section of the tissue sample contacted with the substrate, e.g. array, is prepared using FFPE or other methods of fixing and embedding. In other words the sample may be fixed, e.g. fixed and embedded. In an alternative embodiment of the invention *the tissue sample is prepared by deep-freezing*. In another embodiment a touch imprint of a tissue may be used, according to procedures known in the art. In other embodiments an unfixed sample may be used.” EX1045, 42.

11. Claim 25

291. Claim 25 recites “[t]he composition of claim 16, wherein the target nucleic acid is RNA.”

292. Frisen discloses “wherein the target nucleic acid is RNA.” For example, see analysis for Section VIII.C.5 above, which is herein incorporated by reference.

12. Claim 26

293. Claim 26 recites “[t]he composition of claim 25, wherein the RNA is mRNA.”

294. Frisen discloses “wherein the RNA is mRNA.” For example, see analysis for Section VIII.C.6 above, which is herein incorporated by reference.

13. Claim 30

295. Claim 30 recites “[t]he composition of claim 16, wherein the composition further comprises the target nucleic acid hybridized to a target nucleic-acid binding domain of at least one of the plurality of capture probes.”

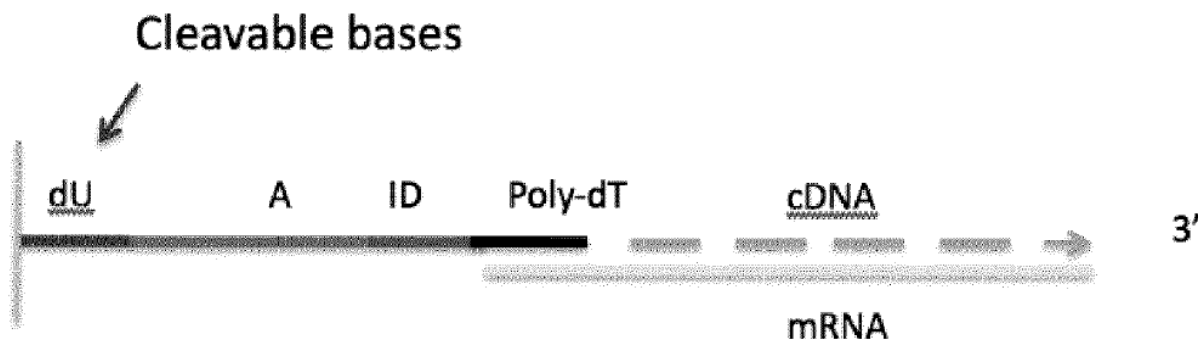
296. Claim 1 of Frisen recites:

A method for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof, comprising:

- (a) providing an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer;
- (b) contacting said substrate with a tissue sample and ***allowing RNA of the tissue sample to hybridise to the capture probes***;
- (c) generating cDNA molecules from the captured RNA molecules using said capture probes as RT primers;
- (d) labelling the cDNA molecules generated in step (c), wherein said labelling step may be contemporaneous with, or subsequent to, said generating step;
- (e) detecting a signal from the labelled cDNA molecules; and optionally
- (f) imaging the tissue sample, wherein the tissue sample is imaged before or after step (c).

297. A POSA would recognize that the RNA of the tissue sample hybridizes to the capture domain of the capture probe. Indeed, Figure 6 in Frisen, reproduced

below, shows the target nucleic acid (mRNA) hybridized to the capture domain (Poly-dT). EX1045, 25 (“Thus, in a preferred embodiment the capture domain comprises a poly-T DNA oligonucleotide, i.e. a series of consecutive deoxythymidine residues linked by phosphodiester bonds, which is capable of hybridizing to the poly-A tail of mRNA.”)



298. Thus, Frisen discloses the composition further comprising the target nucleic acid hybridized to a target nucleic-acid binding domain of at least one of the plurality of capture probes.

IX. Prior Art Anticipates and/or Renders Obvious the Challenged Claims of the '138 Patent

299. I have determined that the Challenged Claims are unpatentable on each of the grounds tabulated below:

IPR2025-00192

IPR2025-00193

Patent No. 11,549,138

Patent No. 11,761,030

Ground	Basis	Priority Date Applied
1	Obviousness over Cantor in view of the knowledge of a POSA	April 5, 2010
2	Obviousness over Cantor in view of Armani and the knowledge of a POSA	April 5, 2010
3	Anticipation by Frisen	February 14, 2019

A. Ground 1: Obvious over Cantor in view of Knowledge of a POSA

1. Claim 1

300. Claim 1 of the '138 Patent recites:

1. A composition comprising an array comprising a substrate and a plurality of capture probes, wherein:

each of the plurality of capture probes comprises: (i) a target-binding domain, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site;

the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain; and

each of the plurality of capture probes are immobilized on the substrate in a random pattern.

301. As described below, Cantor in view of the knowledge of a POSA renders obvious Claim 1.

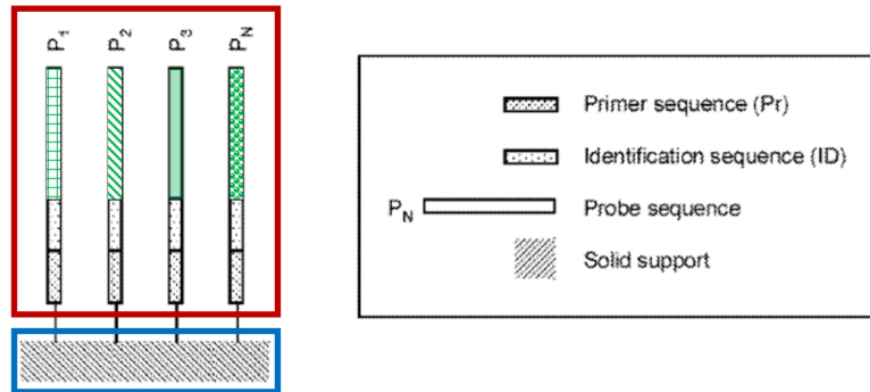
(a) *“A composition comprising an array comprising a substrate and a plurality of capture probes”*

302. Cantor discloses “[a] composition comprising an array comprising a substrate and a plurality of capture probes.” Thus, to extent the preamble is limiting, it is disclosed by Cantor.

303. In short, Cantor discloses arrays comprising a substrate with a plurality of beads that each include a capture probe. In detail, Cantor discloses “[i]mproved solid supports and methods for analyzing target nucleotide sequences....” EX1031, 1. A specific example of Cantor’s disclosure “provides *a substrate* comprising *a collection of beads* oriented in *an array*.” *Id.*, 4. Each bead “is in association with single-stranded nucleic acid.” *Id.* The bead-associated nucleic acids, which constitute the claimed capture probes, include “a probe sequence,” which “hybridizes to” (*i.e.*, captures) “a sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid.” *Id.*

304. Figure 1 below depicts examples of the single-stranded nucleic acids (*i.e.*, the claimed “**capture probes**”) described in the example above. Each of the capture probes include a probe sequence, which is a **target nucleic acid binding domain** that is configured to interact with a target nucleic acid. Figure 1 also shows a solid support, which in the specific example above is a bead attached to a substrate. EX1031, 10 (“Examples of solid supports for use with processes described herein

include, without limitation, beads...”); *see also id.* at 4. I have annotated this figure to show the **capture probes**, **target nucleic acid binding domain**, and the **solid support** (i.e., substrate with attached beads).

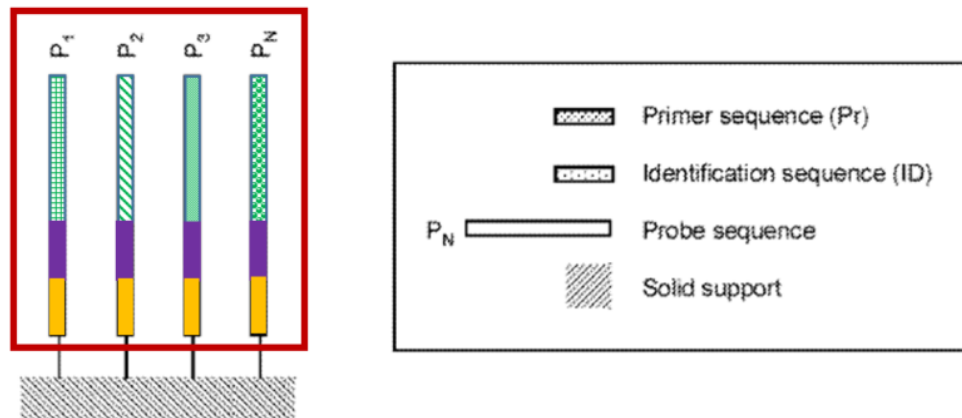


(b) *“each of the plurality of capture probes comprises: (i) a target-binding domain, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain”*

305. Cantor discloses “each of the plurality of capture probes comprises: (i) a target-binding domain, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is

complementary to at least a portion of the nucleic acid that binds to the target-binding domain.”

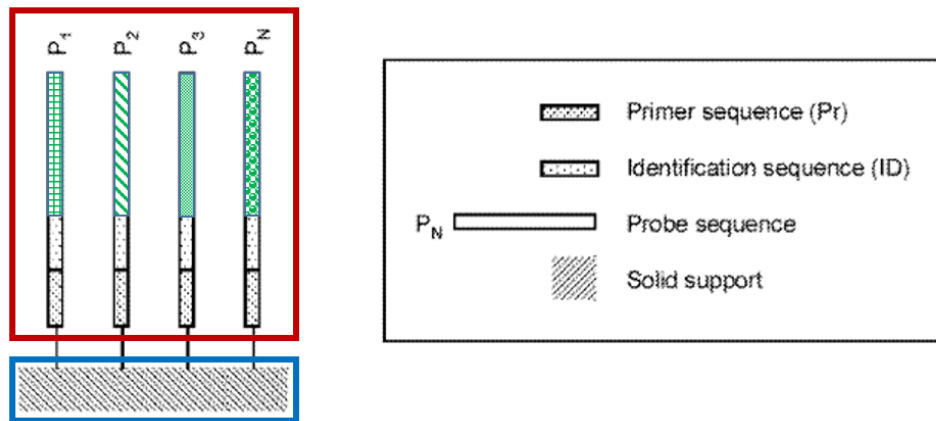
306. As explained in greater detail below, in Figure 1 (annotated below), each single-stranded nucleic acid (i.e., **capture probe**) in Cantor’s arrays comprises (1) **a primer binding site**, (2) **a nucleic acid sequence that identifies a unique location on the substrate** and is not configured to hybridize to a target nucleic acid that binds to a target nucleic acid binding domain, and (3) **a target-binding domain**, wherein the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain.



- (i) ***“each of the capture probes of the plurality of capture probes comprises:... (i) a target-binding domain,... the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain”***

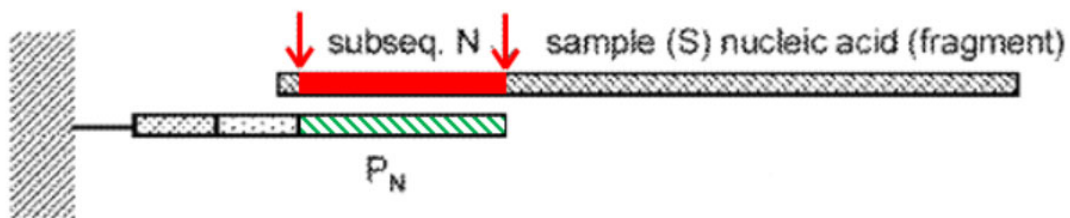
307. Cantor discloses that each of the capture probes has a target-binding domain comprising a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain. As mentioned previously, Cantor discloses that the application is directed to “[i]mproved solid supports and methods for analyzing target nucleotide sequences.... Certain improvements are directed to 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.” EX1031, 1. In particular, Cantor discloses that the capture probes (i.e., single-stranded nucleic acids) contain a sequence that is “complementary to or substantially complementary to a sample nucleic acid nucleotide subsequence and allows solid phase nucleic acid to hybridize to sample nucleic acid. Such a subsequence (e.g., illustrated in Figures 1A-1C) is referred to herein as a ‘probe’ sequence.” *Id.*, 13.

308. This “**probe sequence**” (i.e., **target-binding domain**) is shown below in Figure 1A. This sequence is the third sequence of the **capture probe** from the **solid support (i.e., bead)** surface, as depicted below.



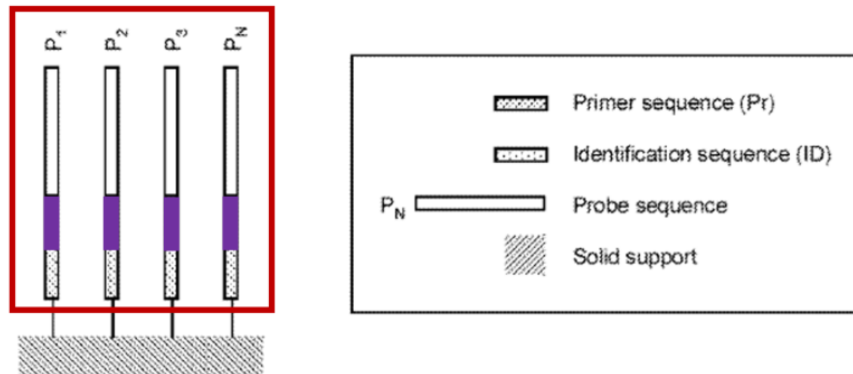
309. Cantor discloses that the target-binding domain is “a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain.” For example, Cantor’s “probe sequences” bind to target nucleic acids (including those from a sample such as a tissue section) because they are “complementary to subsequences in the sample nucleic acid (e.g., probe sequence species P₁, P₂, P₃,...P_n are complementary to sample nucleic acid subsequences 1, 2, 3,...N, respectively).” EX1031, 16. Cantor explains: “*A probe nucleotide sequence is of a length sufficient to specifically hybridize to a sample nucleic acid nucleotide sequence.* In certain embodiments a probe sequence is about 5 to about 100 nucleotides in length, and sometimes is about 5 to about 40

nucleotides in length.” *Id.*, 14; *see also id.* (“Generally, a shorter probe sequence is selected for applications where the target nucleotide sequence is known or partially known and longer probe sequence are selected for applications in which the target nucleotide sequence or portions thereof are not known. In some embodiments, a probe sequence is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000 or 5000 nucleotides in length.”). This is further confirmed in Figure 2, which depicts the target-binding domain hybridized to the target nucleic acid. Cantor explains that in Figure 2 “[s]ample nucleic acid molecule (S) may include a **nucleotide sequence (i.e., subseq.N)** that hybridizes to a **complementary probe sequence (P_N)** of the solid phase nucleic acid.” EX1031, 24. Cantor does not describe any other part of the single-stranded nucleic acid hybridizing (i.e., binding) to the target nucleic acid.



(ii) *“each of the capture probes of the plurality of capture probes comprises:... (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain”*

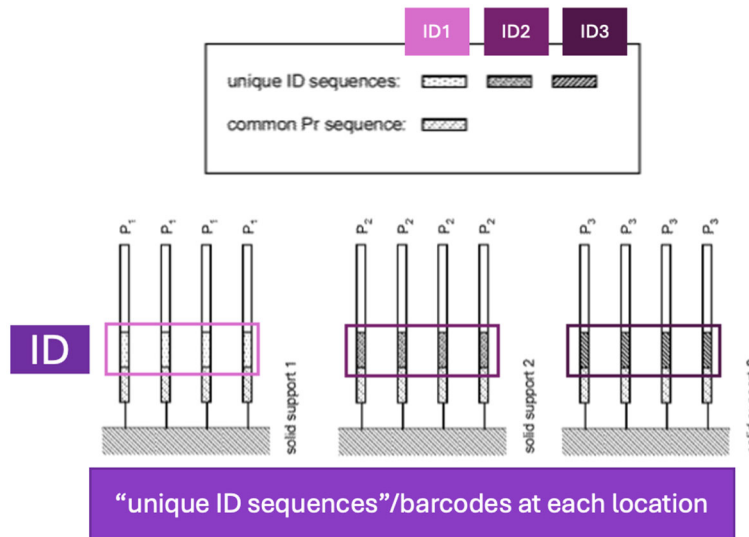
310. Each of the capture probes in Cantor’s arrays (e.g., the single-stranded nucleic acids depicted in Fig. 1) also includes a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a target nucleic acid that binds to a target-binding domain. In particular, Cantor discloses that in its arrays, “each of the solid supports in the collection share a unique identifier sequence different than the identifier sequence of [the] solid phase nucleic acid of the other solid supports.” *Id.*, 2. In other words, each solid support (e.g., bead) is coded with a unique nucleic acid “identifier sequence” that differs from the “identifier sequence” coding of every other bead. This **identifier sequence** is depicted on the **capture probes** shown in Figure 1 below. Since Figure 1 depicts a single solid support, and each capture probe on a given solid support shares a common identifier sequence, each identifier sequence is the same.



311. Additionally, Cantor explains that these “[s]olid supports may be provided in an array” including for example “an ‘X by Y array’ for square or rectangular arrays, where the array includes X number of sample locations or solid supports,” such as beads, “in one dimension and Y number of sample locations or solid supports,” such as beads, “in a perpendicular dimension.” EX1031, 17.

312. Because Cantor discloses an array of beads at which each bead is necessarily deposited at a particular (X, Y) coordinate location, each identification sequence necessarily corresponds to, and thus identifies, the unique location (such as an (X,Y) coordinate) of the bead on the array.

313. Indeed, this is shown in Cantor’s Figure 1C, which depicts separate solid supports, such as beads, each having “unique ID sequences” that are correlated to the specific solid supports “solid support [e.g., bead] 1,” “solid support [e.g., bead] 2,” and “solid support [e.g. bead] 3.”

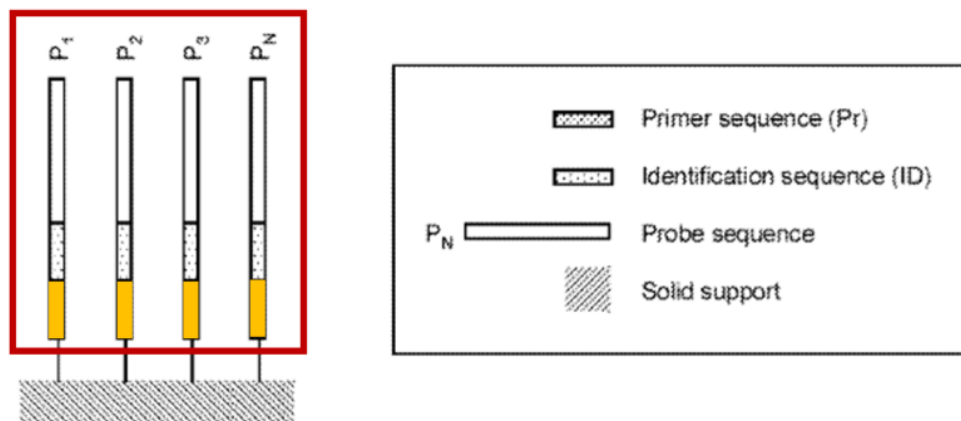


314. Thus, a POSA would understand that the “identification sequence” described in Cantor is a “nucleic acid sequence that identifies a unique location on the substrate.” For example, in the figure annotated above, ID1 identifies the unique location “solid support [e.g., bead] 1,” ID2 identifies the unique location “solid support [e.g., bead] 2,” and ID3 identifies the unique location “solid support [e.g., bead] 3.” *See also* element 1(c), discussed below.

315. Cantor also discloses that the “nucleic acid sequence that identifies a unique location on the substrate” is not configured to hybridize to a nucleic acid that binds to the target-binding domain. Rather, the target nucleic acid binding domain is configured to bind to the target nucleic acid, as described in Section IX.A.1(b)(i) above.

(iii) “each of the capture probes of the plurality of capture probes comprises:... (iii) a primer binding site”

316. Cantor discloses that the single-stranded nucleic acid can “include[] a primer sequence (Pr), which is also referred to herein as a ‘primer hybridization sequence.’ *Primer sequence (Pr) can hybridize [i.e., bind] to a complementary nucleotide sequence in a primer nucleic acid* that can be utilized to amplify extended solid phase nucleic acid previously hybridized to a sample nucleic acid. As used herein, the term ‘primer nucleic acid’ refers to a nucleic acid (e.g., naturally occurring or synthetic) that has a nucleic acid sequence complimentary to a primer hybridization sequence and can hybridize to the primer hybridization sequence under hybridization conditions and can be extended in an amplification process (e.g., primer extension, PCR amplification, and the like).” EX1031, 15. Exemplary **primer sequences** (i.e., **primer binding sites**) on **capture probes** are shown in Figure 1 below.



(c) ***“each of the plurality of capture probes are immobilized on the substrate in a random pattern”***

317. Cantor in view of the knowledge of a POSA renders obvious “each of the plurality of capture probes are immobilized on the substrate in a random pattern.”

In particular, Cantor describes that solid supports (e.g., beads) with attached capture probes can be associated with a substrate to form an array.

318. As just one example, Cantor explains that “the invention in part provides a substrate comprising a collection of beads oriented in an array.” EX1031, 4. The “array may contain one solid support species or multiple solid support species from a collection. ***The array can be arranged on any substrate*** suitable for sequence analysis or manufacture processes described herein. Examples of substrates include without limitation flat substrates, filter substrates, wafer substrates, etched substrates, ***substrates having multiple wells or pits....***” EX1031, 17-18. An exemplary embodiment is described in Cantor’s published claim 48:

48. A substrate comprising a collection of beads oriented in an array, wherein:
- each bead is in association with single-stranded nucleic acid;
 - the solid phase nucleic acid comprises an identifier sequence and a probe sequence;
 - the probe sequence hybridizes to sample nucleic acid when the probe sequence is complementary to a nucleotide sequence in the sample nucleic acid;
 - at least one nucleic acid of the solid phase nucleic acid of each of the solid supports in the collection has a unique probe sequence different than a probe sequence of the solid phase nucleic acid of the other solid supports; and
 - the solid phase nucleic acid of each of the solid supports in the collection share a unique identifier sequence different than the identifier sequence of solid phase nucleic acid of the other solid supports.

319. Cantor explains that the beads can be attached to the substrate by placing the beads in the wells or pits. *Id.*, 18. (“Wells or pits of multiple well and pit substrates may contain one or more solid support units (e.g., each unit being a single bead or particle).”). In addition to this physical attachment, a POSA would also immediately understand, including from references incorporated by reference by Cantor, that Cantor discloses chemically attaching its beads to the substrate through various methods known in the art. Indeed, Cantor incorporates by reference U.S. Patent No. 6,133,436, which is titled “Beads bound to a solid support and to nucleic acids” and discloses methods to attach beads to a substrate. *See, e.g.*, EX1048, 2:56-57 (“FIG. 1 is a schematic showing the covalent attachment of a bead to a solid support and DNA to the bead.”); EX1031, 12 (“Methods for linking solution phase nucleic acid to a solid support also are known (e.g., U.S. Patent No. 6,133,436, naming Koster et al. and entitled ‘Beads bound to a solid support and to nucleic acids’ and WO 91/08307, naming Van Ness and entitled ‘Enhanced capture of target nucleic acid by the use of oligonucleotides covalently attached to polymers’).”).

320. Additionally, a POSA would recognize that Cantor discloses immobilizing capture probes directly onto the substrate surface without using beads. In particular, Cantor discloses that “[m]ethods for linking solution phase nucleic acid to a solid support also are known.... Examples include, without limitation, thioether

linkages (e.g., thiolated nucleic acid); disulfide linkages (e.g., thiol beads, thiolated nucleic acid); amide linkages (e.g., Wang resin, amino-linked nucleic acid);... and the like. Nucleic acid may be linked to a solid support without a linker or with a linker (e.g., S. S. Wong, "Chemistry of Protein Conjugation and Cross-Linking," CRC Press (1991), and G. T. Hermanson, "Bioconjugate Techniques," Academic Press (1995).” EX1031, 12.

321. It also would have been immediately apparent that when attaching the probes to the substrate, there were only a limited number of ways to “arrange” (i.e., “position”) the capture probes on the substrate: (i) selective loading of capture probes to particular locations on the array and/or (ii) randomly loading the capture probes and then decoding the array prior to use. *See* Section V.D.2.

322. Cantor discloses that its “identifier sequence is about 5[-]50 nucleotides in length.” EX1031, 15. In a selective loading procedure, a POSA would have found it obvious to provide beads having, for example, 5 nucleotide identifier sequences that correspond to particular locations in Cantor’s (X, Y) array. A POSA would have reasonably expected success in correlating such sequences with particular (X, Y) coordinates given the disclosure of success in using such systems in the prior work of the named inventor and others. *See* EX1032 (Chee-584), 7:30-33 (beads are spatially arranged in “a regular pattern of sites such that the sites may be addressed

in the X-Y coordinate plane.”); EX1038 (Chee-824), [0044] (“Once the identity of the bioactive agent and its location in the array has been fixed, the array is exposed to samples containing target analytes.”); EX1036, 251 (Barany 1999) (describing 4 nucleotide “zip-code” sequences to define spatial locations on an array in a “checkerboard” (X, Y) array pattern).

323. For example, the below illustration shows an example of a 16 bead array with unique 5-nucleotide identifier sequences at each (X,Y) location. X coordinates are numbered 1-4, and Y coordinates are lettered A-D. In a selective loading technique, to correlate position A1 with the identification sequence AGTGA, a POSA would have found it obvious to deposit a bead (or beads) that was (or were) manufactured to include such sequences according to known methods at the A1 coordinate in the array. *See* EX1038, [0043] (describing “positional decoding” by “targeting the placement of the beads” to predetermined locations on the array). A POSA would have found it obvious to target the placement of the other barcoded beads to the other locations according to similar methods.

	1	2	3	4
A	AGTGA	TGTGC	TCAAA	AGCTA
B	GTACG	TATCC	GCTTG	CTCGA
C	CCTTC	TTCGT	CTACC	TGGGC
D	TGAAT	CCTTA	CATTG	GATTA

324. While the illustration above uses 16 total positions, the use of 5 nucleotide barcodes allows uniquely addressing 1,024 unique positions, and each additional nucleotide added to a barcode would have been known to exponentially increase the number of unique available barcodes and thus the number of beads that can be uniquely addressed and loaded on an array.

325. Selectively loading the beads in a defined pattern becomes more difficult as more beads are added to the array. Thus, alternatively, “the beads may be randomly distributed on the array,” and “can later be ‘decoded’, i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or candidate agent at that particular site can be made.” EX1038, [0042]. A POSA would have been motivated to use such a random loading procedure to simplify and expedite the array generation and fabrication process, and would have found it obvious to decode the array by using any of a number of known decoding procedures. For example, a POSA would have found it obvious to use the decoding

by sequencing procedure disclosed in Chee-824, which allows simultaneous decoding of many bead types in a single experiment. *Id.*, [0046]. For example, Chee-824 discloses “decoding by sequencing” of bead arrays comprising probes structured similarly to those used in Cantor’s bead array, comprising an encoding sequence used to identify the particular bead as well as a gene-specific sequence that may be used to assay target nucleic acids. *Id.*, [0037]-[0038], Figs. 12-13. Moreover, the procedures disclosed in Chee-824 allow the array to be used in analytical assays without issue following the decoding procedure. *Id.*, [0146].

326. Thus, assuming that barcoded beads were randomly deposited on a substrate and landed in the positions illustrated in the table above, decoding by sequencing, such as the methods disclosed in Chee-824, would have been an obvious method for obtaining knowledge of the identification sequence at each location before use in subsequent assays. That is, using such methods, a POSA would have been motivated to decode the array to learn that position A1 had the identification sequence AGTGA, position B1 had the identification sequence GTACG, and so on. A POSA would have reasonably expected success in performing such decoding, as doing so would have involved the mere application of well-known techniques, including those described in Chee-824.

327. Accordingly, Cantor in view of the knowledge of a POSA renders obvious this limitation.

(d) Conclusion on Claim 1

328. As described above, Cantor in view of the knowledge of a POSA renders obvious Claim 1.

2. Claim 5

329. Claim 5 of the '138 Patent recites “[t]he composition of claim 1 wherein the array is deposited on a slide or flow-cell.”

330. Cantor discloses that the array is deposited on either a slide or cell. In particular, Cantor states “[t]he array can be arranged on any substrate suitable for sequence analysis or manufacture processes described herein. Examples of substrates include without limitation 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.” EX1031, 17-18. Cantor also discloses that these “[s]ubstrates can comprise or consist essentially of a suitable material for conducting sequence analysis or manufacture processes described herein, including without limitation, fiber (e.g., fiber filters), glass (e.g., glass surfaces, fiber optic surfaces), metal (e.g., steel, gold, silver, aluminum, silicon and copper; metal coating), plastic

(e.g., polyethylene, polypropylene, polyamide, polyvinylidenedifluoride), silicon and the like.” EX1031, 18; *see also id.*, 53 (“Described hereafter is methodology for performing nucleic acid sequence analyses described herein. Synthesized oligonucleotides containing probe, primer and identification sequences are linked to solid support (beads, *slides*, chips, and the like, and in some embodiments, Dynal® beads) commonly available in the art, via appropriate linkage chemistry.”). Thus, Cantor discloses depositing the array on a slide. *See, e.g.*, ’138 Patent at 30:49-50 (“Synthetic oligonucleotide templates are attached to a glass slide via a 5' amino modification.”).

331. I have been informed by counsel that I should apply the plain and ordinary meaning to a POSA of the terms “slide” and “flow cell” when determining whether Cantor discloses a slide. A POSA would consider the plain and ordinary meaning of “slide” to include all flat substrates, filter substrates, wafer substrates, etched substrates, substrates having multiple wells or pits, and substrates having one or more channels “slides.” Thus, Cantor discloses substrates that are either a “slide” or “flow cell,” and it would be obvious to a POSA to use any one of these substrates with Cantor’s disclosed array.

3. Claim 17

332. Claim 17 of the '138 Patent recites:

17. A composition comprising (i) an array comprising a substrate and a plurality of capture probes, and (ii) a tissue section, wherein:

the tissue section is disposed on the array;

each of the plurality of capture probes comprises: (i) a target-binding domain (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site;

the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence complementary to at least a portion of the nucleic acid that binds to the target-binding domain; and

each of the plurality of capture probes are immobilized on the substrate in a random pattern.

333. As described below, Cantor in view of the knowledge of a POSA renders obvious of Claim 17.

(a) “A composition comprising (i) an array comprising a substrate and a plurality of capture probes, and (ii) a tissue section”

334. As described above in Section IX.A.1(a), Cantor discloses “[a] composition comprising (i) an array comprising a substrate and a plurality of capture probes.” As described below, Cantor also discloses a tissue section.

335. In particular, Cantor explains that its capture probes interact with a “sample nucleic acid” by hybridization. EX1031, 2. (“[T]he solid support and

sample nucleic acid are contacted in the mixture under conditions that allow hybridization of the solid phase nucleic acid,” i.e., the probe, “to the sample nucleic acid.”). Cantor explains that this sample may be “a solid material *such as a tissue...or a biopsy.*” *Id.*, 6. A POSA would have immediately appreciated that a biopsy is a type of tissue section. *See, e.g.*, EX1005, 12:20-22 (“Exemplary biological samples include *tissue sections (e.g., including* whole animal sectioning and *tissue biopsies)*, cell populations on slides or culture dishes, and the like.”). Thus, Cantor discloses a tissue section.

(b) “the tissue section is disposed on the array”

336. Cantor in view of the knowledge of a POSA renders obvious “the tissue section is disposed on the array.” In particular, Cantor explains that its sample may be “a solid material such as a tissue...or a biopsy,” *id.*, 6, and the literature evidenced multiple reasons why a POSA would have been motivated to perform array-based nucleic acid analysis on a tissue sample (such as an intact tissue section or a biopsy sample), including because the analysis of such samples would provide information, such as spatial information, that could not be obtained through the analysis of, for example, a tissue homogenate:

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Variations in DNA status (mutation, epigenetic modification, insertions, deletions) by location within a tissue section are of interest for elucidating their roles in biology and as clinical markers for diseases. However, to study the molecular basis of normal and pathological conditions, many researchers identify genes of interest from tissue specimens by examining cell homogenates (all the cells in the sample mixed together). Since tissue comprises a complex milieu of diverse cell populations that define the local microenvironments, combining the multiple cell populations into one measurement masks a tremendous amount of information. Therefore, to achieve a more complete view, DNA variability within a tissue sample needs to be correlated with the type of cell from which it originates (for example, stroma vs. epithelium), the physical location within the tissue section, and/or histopathological features (for example, a specific disease lesion).

EX1022 (Armani), 3526

When handling cells, which constitute a large percentage of specimens, there are instances in which it is problematic to convert the cell component being measured to a solution state. Since balance of the whole is achieved through mutual interaction between a cell and adjacent cell groups in multi-cellular organisms, pulverizing cell groups will result in a uniform state, causing localized information for each cell to be lost. Further, even within individual cells, mRNA, which is an expressed gene, is localized. Proteins, which are the final product, are also localized within the cell in accordance with their function. These local mRNA and proteins change in accordance with a cell's state of activity, in other words the level of communication between a cell and the adjacent cell group, and the environment in which the cell is placed. Therefore, if an attempt is made to obtain information useful for medically-directed drug discovery, and to describe biological functions more accurately, the mRNA and proteins localized between or inside cells must be quantitatively detected in a state in which local space information is retained.

EX1026 (Okano), [0008]. Thus, a POSA would have been motivated to contact Cantor's array with a tissue section, yielding the claimed combination.

337. Additionally, Cantor explains that its capture probes interact with the target nucleic acid by contacting the nucleic acids with the array to allow for hybridization between the capture probes and the nucleic acids. EX1031, 2. (“[T]he solid support and sample nucleic acid are contacted in the mixture under conditions that allow hybridization of the solid phase nucleic acid,” i.e., the probe, “to the sample nucleic acid.”). Thus, Cantor teaches that the tissue section would need to be contacted with (i.e., disposed on) the array.

338. Cantor further explains in its Example 1, “Sequence analysis methodology,” that “[s]ample nucleic acid may be prepared by any means commonly known in the art, including but not limited to cell lysis procedures using chemical, physical or electrolytic lysis methods.” *Id.*, 53-54. A POSA would have been familiar with, and would have been motivated to apply such techniques to intact tissue or biopsy samples such that the tissue or biopsy sample disposed on the array is then treated such that the capture probes can hybridize to the nucleic acids in the tissue sample.

339. Indeed, these techniques were frequently applied in the art. For example, Southern similarly discloses the spatial analysis of nucleic acid molecules

by contacting tissue-derived nucleic acid molecules with a binding agent affixed to a solid support. EX1033, 16:28-30; 7:3-7. Southern discloses that, “[i]n some methods, *samples are applied directly to the support to generate a spatial arrangement of samples*, as illustrated in Fig. 1. Thus, *when the samples are biological samples comprising cells, the step a) of applying the samples to a support may comprise: (i) applying cells to the support; then (ii) releasing material from the cells.*” *Id.*, 2:17-27. A POSA would recognize that “releasing material from the cells” is synonymous with lysing the cells, as disclosed by Cantor. A POSA would similarly have been familiar with methods for releasing and capturing locally target nucleic acid from cells in a tissue sample disposed on an array, including a bead array, from the background references discussed in Section V.E, above. *See* EX1021-1022 (Armani) (physical pressing and chemical lysis of tissue samples to release target nucleic acid); EX1024 (Shirai) (use of electrophoretic force to lyse cells in tissue sample to locally release target nucleic acid for capture on probes attached to beads); EX1025 (Yasuda) (same); EX1026 (Okano) (same). Thus, a POSA would have been further motivated to dispose the tissue section on the array disclosed in Cantor and lyse the cells such that the probes can interact with the target nucleic acids within the tissue sample.

- (c) ***“each of the plurality of capture probes comprises: (i) a target-binding domain (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence complementary to at least a portion of the nucleic acid that binds to the target-binding domain”***

340. Cantor discloses “each of the plurality of capture probes comprises: (i) a target-binding domain (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence complementary to at least a portion of the nucleic acid that binds to the target-binding domain.” For example, see Section IX.A.1(b) above, which is herein incorporated by reference.

- (d) ***“each of the plurality of capture probes are immobilized on the substrate in a random pattern”***

341. As described above in Section IX.A.1(c), Cantor in view of the knowledge of a POSA renders obvious “each of the plurality of capture probes are immobilized on the substrate in a random pattern.”

4. Claim 20

342. Claim 20 recites “[t]he composition of claim 17, wherein the tissue section is a formalin-fixed paraffin-embedded tissue section or a fresh frozen tissue section.”

343. As mentioned previously, Cantor explains that its target/sample nucleic acid may be “a solid material *such as a tissue...or a biopsy.*” *Id.*, 6. Furthermore, Cantor explains in its Example 1, “Sequence analysis methodology,” that the “[s]ample nucleic acid may be prepared by any means commonly known in the art.” *Id.*, 53-54. As described in Section V.B., there were only a handful of methods for preserving a tissue sample known in the art prior to 2010, including formalin fixation followed by paraffin embedding and freezing a fresh tissue section to create a fresh frozen tissue sample. Thus, a POSA would have found it obvious to use a fresh-frozen tissue sample or formalin-fixed paraffin-embedded tissue sample with the array disclosed in Cantor.

344. Moreover, a POSA would have been motivated to use a fresh-frozen tissue sample with the array disclosed in Cantor. In 2010, fresh-freezing tissue samples was a common method for preserving tissue samples in a laboratory and clinical setting. Given the prominence of fresh-frozen tissue samples in the field, a POSA would have been motivated to try using a fresh frozen tissue sample with

Cantor's array. Additionally, because fresh frozen tissue samples were known to have better RNA/nucleic acid quality than formalin-fixed paraffin embedded tissues, see EX1046, 1229; EX1047, 1933, a POSA would have been motivated to use fresh-frozen samples because their high quality, highly intact nucleic acid content made them highly suitable for use with arrays designed to detect RNA/nucleic acids, like Cantor's.

5. Claim 23

345. Claim 23 recites "[t]he composition of claim 17, wherein the array is deposited on a slide or flow-cell."

346. Cantor discloses "[t]he composition of claim 17, wherein the array is deposited on a slide or flow-cell." See the analysis for Section IX.A.2 above, which is herein incorporated by reference.

6. Claim 28

347. Claim 28 recites "[t]he composition of claim 17, wherein the nucleic acid comprises RNA or DNA."

348. Cantor discloses the target nucleic acid being DNA or RNA. For example, Cantor discloses that the "[s]ample [*i.e.*, target] nucleic acid may comprise or consist essentially of any type of nucleic acid suitable for use with processes of the invention, such as sample nucleic acid that can hybridize to solid phase nucleic

acid (described hereafter), for example. A sample nucleic in certain embodiments can comprise or consist essentially of DNA (e.g., complementary DNA (cDNA), genomic DNA (gDNA) and the like), RNA (e.g., message RNA (mRNA), short inhibitory RNA (siRNA), ribosomal RNA (rRNA), tRNA and the like), and/or DNA or RNA analogs (e.g., containing base analogs, sugar analogs and/or a non-native backbone and the like).” EX1031, 6; *see also id.*, cl. 10 (“A method according to any one of claims 1-7, wherein the sample nucleic acid comprises RNA.”); 5 (“Sample nucleic acid may be derived from one or more samples or sources. As used herein, ‘nucleic acid’ refers to polynucleotides such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).”).

7. Claim 29

349. Claim 29 recites “[t]he composition of claim 28, wherein the RNA is mRNA.”

350. As described above in relation to Claim 28, which is herein incorporated by reference, Cantor discloses the RNA being mRNA. For example, Cantor discloses that the “[s]ample [i.e, target] nucleic acid may comprise or consist essentially of any type of nucleic acid suitable for use with processes of the invention, such as sample nucleic acid that can hybridize to solid phase nucleic acid (described hereafter), for example. A sample nucleic in certain embodiments can

comprise or consist essentially of DNA (e.g., complementary DNA (cDNA), genomic DNA (gDNA) and the like), *RNA (e.g., message RNA (mRNA)*, short inhibitory RNA (siRNA), ribosomal RNA (rRNA), tRNA and the like), and/or DNA or RNA analogs (e.g., containing base analogs, sugar analogs and/or a non-native backbone and the like).” EX1031, 6.

B. Ground 2: Obvious over Cantor in view of Armani and the Knowledge of a POSA

1. Claim 1 and Claim 17

351. I incorporate by reference my analysis in Ground 1 above, which outlines why the Challenged Claims are obvious over Cantor in view of the knowledge of a POSA. Armani provides additional information rendering obvious disposing the tissue section on the array as recited by Claim 17 and wherein the tissue section is a fresh-frozen tissue section as recited by Claim 20. Nevertheless, all challenged claims are still rendered obvious for the reasons laid out in Ground 1 and said reasoning applies to this ground with equal force. Moreover, because Claim 17 recites the same features as claim 1 combined with “(ii) a tissue section, wherein: the tissue section is disposed on the array,” claim 1 is rendered obvious over Cantor in view of Armani and Knowledge of a POSA for the same reasons as claim 17. Thus, all challenged claims are rendered obvious under this ground as well.

352. Claim 17 of the ’138 Patent is reproduced below.

17. A composition comprising (i) an array comprising a substrate and a plurality of capture probes, and (ii) a tissue section, wherein:

the tissue section is disposed on the array;

each of the plurality of capture probes comprises: (i) a target-binding domain (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site;

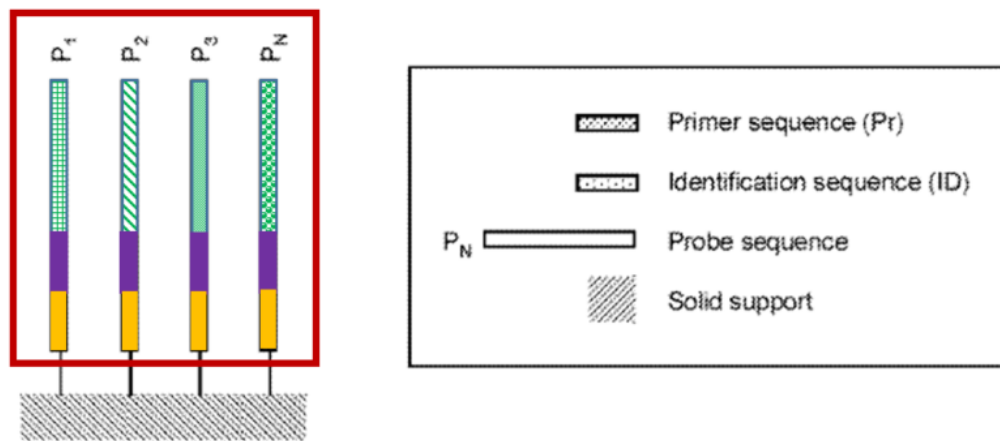
the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence complementary to at least a portion of the nucleic acid that binds to the target-binding domain; and

each of the plurality of capture probes are immobilized on the substrate in a random pattern.

353. Cantor in view of Armani and the knowledge of a POSA further renders obvious the composition of claim 17 wherein “the tissue section is disposed on the array.”

354. As explained above, Cantor describes a device for detecting nucleic acids using nucleic acid arrays that capture, extend, amplify, and detect target nucleic acids with sequencing. In summary, Cantor discloses providing a substrate with a plurality of the claimed “**capture probes**” in the form of nucleic acid constructs that include a “**probe sequence**” that is configured to hybridize with a target nucleic acid, as well as a “**coding identifier**” or “**identification sequence**” that may be used to identify a unique location on the substrate. Cantor’s Figure 1A shows an example of its solid support, such as a substrate, with a plurality of

“**capture probes**,” each having the same “**primer sequence**” and “**identification sequence**” and different “**probe sequences**.” I have annotated this figure to show the correspondence between Cantor’s Figure 1 and the requirements of the claimed “capture probes” below.



355. Cantor further explains that its “array can be arranged on any substrate suitable for sequence analysis or manufacture processes described herein,” including “substrates having multiple wells or pits,” with varying volumes ranging from the microliter to picoliter scale. EX1031, 17-18. Cantor explains that “[w]ells or pits of multiple well and pit substrates may contain one or more solid support units (e.g., each unit being a single bead or particle.” *Id.*, 18; *see also id.*, 19 (“In some embodiments, the microreactor volume is large enough to accommodate one solid support bead in the microreactor and small enough to exclude the presence of two or more beads in the microreactor.”). Thus, Cantor discloses providing a plurality of

beads containing the claimed binding agents by arranging the beads in a multiple well plate. This arrangement of binding agents in wells motivates combining the teachings of Cantor with the teachings of Armani.

356. Armani further discloses apparatuses/methods for the analysis of nucleic acids in a tissue section. Using Armani's apparatus, (1) a "tissue section is transferred onto the surface of the multi-well array (step 1A) and the pressed onto the surface (1B)," after which "[t]he contents of the well are dehydrated to enable the addition of lysis reagents (1C); (2) "the tissue is lysed to release genomic DNA into solution"; (3) the "DNA is amplified" using PCR; and (4) "the DNA is imaged" using fluorescent detection techniques. EX1022, 3527. This is depicted in Armani's Figure 2.

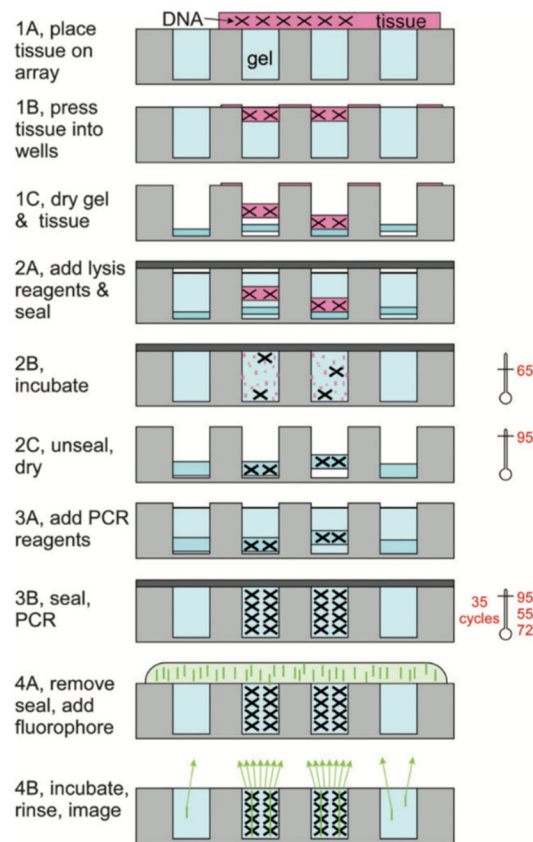


Fig. 2 Experimental protocol used to demonstrate the 2D-PCR concept. Tissue is transferred into wells, isolated, and lysed to extract the genomic DNA (represented by "x"s). PCR is performed to amplify the target DNA, and the targets are visualized with fluorescent dye (indicated by vertical lines "I").

357. A POSA would have found it obvious to combine the teachings of Cantor and Armani by providing Cantor's bead array with the plurality of beads disposed in wells (as noted above, an embodiment expressly contemplated by Cantor), contacting that array with a tissue slice as disclosed by Armani, and then pressing the tissue sample into the wells and lysing the tissue as described by Armani. EX1022, 3531 (describing "loading the tissue vertically, pressing it directly into the array of wells to isolate tissue subregions while preserving the overall 2D

spatial relationship.... This tissue loading technique also has the advantage of beginning with traditional histological sectioning and transfer steps, which are familiar to investigators and clinicians who may wish to adopt this technique for research or diagnosis.”).

358. A POSA reviewing Cantor and Armani would have been motivated to combine their teachings for several reasons. Cantor’s protocols facilitate the capture, amplification, and detection of mRNA by sequencing, which Armani indicates was a desirable application for spatial analysis. By using sequencing as a detection technique, rather than visualization (e.g., fluorescence), the combination would allow the detection of even low abundance mRNAs. *See* EX1022, 3532 (“We are currently working on mapping RNA from tissue, since current visualization techniques for RNA cannot reliably detect below a few dozen copies per cell in situ.”). Moreover, the use of Cantor’s methods to generate cDNA libraries of target nucleic acids coded with location information (via Cantors’ identification sequences) would simplify Armani’s detection procedure by eliminating at least the steps of unsealing the array, adding a fluorophore, incubating, rinsing, and imaging in Armani’s steps 4A-4B. Instead, using sequencing as a detection method would allow a POSA to simply pool the contents of all wells together in a multiplexed

sequencing procedure, where the data can then be deconvoluted and spatially mapped based on the read out of location-correlated identification sequences.

359. A POSA would have reasonably expected success in combining the teachings of Cantor and Armani. Indeed, the combination would have involved using Cantor's device and methods in the manner they are disclosed—to detect nucleic acids from a tissue sample, while specifically using the identification probes to identify the location at which beads were provided. Armani further provides additional specific implementations of how to successfully prepare and process effectively a nucleic acid tissue sample for localized detection, methods not claimed in the '138 Patent, but the practice of which confirms the obviousness of the claimed arrangement.

2. Claim 20

360. Claim 20 recites “[t]he composition of claim 17, wherein the tissue section is a formalin-fixed paraffin-embedded tissue section or a fresh frozen tissue section.”

361. As mentioned previously, Cantor explains that its target/sample nucleic acid may be “a solid material *such as a tissue...or a biopsy*.” *Id.*, 6. Furthermore, Cantor explains in its Example 1, “Sequence analysis methodology,” that “[s]ample nucleic acid may be prepared by any means commonly known in the art.” *Id.*, 53-

54. A POSA would have been familiar with, and would have been motivated to apply such techniques to the tissue or biopsy samples disclosed by Cantor such that the tissue or biopsy sample could be disposed on the array and then treated such that the capture probes can hybridize to the nucleic acids in the tissue sample.

362. Armani describes an apparatus for the analysis of nucleic acids in a frozen tissue section. In particular, Armani discloses placing a fresh-frozen tissue section on a multi-well array as shown below to detect the presence of a target nucleic acid at various location throughout the tissue sample. EX1022, 3528 (“Two types of tissue were used in these studies: frozen tissue samples from a human prostate with benign hyperplasia and breast tissue with lobular carcinoma....Tissue specimens were snap frozen and embedded in O.C.T. compound embedding medium (Sakura Fine-Tek, Torrance, CA).”); *id.* (“Tissue sections were transferred to the multi-well device immediately after sectioning while still frozen[.]”). After “the tissue section is transferred onto the surface of the multi-well array (step 1A),” (1) the tissue section is “pressed onto the surface (1B),” (2) “[t]he contents of the well are dehydrated to enable the addition of lysis reagents (1C); (3) “the tissue is lysed to release genomic DNA into solution”; (4) the “DNA is amplified” using PCR; and (5) “the DNA is imaged” using fluorescent detection techniques. EX1022, 3527. This is depicted in Armani’s Figure 2.

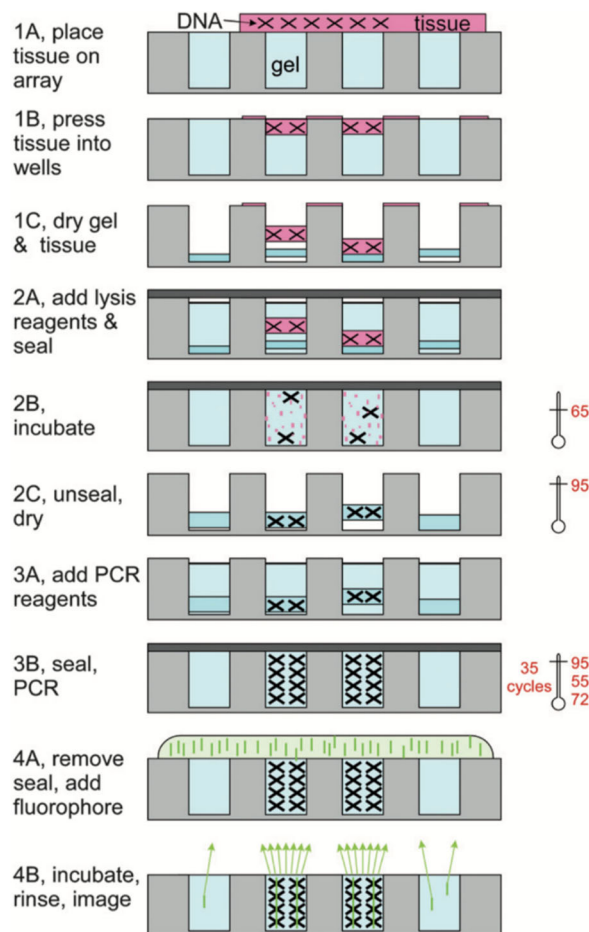


Fig. 2 Experimental protocol used to demonstrate the 2D-PCR concept. Tissue is transferred into wells, isolated, and lysed to extract the genomic DNA (represented by “x”s). PCR is performed to amplify the target DNA, and the targets are visualized with fluorescent dye (indicated by vertical lines “|”).

363. A POSA would have found it obvious to combine the teachings of Cantor and Armani by providing Cantor’s bead array with the plurality of beads disposed in wells (as noted above, an embodiment expressly contemplated by Cantor), contacting that array with a fresh-frozen tissue slice as disclosed by both Cantor and Armani, and then pressing the tissue sample into the wells and lysing the tissue as described by Armani. EX1022, 3531 (describing “loading the tissue

vertically, pressing it directly into the array of wells to isolate tissue subregions while preserving the overall 2D spatial relationship.... This tissue loading technique also has the advantage of beginning with traditional histological sectioning and transfer steps, which are familiar to investigators and clinicians who may wish to adopt this technique for research or diagnosis.”).

364. A POSA reviewing Cantor and Armani would have been motivated to combine their teachings for several reasons. Cantor’s protocols facilitate the capture, amplification, and detection of mRNA by sequencing, which Armani indicates was a desirable application for spatial analysis. By using sequencing as a detection technique, rather than visualization (e.g., fluorescence), the combination would allow the detection of even low abundance mRNAs. *See* EX1022, 3532 (“We are currently working on mapping RNA from tissue, since current visualization techniques for RNA cannot reliably detect below a few dozen copies per cell in situ.”). Moreover, the use of Cantor’s methods to generate cDNA libraries of target nucleic acids coded with location information (via Cantors’ identification sequences) would simplify Armani’s detection procedure by eliminating at least the steps of unsealing the array, adding a fluorophore, incubating, rinsing, and imaging in Armani’s steps 4A-4B. Instead, using sequencing as a detection method would allow a POSA to simply pool the contents of all wells together in a multiplexed

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sequencing procedure, where the data can then be deconvoluted and spatially mapped based on the read out of location-correlated identification sequences.

365. A POSA would have reasonably expected success in combining the teachings of Cantor and Armani. Indeed, the combination would have involved using Cantor's device and methods in the manner they are disclosed—to detect nucleic acids from a tissue sample, while specifically using the identification probes to identify the location at which beads were provided. Armani further provides additional specific implementations of how to successfully prepare and process effectively a nucleic acid tissue sample for localized detection.

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C. Ground 3: Anticipation by Frisen

1. Priority Date

366. The '138 Patent issued from Application No. 17/825,719 (“the '719 Application”), which was filed on May 26, 2022. I understand that the '138 Patent/'719 Application purports to claim priority to the following applications:

Related U.S. Application Data

- (63) Continuation of application No. 17/030,230, filed on Sep. 23, 2020, now Pat. No. 11,384,386, which is a continuation of application No. 16/988,284, filed on Aug. 7, 2020, now Pat. No. 10,961,566, which is a continuation of application No. 16/414,213, filed on May 16, 2019, now Pat. No. 10,787,701, which is a continuation of application No. 16/402,098, filed on May 2, 2019, now Pat. No. 10,472,669, which is a continuation of application No. 16/276,235, filed on Feb. 14, 2019, now Pat. No. 10,480,022, which is a continuation of application No. 15/187,661, filed on Jun. 20, 2016, now Pat. No. 10,308,982, which is a continuation of application No. 13/080,616, filed on Apr. 5, 2011, now Pat. No. 9,371,598.
- (60) Provisional application No. 61/321,124, filed on Apr. 5, 2010.

EX1004, Codes (63), (60). These purported priority documents are tabulated below. Because the '661 Application (filed on Jun. 20, 2016), '616 Application (filed on Apr. 5, 2011), and '124 Application (filed on Apr. 5, 2010) do not provide written description support for the challenged claims, the priority date for the '138 Patent is no earlier than the February 14, 2019 filing date of the '235 Application.¹⁷

¹⁷ The '235 Application fails to support the challenged claims for the same reasons discussed herein. However, I understand that for purposes of determining the priority date to which the Challenged Claims are entitled, Frisen is eligible as

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Accordingly, Frisen, which was published on April 24, 2014, is prior art to the '138 Patent. EX1045, Code (43).

'138 Patent Priority Documents	Filing Date	Exhibit
17/030,230 (the "'230 Application")	September 23, 2020	EX1060
16/988,284 (the "'284 Application")	August 7, 2020	EX1059
16/414,213 (the "'213 Application")	May 16, 2019	EX1058
16/402,098 (the "'098 Application")	May 2, 2019	EX1057
16/276,235 (the "'235 Application")	February 14, 2019	EX1010
15/187,661 (the "'661 Application")	June 20, 2016	EX1011
13/080,616 (the "'616 Application")	April 5, 2011	EX1012
61/321,124 (the "'124 Provisional")	April 5, 2010	EX1009

367. Based on my review, and as discussed in detail below, the challenged claims of the '138 Patent are not entitled to a priority date earlier than February 14, 2019, the filing date of the '235 Application. In my opinion, the challenged claims of the '138 patent are not supported by sufficient written description in the Specification of the '661 Application, the next priority application in the chain, such

prior art based on the break in the chain of priority at the '661 Application. Because Frisen is eligible as prior art based on this analysis, I have not formed an opinion as to whether any subsequently filed application supports the challenged claims, and reserve the right to perform that analysis if raised by Patent Owner.

that a POSA would have understood the inventor to be in possession of the subject matter recited in the challenged claims. Accordingly, I understand that since the '661 Application does not support the challenged claims, they are not entitled to a priority date earlier than the February 14, 2019 filing date of the '235 Application.¹⁸

368. As I explain below, the '661 Application fails to disclose the subject matter recited in the claims of the '138 patent. The preceding '616 Application shares the same specification as the '661 application, and thus fails to provide adequate description of the subject matter recited in the challenged claims for the same reasons. Finally, and as discussed further below, the passages of the '124 Provisional that were omitted from the '661 and '616 Applications also fail to describe the subject matter of the challenged claims.

(a) *The '661 Application discloses delivering probes to a fixed tissue sample; not delivering a tissue sample to a previously generated array of immobilized nucleic acid probes.*

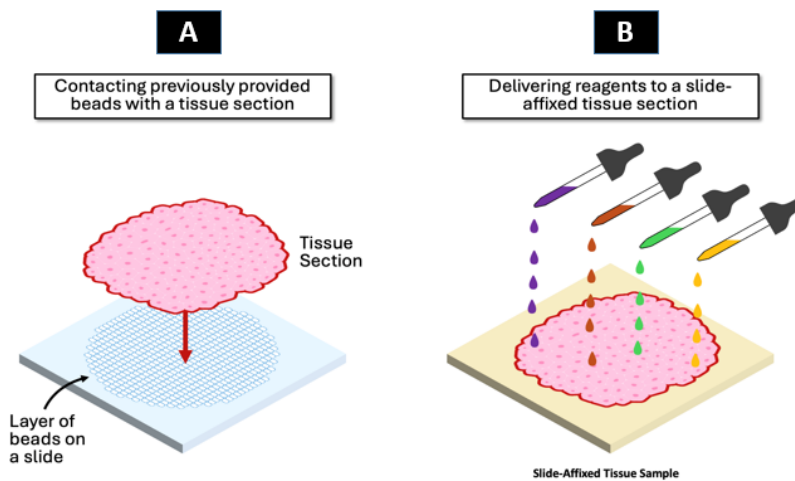
369. Claims 1 and 17 of the '138 Patent recite “[a] composition comprising an array comprising a substrate and a plurality of capture probes, wherein...each of

¹⁸ The '235 Application fails to support the challenged claims for the same reasons discussed herein. However, I understand that for purposes of determining the priority date to which the Challenged Claims are entitled, Frisen is eligible as prior art based on the break in the chain of priority at the '661 Application.

the plurality of capture probes are immobilized on the substrate in a random pattern.”

Additionally, Claim 17 recites wherein “the tissue section is disposed on the array.”

In my opinion, the ’661 Application (including the ’124 Provisional) fails to disclose these limitations. The claims as drafted and as read by Patent Owner to assert infringement require an array of nucleic acids attached to a substrate in a random pattern (either directly, or through an intermediary substrate, such as a bead), where the substrate comprises a capture probe that binds a target nucleic acid, as shown in Figure A below. Claim 17 as read by Patent Owner to assert infringement then recites disposing a tissue on top of this array, as depicted in Figure A below. But the ’661 Application focuses exclusively on providing a tissue sample affixed to a solid support, and then delivering reagents, such as capture probes, to the top of the support-affixed tissue sample according to a known (i.e., not random) spatial pattern, as depicted in Figure B below.



In fact, the specification emphasizes that the novelty of the “invention” lies in the delivery instrumentation used in the system of Figure B. “The assay system further provides instrumentation with an ability to deliver reagents in a spatially-defined pattern. This instrumentation, together with software, reagents and protocols, provides a key component of the highly innovative assay system of the invention....” EX1004, 8:63-67. “In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology.” *Id.*, 18:3-4. The claims of the ’138 patent do not cover the allegedly “highly innovative assay system of the invention” as depicted in Figure B.

370. The differences in these methodologies are not trivial, and the implementation of each requires different sophisticated laboratory instrumentation, techniques, and surface chemistries. For example, in the methods described in the

'138 Patent and its priority applications, probes are delivered to the tissue in solution, as illustrated in Figure B. Such techniques require, as discussed below, precise liquid handling and dispensing techniques. And because the probes are provided in solution, the disclosed embodiments repeatedly include a step of "washing" or separating encoded probes that did not bind to a target away from the sample. EX1011, Figs. 1-2; [0028] ("Once encoded probes interact with the biological targets, the encoded probes that interacted with the biological targets must be separated from the encoded probes that did not interact with the biological targets at step 140. In the case where the biological targets are nucleic acids and the encoded probes are oligonucleotides, the separation can be accomplished by, e.g., washing the unhybridized encoded probes from the sample."). The removal of unhybridized probes is important in the Figure B approach, because if such probes are not washed away, they contribute background "noise" to the readout signal, such as sequencing, making it difficult to detect the interactions of interest. By contrast, the claims as read by patent owner to assert infringement against Petitioner purport to encompass probes provided in a solid phase. Such techniques require fabrication technologies and chemistries to create substrates with the nucleic acids (such as probes) affixed to the substrate randomly. A step of separating or washing encoded probes that did not bind to a target would not make sense in the context of these chemistries, because

each spot on the array would typically consist of multiple probes, some of which would bind a sample nucleic acid and some would not. But because such probes are covalently bound to the location on the substrate, it would be extremely difficult and unfeasible to remove unhybridized probes from the array-sample combination without also disrupting the interactions between hybridized probes and the sample.

371. Moreover, the '138 Patent criticizes Armani, which provides a variation of the Figure A approach, stating that its reliance on the transfer of tissue into wells, “prevents random access to tissue samples and prevents high levels of multiplexing.” *See* EX1004, 2:2-4. To the extent that the disclosures of the '138 Patent (but not its claims) purports to address these alleged deficiencies, it is through the delivery of reagents from above the tissue sample. EX1004, 16:19-21 (“Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto* the biological sample.”).

372. A POSA reviewing the '661 Application (including the '124 Provisional) would not have understood it to demonstrate that the inventor possessed methods of contacting a pre-existing array or arrangement of binding agents, such as probes, with a tissue sample. Instead, a POSA would have understood the disclosure of the '124 Provisional to have been limited to the delivery of reagents in

a spatially-defined pattern, including binding agents and encoded probes, to a tissue sample already affixed to the solid support.

373. For example, the **SUMMARY OF THE INVENTION** explains that “[t]he assay system comprises an assay capable of high levels of multiplexing where encoded probes *are provided to a biological sample in a defined spatial patterns....*” EX1011 (’661 Application), [0007].¹⁹

374. The first “embodiment” disclosed is one in which “the assay system performs the following steps: *providing a sample affixed to a support; delivering encoded probes* for the multiple biological targets to the multiple sites in the sample in a known spatial pattern” *Id.*, [0008]. The next “embodiment” is one in which “there is provided an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample, where the assay system performs the following steps: *providing a sample affixed to a support; delivering oligonucleotide probes* for multiple nucleic acid targets *to the multiple sites in the sample in a known spatial pattern.*” *Id.* [00014]. “Other embodiments of the invention provide an assay system” that “performs the following steps: *providing a sample affixed to support; delivering encoded probes* for the

¹⁹ I note that the ’661 Application’s paragraph numbering re-starts following the **SUMMARY OF THE INVENTION** section.

multiple protein targets to the multiple sites in the sample *in a known spatial pattern.*” *Id.*, [00015]. The final “embodiment” described in the **SUMMARY OF THE INVENTION** section similarly involves “*providing a sample affixed to a support; delivering encoded probes* for the multiple biological targets at multiple sites in a sample in a known spatial pattern” *Id.*, [00016].

375. Thus, a POSA reviewing the ’661 Application would understand from the inventor’s **SUMMARY OF THE INVENTION** that the alleged invention related to (i) providing a sample affixed to a support and (ii) delivering probes to such a sample in a known (i.e., non-random) spatial pattern. A POSA would not understand these disclosures to demonstrate that the inventor possessed methods of creating arrays of encoded probes by randomly attaching said probes to a substrate, and subsequently disposing a tissue sample on top of such an array. Such an approach inverts the workflow of the methods disclosed in the ’661 Application’s **SUMMARY OF THE INVENTION**, requires a structure that is not disclosed, and is not mentioned in that section (or elsewhere in the Application).

376. Turning to the '661 Application's figures, "Figure 1 provides a simplified overview of *the assay system of the present invention*." EX1011, [0001]. This overview is explicit that the "assay system of the present invention" involves first "provid[ing] biological sample affixed to a support" and then "deliver[ing] encoded probes to the biological sample according to a known spatial pattern."

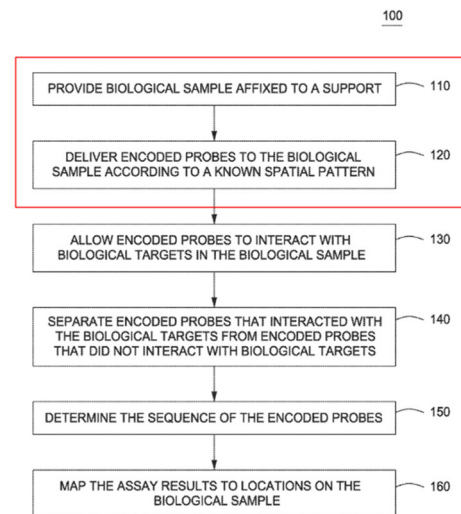


FIG. 1

EX1011, Fig. 1; *see also id.*, [00025] (describing Fig. 1).

377. Thus, a POSA reviewing Figure 1 and its caption, which provides an "overview of *the assay system of the present invention*" would understand the inventor considered the methods of the alleged invention to require fixing a sample to a support, followed by delivering encoded probes to the sample affixed to the support in a known spatial pattern.

378. Similarly, Figure 2 "provides a simplified overview of one embodiment of *the assay of the present invention* for detecting nucleic acids." *Id.*, [0002]. This disclosure again requires "provid[ing] biological sample affixed to a support" and

then “deliver[ing] probes” and “deliver[ing] encoding oligonucleotides” to the biological sample.” EX1011, Fig. 2. *see also id.*, [00030], [00031] (describing Fig. 2).

379. A POSA would not have understood Figures 1-2 to describe providing an array of probes, and then delivering a tissue sample to that array.

380. The remainder of the '661

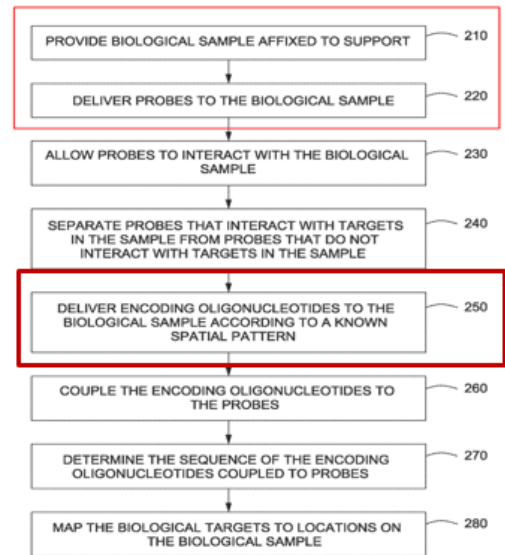


FIG. 2

Application’s Specification is replete with additional statements confirming that the “invention” requires (i) providing a sample affixed to a support before (ii) delivering probes to the sample in a known spatial pattern.

381. For example, the **DETAILED DESCRIPTION OF THE INVENTION** section begins with a subsection headed, “**The Invention in General.**” EX1011, [00023].

This section begins:

[00023] *The assay systems of the invention provide* spatially-encoded, multiplexed assays comprising 1) an assay capable of high levels of multiplexing with an efficient spatial encoding scheme; 2) *instrumentation capable of delivering reagents according to a spatial pattern*; and 3) decoding determined by a readout that is digital in nature. The assay systems of the invention detect the presence or absence and relative amount of a biological target or biological activity indicative of a biological target, as well as the location of the biological target or activity in a biological sample, *e.g., a tissue section or other*

biological structure disposed upon a support such as a microscope slide or culture dish.

EX1011, [00023]; *see also id.*, [00024] (“The assay system further provides ***instrumentation with an ability to deliver reagents*** in a spatially-defined pattern. ***This instrumentation***, together with software, reagents and protocols, ***provides a key component*** of the highly innovative assay system ***of the invention....***”).

382. Confirming that the '661 Application discloses only the delivery of reagents, including probes, to a sample affixed to a solid support, the Specification further notes that the above-referenced “instrumentation capable of delivering reagents according to a spatial pattern” “onto the biological sample” is “***integral to the assay system of the invention.***” EX1011, [00055] (“Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents ***onto the biological sample.***”); *see also, e.g., id.*, [00046] (“Coding tag a1, for example, ***is deposited on the biological sample*** in ten discrete areas or spots.... Coding tag a2 ***is deposited on the biological sample*** in ten spots on the second horizontal line.... Coding tag a3 ***is deposited on the biological sample....***”); *id.*, [00048] (“A deposit or dispensing scheme is shown at 530. Like the grid exemplified in Figure 4, encoding oligonucleotides a1 through a4 ***are deposited*** in spots in a pattern.... The grid though shown as a square with spots is actually ***a deposition pattern on a***

biological sample (not shown) such as tissue Section 416 shown in Figure 4.”); *id.*, [00049] (“The target-specific oligonucleotides *are delivered to the biological sample*....”).

383. Under the subsection headed, “**Assays**” the ’661 Application states that “[t]he assay portion of the assay systems of the present invention comprise the following general steps: *delivering probes and encoding agents* where the encoding agents (in some embodiments pre-coupled to the probes) are *delivered to the sample according to a known spatial pattern*....” EX1011, [00034]. Further confirming that the invention as disclosed by the priority documents requires delivering probes or reagents to a sample, this section goes on to state that “[t]he samples of the present invention include virtually any biological sample or samples *that can be affixed to a support* or provided essentially in a two-dimensional manner....” *Id.*, [00035].

384. Because the ’661 Application’s alleged inventions require the delivery of reagents to samples affixed to a support, the **DETAILED DESCRIPTION OF THE INVENTION** section contains a subsection titled, “**Reagent Delivery Systems.**” EX1011, [00054]. “The reagent delivery system *of the invention* includes instrumentation that allows the delivery of reagents to discrete portions of the biological sample.” *Id.* The ’661 Application goes on to note that “reagent delivery may be to many different biological samples at one time,” although “[a] single tissue

section has been exemplified herein.” *Id.* The Specification does not, however, contain any disclosure indicating that the system “of the invention” may operate by delivering a tissue section to an array of probes.

385. As noted, the ’661 Application provides that “[i]ntegral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto* the biological sample.” *Id.*, [00055]. Such techniques are disclosed to include, for example, “ink jet spotting; mechanical spotting...; micro contact printing; photochemical or photolithographic methods; and the like.” *Id.*, [00056]; *see also id.*, [00064] (“[T]he reagent delivery system controls the delivery of reagents to specific patterns *on a biological sample surface* using semiconductor techniques such as masking and spraying.”); *id.*, [00065] (“In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology.”).

386. A POSA would not have understood such disclosures, which necessarily involve delivering reagents on top of a substrate-affixed tissue sample in a known pattern, to demonstrate possession of methods in which a tissue sample is placed on top of an array of randomly immobilized capture probes, or the devices required to perform such methods, as claimed.

387. Accordingly, the '661 Application (including the '124 Provisional) fails to support the challenged claims of the '138 patent. The additional limitations of the challenged dependent claims do not narrow claim 1 or 17 in a manner that resolves these deficiencies, and thus all challenged claims are unsupported for the same reasons.

(b) Conclusion on Priority Date

388. For the reasons set forth above, the challenged claims are not supported by the '661 Application filed on June 20, 2016, and thus are entitled to a priority date no earlier than the February 14, 2019 filing date of the '235 Application. I reserve the right to respond to any arguments advanced by Patent Owner suggesting that the challenged claims are supported by one or more of the '138 Patent's priority applications.

2. Claim 1

389. Claim 1 of the '138 Patent recites:

1. A composition comprising an array comprising a substrate and a plurality of capture probes, wherein:

each of the plurality of capture probes comprises: (i) a target-binding domain, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site;

the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain; and

each of the plurality of capture probes are immobilized on the substrate in a random pattern.

390. Frisen discloses each limitation of Claim 1.

(a) “*A composition comprising an array comprising a substrate and a plurality of capture probes*”

391. Frisen discloses “[a] composition comprising an array comprising a substrate and a plurality of capture probes.” Thus, to extent the preamble is limiting, it is anticipated by Claim 14 of Frisen.

392. Frisen discloses “methods and products for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof.” EX1045, Abstract. In particular, Frisen claims methods “for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof” by utilizing a composition comprising an array comprising a substrate and a plurality of capture probes.

393. For example, Claims 1, 13 and 14 in Frisen recite:

1. A method for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof, comprising:

(a) providing *an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer;*

(b) contacting said substrate with a tissue sample and allowing RNA of the tissue sample to hybridise to the capture probes;

(c) generating cDNA molecules from the captured RNA molecules using said capture probes as RT primers;

(d) labelling the cDNA molecules generated in step (c), wherein said labelling step may be contemporaneous with, or subsequent to, said generating step;

(e) detecting a signal from the labelled cDNA molecules; and optionally

(f) imaging the tissue sample, wherein the tissue sample is imaged before or after step (c).

13. The method of any one of claims 1 to 13, wherein the capture probes further comprise a positional domain which is 5' relative to the capture domain, wherein said positional domain comprises a sequence that corresponds to the position of the capture probe on the object substrate, preferably wherein the positional domain of each species of capture probe comprises a unique barcode sequence.

14. The method of any one of claims 1 to 13, wherein the capture probes further comprise a universal domain which is 5' relative to the capture domain or, if present the positional domain, wherein said universal domain comprises:

(i) an amplification domain, for amplifying the generated DNA molecules; and/or

(ii) a cleavage domain for releasing the generated DNA molecules from the surface of the object substrate.

394. Of particular relevance here is the limitation of Claim 1²⁰ requiring “an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer.” *See also* EX1045, 6 (“[T]he methods and arrays of the invention may be used to determine the optimum conditions to capture the nucleic acid molecules from a tissue sample on an *object substrate, e.g. an array.*”). Thus, Frisen discloses an array comprised of *a substrate* with *a plurality of capture probes* being immobilized thereon.

²⁰ I have been informed by counsel that because Claims 13 and 14 depend from Claim 1, this limitation is also a limitation in Claims 13 and 14.

- (b) “each of the plurality of capture probes comprises: (i) a target-binding domain, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain”**

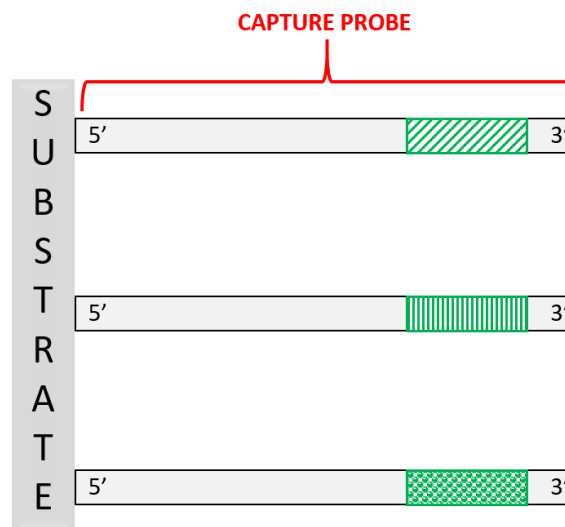
395. Frisen discloses “each of the plurality of capture probes comprises: (i) a target-binding domain, (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain.”

- (i) “each of the plurality of capture probes comprises: (i) a target-binding domain,...the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence that is complementary to at least a portion of the nucleic acid that binds to the target-binding domain”**

396. As described previously, Claim 1 of Frisen requires “an object substrate on which at least one species of capture probe, comprising a capture domain, is

directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer.”

397. A POSA would recognize that Claim 1 is describing capture probes attached to a substrate by their 5' ends such that their 3' ends are free. Thus, a POSA would understand Frisen to disclose a substrate with a plurality of **capture probes** with **target-binding domains**, wherein the **capture probes** are attached to the substrate by their 5' ends, as shown below.



398. Additionally, Frisen explains that the disclosed “capture domain comprises a nucleotide sequence that is capable of hybridizing to nucleic acid, e.g. RNA (preferably mRNA) present in the cells of the tissue sample contacted with the array.” EX1045, 24-25. Moreover, “the capture domain may be selected or designed to bind (or put more generally may be capable of binding) selectively or specifically

to the particular nucleic acid, e.g. RNA, it is desired to detect or analyse.” EX1045, 25. For example, Frisen discloses that the capture domain may be designed for the “selective capture of mRNA,” as opposed to other classes of nucleic acids (e.g., DNA, rRNA, tRNA), using poly-T capture domains:

As is well known in the art, this may be on the basis of hybridisation to the poly-A tail of mRNA. Thus, in a preferred embodiment the capture domain comprises a poly-T DNA oligonucleotide, i.e. a series of consecutive deoxythymidine residues linked by phosphodiester bonds, which is capable of hybridizing to the poly-A tail of mRNA.

*Id.*²¹ As another example, Frisen discloses that “[t]he capture domain may be capable of binding selectively to a desired subtype or subset of nucleic acid, e.g. RNA, for example a particular type of RNA such mRNA or rRNA etc. as listed above, or to a particular subset of a given type of RNA, for example, a particular mRNA species e.g. corresponding to a particular gene or group of genes. Such a capture probe may be selected or designed based on sequence of the RNA it is desired to capture. Thus it may be a sequence-specific capture probe, specific for a particular RNA target or group of targets (target group etc). Thus, it may be based on a particular gene

²¹ I understand that in the District Court litigation, 10X Genomics and Prognosys have taken the position that polyT domains are “target nucleic acid binding domains.” Thus, Frisen’s disclosure satisfies the claim for the same reasons.

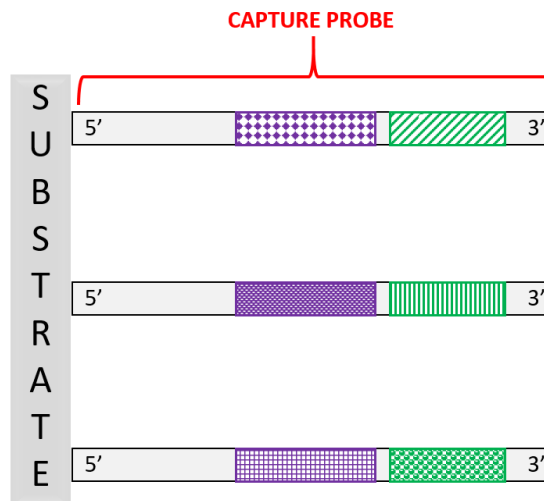
sequence or particular motif sequence or common/conserved sequence etc., according to principles well known in the art.” *Id.* at 26.

399. Thus, a POSA would understand that the capture domain (i.e., target nucleic acid-binding domain) of each of the plurality of capture probes described in Claim 1 of Frisen comprises a nucleic acid sequence that is complementary to at least a portion of the target nucleic acid that binds to the target-binding domain.

(ii) “each of the plurality of capture probes comprises:...(ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain”

400. Moreover, Published Claim 13 of Frisen depends from Claim 1 and recites “wherein the capture probes further comprise a positional domain which is 5' relative to the capture domain, wherein said positional domain comprises a sequence that corresponds to the position of the capture probe on the object substrate, preferably wherein the positional domain of each species of capture probe comprises a unique barcode sequence.” *See also id.*, 26 (“The positional domain (feature identification domain or tag) of the capture probe, if present, is located directly or indirectly upstream, i.e. closer to the 5' end of the capture probe nucleic acid molecule, of the capture domain.”)

401. I have been informed by counsel that a dependent claim incorporates all of the limitations from the claim from which it depends. Thus, Claim 13 of Frisen requires the substrate and **capture probes** of Claim 1 in addition to the capture probes comprising “a **positional domain** which is 5' relative to the **capture domain**, wherein said **positional domain** comprises a sequence that corresponds to the position of the **capture probe** on the object substrate, preferably wherein the **positional domain** of each species of **capture probe** comprises a unique barcode sequence,” as shown below.



402. Frisen explains that “[t]he positional domain (or tag),” when present in the disclosed capture probes, “comprises the sequence which is unique to each feature or a group of directly or indirectly adjacent features, and acts as a positional or spatial marker (the identification tag). In this way, each region or domain of the

tissue sample, e.g. each cell in the tissue, may be identified by spatial resolution across the array linking the nucleic acid, e.g. RNA (e.g. the transcripts) from a certain cell to a unique positional domain sequence in the capture probe. The positional domain of a capture probe in the array is one aspect of the methods of the invention that allows a specific transcript (or group of transcripts) to be correlated to a position in the tissue sample, for example it may be correlated to a cell in the sample. Thus, the positional domain of the capture domain may be seen as a nucleic acid tag (identification tag) and enables the position of a transcript to be correlated to a position in the sample indirectly, e.g. by analysis of the sequence of the captured transcript.” *Id.*, 28.

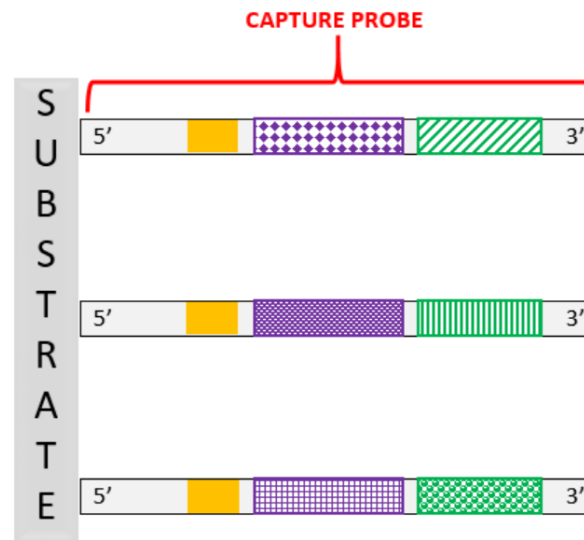
403. Frisen also explains that while “[a]ny suitable sequence may be used as the positional domain in the capture probes of the invention,” “the positional domain should not interfere with (i.e. inhibit or distort) the interaction between the RNA of the tissue sample and the capture domain of the capture probe. For example, the positional domain should be designed such that nucleic acid molecules in the tissue sample do not hybridize specifically to the positional domain. Preferably, the nucleic acid sequence of the positional domain of the capture probes has less than 80% sequence identity to the nucleic acid sequences in the tissue sample. Preferably, the positional domain of the capture probe has less than 70%, 60%, 50% or less than

40% sequence identity across a substantial part of the nucleic acids molecules in the tissue sample. Sequence identity may be determined by any appropriate method known in the art, e.g. using the BLAST alignment algorithm.” *Id.*, 29-30.

404. Thus, Frisen discloses that “each of the capture probes of the plurality of capture probes comprises...(ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain.”

(iii) “each of the plurality of capture probes comprises:...(iii) a primer binding site”

405. Lastly, Claim 14 of Frisen, which depends from Claim 13, further requires “wherein the **capture probes** further comprise a universal domain which is 5' relative to the **capture domain** or, if present the **positional domain**, wherein said universal domain comprises: (i) **an amplification domain**, for amplifying the generated DNA molecules,” as shown below:



406. When describing the amplification domain in detail, Frisen explains that “it may be advantageous to amplify the nucleic acid, e.g. cDNA molecules, for example after they have been released (e.g. removed or cleaved) from the substrate. It will be appreciated, however, that the initial cycle of amplification, or indeed any or all further cycles of amplification may also take place in situ on the substrate. ***The amplification domain comprises a distinct sequence to which an amplification primer may hybridize.*** The amplification domain of the universal domain of the capture probe is preferably identical for each species of capture probe. Hence a single amplification reaction will be sufficient to amplify all of the nucleic acid, e.g. cDNA, molecules (which may or may not be released from the substrate prior to amplification).” *Id.*, 32.

407. Thus, the “amplification domain” disclosed by Frisen is a “primer binding site” and “each of the plurality of capture probes comprises...(iii) a primer binding site.”

(c) “each of the plurality of capture probes are immobilized on the substrate in a random pattern”

408. Frisen discloses “each of the plurality of capture probes are immobilized on the substrate in a random pattern.” For example, Frisen states that “the probes may be immobilized on beads, e.g. plastic microbeads, which can be used to immobilize the probes on the substrate. Suitable techniques for immobilizing the nucleic acid molecules on beads may be selected from the techniques discussed above or selected from methods known in the art. The beads may be contacted with, and immobilized on, an object substrate, thereby indirectly immobilizing the probes on the surface of the substrate.” EX1045, 20. A POSA would appreciate from this disclosure that as known in the art the beads could either be (1) linked randomly to the substrate, such as described in Chee-824,²² or (2) linked in a predetermined

²² Ex. 1038, [0013] (“Since the beads go down randomly, a unique optical signature is needed to ‘decode’ the array; i.e., after the array is made, a correlation of the location of an individual site in the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the in situ synthesis or spotting techniques of the prior art. Once the array is loaded with

manner, such as described in Chee-584.²³ Thus, a POSA would immediately envisage the plurality of beads/capture probes being immobilized on the substrate in a random pattern.

3. Claim 5

409. Claim 5 of the '138 Patent recites “[t]he composition of claim 1 wherein the array is deposited on a slide or flow-cell.”

410. As described previously, Claim 1 requires “an *object substrate* on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized.” Frisen explains that “[t]he ‘object substrate’ or ‘substrate’ of the invention may be any solid substrate on which nucleic acid molecules can be immobilized directly or indirectly, e.g. *a slide* or chip. In preferred embodiments the object substrate may be viewed as being an array substrate, i.e. any

beads, the array can be decoded, or can be used, with full or partial decoding occurring after testing, as is more fully outlined below.”)

²³ Ex. 1032, 28:6-19 (“[S]ub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an “area code” that can have the same tags (i.e. telephone numbers) of other subarrays, that are separated by virtue of the location of the subarray. Thus, for example, the same unique tags can be reused from bundle to bundle. Thus, the use of 50 unique tags in combination with 100 different subarrays can form an array of 5000 different bioactive agents. In this embodiment, it becomes important to be able to identify one bundle from another; in general, this is done either manually or through the use of marker beads, i.e. beads containing unique tags for each subarray.”).

substrate that could be used to generate a nucleic acid array, e.g. a microarray substrate.” EX1045, 17; *see also id.*, 1 (“The methods of the invention allow the nucleic acid molecules (e.g. RNA molecules) in the tissue sample, particularly mRNA, to be captured on an object substrate (e.g. a slide or chip, which may be an array).”)

411. Thus, a POSA would recognize that Frisen discloses “[t]he composition of claim 1 wherein the array is deposited on a slide or flow-cell.”

4. Claim 17

412. Claim 17 of the ’138 Patent recites:

17. A composition comprising (i) an array comprising a substrate and a plurality of capture probes, and (ii) a tissue section, wherein:

the tissue section is disposed on the array;

each of the plurality of capture probes comprises: (i) a target-binding domain (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site;

the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence complementary to at least a portion of the nucleic acid that binds to the target-binding domain; and

each of the plurality of capture probes are immobilized on the substrate in a random pattern.

413. As described below, Frisen anticipates the composition of Claim 17.

(a) “A composition comprising (i) an array comprising a substrate and a plurality of capture probes, and (ii) a tissue section”

414. Frisen discloses “[a] composition comprising (i) an array comprising a substrate and a plurality of capture probes, and (ii) a tissue section.”

415. As described above in Section IX.C.2(a) and herein incorporated by reference, Frisen discloses “[a] composition comprising (i) an array comprising a substrate and a plurality of capture probes.” Frisen also discloses a tissue section.

416. For example, Claim 1 in Frisen recites:

A method for localized or spatial detection and/or analysis of RNA in a tissue sample or a portion thereof, comprising:

- (a) providing an object substrate on which at least one species of capture probe, comprising a capture domain, is directly or indirectly immobilized such that the probes are oriented to have a free 3' end to enable said probe to function as a reverse transcriptase (RT) primer;
- (b) contacting said substrate with *a tissue sample* and allowing RNA of the tissue sample to hybridise to the capture probes;
- (c) generating cDNA molecules from the captured RNA molecules using said capture probes as RT primers;
- (d) labelling the cDNA molecules generated in step (c), wherein said labelling step may be contemporaneous with, or subsequent to, said generating step;
- (e) detecting a signal from the labelled cDNA molecules; and optionally

(f) imaging the tissue sample, wherein the tissue sample is imaged before or after step (c).

A POSA would understand that a tissue sample is synonymous with a tissue section. *See also* EX1045, 6 (“In its simplest form, the invention may be illustrated by the following summary. The invention requires reverse transcription (RT) primers to be immobilised on an object substrate, e.g. a glass slide or chip. ***Thin tissue sections*** are placed onto the substrate and a reverse transcription reaction is performed in the tissue section on the substrate.”)

(b) “the tissue section is disposed on the array

417. Frisen discloses the tissue section being disposed on the array. For example, Claim 1 above requires “contacting said substrate [i.e., array] with a tissue sample.”

418. Frisen also explains that “[i]n its simplest form, the invention may be illustrated by the following summary. The invention requires reverse transcription (RT) primers to be immobilised on an object substrate, e.g. a glass slide or chip. ***Thin tissue sections are placed onto the substrate*** and a reverse transcription reaction is performed in the tissue section on the substrate.” *Id.*, 6.

- (c) ***“each of the plurality of capture probes comprises: (i) a target-binding domain (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence complementary to at least a portion of the nucleic acid that binds to the target-binding domain”***

419. Frisen discloses “each of the plurality of capture probes comprises: (i) a target-binding domain (ii) a nucleic acid sequence that identifies a unique location on the substrate and is not configured to hybridize to a nucleic acid that binds to the target-binding domain, and (iii) a primer binding site; the target-binding domain of each of the plurality of capture probes comprises a nucleic acid sequence complementary to at least a portion of the nucleic acid that binds to the target-binding domain.” For example, see the analysis above for Section IX.C.2(b), which is herein incorporated by reference.

- (d) ***“each of the plurality of capture probes are immobilized on the substrate in a random pattern”***

420. Frisen discloses “each of the plurality of capture probes are immobilized on the substrate in a random pattern.” For example, see Section IX.C.2(c) above, which is herein incorporated by reference.

5. Claim 20

421. Claim 20 recites “[t]he composition of claim 17, wherein the tissue section is a formalin-fixed paraffin-embedded tissue section or a fresh frozen tissue section.”

422. Frisen discloses “the composition of claim 17, wherein the tissue section is a formalin-fixed paraffin-embedded tissue section or a fresh frozen tissue section.” For example, Frisen discloses a few types of tissue samples that may be used with the disclosed array, including formalin-fixed paraffin-embedded (“FFPE”) tissue sections and fresh frozen tissue sections. EX1045, 42 (“[I]n one embodiment of the invention, the tissue sample, i.e. the section of the tissue sample contacted with the substrate, e.g. array, is prepared using FFPE or other methods of fixing and embedding. In other words the sample may be fixed, e.g. fixed and embedded. In an alternative embodiment of the invention the tissue sample is prepared by deep-freezing. In another embodiment a touch imprint of a tissue may be used, according to procedures known in the art. In other embodiments an unfixed sample may be used.”); 41 (“As a first representative example of a tissue sample for use in the invention, the tissue may prepared by deep freezing at temperature suitable to maintain or preserve the integrity (i.e. the physical characteristics) of the tissue structure....”); 41 (“In a second representative example, the tissue may be prepared

using standard methods of formalin-fixation and paraffin-embedding (FFPE), which are well-established in the art.”).

423. Thus, a POSA would recognize that the tissue section in Claim 17 is a formalin-fixed paraffin-embedded tissue section or a fresh frozen tissue section.

6. Claim 23

424. Claim 23 recites “[t]he composition of claim 17, wherein the array is deposited on a slide or flow-cell.”

425. Frisen discloses “[t]he composition of claim 17, wherein the array is deposited on a slide or flow-cell.” For example, see the analysis for Section IX.C.3 above, which is herein incorporated by reference.

7. Claim 28

426. Claim 28 recites “[t]he composition of claim 17, wherein the nucleic acid comprises RNA or DNA.”

427. Frisen discloses “[t]he composition of claim 17, wherein the nucleic acid comprises RNA or DNA.” For example, Frisen explains that the disclosed “capture domain comprises a nucleotide sequence that is capable of hybridizing to nucleic acid, e.g. RNA (preferably mRNA) present in the cells of the tissue sample contacted with the array.” EX1045, 24-25. Moreover, “the capture domain may be selected or designed to bind (or put more generally may be capable of binding)

selectively or specifically to the particular nucleic acid, e.g. RNA, it is desired to detect or analyse.” EX1045, 25. For example, Frisen discloses that the capture domain may be designed for the “selective capture of mRNA” using poly-T capture domains:

As is well known in the art, this may be on the basis of hybridisation to the poly-A tail of mRNA. Thus, in a preferred embodiment the capture domain comprises a poly-T DNA oligonucleotide, i.e. a series of consecutive deoxythymidine residues linked by phosphodiester bonds, which is capable of hybridizing to the poly-A tail of mRNA.

Id.

428. Thus, a POSA would understand that Frisen discloses the target nucleic acid being DNA or RNA.

8. Claim 29

429. Claim 29 recites “[t]he composition of claim 28, wherein the RNA is mRNA.”

430. Frisen discloses “[t]he composition of claim 28, wherein the RNA is mRNA.” For example, Frisen explains that the disclosed “capture domain comprises a nucleotide sequence that is capable of hybridizing to nucleic acid, e.g. RNA (preferably mRNA) present in the cells of the tissue sample contacted with the array.” EX1045, 24-25. Moreover, “the capture domain may be selected or designed to bind (or put more generally may be capable of binding) selectively or specifically

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to the particular nucleic acid, e.g. RNA, it is desired to detect or analyse.” EX1045,

25. For example, Frisen discloses that the capture domain may be designed for the

“selective capture of mRNA” using poly-T capture domains:

As is well known in the art, this may be on the basis of hybridisation to the poly-A tail of mRNA. Thus, in a preferred embodiment the capture domain comprises a poly-T DNA oligonucleotide, i.e. a series of consecutive deoxythymidine residues linked by phosphodiester bonds, which is capable of hybridizing to the poly-A tail of mRNA.

Id.

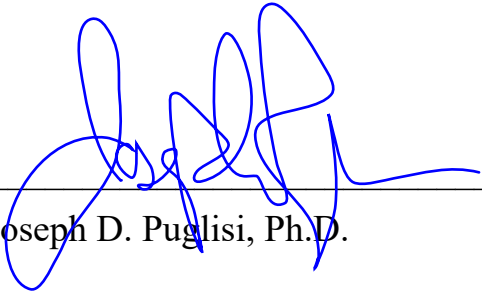
431. Thus, a POSA would understand that Frisen discloses the target nucleic acid being mRNA.

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I hereby declare that all statement 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.

Dated: November 14, 2024

BY:



Joseph D. Puglisi, Ph.D.