

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.

IPR2024-01289
Patent No. 10,480,022

PETITION FOR *INTER PARTES* REVIEW
OF U.S. PATENT NO. 10,480,022

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EX1001	U.S. Patent No. 10,480,022 issued to Chee
EX1002	U.S. Patent No. 10,662,468 issued to Chee
EX1003	U.S. Patent No. 11,001,879 issued to Chee
EX1004	U.S. Patent No. 11,549,138 issued to Chee
EX1005	U.S. Patent No. 11,761,030 issued to Chee
EX1006	10x Genomics' Complaint for Patent Infringement, Case 1:23-cv-01375, Doc. 1, filed December 1, 2023
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EX1009	U.S. Provisional Application No. 61/321,124
EX1010	File History for U.S. Application No. 16/276,235 (2 parts)
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EX1013	Nick J. Proudfoot, <i>et al.</i> , <i>Integrating mRNA Processing with Transcription</i> , Cell, Vol. 108, 501-512, February 22, 2002
EX1014	Uwe Kuhn <i>et al.</i> , <i>Poly(A) Tail Length Is Controlled by the Nuclear Poly(A)-binding Protein Regulating the Interaction between Poly(A) Polymerase and the Cleavage and Poladenylation Specificity Factor</i> , The Journal of Biologic Chemistry VOL. 284, NO. 34, pp 22803-22814, August 21, 2009
EX1015	James Eberwine <i>et al.</i> , <i>Complementary DNA Synthesis in Situ: Methods and Applications</i> , Methods in Enzymology, VOL. 216, 1992

EX1016	Laurence H. Tecott <i>et al.</i> , <i>In Situ Transcription: Specific Synthesis of Complementary DNA in Fixed Tissue Sections</i> , Science, VOL. 240, pp. 1661-1664, 1988
EX1017	Anna Ligasová and Karel Koberna, <i>In situ reverse transcription: the magic of strength and anonymity</i> , Nucleic Acids Research, 2010, Vol. 38, No. 16
EX1018	Victor Trevino <i>et al.</i> , <i>DNA Microarrays: a Powerful Genomic Tool for Biomedical and Clinical Research</i> , Mol Med 13 (9-10) 527-541, September-Doctor 2007
EX1019	Stahl <i>et al.</i> , “Visualization and analysis of gene expression in tissue selections [sic: sections] by spatial transcriptomics,” <i>Science</i> 353(6294):78-82 (July 1, 2016)
EX1020	WO 2012/140224 A1 to Frisen
EX1021	WO 2010/088517 A1 to Emmert-Buck
EX1022	Michael Armani <i>et al.</i> , <i>2D-PCR: a method of mapping DNA in tissue sections</i> , Lab Chip, 2009, 9(24), 3526-3534; NIH Public Access Author Manuscript
EX1023	Lambda Moses and Lior Pachter, <i>Museum of spatial transcriptomics</i> , Nature Methods, VOL 19, May 2022 [534-546]
EX1024	U.S. Patent No. 11,214,796 issued to Shirai
EX1025	JP2007-14297 to Okano
EX1026	EP 2 130 913 A1 to Okano
EX1027	Franklin M. Harold, <i>Molecules into Cells: Specifying Spatial Architecture</i> , Microbiology and Molecular Biology Reviews, Dec. 2005, p. 544-564
EX1028	Vanessa M. Brown <i>et al.</i> , <i>Multiplex Three-Dimensional Brain Gene Expression Mapping in a Mouse Model of Parkinson's Disease</i> , Genome Research, 12:868-884, 2002

EX1029	Michael L. Metzker, <i>Sequencing technologies-the next generation</i> , Nature Reviews Genetics, Volume 11, January 2010
EX1030	Poornima Parameswaran, <i>et al.</i> , <i>A pyrosequencing-tailored nucleotide barcode design unveils opportunities for largescale sample multiplexing</i> , Nucleic Acids Research, 2007, Vol. 35, No. 19
EX1031	WO 2009/091934 A1 Cantor
EX1032	U.S. Patent No. 6,620,584 issued to Chee
EX1033	WO 2008/075086 A1 Southern <i>et al.</i>
EX1034	Spellman Declaration EX1002 from IPR2023-01298
EX1035	10x Genomics' Opening Brief in support of Motion to Dismiss, Case 1:18-cv-01800 (D.Del), Doc. 19, filed March 1, 2019
EX1036	Norman P. Gerry, <i>et al.</i> , <i>Universal DNA Microarray Method for Multiplex Detection of Low Abundance Point Mutations</i> , J. Mol. Biol. (1999) 292, 251-262
EX1037	U.S. Patent No. 8,835,358 issued to Fodor
EX1038	U.S. No. 2007/0231824 A1 to Chee
EX1039	Jian-Bing Fan, <i>et al.</i> , <i>Illumina Universal Bead Arrays</i> , Methods in Enzymology, VOL. 410, 2006
EX1040	10X Genomics' Petition for IPR Review of Nanostring Technologies' Patent No. 11,377,689 (IPR2023-01298)
EX1041	July 24, 2024 Email from Counsel for Patent Owner to Counsel for Petitioner
EX1042	July 16, 2024 Email from Counsel for Patent Owner to Counsel for Petitioner
EX1043	Scheduling Order, Doc #25, C.A. 23-cv-1375 (D.Del.)

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EX1044	Leslie P. Gartner, Ph.D. and James L. Hiatt Ph.D., <i>Color Textbook of Histology</i> , Third Edition, Saunders Elsevier
EX1045- EX1099	Reserved
EX1100	Declaration of Joseph D. Puglisi, Ph.D.
EX1101	Curriculum Vitae of Joseph D. Puglisi, Ph.D.

LISTING OF CHALLENGED CLAIMS

1. A method for determining a spatial location of a biological molecule of a tissue sample comprising
 - (a) providing a plurality of beads, wherein the plurality of beads comprise a plurality of binding agents, wherein a binding agent of the plurality of binding agents (i) comprises a coding identifier having a nucleic acid sequence and (ii) is configured to interact with a biological molecule of a tissue sample, wherein the coding identifier corresponds to a location at which the binding agent interacts with a biological molecule of a tissue sample;
 - (b) contacting the plurality of beads with the tissue sample such that the binding agent interacts with the biological molecule; and
 - (c) using the coding identifier to identify the location where the binding agent interacted with the biological molecule, thereby determining a spatial location of the biological molecule of the tissue sample.
2. The method of claim 1, wherein the plurality of beads is provided on a substrate surface.
3. The method of claim 2, wherein the plurality of beads is provided in wells on the substrate surface.
4. The method of claim 1, wherein the biological molecule is a nucleic

acid.

5. The method of claim 4, wherein the binding agent of the plurality of binding agents comprises a nucleic acid.

6. The method of claim 5, wherein the binding agent 5 interacts with the biological molecule via hybridization.

7. The method of claim 1, wherein the binding agent and coding identifier are provided as a single molecule. The method of claim 1, wherein said tissue sample comprises a metastatic tissue sample, a mixed tissue sample, 30 a biopsy, or a core needle biopsy.

11. The method of claim 1, wherein the plurality of binding agents comprises an additional binding agent, which (i) comprises an additional coding identifier and (ii) is configured to interact with another biological molecule of the tissue sample, wherein the additional coding identifier is different from the coding identifier of step (a)(i).

12. The method of claim 11, wherein the additional coding identifier corresponds to a location at which the additional binding agent of the plurality of binding agents interacts with said another biological molecule of the tissue sample.

13. The method of claim 12, wherein the location at which the binding agent interacts with the biological molecule and the additional binding agent interacts with said another biological molecule is different.

14. The method of claim 13, wherein the location at which the binding agent interacts with the biological molecule and said another biological molecule of the tissue sample is different.

15. The method of claim 1, wherein step (c) comprises determining the spatial locations of a plurality of biological molecules of the tissue sample simultaneously.

17. The method of claim 1, wherein step (c) comprises determining the relative amount of the biological molecule at the location at which it is identified.

19. The method of claim 1, wherein step (c) comprises sequencing all or a portion of the nucleic acid sequence of the coding identifier.

27. The method of claim 1, further comprising analyzing gene expression in the tissue sample.

28. The method of claim 1, wherein the location of the binding agent is known prior to step (b).

29. The method of claim 1, wherein the binding agent is immobilized directly or indirectly onto a bead of the plurality of beads.

Curio Bioscience, Inc. (“Petitioner”) requests *inter partes* review and cancellation of claims 1-7, 11-15, 17, 19, and 27-29 of U.S. Patent No. 10,480,022 (“Chee-022”), entitled “Spatially Encoded Biological Assays.” Chee-022 is owned by Prognosys, and was exclusively licensed to real-party-in-interest 10x Genomics (“10x”) in November 2018.¹

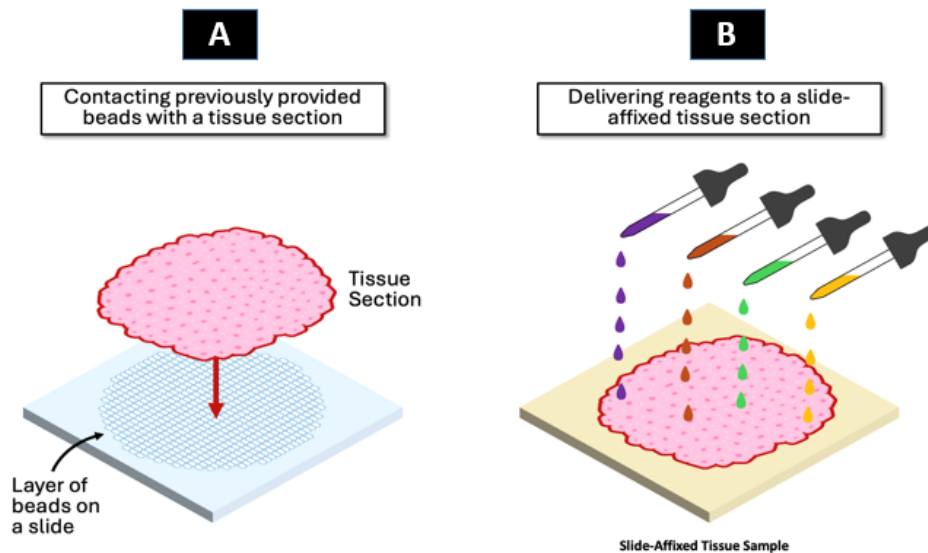
I. INTRODUCTION

The claims of Chee-022 recite “a method for determining a spatial location of a biological molecule of a tissue sample” comprising contacting nucleic acid “binding agents” and “coding identifiers” with a tissue sample, and “using the coding identifier to identify the location where the binding agent interacted with the biological molecule.” EX1001, cl. 1. In co-pending litigation, PO has read the claims of Chee-022 to cover methods of using nucleic acid bead arrays to analyze the spatial organization of nucleic acids in a tissue sample placed on top of the array, and using the array to tag captured mRNA with “spatial barcodes.” *See* EX1006.

Such methods are not described by Chee-022 or its priority documents. Rather, Chee-022’s specification focuses on the patterned delivery of probes to a slide-affixed tissue sample using allegedly novel “reagent delivery systems” such as inkjet printing or photolithography, techniques Chee-022 calls “integral to the assay

¹ Patent Owner (“PO”) collectively refers to 10x and Prognosys. 10x and Prognosys refer to the separate actions of each.

system of the invention.” The stark contrast between the approach PO now claims (Figure A) and the approach actually described in the specification (Figure B) is illustrated below.



This disconnect exists because 10x drafted the Chee-022 claims in February 2019 to cover implementations of technology it acquired from Spatial Transcriptomics AB (“ST”). ST is a company 10x acquired in late 2018, around the same time it licensed Chee-022 from Prognosis. In proceedings not involving Prognosis, 10x has stated:

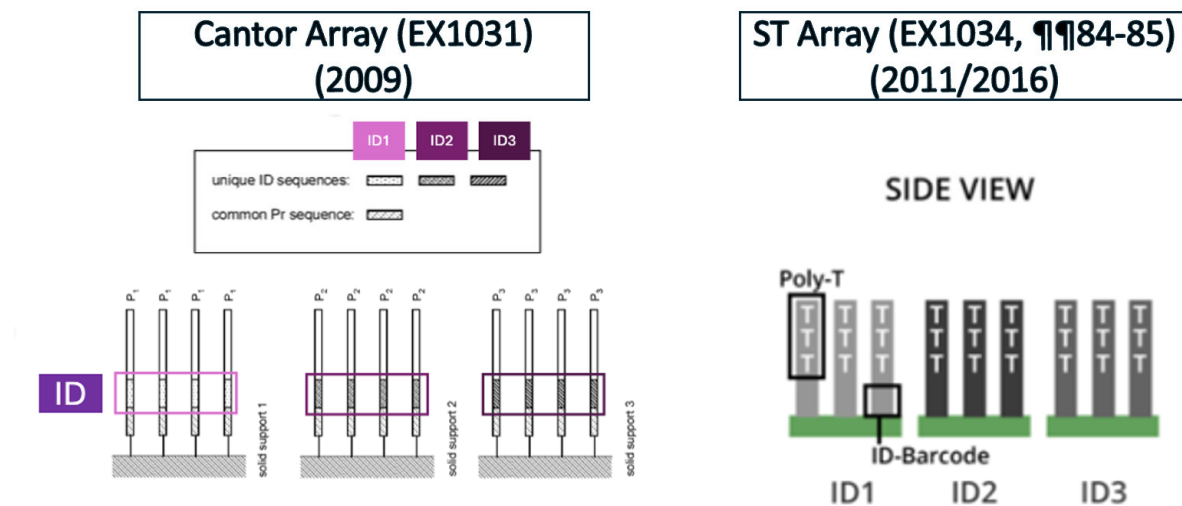
As early as 2011, Stahl et al. *developed a pioneering spatially barcoded array paradigm* for determining the locations of target mRNAs in a biological sample. *The scientists disclosed this foundational technology in a patent application filed in 2011* (WO 2012/140224; [EX1020]) and subsequently published it in a 2016 article [EX1019].

EX1034, ¶82. 10x's claim that Frisen's work—filed over one year after what PO contends is Chee-022's priority date (February 2010)—was “foundational” and “pioneering” is a telling sign that Chee-022 does not support claims to spatially barcoded arrays for nucleic acid analysis. Nevertheless, shortly after taking over prosecution of the Chee-022 family in early 2019, 10x filed a substitute specification and began seeking claims to cover the “pioneering” work of ST.

The written description requirement exists precisely “to prevent an applicant from later asserting that he invented that which he did not, [which] is particularly important when, as here, claims are added later during prosecution in response to development by others.” *Quake v. Lo*, 928 F.3d 1365, 1373 (Fed. Cir. 2019) (internal quotation omitted). In this case, the lack of support for the claims in Chee-022's priority documents means that it is entitled to, at the earliest, the 2019 filing date of the claims. Accordingly, Chee-022 is anticipated by Frisen.

To be sure, before Chee-022's earliest priority date, there was nothing new about either its claims or the later-filed yet still somehow “pioneering” and “foundational” ST array designs it purports to cover. By 2009, noted geneticist Charles Cantor had already published nucleic acid bead arrays in which each bead comprises (i) a probe sequence that binds a target nucleic acid in a tissue sample and (ii) an identification sequence (ID) (or “barcode”) that is unique for each bead. Because each bead necessarily occupies a different spatial location on a given array,

each barcode necessarily corresponds to a particular location in the array. As illustrated below, Cantor's 2009 array consists of the exact same [solid support (e.g., bead)]—[barcode]—[probe] structure as the subsequently-disclosed ST embodiment—which is claimed but not disclosed in Chee-022. EX1100, ¶114.



In Cantor's methods, after the sample nucleic acid hybridizes to a probe at a specific location in the array, it undergoes a process of extension and amplification to generate a population of "barcoded" nucleic acids in which its bead ID/barcode sequences are appended to the captured sample nucleic acids. These nucleic acids are then sequenced, which provides the identity and spatial location of the detected molecule. Accordingly, Cantor renders the challenged claims unpatentable.

As explained further below, and in the accompanying declaration of Joseph Puglisi, Ph.D., the Board should institute IPR and cancel the challenged claims.

II. GROUNDS FOR STANDING

Petitioner certifies that the '022 patent is available for *inter partes* review and that Petitioner is not barred or estopped from requesting an *inter partes* review challenging its claims on the grounds identified in this petition.

III. MANDATORY NOTICES

A. Real Party-In-Interest

Petitioner Curio Bioscience, Inc. is the real party-in-interest with respect to this petition for *inter partes* review.

B. Related Matters

On December 1, 2023, Prognosys and 10x Genomics filed a lawsuit against Petitioner in the District of Delaware. *10x Genomics, Inc. and Prognosys Biosciences Inc. v. Curio Bioscience, Inc.*, No. 23-cv-01375-MN (D. Del.).

The '022 patent issued from application No. 16/276,235, which claims priority to continuation application No. 15/187,661, filed on June 20, 2016, now Patent No. 10,308,982, which is a continuation application No. 13/080,616, filed on April 5, 2011, now Patent No. 9,371,598, and provisional Application No. 61,321,124, filed on April 5, 2010.

C. Lead and Back-up Counsel and Service Information

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D. Additional Service Information

Please address all correspondence to the lead and back-up counsel as shown above. Petitioner consents to electronic service at: CurioIPRs@irell.com.

IV. GROUNDS FOR THE CHALLENGES

Petitioner challenges the claims of Chee-022 on grounds tabulated below. Grounds 1 and 2 are based on references that are prior art even if Chee-022 is entitled to the filing dates of its priority applications.

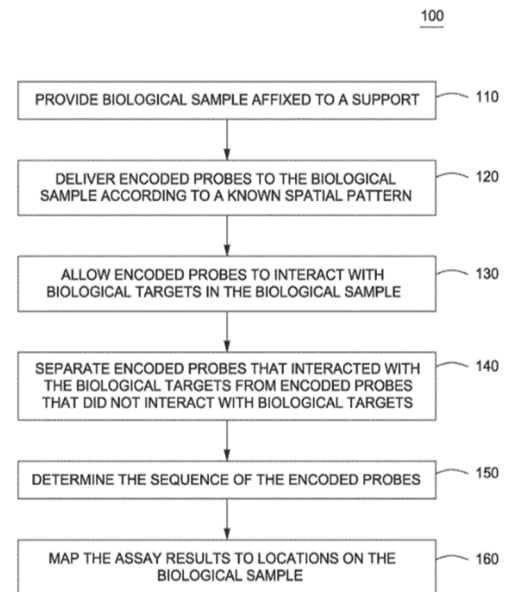
	Challenged Claims	Prior Art
1	1-2, 4-7, 11-15, 17, 19, 27-29	Obviousness (pre-AIA §103) over Cantor in view of knowledge of a POSA
2	1-7, 11-15, 17, 19, 27-29	Obviousness (pre-AIA §103) over Cantor in view of Armani and knowledge of a POSA
3	1-2, 4-7, 11-15, 17, 19, 27-29	Anticipation (AIA § 102) by Frisen

V. THE '022 PATENT

According to its face, Chee-022 issued from Application No. 16/276,235, which was filed on February 14, 2019. EX1001, Code (22). The '022 Patent claims priority to “Provisional application No. 61/321,124, filed on Apr. 5, 2010.” *Id.*, Code (60). The only Office Action issued during prosecution provisionally rejected the claims based on non-statutory double-patenting. EX1010, 444-447. The Examiner did not raise prior art or Section 112 issues. Additionally, the Examiner did not indicate any substantive basis for their “Reasons for Allowance” in a Notice of Allowability. *See id.*, 897-900; 943-950.

The **ABSTRACT** of the '022 Patent states 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 readout that is digital in nature.” *Id.*

To the extent any inventive subject matter is disclosed in Chee-022, it is the fluid delivery systems for spatial patterning of reagents that the specification repeatedly emphasizes as “the invention.” For example, in the section headed “The Invention in General,” the inventor wrote: “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.” EX1001, 8:39-44. It continues: “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.” Id., 8:51-55.

When Chee-022 discusses arrays, including bead arrays, its disclosures are unrelated to the subject matter that it now seeks to claim. Instead, it discusses them as conventional genotyping techniques; not as tools for performing “the invention.” See EX1001, 24:38-25:4 (“Genotyping may be performed using any technique known to those skilled in the art.... Some examples of suitable techniques involve...nucleic acid arrays, bead arrays....”). Moreover, when nucleic acid arrays are discussed in the (exclusively prophetic) examples, they are not used to demonstrate “the invention”; rather, “[a] microarray is used as *a proxy for a tissue section.*” EX1001, 30:30. The examples do *not* then describe placing a tissue section on top of “prox[ies] for a tissue section” to perform spatial analysis. Instead, they describe, for example, “delivery of a 10 micron diameter reagent droplet to an individual cell,” consistent with Chee-022’s repeated characterization of “the invention” as methods for delivering reagents onto a substrate-affixed biological sample (as illustrated in Figure B, above). *Id.*, 30:47-49.

VI. CITED PRIOR ART

A. Cantor

Cantor, WO 2009/091934, is WIPO application that was published on July 23, 2009. EX1031; EX1100, ¶¶106-108.

B. Armani

Armani et al., “2D-PCR: a method of mapping DNA in tissue sections,” is an article that was published in *Lab on a Chip* on October 12, 2009. EX1022; EX1100, ¶109.

C. Frisen

Frisen et al., WO 2012/140224, is a WIPO application that was published on October 18, 2012. EX1020, EX1100, ¶¶110-114.

VII. LEVEL OF ORDINARY SKILL IN THE ART

As Dr. Puglisi explains, a POSA in the field of Chee-022 in February 2010:

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

EX1100, ¶¶30-33. As of the February 2019 priority date applied in Ground 3, “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.” *Id.*, ¶34.

VIII. BACKGROUND KNOWLEDGE OF A POSA

A. Molecular Biology

The claims of the '022 Patent refer to the analysis and detection of “biological molecules,” and in particular nucleic acids. The '022 Patent’s “**BACKGROUND OF THE INVENTION**” admits that prior art “gene expression 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.” EX1001, 1:16-35. The concept of “gene expression” refers to the process by which a gene encoded by DNA gets turned on in a cell to make RNA and proteins. EX1100, ¶¶36-44.

B. Cells and Tissues

Tissues are groups of cells that function together as a unit. Important to many aspects of biological research, tissues can be in a normal, healthy state, or in a disease state, and a POSA would have been familiar with many techniques for isolating and preparing tissue samples for further investigation. EX1100, ¶¶45-50.

C. Spatial Analysis

Chee-022 admits that “conventional methods” were used in the prior art to “reveal[] spatial patterns of gene expression and have helped shed light on the molecular basis of development and disease.” EX1001, 1:26-35.

As Dr. Puglisi explains, by April 2010 biologists found 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). Accordingly, scientists had long been devising ways to analyze the spatial relationships of biological molecules, developing numerous tools and techniques 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.” EX1027, 552. EX1100, ¶¶51-56.

1. Detection of Nucleic Acids

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. For example, nucleic acids are readily labeled with fluorescent dyes to form labelled probes, and when such probes are hybridized with a target, will cause the target-probe complex to be fluorescent upon exposure to an appropriate light source, allowing detection with simple imaging or spectroscopy methods. EX1100, ¶¶57-59.

Sequencing generally refers to techniques used to determine the order of nucleotide bases present in the DNA or RNA of a biological sample. Such techniques include high-throughput “Next Generation Sequencing” (NGS) as well as traditional Sanger sequencing. Some applications of these techniques included the use of “custom-designed oligonucleotide microarrays...for targeting regions of interest.” EX1029, 41. Such techniques may be applied in conjunction with NGS to sequence nucleic acids of interest captured using microarrays. *Id.*, 41. EX1100, ¶¶60-61.

2. Nucleic Acid Arrays

Nucleic acid arrays are a class of technologies in which DNA or other nucleic acid sequences are provided on a solid support. 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,” and hybridization is then detected by fluorescence or sequencing. EX1018, 528. EX1100, ¶¶62-63.

Around the priority date, there were two main classes of solid supports on which the hybridization probes, sometimes called capture probes, were typically affixed: glass slides, similar to microscope slides, and beads. EX1018, 528. In bead arrays, the beads would then be deposited on a support, and the position of each bead

and the sequence it carried would be determined by a process called “decoding.” *Id.* EX1100, ¶64.

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 Dr. Chee explained that “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.” EX1038, [0013]. Known processes for such decoding included “positional decoding” methods in which the beads carrying known probes were selectively placed at particular locations. *Id.*, [0043]. Other known techniques involved analyzing “identification sequences” affixed to the beads. *Id.*, [0042]. This facilitated the use of “decoding by sequencing” methods to map the location of particular beads in the array, and which “allow[] decoding to be accomplished independent of the” capture agent on the molecule. *Id.*, [0146]. EX1100, ¶¶65-70.

3. Sequence Barcoding

Multiplexing, which allows higher throughput analysis of many samples in parallel, was a key development for nucleic acid sequence analysis before 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, 3:55-64. Consistent with this knowledge, it is notable that in a prior proceeding involving EX1037, 10x asserted that the concept of nucleic acid barcoding “is as old as the scientific method and is familiar to every student in any introductory science class.” EX1035, 4-5. As such, 10x asserted that the use of barcodes or tags in “the particular technological environment of DNA (and other nucleic acids) by adding ‘attaching,’ ‘amplifying,’ and ‘detecting steps and other similarly generic techniques were *well-known and conventional before 2009.*” *Id.* EX1100, ¶¶71-72.

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 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. 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. EX1100, ¶¶73-74.

	2nd two bases																			
1st two bases	TT	TC	TG	TA	CT	CC	CG	CA	GT	GC	GG	GA	AT	AC	AG	AA				
TT							16'			23'		TTGA 6			TTAG 8					
TC			TCTG 1		30'	TCCC 3			TCGT 5							6'				
TG		TGTC 2		36'			TGCG 4						TGAT 7			11'				
TA					18'			TACA 36			33'									
CT	32'		CTTG 9					CTCA 11	CTGT 13							8'				
CC				CCTA 33					29'					CCAT 15						
CG	CGTT 10		12'					4'							28'		CGAA 16			
CA		34'			25'		CACG 12			CAGC 14		1'				9'				
GT					GTCT 19	24'				GTGC 22			31'							
GC	GCTT 17		14'												22'		GCAA 23			
GG		20'		GGTA 18	35'							3'		GGAC 24						
GA			GATG 34				GACC 20		2'	GAGT 21										
AT							ATCG 28	7'			15'			ATAC 31						
AC		21'			ACCT 27						ACGG 29	5'				13'				
AG			AGTG 25				AGCC 35		27'			AGGA 30			19'					
AA	AATC 26							10'		17'						AAAG 32				

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 GGTC (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).

Similarly, Chee-824 discloses barcoded bead arrays in which “a spatial or positional coding system is done.” EX1032, 28:6-7; *see also* EX1038, [00146] (“In this way, beads are tagged with fluorescent labels, i.e., barcodes of sorts, their location or identity deciphered and recorded.”). EX1100, ¶¶75-77.

D. Overview of Spatial Analysis Technologies

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.* For example, Armani observed 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 their spatial organization. EX1100, ¶¶78-84.

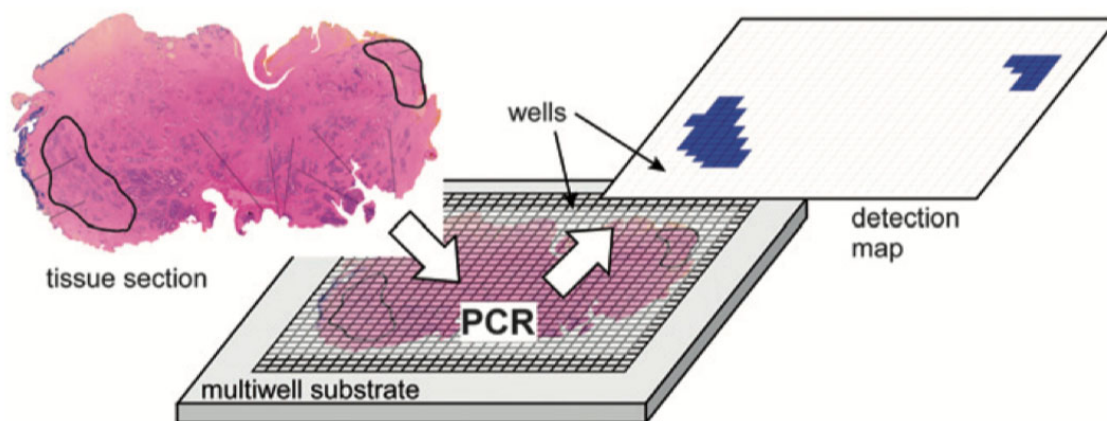


Fig. 1 2D-PCR is achieved by transferring a tissue section vertically into a multi-well array device, isolating tissue subregions. The DNA is extracted and then amplified by the polymerase chain reaction. A detection map is created by imaging the amplified DNA, for example using a DNA-intercalating dye.

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, including after reverse-transcription processes. EX1100, ¶¶85-91.

Shirai discloses an example of such techniques, 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 . 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. 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. EX1100, ¶¶92-96. Shirai then discloses detecting gene expression using fluorescent

probes to generate a spatial map. EX1024, 10:46-55; EX1100, ¶¶97. *See also id.* EX ¶¶98-101 (describing similar methods); EX1025.

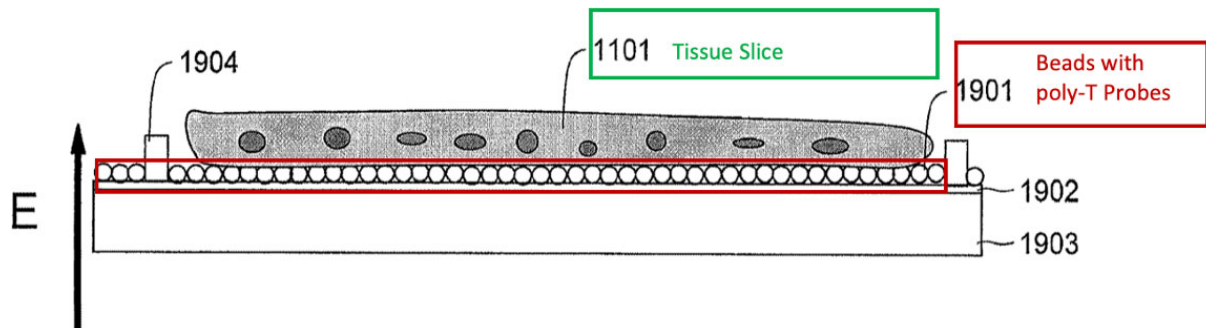


FIG. 19

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 [may] 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 complementary cDNA by extending the probe, using the captured mRNA as a template for *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. EX1030, 27:12-28. In addition to detection by

fluorescence, Southern continues by noting that “single molecule sequencing” is also a suitable readout. *Id.*, 39:25-28. EX1100, ¶¶102-103.

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\ in prior litigation, 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 concept of “serial numbers” to nucleic acids. EX1035, 4-5. Such approaches are illustrated in Cantor and Frisen, as discussed in the Grounds below. EX1100, ¶¶104-105.

IX. CLAIM CONSTRUCTION

The Petition applies the understanding of the claim terms applied by PO to assert infringement against Curio. PO has asserted that no claim terms require construction. EX1041-1042.

Chee-022 defines the claimed “binding agent” as an “agent that specifically binds to a biological molecule of interest.” EX1001, 4:56-58. PO contends that this limitation is satisfied in Curio’s accused products by the interaction of poly-T probes

and the poly-A tails present on typical mRNA molecules. Curio disputes that such interactions constitute the “specific binding” required by the claims. However, the Board need not address this dispute 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.

X. THE BOARD SHOULD CANCEL THE CHALLENGED CLAIMS

A. Ground 1: Obviousness over Cantor

1. Claim 1

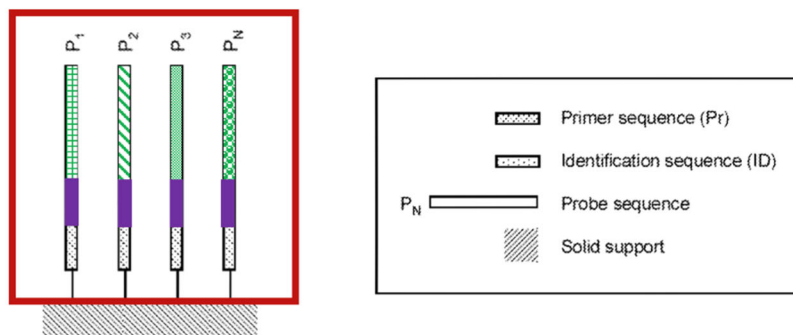
Cantor in view of the knowledge of a POSA renders obvious a “method for determining a spatial location of a biological molecule of a tissue sample” by conducting each of the claimed steps. Thus, to the extent the preamble is limiting, Cantor renders it obvious. EX1100, ¶¶118-122.

Cantor discloses methods for analyzing biological samples using binding agents affixed to solid supports such as beads. EX1031, 13 (a “solid support unit (e.g., one bead) can be selected”). Individual solid supports “may be in a collection of solid supports,” such as an array wherein each “solid support unit” is “one bead.” *Id.*, 4, 13. Cantor explains that such arrays are arranged on a substrate, for example in an “X by Y array,” with one bead at each X,Y coordinate. EX1031, 17.

Tracking the claims, Cantor discloses that “[t]he biological sample can be in any form, including without limitation a solid material such as a tissue.” EX1031, 6.

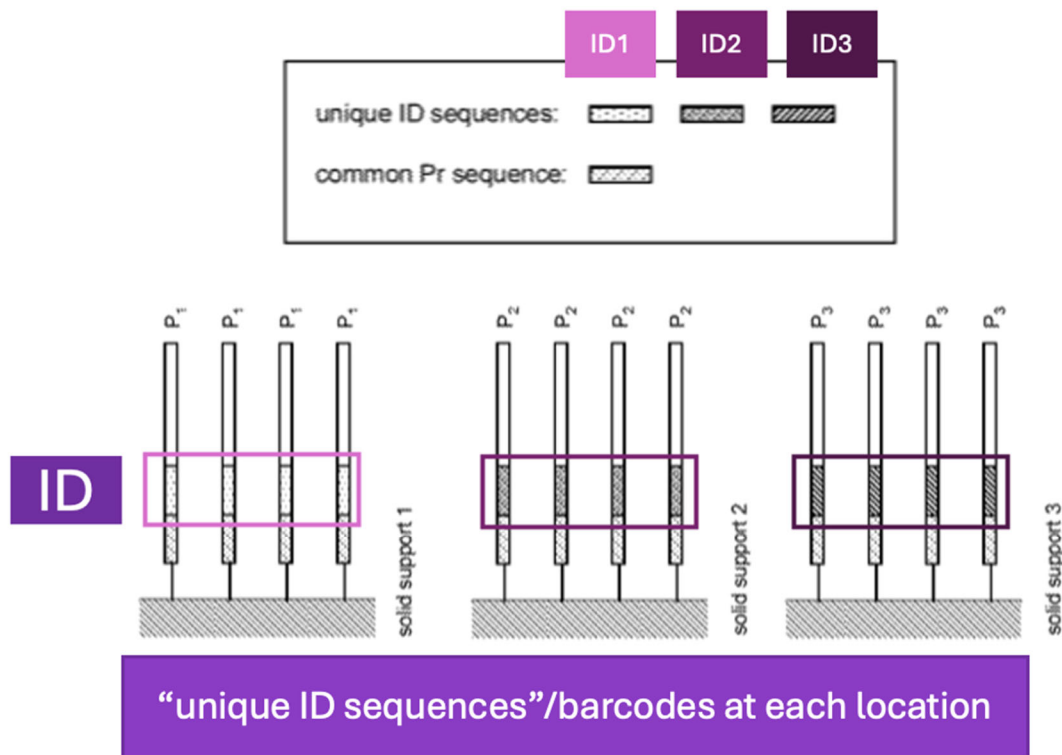
And Cantor's plurality of beads comprise nucleic acids that constitute the claimed **"binding agents"** because they are nucleic acid constructs that include (a) a **"probe sequence"** that is **"configured to interact"** with (e.g., hybridize to) a target nucleic acid of the sample, as well as (b) a **"coding identifier"** – Cantor's **"identification sequence"** – that uniquely identifies the specific bead, and thus corresponds to the location at which that particular bead's binding agent interacted with the target nucleic acid of the sample. EX1100, ¶¶124-125. Accordingly, Cantor discloses bead arrays comprising every structural element required by the claim.

Cantor's Figure 1A shows an example of a single solid support, such as a bead, with a plurality of **"binding agents,"** each having an **"identification sequence (ID)"** and a **"probe sequence."** Cantor discloses that each binding agent on the same solid support (such as a bead) has the same identification sequence. The annotations below show the presence of each feature of the claimed binding agents in Cantor's Figure 1 and the requirements of the claimed **"binding agents"**. EX1100, ¶123.



Cantor explains that its “[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. In such an array, “each of the solid supports” (i.e., each “bead”) has a “unique identifier sequence different than the identifier sequence” of the “other solid supports.” *Id.*, 2. Thus, a POSA would have understood Cantor to disclose an array of beads at which each bead is necessarily deposited at a particular (X, Y) coordinate location, and each such bead would have its own unique identification sequence as part of its binding agent. Such binding agents would also include a “probe” sequence as part of the same single-stranded nucleic acid. Thus, the “identification sequence” corresponds to the location at which the “probe” portion of the binding agent interacts with the tissue sample. EX1100, ¶125.

For example, Cantor’s Figure 1C depicts three solid supports (1, 2, and 3), such as three beads. Each support/bead has a “unique ID sequence[]” that is 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.” In other words, each binding agent on support/bead 1 has an ID/bead barcode that is different than the ID/bead barcode present on each of the other supports/beads. EX1100, ¶126.



Because each unique ID sequence corresponds to a particular bead and thus “barcodes” a given bead (and thus its location in the array), the existing knowledge of a POSA would have further motivated the use of Cantor’s bead arrays to determine the spatial location of biological molecules in tissue samples for a variety of reasons. For example, Southern, like Cantor, discloses arrays of target-binding nucleic acid probes and expressly instructs the use of such arrays to determine “spatial arrangements” of target nucleic acids. EX1033, Abstract. Southern discloses that the samples suitable for analysis using its methods include “samples containing different types of human cells” such as “tissue samples.” EX1033, 16:28-30. As Dr. Puglisi explains, multiple additional references documented a POSA’s motivation to

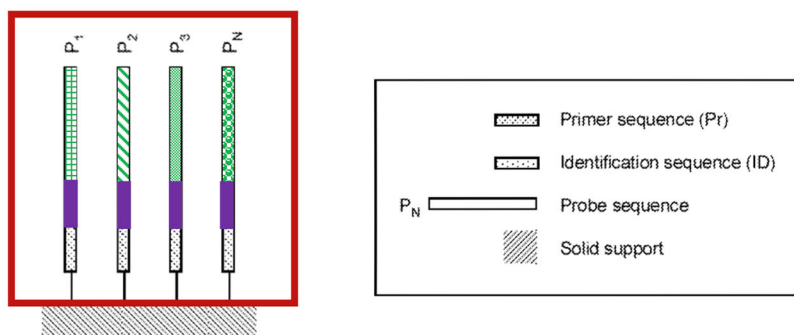
analyze tissue samples with methods that “preserv[e] and map[] the spatial distribution of nucleic acids at the tissue and cellular levels.” EX1021, 1:13-15; EX1022, 3526 (important to analyze “DNA variability within a tissue sample” including its “ physical location within the tissue section”); EX1025, [0008]-[0009] (showing POSA’s motivation to implement techniques that “quantitatively measure the mRNA and proteins localized between or inside cells without losing local space data.”) EX1025, [0008]-[0009]. EX1100, ¶127.

a) “providing a plurality of beads....”

First, Cantor discloses “providing a plurality of beads, wherein the plurality of beads comprise a plurality of binding agents.” Cantor discloses nucleic acid binding arrays in which the claimed binding agents are attached to a solid support, such as a bead. EX1031, 4 (“[T]he invention in part provides a substrate comprising a collection of beads oriented in an array....”). “Each bead is in association with single-stranded nucleic acid,” which “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.” *Id.* The single-stranded nucleic acid on each bead also contains “a unique identifier sequence different than the identifier sequence” associated with the other beads in the array. EX1100, ¶¶128-130, 132.

Cantor explains its beads contain “[p]robe, identification and primer hybridization sequences in solid phase nucleic acid [that] can be arranged in any suitable orientation with respect to one another,” and that “[i]n certain embodiments, the sequences are contiguous and in the following orientation: 5’-(primer sequence)-(identification sequence)-(probe sequence)-3’.” EX1031, 17. Such a single stranded nucleic acid construct constitutes one of the claimed “binding agents.” EX1100, ¶131. Cantor also discloses including a plurality of binding agents on each bead, as “[t]he density of solid phase nucleic acid molecules per solid support unit (e.g., one bead) can be selected,” including at a range of 5-10,000 nucleic acids per bead. *Id.*, 13. EX1100, ¶133. Alternatively, “[i]n certain embodiments the density of the solid phase nucleic acid per unit solid support is about 1 to 1 (e.g., one molecule of solid phase nucleic acid to one bead).” *Id.*

Second, Cantor discloses that each “binding agent of the plurality of binding agents (i) comprises a coding identifier having a nucleic acid sequence.” EX1031, 4 (“each bead is in association with **single-stranded nucleic acid**; the solid phase nucleic acid comprises an **identifier sequence** and a **probe sequence**....”). EX1100, ¶¶134-135.



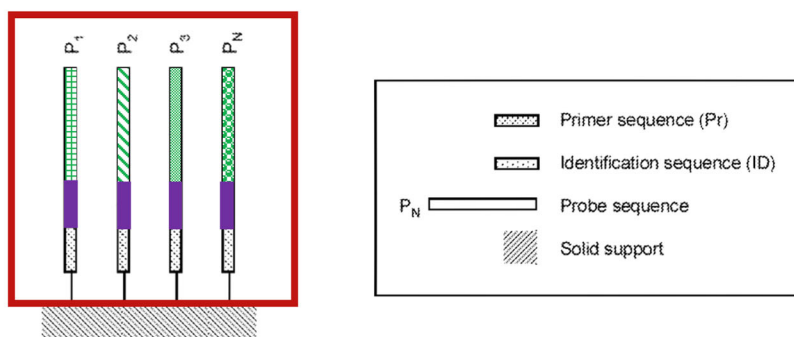
Cantor discloses that each of its single-stranded nucleic acid binding agents includes an “**identification sequence**” which constitutes the claimed “**coding identifier** having a nucleic acid sequence.” EX1100, ¶136.

For example, Cantor states that “the solid phase nucleic acid of each of the solid supports [e.g., beads], in the collection [e.g., an array] share a unique identifier sequence different than the identifier sequence of the solid phase nucleic acid of the other solid supports [e.g., beads].” EX1031, 4. A POSA would have understood these disclosures to mean that when the solid supports are beads, each binding agent attached to a particular bead included the same “identification sequence,” while the binding agents attached to other beads would have a different “identification sequence.” These differing “identification sequences” would therefore “be useful in part for constructing partial sequence reads into larger sequence constructions,” for example to discern which specific bead in the X, Y array a particular sequence read came from. *Id.*, 15. EX1100, ¶137.

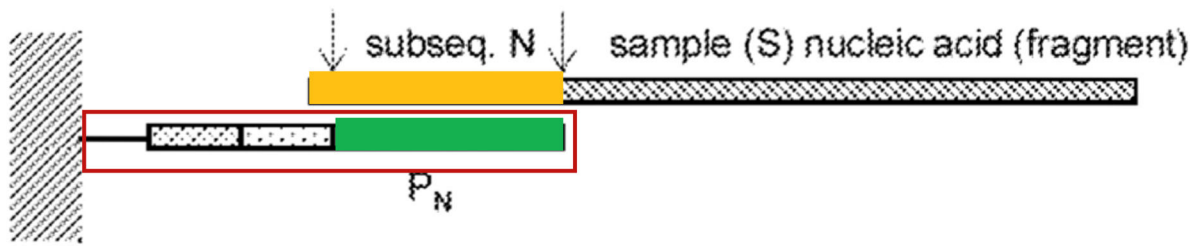
Cantor explains that “one identification sequence species may differ from a second identification sequence species by one or more nucleotides when the first and second identification sequences are aligned with one another....” EX1031, 15. These identification sequences “may be of any length suitable for analyzing the nucleotide sequence or partial nucleotide sequence of sample nucleic acid,” and “[i]n some embodiments, an identifier sequence is about 5 to about 50 contiguous nucleotides in length, sometimes about 5 to about 20 nucleotides in length and at times about 10 nucleotides in length.” *Id.* A barcode X nucleotides in length generates barcodes for 4^X unique beads, thus, depending on the length, Cantor’s ID sequences provide barcodes for at least 1,024 (5 nt) to 1×10^{12} (20 nt) unique beads. EX1100, ¶¶73-75, 138.

Third, Cantor discloses that each “binding agent of the plurality of binding agents (ii) is configured to interact with a biological molecule of a tissue sample.”

Cantor’s “**binding agents**” are “configured to interact with a **biological molecule of a tissue sample**” through their “**probe sequence**.” EX1100, ¶140.



For example, Cantor explains that “[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, 24.² This is depicted in Figure 2, excerpted below to show interaction between the “**probe sequence**” of the “**binding agent**” and a portion of a “**biological molecule**,” here a subsequence (N) of sample nucleic acid (S). EX1100, ¶141.

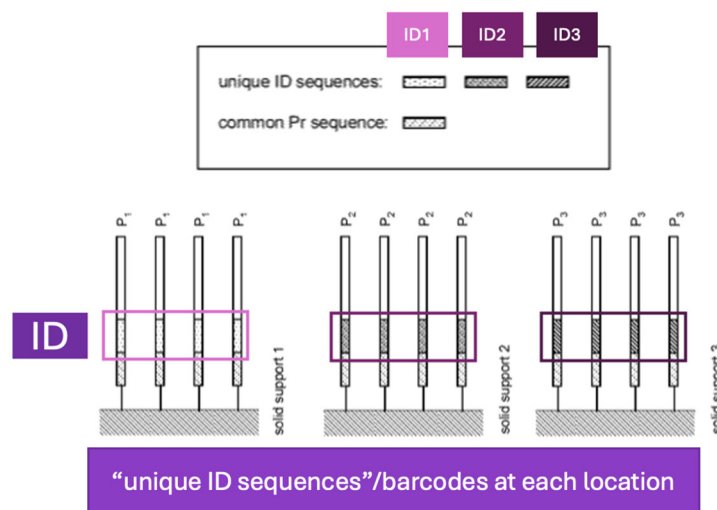


Cantor expressly discloses that the target biological molecules in the “‘biological sample’ can be in any form, including without limitation a solid material such as a tissue.” *Id.*, 6.

Fourth, Cantor renders obvious “the coding identifier corresponds to a location at which the binding agent interacts with a biological molecule of a tissue sample.” Cantor explains that its “[s]olid supports may be provided in an array”

² Cantor discloses both arrays in which the probe sequence is the same at each position, and arrays in which the probe sequence is not the same at each position. *See* EX1031, Claims 31-32.

Because Cantor's binding agents include a "probe" sequence as part of the same single-stranded nucleic acid as the "identification sequence," Cantor's "identification sequence" necessarily corresponds to the location at which the "probe" portion of the binding agent hybridizes with sample nucleic acid present in a tissue sample. For example, Cantor's Figure 1C 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." EX1100, ¶¶145-146.



Moreover, as explained further in connection with step 1(c), Cantor in view of the knowledge of a POSA renders obvious using Cantor's identification sequence to identify the location at which the binding agent interacted with the biological molecule. EX1100, ¶147.

b) “contacting the plurality of beads with the tissue sample...”

Cantor explains that its binding agents interact with the target 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 its sample may be “a solid material such as a tissue...or a biopsy.” *Id.*, 6. Thus, Cantor discloses contacting the plurality of beads with a tissue sample such that the binding agents affixed to its beads interact with biological molecules, such as nucleic acids, in those samples. EX1100, ¶148.

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.” EX1031, 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. EX1100, ¶149.

For example, Southern similarly discloses the spatial analysis of nucleic acid molecules by contacting tissue samples 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 target nucleic acid from cells in a tissue sample disposed on an array, including a bead array. *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-EX1026 (same). EX1100, ¶150.

c) “using the coding identifier to identify the location....”

Cantor renders obvious using the bead-specific “identification sequences” present in its “binding agents,” to identify the location where the “probe” region of each “binding agent” bound to a target nucleic acid molecule from a tissue sample.

Specifically, Cantor's Figure 2 and associated discussion describes how to detect target nucleic acids and determine the identity of both the target nucleic acid sequence and the identification sequence of the bead on which it was captured. Since Cantor discloses that each identification sequence corresponds to a unique bead at a unique (X,Y) position, when the identification sequence is determined as part of the sequenced amplification product, the presence of a particular identification sequence (or its complement) in a given sequence read identifies the location where the probe portion of the binding agent interacted with the captured sample nucleic acid. EX1100, ¶151.

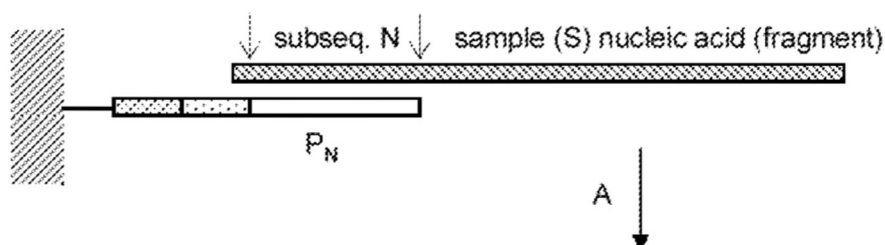
Specifically, Cantor discloses methods for extending and amplifying captured target nucleic acids to generate amplified populations of nucleic acids, which contain the target nucleic acid (extended from the probe region which captured it) and the identification sequence, as well as their complements. These populations are then sequenced to determine the identity of the target sequence as well as the identification sequence (i.e., the bead barcode). For example, Cantor depicts the "released amplification product," which is then subjected to "sequence determination of amplification product." The result of such sequencing would readout, from left-to-right, the sequence of the **primer**, the complement of the **identification sequence**, and the **target sequence**. EX1100, ¶152.



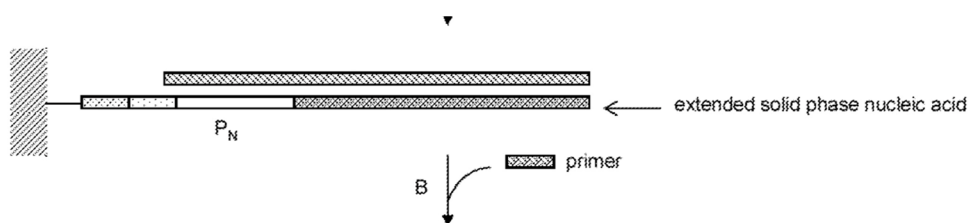
Thus, sequencing the released amplification product would necessarily determine the sequence of a target nucleic acid and at least the complement of the identification sequence corresponding to a particular (X,Y) location of a bead in the array, thereby determining the spatial location at which the binding agent interacted with the tissue sample. *Id.*, ¶153.

When the target nucleic acid is mRNA, a POSA would have found it obvious to implement this extension and amplification procedure using reverse transcription techniques. EX1031, 22 (“Conditions under which nucleic acids can be extended and/or amplified by [“extension agents” and “extension reagents”] are known.”). Cantor explains that “extension agents may include...extension nucleotides, a polymerase and a primer that can hybridize to a primer sequence in solid phase nucleic acid,” such as “polymerases for thermostable amplification (e.g., RNA polymerase for transcription-mediated amplification (TMA),” as well as components “such as reverse transcriptase for TMA reactions, for example.” *Id.*, 22. *See also id.*, 23. EX1100, ¶154.

The first panel of Figure 2 shows the capture of a target nucleic acid from the biological sample by the probe region, as discussed above. EX1100, ¶155.

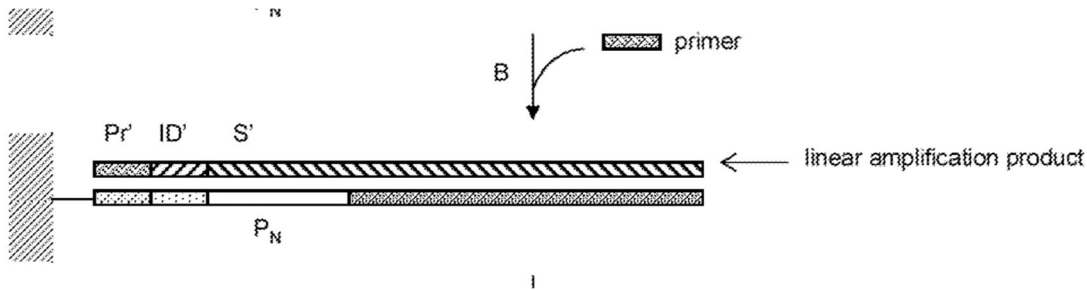


The next panel shows the “extension” of the binding agent to generate a second strand complementary to the bound target nucleic acid. EX1031, 24 (“Solid phase nucleic acid hybridized to sample nucleic acid (S) can be extended (e.g., illustrated in Figure 2 as A) and “extended solid phase nucleic acid’ is generated.”). The nucleic acid depicted to the right of the probe sequence P_N in the figure below shows the extension of the binding agent to include “a nucleotide subsequence complementary to a target nucleotide sequence in the sample nucleic acid molecule.” *Id.* EX1100, ¶156.



Next, “[e]xtended solid phase nucleic acid may be amplified by hybridizing primer (Pr’), which is complementary to primer sequence (Pr) of the extended solid phase nucleic acid, in the presence of amplification/extension reagents.” EX1031, 24. “The hybridized primer [Pr’] is extended thereby generating an amplification product.” *Id.* This amplification product is the top strand shown in the figure panel

below. “The amplification product contains the primer nucleotide sequence (Pr’), a nucleotide sequence complementary to the identification sequence in solid phase nucleic acid, and a nucleotide sequence identical to or substantially identical to a target nucleotide sequence in the sample nucleic acid molecule.” *Id.* EX1100, ¶157.



Cantor further discloses that “[t]his linear amplification product can be released from the solid support for sequence analysis (e.g., illustrated in Figure 2 as C).” EX1031, 24-25. EX1100, ¶158.

Following release, Cantor discloses that its “[a]mplification products...may be subject to sequence analysis,” that is, the use of sequencing “to determin[e] a nucleotide sequence of an amplification product.” *Id.*, 25. Cantor explains that “[t]he entire sequence or partial sequence of an amplification product can be determined, and the determined nucleotide sequence is referred to...as a ‘read.’” *Id.* “A read may be obtained with or without further amplification,” and accordingly, Cantor discloses determining both the sequence of the strand including the original target nucleic acid molecule, as well as its complement. As explained above, this facilitates the determination of the location at which the bead and target nucleic acid molecule

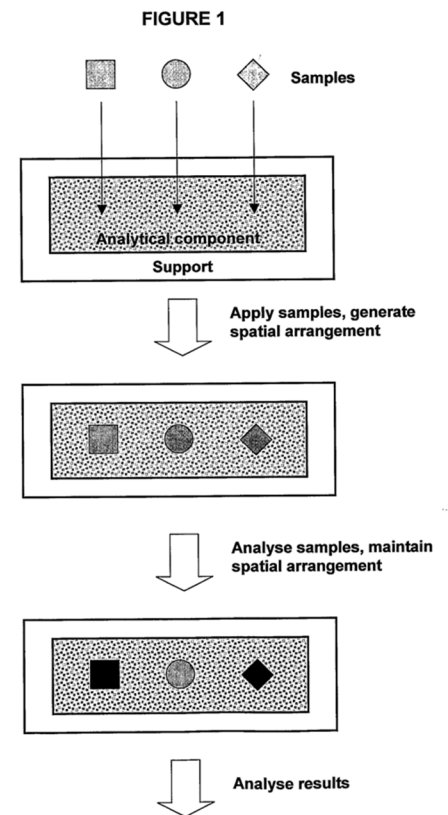
interacted by correlation of identification sequences (or their complements), which are present in the amplification products, with the (X,Y) coordinates of the bead on which such identification sequences were present. EX1100, ¶159.

d) A POSA's knowledge would have further confirmed a POSA's motivation and reasonable expectation of success for using Cantor's arrays to identify the spatial location of nucleic acids in a tissue sample.

A POSA's motivation to correlate Cantor's bead "identification sequences" with the bead's location in array, is further confirmed by the knowledge of a POSA. As Dr. Puglisi explains, a POSA would have been familiar with additional examples documenting the use of nucleic acid arrays, such as bead arrays, to (1) capture or hybridize nucleic acids from a tissue sample through the use of complementary probes; (2) amplify the captured nucleic acid; and (3) determine the identity of the captured nucleic acid and the location at which it interacted with the complementary probe, and thus its location in a tissue sample. EX1100, ¶160.

This is evidenced, for example, by Southern, which discloses methods of using nucleic acid probe arrays to perform spatial analysis of "samples containing different types of human cells" such as "tissue samples." EX1033, 16:28-30. The arrays and extension, amplification, and detection procedures disclosed in Southern are analogous to those described in Cantor. EX1100, ¶161.

Southern explains that its “samples are analysed by allowing them to interact with an analytical component on a support. “Target analytes,” such as nucleic acids, “in the samples are detected when the sample interacts with the analytical component.” EX1030, 1:19-21. Southern explains that its samples “are applied in step a) to different areas of the support to produce a spatial arrangement,” which is maintained, “permitting the results of the analysis to be matched to individual samples,” thus determining the spatial location of target nucleic acids. EX1100, ¶162.



Like Cantor, Southern discloses the detection of target nucleic acid by creating a nucleic acid strand complementary to sample nucleic acid. For example, with mRNA, Southern discloses creating a complementary cDNA by using the captured mRNA as a template for reverse transcription. Southern discloses that its hybridization events can be detected “by any of the familiar means of fluorescence detection,” and that “even low-abundance mRNA can be detected.” EX1033, 27:12-28. Additionally, Southern discloses that its detection methods 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. EX1011, ¶163.

As Dr. Puglisi explains, additional references documenting a POSA's knowledge similarly used array-based probes to capture and amplify target nucleic acids from tissue samples to preserve and determine the spatial location of the captured target nucleic acids in a tissue sample. *See, e.g.*, EX1024, Abstract, Figure 19 (reproduced below); EX1025, [0072]. EX1100, ¶164.

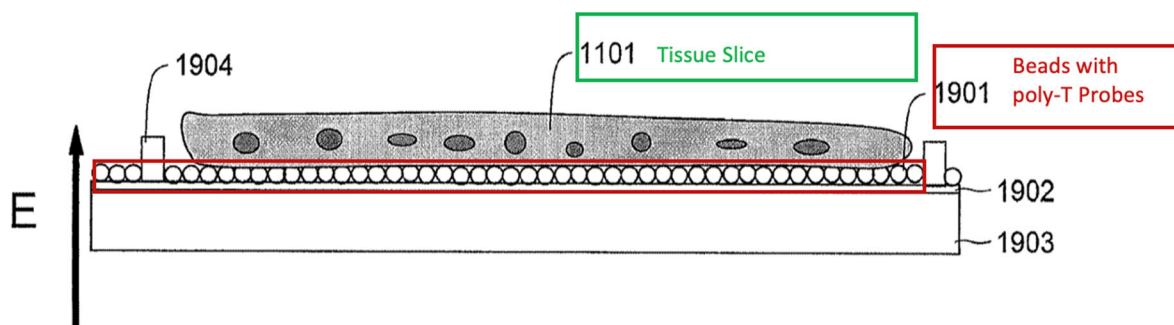


FIG. 19

- e) **A POSA's knowledge would have further confirmed a POSA's motivation and reasonable expectation of success in using Cantor's identification sequences to determine the location at which a probe interacted with a target nucleic acid from a tissue sample.**

A POSA would have appreciated that Cantor's disclosure of a unique identification sequence for each bead constituted a unique "barcode" nucleic acid sequence to distinguish one bead from another. In addition to Cantor's express disclosures of providing the beads according to an (X, Y) array, a POSA would have found it obvious to determine the identity and location of each bead in the array before using it in Cantor's analytical procedures at least by (i) selective loading of

particular beads to particular locations on the array and/or (ii) decoding the array.

EX1100, ¶¶65-66 (discussing EX1038); ¶¶165-166.

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 art. *See* EX1032, 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, [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 (describing 4 nucleotide “zip-code” sequences to define spatial locations on an array in a “checkerboard” (X, Y) array pattern). EX1100, ¶¶167-169.

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]. As Dr. Puglisi explains, a POSA would have been familiar with multiple well-established techniques for decoding bead arrays, for example by sequencing or using fluorescent probes to “correlat[e] the location of an

individual site in the array with the bead,” at that location. EX1038, [0013]. EX1100, ¶¶65-70. A POSA would have been motivated to use 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. *Id.*, [0037]-[0038], [0046], Figs. 12-13. For example, Chee-824 discloses “decoding by sequencing” of bead arrays comprising probes structured similarly to those used in Cantor’s bead array, using procedures that allow the array to be used in analytical assays without issue following the decoding procedure. *Id.*, [0146]. EX1100, ¶¶170-171.

When the “identification sequence” of a particular bead location is produced as a sequencing readout, a necessary result of Cantor’s workflow, a POSA would then have found it obvious to map the rest of the sequence readout (i.e., the sequence of the target nucleic acid or a complement thereof) to the bead location, thereby determining a spatial location of the biological molecule of the tissue sample. A POSA would have reasonably expected success in correlating Cantor’s “identification sequences” with the location of the beads to which they are bound on an array, at least based on the use of known loading and/or decoding procedures. Indeed, a POSA would made this correlation without any modifications to Cantor’s device, or the process in which it is used to capture, amplify, extend and sequence target nucleic acid sequences. Instead, correlating a particular sequence to a

particular location involves (at most) only the mental step of associating a particular “identification sequence” with a “particular bead” – features already present in Cantor’s device and methods – based on the routine and predictable use of well-known array decoding techniques. EX1100, ¶¶172-173.

f) Conclusion

Cantor in view of a POSA’s knowledge renders claim 1 obvious.

2. Claim 2³

Cantor discloses “the plurality of beads is provided on a substrate surface.” Cantor states: “the invention in part provides a substrate comprising a collection of beads oriented in an array.” EX1031, 4. *See also id.*, 17-18; cl. 48. EX1100, ¶¶175-178.

3. Claims 4-6

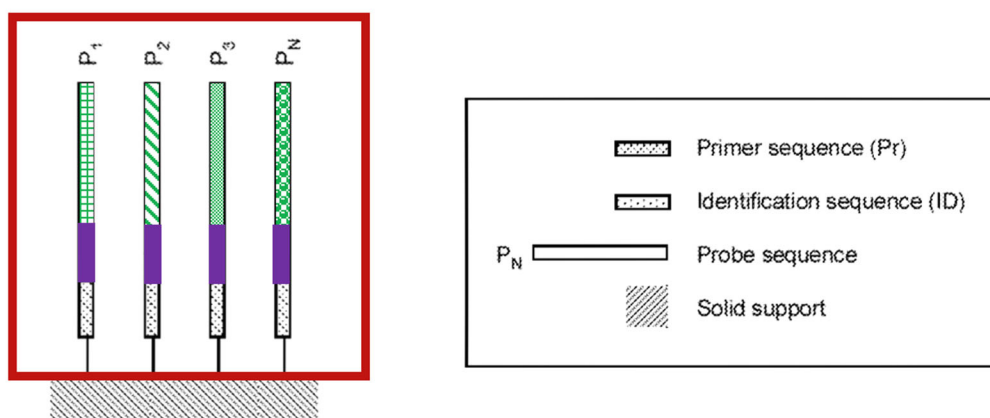
Cantor discloses “the biological molecule is a nucleic acid,” “the binding agent of the plurality of binding agents comprises a nucleic acid,” and “the binding agent interacts with the biological molecule via hybridization.” Cantor discloses that “solid phase nucleic acid generally is single-stranded and is of any type suitable for hybridizing sample nucleic acid,” and thus the binding agent interacts with a target

³ The analysis of claim 1 is incorporated into the analysis of each dependent claim.

nucleic acid via hybridization. *See* EX1031, 4, 6, 11-12, Figs. 1-2. EX1100, ¶¶179-187.

4. Claim 7

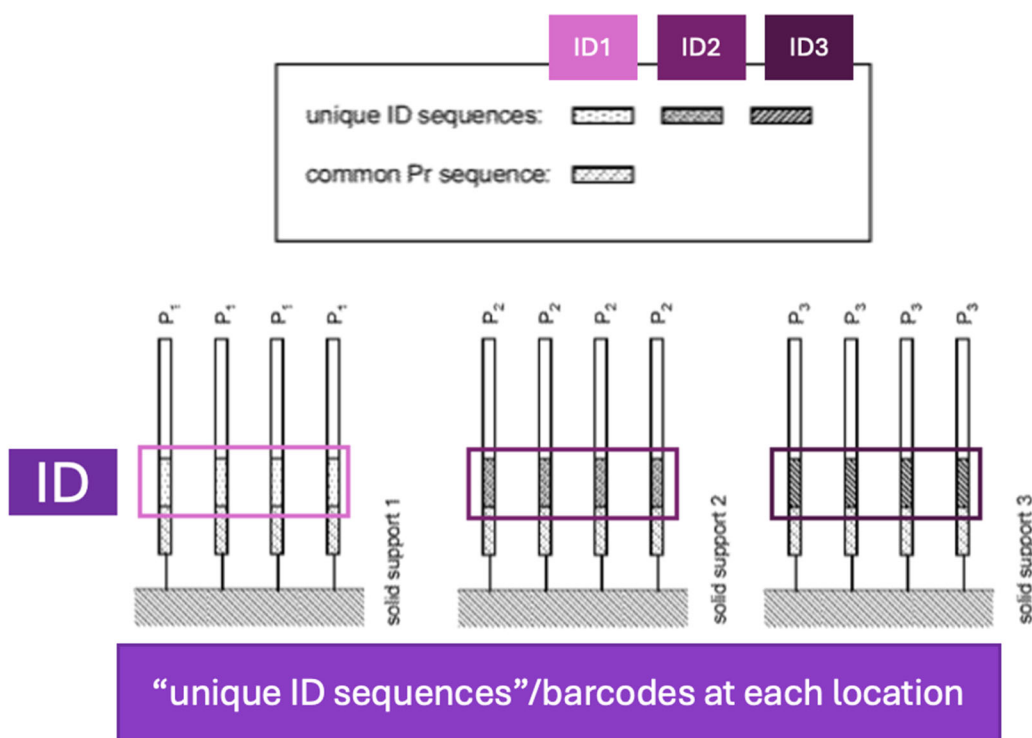
Cantor discloses “the binding agent and coding identifier are provided as a single molecule.” *See* EX1031, 4 (“each bead is in association with **single-stranded nucleic acid**; the solid phase nucleic acid comprises an **identifier sequence** and a **probe sequence**....”); *see also id.*, 16-17, Fig. 1A. EX1100, ¶¶188-191.



5. Claims 11-14

Cantor discloses the claimed additional binding agents comprising an additional coding identifier, wherein the additional binding agents interact with another biological molecule of the tissue sample at a different location, as recited in claims 11-14.

Claim 11: Cantor discloses that the plurality of binding agents may be provided on separate solid supports, such as beads, where the coding identifier differs from one bead to another. *See* Claim 1(a). For example, Figure 1C depicts three distinct beads: “solid support 1,” “solid support 2,” and “solid support 3.” EX1031, FIG 1C. EX1100, ¶¶192-194.



The coding identifier, or “unique ID sequences” for each of the solid supports or beads depicted in Figure 1C is different. As explained above, each bead is provided at a different (X,Y) coordinate. Thus, each of the binding agents of Cantor’s array “is configured to interact with another biological molecule of a tissue

sample” namely the nucleic acids present at the various different (X,Y) coordinates of the array and tissue sample placed thereon. EX1100, ¶195.

Additionally, Cantor further discloses “binding agents” that are “configured to interact with another biological molecule of the tissue sample” by disclosing embodiments in which each solid support or bead may have a probe sequence that is distinct from the probe sequences of other beads. *See* Figure 1C (depicting “probe” sequences P_1 , P_2 , and P_3 as different for each location). EX1100, ¶¶196-198.

Claims 12-14: Because Cantor in view of the knowledge of a POSA renders obvious correlating each of Cantor’s “identification sequences,” which constitute the claimed “coding identifiers,” with the (X,Y) coordinates of the bead in array, the coding identifier corresponds to the location at which a given binding agent (such as the claimed “additional binding agent”) interacts with another nucleic acid from a different spatial location of the tissue sample. EX1100, ¶¶199-201. For example, when “the location at which the binding agent interacts with the biological molecule” is at Cantor’s “solid support 1,” a bead having an (X,Y) coordinate (X_1 , Y_1), and the “additional binding agent” that “interacts with said another biological molecule” is on Cantor’s “solid support 2,” a bead having an (X,Y) coordinate (X_2 , Y_2), the location at which the first and second binding agents interacted with their respective biological molecules is necessarily different. Because each bead is provided at a unique spatial address and interacts with the tissue sample disposed thereon, the

spatial location of a biological molecule that interacts with a binding agent associated with a given bead is necessarily different than the spatial location of a biological molecule of the tissue sample that interacts with a binding agent associated with a different bead at a different location. EX1100, ¶¶202-207.

6. Claim 15

Cantor discloses “determining the spatial locations” of multiple nucleic acids simultaneously. Cantor renders obvious determining the spatial locations of nucleic acids detected on each of its beads by sequencing. *See* Claim 1(c). This allows for the simultaneous detection of the location of different biological molecules that were detected by sequencing all of the amplification products together, and analyzing the frequency at which a given “identification sequence” is read out. EX1100, ¶¶208-209. Cantor refers to such analysis as “multiplexing.” EX1031, 50. EX1100, ¶¶210-211.

7. Claim 17

Cantor renders obvious “determining the relative amounts” of nucleic acid at a location. For example, Cantor discloses certain instances in which the probe sequences are the same at each position on its array. *See* Claim 1(a). In such instances, determining that a nucleic acid bound to the probe sequence at a particular bead (based on the presence of sequencing reads containing that bead’s identification sequence) but not at another bead (based on the absence of sequencing reads

containing that bead's identification sequence) indicates a relative abundance of the biological molecule at the first bead relative to the second bead. EX1100, ¶¶212-213. Additionally, a POSA would have found it obvious to quantitatively analyze the sequencing data generated in claim step 1(c), for example by tallying the readcounts—which are proportional to the amount of a given sample nucleic acid—at different positions across the array. *Id.*, ¶¶214-215.

8. Claim 19

Cantor discloses “wherein step [1](c) comprises sequencing...the coding identifier.” Cantor's methods generate amplification products that include the entirety of the “**identification sequence**,” which corresponds to the claimed “**coding identifier**,” or its complement. EX1031, 25.



Cantor explains that “[t]he entire sequence or partial sequence of an amplification product,” which includes the “**identification sequence**,” “can be determined, and the determined nucleotide sequence is referred to...as a ‘read.’” *Id.* “As explained above, this facilitates the determination of the location at which the bead and target nucleic acid molecule interacted by correlation of identification sequences (or their

complements) with the (X,Y) coordinates of the bead on which such identification sequences were present. EX1100, ¶¶216-219.

9. Claim 27

Cantor discloses “analyzing gene expression in the tissue sample.” First, Cantor explains that the target nucleic acid may be mRNA. EX1031, 6. A POSA would have known that analyzing the presence or absence of mRNA constitutes an analysis of gene expression. See EX1040 (“The fundamental question in the analysis of gene expression at the RNA level is whether RNA sequences derived from a gene of interest are present in cells or tissues.”). Cantor further discloses that the sequencing data generated in its detection methods may be used for “detection of allelic variations in gene expression.” EX1031, 55. EX1100, ¶¶220-224.

10. Claim 28

Cantor renders obvious “the location of the binding agent is known prior to step [1](b).” As discussed in Section X.A.1.e), a POSA would have found it obvious to decode Cantor’s array using known methods before using it to assay target nucleic acids from a tissue sample. Thus, the location of each binding agent and the bead to which it is affixed is known prior to step 1(b). EX1100, ¶¶225-228.

11. Claim 29

Cantor discloses “the binding agent is immobilized.” For example, Cantor explains that its binding agents may be affixed to beads by covalent bonding, with

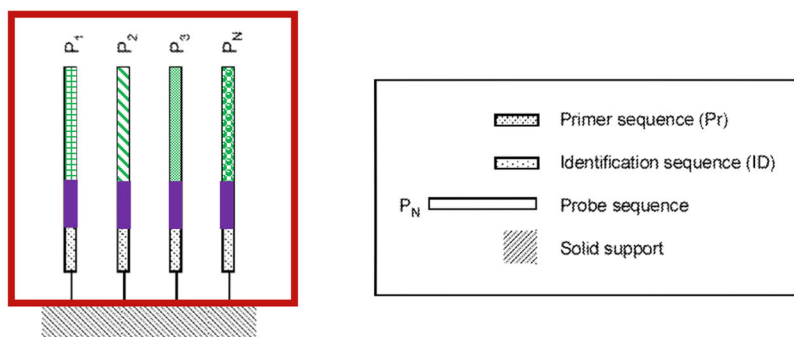
or without a linker, thereby disclosing both direct and indirect immobilization onto a bead. EX1031, 11-12. EX1100, ¶¶229-231.

B. Ground 2: Obviousness over Cantor in view of Armani

Ground 2 incorporates the analysis of Ground 1. Armani provides further evidence of a POSA's motivation and reasonable expectation of success in using Cantor's device and methods to determine the spatial location of biological molecules, including mRNA, in a tissue sample. Moreover, Armani provides additional information rendering obvious the step of contacting the plurality of beads with a tissue section such that the binding agents interact with nucleic acids of the tissue sample. EX1100, ¶¶232-235.

1. Claim 1

As explained above, Cantor describes a device and methods 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 plurality of beads with the claimed “**binding agents**” in the form of nucleic acid constructs that include a “**probe sequence**” that is “**configured to interact**” with a target nucleic acid, as well as a “**coding identifier**” or “**identification sequence**” that may be used to identify the specific bead, and thus location, at which a particular binding interaction occurred. EX1100, ¶236.



Cantor further explains that its “array can be arranged on any substrate,” 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. EX1100, ¶237.

Thus, Cantor discloses providing a plurality of beads containing the claimed binding agents by arranging the beads in a multiple well plate. This arrangement is expressly encompassed by the challenged claims. *See* EX1001, cl. 3 (“the plurality of beads is provided in wells on the substrate surface.”). This arrangement of binding agents in wells also motivates combining the teachings of Cantor with the teachings of Armani. EX1100, ¶237.

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 then 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. EX1100, ¶238.

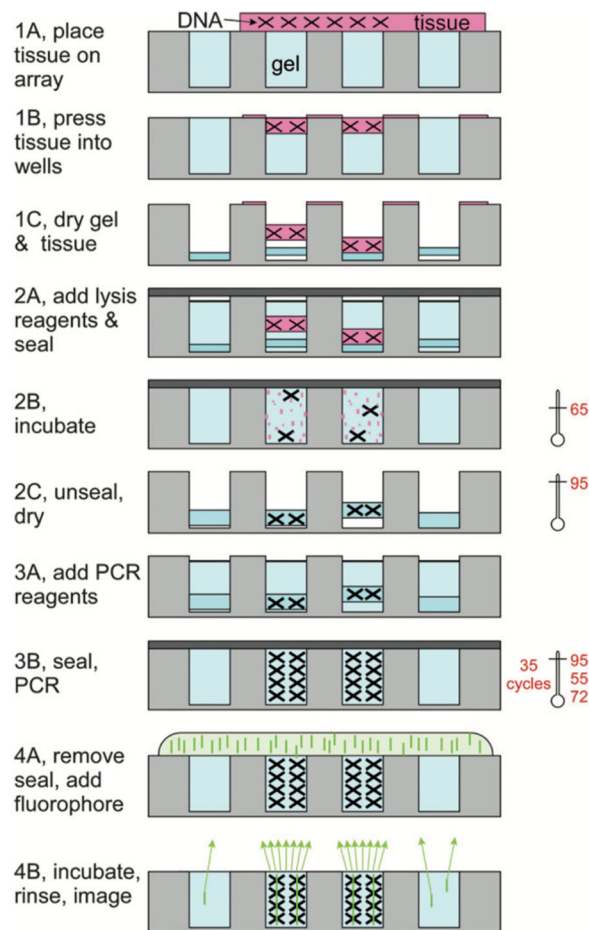


Fig. 2 Experimental protocol used to demonstrate the 2D-PCR concept. Tissue is transferred into wells, isolated, and lysed to extract the genomic DNA (represented by “x”s). PCR is performed to amplify the target DNA, and the targets are visualized with fluorescent dye (indicated by vertical lines “|”).

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 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...”). EX1100, ¶239.

A POSA would then have proceeded with the nucleic acid extension, amplification, and detection by sequencing steps disclosed by Cantor. *Id.*, ¶240.

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. *See* Ground 1, claim 15. EX1100, ¶241.

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 expressly motivates this mental step, and further provides additional specific implementations of how to successfully prepare and process effectively a tissue sample for localized nucleic acid detection. EX1100, ¶242.

2. Claims 2-3

Cantor in view of Armani renders obvious “the plurality of beads is provided in wells on the substrate surface,” as claimed. As set forth above, the combination of Cantor and Armani renders obvious providing Cantor's barcoded beads in in wells on the substrate surface. EX1100, ¶¶243-246.

3. Claims 4-7, 11-15, 17, 19, 27-29

The additional features of these claims are disclosed by the combination of Cantor and Armani for the same reasons they are disclosed in Cantor alone. EX1100, ¶247.

C. Ground 3: Anticipation by Frisen

1. Priority Date

The priority applications leading to Chee-022 are tabulated below:

'022 Patent Priority Documents	Filing Date	Exhibit
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

The challenged claims are not entitled to a priority date earlier than the February 14, 2019 filing date of the '235 Application because the claimed invention is not disclosed "in the manner provided by §112(a)" in earlier applications. 35 U.S.C. §§119(e), 120. As explained below, the '616 Application, which is the first step back in the priority chain, does not support the challenged claims. Since "there has to be a continuous chain of copending applications each of which satisfies the requirements of §112 with respect to the subject matter claimed," this break in the

priority chain precludes PO from relying on any earlier date.⁴ *Holmer v. Harari*, 681 F.3d 1351, 1355 (quotation omitted). EX1100, ¶286.

As set forth below, the Chee-022 priority applications at most contain ambiguous disclosures of isolated aspects of the claims, with no explanation of how those disclosures are linked to other aspects of the specification, let alone in the way the elements are arranged in the claims. Because “[a] patent owner cannot show written description support by picking and choosing claim elements from different embodiments that are never linked together in the specification,” Chee-022 is not entitled to the filing dates of its priority applications. *Flash-Control, LLC v. Intel Corp.*, No. 2020-2141, 2021 WL 2944592, at *4 (Fed. Cir. July 14, 2021); *Novozymes A/S v. DuPont Nutrition Biosciences APS*, 723 F.3d 1336, 1349 (Fed. Cir. 2013) (“pointing to an ‘amalgam of disclosures’ from which an artisan could have created the claimed invention does not satisfy this requirement.”).

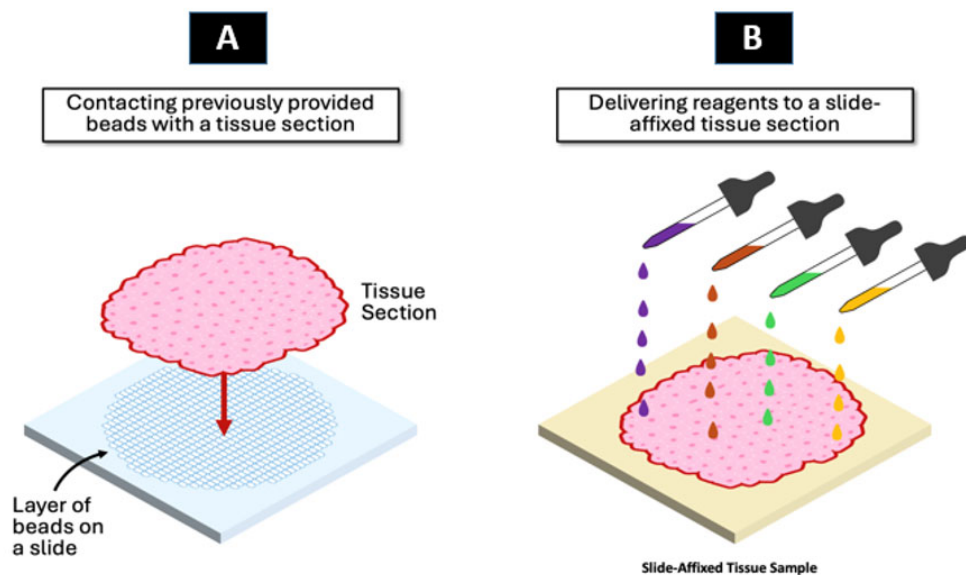
There are at least three key elements of independent claim 1 that are not supported by the ’661 application, and which are not further limited by the challenged dependent claims. Specifically, there is no disclosure of each aspect of step 1(b): “[a]contacting the plurality of beads with the tissue sample [b] such that

⁴ Curio does not concede support in the ’235 Application for the challenged claims.

the binding agent interacts with the biological molecule.” Nor is there disclosure of [c] “providing a plurality of beads, wherein the plurality of beads...(i) comprises a coding identifier having a nucleic acid sequence” as required by step 1(a).

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.

The claims as drafted and as read by PO to assert infringement require first providing bead array, where each bead is functionalized with the claimed binding agents, before contacting such an array with a tissue sample. *See* Figure A, *supra*. The '661 Application discloses a different set of methods, which uniformly require providing a tissue sample affixed to a solid support, and then delivering reagents, such as binding agents, to the support-affixed tissue sample according to a spatial pattern. *See* Figure B, *supra*. EX1100 ¶¶251-252.



As Dr. Puglisi explains, the differences between these methodologies are not trivial. For example, consistent with the '661 Application's focus on fluid handling techniques for delivery of probes, it repeatedly describes the delivery of probes to tissue in solution. As a result, Chee-022 repeatedly emphasizes a step of "washing" or separating encoded probes that did not bind to a target away from the sample, which is important for eliminating "noise" from the sequencing readout. EX1011, Figs. 1-2, [0028]. By contrast, the claims purport to encompass providing an array of solid phase probes to which a tissue sample is subsequently delivered. 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. Here, 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 the probes are covalently bound to the beads. 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. EX1100, ¶¶252, 79.

Moreover, Chee-022 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 degrees of multiplexing." *See* EX1001, 1:50-56. To the extent that the disclosures of Chee-022 (but not its claims) purport to address these alleged deficiencies, it is through the

delivery of reagents from above the tissue sample. EX1001, 16:3-5 (“Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents *onto* the biological sample.”). EX1100, ¶253.

A POSA reviewing the ’661 Application 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, 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. EX1100, ¶254.

The ’661 Application’s Specification and Drawings are replete with statements confirming that its “invention” requires (i) providing a sample affixed to a support before (ii) delivering probes to the sample. These include:

- **SUMMARY OF THE INVENTION:** “The 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, [0007]. The other embodiments disclosed in this section similarly involve “providing a sample affixed to a support” and “delivering encoded probes.” *E.g., id.*, [00015]. EX1100, ¶¶255-257.

- **FIGURES:** The figures require (i) providing biological samples affixed to a support, and (ii) delivering encoded probes to the biological sample. EX1011, Figs. 1-2. EX1100, ¶¶258-261.
- **“The Invention in General”:** “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....*” EX1011, [00023]-[00024]. EX1100, ¶263.
- **“Assays”:** “The assay portion of the assay systems of *the present invention* comprise the following general steps: *delivering probes and encoding agents*” to samples, where “[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....” EX1011, [00035]. EX1100, ¶265.

Further confirming that 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 “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]. Such techniques are disclosed to include, for example, ink jet printing and photolithographic processes. *Id.*, [00056], [00064]-

[00065]; *see also* [00054] (“The reagent delivery system *of the invention* includes instrumentation that allows the *delivery* of reagents to discrete portions of the biological sample.”). 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, such as an array of functionalized beads, as claimed. EX1100, ¶¶264, 266-267.

The only disclosures in the '661 Application pertaining to beads (which, as discussed further below, are at most incorporated from the '124 Provisional) give no indication that they refer to providing beads in an array as claimed, as opposed to depositing beads comprising a binding agent to a tissue sample that was previously affixed to a solid support using the disclosed “reagent delivery systems.” EX1009, [00018]. Indeed, the only time the '124 Provisional discloses a “bead array” is to refer to them as part of a laundry list of well-known prior art genotyping techniques. EX1009, [0052]. A POSA would not have understood this single reference to bead arrays as one of many 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. EX1100, ¶¶267-270.

Such references “in the context of this patent would be a highly elliptical, cryptic way to communicate a second method of” contacting an existing bead array with a tissue sample. *Quake*, 928 F.3d at 1376. Even if these disclosures “are (at most) faint ‘blaze marks’” for performing spatial analysis on bead arrays – which Petitioner does not concede – “the rest of the specification marks a clear trail to” spatial delivery of reagents to an already affixed sample, and thus does not convey possession of the claimed methods. *Id.* See also *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473, 1479 (Fed. Cir. 1998) (finding failure of written description for claims directed to furniture with controls off the console where “the original disclosure clearly identifies the console as the only possible location for the controls”); *Rivera v. Int’l Trade Comm’n*, 857 F.3d 1315, 1321 (Fed. Cir. 2017) (The “question is whether a pod adaptor assembly intended to allow compatibility between distinct brewing systems, also supports an undisclosed configuration that eliminates a fundamental component of one of those systems (i.e., the ‘pod’) through integration. It does not.”); *ICU Med., Inc. v. Alaris Med. Sys., Inc.*, 558 F.3d 1368, 1378 (Fed. Cir. 2009) (The “specification describes only medical valves with spikes... [A POSA] would not understand the inventor...to have invented a spikeless medical valve”).

b) The '661 Application does not disclose contacting a plurality of beads with a tissue section “such that the binding agent interacts with the biological molecule.”

The '661 Application criticizes the tissue preparation procedures disclosed in Armani:

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

EX1011, [0004]. Yet the subsequent disclosures of the '661 Application contain no teaching on how to prepare the tissue section “such that the binding agent interacts with the biological molecule” when a tissue section is contacted with a plurality of beads. EX1100, ¶271.

In light of this criticism, a POSA would understand that the inventor did not envision or possess providing access to the nucleic acids in a tissue sample through physical transference and lysis as in Armani. But the '661 Application does not disclose any alternative techniques for “contacting” an array “such that the binding agent interacts with the biological molecule.” Thus, a POSA would have understood the '661 Application as having disclosed at most a hope or a wish to obtain access to tissue samples without relying on transference and lysis techniques as disclosed in Armani. While, as discussed above, techniques for local release of nucleic acids

or other biological molecules from tissue samples for capture on probes in bead or other arrays were known, the '661 Application does not point to them. EX1100, ¶¶272-274. Even if it did, pointing to references that render the claimed subject matter obvious is insufficient to establish possession of the claimed invention in the priority documents. *See, e.g., Novozymes A/S*, 723 F.3d at 1349.

c) The '661 Application does not disclose “providing a plurality of beads, wherein the plurality of beads...(i) comprises a coding identifier having a nucleic acid sequence.”

The '661 Application itself does not refer to or mention “beads.” However, it purports to incorporate by reference the '124 Provisional. EX1011, [0001]. Evaluation of the '124 Provisional confirms that, although it makes a handful of references to beads, it fails to disclose beads comprising nucleic acid binding agents having a nucleic acid coding identifier. EX1100, ¶¶275-285.

The '124 Provisional references beads, for example, in describing embodiments in which “binding agents are immobilized onto beads....that are then provided in known locations on the substrate surface.” EX1009, [0017]-[0018], [0021]. EX1100, ¶277. The 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 '022 Patent do not expressly recite providing beads at known locations (notwithstanding the written description of the

invention); instead, as claimed in the '022 Patent, it is the “coding identifier” that identifies the location where the bead-bound binding agent interacted with the biological molecule. 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 coding identifiers as claimed in the '022 Patent. EX1100, ¶278.

Paragraph [0017] 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.” Paragraph [0018] specifies that “the binding agents are nucleic acids,” but does not disclose to a POSA a bead comprising a nucleic acid binding agent *plus* a nucleic coding identifier. EX1100, ¶¶279-280, ¶282.

Additionally, a POSA would not understand the references in the Provisional to nucleic acid “binding agents” affixed to beads to necessarily encompass including “coding identifiers” on such beads. Indeed, the specification repeatedly refers to “binding agents” and “coding identifiers” as separate structures. *See, e.g.*, EX1009,

[0007] (“This encoding scheme uses **both** molecule specific binding agents **and** coding identifiers....”). EX1100, ¶281.

These disclosures do not demonstrate possession of beads comprising nucleic acid binding agents **and** coding identifiers. To the extent PO points to separate disclosures of coding identifiers in other aspects of the priority documents as purporting to support the claims, such unlinked disclosures are insufficient to establish written description of the claimed combination. *Novozymes A/S* 723 F.3d at 1349 (“pointing to an ‘amalgam of disclosures’ from which an artisan could have created the claimed invention does not satisfy this requirement.”).

2. Claim 1

Frisen anticipates claim 1. Frisen discloses the claimed “method for determining a spatial location of a biological molecule of a tissue sample,” which it describes as “a method for localized detection of nucleic acid in a tissue sample.” EX1020, 7-8, cls. 1, 40, 42. EX1100, ¶¶287-288.

Frisen further explains that its methods involve the implementation “of standard arrays [that] are commercially available and both the number and size of the features may be varied.” EX1020, 15:21-24. Frisen explains that the arrays suitable for its methods include “[c]ommercial arrays” such as “e.g. an Illumina bead array.” *Id.*, 15:30-33. Thus, Frisen discloses providing its array of capture probes or binding agents by providing a plurality of beads. EX1100, ¶¶289-290.

a) “providing a plurality of beads....”

Frisen discloses that its array surface, such as a plurality of beads, comprises a plurality of binding agents. These binding agents “(i) comprise[] a coding identifier having a nucleic acid sequence...wherein the coding identifier corresponds to a location at which the binding agent interacts with a biological molecule of a tissue sample.” Specifically, Frisen discloses “capture probes” immobilized on its solid supports, such as beads, “wherein each species of said capture probe comprises a nucleic acid molecule from 5’ to 3’ (i) a positional domain that corresponds to the position of the capture probe on the array.” EX1020, 7:16-21. EX1100, ¶¶291-292.

Frisen further discloses that “each species” of capture probe comprises “(ii) a capture domain.” *Id.*, 7:24. Frisen discloses that the “capture domain” may be poly-T, EX1020, 18:24-26, or that “[t]he capture domain may be capable of binding selectively to a desired subtype or subset of nucleic acid,” such as “a particular mRNA species e.g. corresponding to a particular gene or group of genes.” *Id.*, 19:15-24. EX1100, ¶¶293-294.

b) “contacting the plurality of beads with a tissue sample....”

Frisen discloses “(b) contacting said array with a tissue sample such that the position of a capture probe on the array may be correlated with a position in the

tissue sample and allowing nucleic acid of the tissue sample to hybridise to the capture domain in said capture probes.” EX1020, 7. EX1100, ¶¶295-296.

c) “using the coding identifier to identify the location....”

After step (b), Frisen discloses “(c) generating DNA molecules from the captured nucleic acid molecules using said capture probes as extension or ligation primers, wherein said extended or ligated DNA molecules are tagged by virtue of their positional domain,” “(e) releasing at least part of the tagged DNA molecules and/or their complements or amplicons from the surface of the array, wherein part includes the positional domain or a complement thereof;” and “(f) directly or indirectly analyzing the sequence of the released DNA molecules.” EX1020., 7:29-8:5. For example, when the released DNA is sequenced this “results in a transcriptome with exact positional information” because each positional domain “correlated with a position in the tissue sample.” *Id.*, 6:2-16; Figure 1. EX1100, ¶297.

Thus, Frisen anticipates claim 1. *Id.*, ¶298.

3. Claim 2

Frisen discloses “the plurality of beads is provided on a substrate surface.” Frisen explains that “[i]n standard microarrays, the probes are attached to a solid surface or substrate,” and that “[t]he substrate typically is a glass, plastic or silicon

chip or slide, although other microarray platforms are known, e.g., microscopic beads.” EX1020, 13:24-27. Frisen expressly states that “an Illumina bead array” “may also be used as the array surface in the context of the present invention.” *Id.*, 15:30-33. A POSA would understand that an Illumina bead array provides beads on a substrate surface. *See* EX1039, 58 (“BeadArray technology is based on the random self-assembly of a bead pool onto a patterned substrate.”). EX1100, ¶¶299-301.

4. Claims 4-6

Frisen discloses “the biological molecule is a nucleic acid,” “the binding agent of the plurality of binding agents comprises a nucleic acid,” and “the binding agent interacts with the biological molecule via hybridization.” Specifically, Frisen discloses nucleic acid binding agents that interact with target biological molecules, such as mRNA, by hybridization. *See* EX1020, 7-8; cls. 1, 40, 42. EX1100, ¶¶302-306.

5. Claim 7

Frisen discloses “the binding agent and coding identifier are provided as a single molecule.” Frisen discloses that “each species of said capture probe comprises a nucleic acid molecule with 5’ to 3’: (i) a positional domain that corresponds to the position of the capture probe on the array, and (ii) a capture domain.” EX1020, 7:20-24. EX1100, ¶¶307-309.

6. Claims 11-14

Frisen discloses the claimed additional binding agents comprising an additional coding identifier, wherein the additional binding agents interact with another biological molecule of the tissue sample at a different location, as recited in claims 11-14. EX1100, ¶¶310-313.

Frisen explains that “each feature or position of the array carries a capture probe of a single species (specifically each feature or position carries a capture probe which has an identical positional tag, i.e. there is a single positional domain at each feature or position).” EX1020, 20:17-20. “Each species has a different positional domain which identifies the species.” *Id.*, 20:20-21. Thus, the coding identifiers at different locations are different depending on the location. EX1100, ¶314.

Moreover, Frisen discloses that “the nucleotide sequence of the probes at each feature is different, distinct or distinguishable from the probes immobilized at every other feature,” and that “[p]referably each feature comprises a different species of probe.” EX1020, 20:29-31. Thus, Frisen discloses binding agents at different locations that are configured to interact with different biological molecules, as claimed. EX1100, ¶315.

7. Claim 15

Frisen discloses “step [1](c) comprises determining the spatial locations” of nucleic acids simultaneously. Specifically, Frisen discloses that “the methods of the

present invention may be used to determine simultaneously the expression of one or more genes at one or more positions within a tissue sample.” EX1020, 11:22-33; 8:14-15. EX1100, ¶¶317-319.

8. Claim 17

Frisen discloses “determining the relative amount of the biological molecule at the location.” Frisen explains that “[t]he global gene expression information obtainable from the methods of the invention also allows co-expression information and *quantitative estimates of transcript abundance*.” EX1020, 6:18-21. Similarly, Frisen explains that “the methods of the present invention make it possible to detect and/or quantify the spatial expression of all genes in [a] single tissue sample.” *Id.*, 8:11-17. In such analyses, “the expression of every gene can be quantified for each position or feature on the array,” thereby determining the relative amounts of target nucleic acids at each location. *Id.*, 8:9-11. EX1100, ¶¶320-322.

9. Claim 19

Frisen discloses “sequencing all or a portion...of the coding identifier.” Frisen’s methods involve sequencing each positional domain, which is “correlated with a position in the tissue sample.” EX1020, 6:2-16; Figure 1. “The step of sequence analysis may identify the positional domain and hence allow the analysed molecule to be localized to a position in the tissue sample.” *Id.*, 45:20-26. EX1100, ¶¶323-325.

10. Claim 27

Frisen discloses “analyzing gene expression in the tissue sample.” Frisen explains that “[t]he global gene expression information obtainable from the methods of the invention also allows co-expression information and quantitative estimates of transcript abundance.” EX1020, 6:18-21. In such analyses, “the expression of every gene can be quantified for each position or feature on the array.” *Id.*, 8:9-11. EX1100, ¶¶326-328.

11. Claim 28

Frisen discloses “the location of the binding agent is known prior to step [1](b).” Frisen explains that “each feature (distinct position) of the array comprises a spot of a species of nucleic acid probe, wherein the positional domain at each feature is unique.” EX1020, 20:5-7. A POSA would at once envisage determining the location of each binding agent based on the location of the binding domain before applying a tissue sample. Frisen expressly discloses doing so using fluorescence to decode the positional domains before applying a tissue sample. *Id.*, 76:3-9. EX1100, ¶¶329-332.

12. Claim 29

Frisen discloses “the binding agent is immobilized...onto a bead.” Frisen explains that “[t]he covalent linkage used to couple a nucleic acid probe on an array substrate may be viewed as both a direct and indirect linkage, in that although the

probe is attached by a ‘direct’ covalent bond there may be a chemical moiety or linker separating the ‘first’ nucleotide of the nucleic acid probe from the, e.g, glass or silicon, substrate, i.e. an indirect linkage.” *Id.*, 16:10-17. EX1100, ¶¶333-335.

XI. SECONDARY CONSIDERATIONS

Petitioner is not aware of any secondary considerations of non-obviousness, and reserves the right to respond to any such issues raised by PO.

Even if Chee-022 were entitled to its provisional or non-provisional filing date, the contemporaneous work of Frisen, which was filed on April 13, 2011, is “strong evidence of what constitutes the level of ordinary skill in the art.” *Geo M. Martin Co. v. Alliance Mach. Sys. Int’l LLC*, 618 F.3d 1294, 1306 (Fed. Cir. 2010). As illustrated in Ground 3, Frisen discloses all elements of the claims, and thus developed the claimed subject matter totally independent of the original applicant. This simultaneous invention and development is strong evidence of obviousness. *Id.* at 1305 (“Independently made, simultaneous inventions, made ‘within a comparatively short space of time,’ are persuasive evidence that the claimed apparatus was the product only of ordinary mechanical or engineering skill.”).

XII. NO BASIS FOR DISCRETIONARY DENIAL

A. 314(a) Does Not Warrant Denial

Apple Inc. v. Fintiv, Inc., IPR2020-00019, Paper 11 at 6 (PTAB March 20, 2020) does not support discretionary denial. Here, the Chee-022 district court

litigation is still in the early stages, and the parties have not invested significantly in litigation. Trial is scheduled for May 2026. EX1043. If instituted, the Board's FWD would be expected in March 2026, well in advance of trial. Moreover, many key case events, such as claim construction, would not occur until after the Board's anticipated Institution Decision. *Id. See* EX1040, 77 (10x (as Petitioner) asserting that "*Fintiv* simply does not apply" even in IPR proceeding where trial was scheduled "approximately two months before a Final Written Decision would be reached").

B. 325(d) Does Not Warrant Denial

This Petition is not based on "the same or substantially the same arguments previously presented to the Office." *Advanced Bionics, LLC v. MED-EL Elektromedizinische Gerate GmbH*, IPR2019-01469, Paper 6 at 7. *See* Section V. Thus, denial under 325(d) is inappropriate.

Grounds 1-2: Cantor was neither considered during prosecution nor cited in an IDS. *See Yita LLC v. MacNeil IP LLC*, IPR2023-00172, Paper No. 12 at 47 (when reference not previously presented, "the first prong of the *Advanced Bionics* framework is not satisfied and we need not consider the second prong.").

Ground 3: The prosecution history does not indicate that the Examiner critically evaluated Chee-022's claims for section 112 support in the priority documents or otherwise meaningfully evaluated the claims against Frisen. Thus, the

Office materially erred, because had the Examiner analyzed the lack of support for the claims filed on February 14, 2019 in the priority documents, they would have then appreciated the relevance of Frisen as anticipatory prior art.

Moreover, the Office did not have the benefit of the detailed expert evidence submitted with this Petition, which shows why the claims are not supported and how Cantor, Frisen, and Armani confirm their unpatentability. *See* EX1100; *see also Mylan Pharmaceuticals Inc. v. Merck Sharp & Dohme Corp.*, IPR2020-00040, Paper 21 at 20 (PTAB May 12, 2020)..

XIII. CONCLUSION

Petitioner respectfully submits that the Board should institute IPR and cancel the challenged claims.

Dated: September 10, 2024

Respectfully submitted,

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CERTIFICATE OF COMPLIANCE WITH 37 C.F.R. § 42.24

I hereby certify, pursuant to 37 C.F.R. § 42.24, that the petition contains 13,866 words, excluding the words in the table of contents, exhibit list, claim listing, mandatory notices under § 42.8, this certificate of word count, and the certificate of service. The word count is based on the word count of the Microsoft Word program used to prepare the petition.

Dated: September 10, 2024

Respectfully submitted,

/Andrew E. Krause/
Andrew E. Krause

CERTIFICATE OF SERVICE

I hereby certify, pursuant to 37 C.F.R. sections 42.6 and 42.105, that a complete copy of the Petition for *Inter Partes* Review of U.S. Patent No. 10,480,022 and Exhibits are being served via Express Mail on September 10, 2024, the same day as the filing of the above-identified documents in the United States Patent and Trademark Office/Patent Trial and Appeal Board, upon the patent prosecution counsel of record for the Patent Owner:

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Courtesy copies were also sent via ShareSite to Patent Owner's counsel of record in the related district court litigation at the following addresses:

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