FISH.
FISH & RICHARDSON

Street Address
Fish & Richardson P.C.
One Marina Park Drive
Boston, MA 02210-1878

Mail Address
P.O. Box 1022
Minneapolis, MN 55440-1022

617 542 5070 main
877 769 7945 fax

February 14, 2019

Attorney Docket No.: 47706-0004003

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Presented for filing is a new continuation patent application for prioritized examination:

Inventor(s): MARK S. CHEE

Title: SPATIALLY ENCODED BIOLOGICAL ASSAYS

Enclosed are the following papers, including those required to receive a filing date under 37 C.F.R. § 1.53(b):

	Pages
Specification	34
Claims	1
Abstract	1
Declaration	1
Drawing(s)	5

Enclosures:

— Application Data Sheet, 8 pages.
— Certification and Request for Prioritized Examination (Track I), 2 pages

The applicant claims small entity status under 37 C.F.R. § 1.27.

Curio EX1010-p. 1
Curio v Prognosys
IPR2024-01289

Basic Filing Fee			$75
Search Fee			$330
Examination Fee			$380
Publication fee			$0
Track I processing fee			$70
Track I prioritized examination fee			$2000
Total Claims 1	over 20	**0 x $50**	$0
Independent Claims 1	over 3	**0 x $230**	$0
Fee for Multiple Dependent claims			$0
Application size fee for each 50 pages over 100 Total Sheets: 41 x .75 - 100/50 = 0 x			$0
Total Filing fee			$2855

Please apply any necessary charges or credits to Deposit Account 06-1050, referencing the attorney docket number shown above.

I hereby authorize the USPTO to communicate in response to email communications from the undersigned and practitioners under Customer No. 159367 in accordance with 37 C.F.R. § 1.33 and 37 C.F.R. § 1.34 concerning any subject matter of this application via email. I understand that a copy of these communications will be made of record in the application file. (MPEP 502.03).

If this application is considered to be incomplete, or if a telephone conference would otherwise be helpful, call the undersigned at (617) 368-2124.

Send all correspondence to:

159367
Customer Number

Respectfully submitted,

/Peng Lin/

Peng Lin
Registration No. 74,999

Enclosures

PCL/jaw
61846718.doc

SPATIALLY ENCODED BIOLOGICAL ASSAYS

Abstract of the Disclosure

The present invention provides assays and assay systems for use in spatially encoded biological assays. The invention provides an assay system comprising an assay 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.

Curio EX1010-p. 4
Curio v Prognosys
IPR2024-01289

I claim:

1. An assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the assay system performs the following steps:

providing a sample affixed to a support;

delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered;

allowing the encoded probes to interact with the biological targets;

separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets;

determining all or a portion of a sequence of the encoded probes, and

associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

Curio EX1010-p. 5
Curio v Prognosys
IPR2024-01289

SPATIALLY ENCODED BIOLOGICAL ASSAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Patent Application No. 61/321,124, filed April 5, 2010 and is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention relates to assays of biological molecules, and more particularly to assays for determining spatial distributions of a large number of biological molecules in a solid sample simultaneously.

BACKGROUND OF THE INVENTION

[0003] In the following discussion certain articles and methods will be described for background and introductory purposes. Nothing contained herein is to be construed as an "admission" of prior art. Applicant expressly reserves the right to demonstrate, where appropriate, that the articles and methods referenced herein do not constitute prior art under the applicable statutory provisions.

[0004] Comprehensive gene expression analysis and protein analysis have been useful tools in understanding mechanisms of biology. Use of these tools has allowed the identification of genes and proteins involved in development and in various diseases such as cancer and autoimmune disease. Conventional 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. Other technologies that have enabled the quantitative analysis of many RNA sequences per sample include microarrays (see Shi, et al., Nature Biotechnology, 24(9):1151-61 (2006); and Slonim and Yanai, Plos Computational Biology, 5(10):e1000543 (2009)); serial analysis of gene expression (SAGE) (see Velculescu, et al.,

Curio EX1010-p. 6
Curio v Prognosys
IPR2024-01289

Science, 270(5235):484-87 (1995)), high-throughput implementations of qPCR (see Spurgeon, et al., Plos ONE, 3(2):e1662 (2008)) and *in situ* PCR (see Nuovo, Genome Res., 4:151-67 (1995)). As useful as these methods are, however, they do not enable simultaneous measurement of the expression of many genes or the presence and/or activity of multiple proteins at many spatial locations in a sample. Laser capture microdissection has permitted the analysis of many genes at a small number of locations, but it is very expensive, laborious, and does not scale well. 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.

[0005] At present, no practical method exists to analyze at high resolution the spatial expression patterns of large numbers of genes, proteins, or other biologically active molecules simultaneously. There is thus a need for reproducible, high-resolution spatial maps of biological molecules in tissues. The present invention addresses this need.

SUMMARY OF THE INVENTION

[0006] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the following written Detailed Description including those aspects illustrated in the accompanying drawings and defined in the appended claims.

[0007] The invention encompasses assay systems that provide high-resolution spatial maps of biological activity in tissues. The assay system comprises an assay capable of high levels of multiplexing where encoded probes 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. In short, the present invention provides the ability to look at many biological

targets in many locations, providing the resolution of in situ hybridization with the highly-parallel data analysis of sequencing.

[0008] Thus, in some embodiments, the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered; allowing the encoded probes to interact with the biological targets; separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets; determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

[0009] In particular aspects of the invention the biological targets comprise nucleic acids and the encoded probes are oligonucleotides, and in some aspects, there are two encoded probes for each of the multiple nucleic acid targets. In some aspects, the multiple biological targets comprise proteins, the probe regions of the encoding probes are proteins and the coding tags comprise oligonucleotides. In some aspects the multiple biological targets comprise enzymes. In some aspects the probe regions of the encoded probes comprise antibodies, aptamers or small molecules.

[00010] Some aspects of the assay system further comprise an amplification step between the separating step and the determining step. In some aspects, the determining step is performed by nucleic acid sequencing, and in preferred aspects, the sequencing is high-throughput digital nucleic acid sequencing.

[00011] In some aspects of the invention, the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 20, in some aspects product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 50, in some aspects the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 75, 100, 150, 500, 750, 1,000, 5,000, 10,000, 25,000, 50,000, 100,000, 500,000, or 1,000,000 or more. In other

aspects, the sequence of at least fifty thousand encoding probes are determined in parallel, in other aspects the sequence of at least one hundred thousand encoding probes are determined in parallel, in some aspects the sequence of at least five hundred thousand encoding probes are determined in parallel, and in some aspects the sequence of at least one million, ten million, one hundred million, one billion, ten billion, one hundred billion or more encoding probes are determined in parallel.

[00012] In some aspects, the known spatial pattern is determined by histological features of the sample. Also in some aspects, software programmed hardware performs at least two steps of the delivering step, the separation step, the determining step and the associating step.

[00013] In some aspects, the probe regions of the encoded probes are proteins and the separating step is accomplished by encoded probes that interact with the biological targets being captured by an affinity capture agent. In some aspects the probe regions of the encoding probes are nucleic acids and the separating step is accomplished by a washing of the sample.

[00014] In other embodiments there is provided an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering oligonucleotide probes for multiple nucleic acid targets to the multiple sites in the sample in a known spatial pattern; allowing the oligonucleotide probes to hybridize with the nucleic acid targets; washing unhybridized encoded oligonucleotide probes from the sample; delivering one or more encoding agents to locations of the multiple sites in the sample according to a known spatial pattern, where the combination of encoding agents delivered to each site is different; coupling the encoding agents and the oligonucleotide probes to form encoded probes; determining all or a portion of a sequence of the encoded probes using high-throughput sequencing, and associating the abundance or activity or both of multiple biological targets to the locations of multiple sites in the sample.

[00015] Other embodiments of the invention provide an assay system to determine spatial patterns of abundance or activity or both of multiple protein targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed

Curio EX1010-p. 9
Curio v Prognosys
IPR2024-01289

to a support; delivering encoded probes for the multiple protein targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a protein probe region that may interact with the protein targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and the protein probe region of the encoding probe of which the coding tag is part; allowing the encoded probes to interact with the protein targets; separating encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets; determining all or a portion of a sequence of the encoded probes by high throughput sequencing, and associating the abundance or activity or both of the multiple protein targets to the locations of the multiple sites in the sample.

[00016] Other embodiments provide an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and identifies the biological target; allowing the encoded probes to interact with the biological targets; determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

[00017] The assay system of the invention can utilize various detection mechanisms, based on the molecules to be detected and the reagents needed for such detection system. Exemplary methods that can be used with the assay systems of the invention are described in more detail below.

DESCRIPTION OF THE FIGURES

[0001] Figure 1 provides a simplified overview of the assay system of the present invention.

[0002] Figure 2 provides a simplified overview of one embodiment of the assay system of the present invention for detecting nucleic acids.

Curio EX1010-p. 10
Curio v Prognosys
IPR2024-01289

[0003] Figure 3 is a representational depiction of one embodiment of the assay overviewed in Figure 2.

[0004] Figure 4 illustrates a general mechanism for one embodiment of a combinatorial encoding scheme of the assay systems of the invention.

[0005] Figure 5 provides a simplified, specific example of the embodiment of a combinatorial encoding scheme shown in Figure 4.

DEFINITIONS

[0006] The terms used herein are intended to have the plain and ordinary meaning as understood by those of ordinary skill in the art. The following definitions are intended to aid the reader in understanding the present invention, but are not intended to vary or otherwise limit the meaning of such terms unless specifically indicated.

[0007] The term "antibody" as used herein is intended to refer to an entire immunoglobulin or antibody or any functional fragment of an immunoglobulin molecule which is capable of specific binding to an antigen (antibodies and antigens are "binding partners" as defined herein). "Antibody" as used herein is meant to include the entire antibody as well as any antibody fragments capable of binding the antigen or antigenic fragment of interest. Examples of such peptides include complete antibody molecules, antibody fragments, such as Fab, F(ab')2, CDRS, VL, VH, and any other portion of an antibody which is capable of specifically binding to an antigen. Antibodies for assays of the invention are immunoreactive or immunospecific for, and therefore specifically and selectively bind to, proteins either detected (*i.e.,* biological targets) or used for detection (*i.e.*, probes) in the assays of the invention.

[0008] The term "binding agent" as used herein refers to any agent that specifically binds to a biological molecule of interest.

[0009] "Complementary" or "substantially complementary" refers to the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, such as, for instance, between the two strands of a double-stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single-stranded nucleic acid. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two

Curio EX1010-p. 11
Curio v Prognosys
IPR2024-01289

single-stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the other strand, usually at least about 90% to about 95%, and even about 98% to about 100%.

[00010] "Hybridization" refers to the process in which two single-stranded polynucleotides bind non-covalently to form a stable double-stranded polynucleotide. The resulting (usually) double-stranded polynucleotide is a "hybrid" or "duplex." "Hybridization conditions" will typically include salt concentrations of approximately less than 1M, often less than about 500 mM and may be less than about 200 mM. A "hybridization buffer" is a buffered salt solution such as 5% SSPE, or other such buffers known in the art. Hybridization temperatures can be as low as 5°C, but are typically greater than 22°C, and more typically greater than about 30°C, and typically in excess of 37°C. Hybridizations are often performed under stringent conditions, *i.e.*, conditions under which a primer will hybridize to its target subsequence but will not hybridize to the other, non-complementary sequences. Stringent conditions are sequence-dependent and are different in different circumstances. For example, longer fragments may require higher hybridization temperatures for specific hybridization than short fragments. As other factors may affect the stringency of hybridization, including base composition and length of the complementary strands, presence of organic solvents, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one parameter alone. Generally stringent conditions are selected to be about 5°C lower than the T_m for the specific sequence at a defined ionic strength and pH. Exemplary stringent conditions include a salt concentration of at least 0.01M to no more than 1M sodium ion concentration (or other salt) at a pH of about 7.0 to about 8.3 and a temperature of at least 25°C. For example, conditions of 5xSSPE (750 mM NaCl, 50 mM sodium phosphate, 5 mM EDTA at pH 7.4) and a temperature of approximately 30°C are suitable for allele-specific hybridizations, though a suitable temperature depends on the length and/or GC content of the region hybridized.

[00011] "Ligation" means to form a covalent bond or linkage between the termini of two or more nucleic acids, *e.g.*, oligonucleotides and/or polynucleotides, in a template-driven reaction. The nature of the bond or linkage may vary widely and the ligation may be

carried out enzymatically or chemically. As used herein, ligations are usually carried out enzymatically to form a phosphodiester linkage between a 5' carbon terminal nucleotide of one oligonucleotide with a 3' carbon of another nucleotide.

[00012] "Nucleic acid", "oligonucleotide", "oligo" or grammatical equivalents used herein refers generally to at least two nucleotides covalently linked together. A nucleic acid generally will contain phosphodiester bonds, although in some cases nucleic acid analogs may be included that have alternative backbones such as phosphoramidite, phosphorodithioate, or methylphophoroamidite linkages; or peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with bicyclic structures including locked nucleic acids, positive backbones, non-ionic backbones and non-ribose backbones. Modifications of the ribose-phosphate backbone may be done to increase the stability of the molecules; for example, PNA:DNA hybrids can exhibit higher stability in some environments.

[00013] "Primer" means an oligonucleotide, either natural or synthetic, that is capable, upon forming a duplex with a polynucleotide template, of acting as a point of initiation of nucleic acid synthesis and being extended from its 3' end along the template so that an extended duplex is formed. The sequence of nucleotides added during the extension process is determined by the sequence of the template polynucleotide. Primers usually are extended by a DNA polymerase.

[00014] The term "SNP" or "single nucleotide polymorphism" refers to a genetic variation between individuals; *e.g.*, a single nitrogenous base position in the DNA of organisms that is variable. SNPs are found across the genome; much of the genetic variation between individuals is due to variation at SNP loci, and often this genetic variation results in phenotypic variation between individuals. SNPs for use in the present invention and their respective alleles may be derived from any number of sources, such as public databases (U.C. Santa Cruz Human Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway) or the NCBI dbSNP website (http://www.ncbi.nlm.nih.gov/SNP/), or may be experimentally determined as described in U.S. Pat. No. 6,969,589; and US Pub. No. 2006/0188875 entitled "Human Genomic Polymorphisms." Although the use of SNPs is described in some of the embodiments presented herein, it will be understood that other biallelic or multi-allelic genetic markers may also be used. A biallelic genetic marker is

one that has two polymorphic forms, or alleles. As mentioned above, for a biallelic genetic marker that is associated with a trait, the allele that is more abundant in the genetic composition of a case group as compared to a control group is termed the "associated allele," and the other allele may be referred to as the "unassociated allele." Thus, for each biallelic polymorphism that is associated with a given trait (*e.g.*, a disease or drug response), there is a corresponding associated allele. Other biallelic polymorphisms that may be used with the methods presented herein include, but are not limited to multinucleotide changes, insertions, deletions, and translocations. It will be further appreciated that references to DNA herein may include genomic DNA, mitochondrial DNA, episomal DNA, and/or derivatives of DNA such as amplicons, RNA transcripts, cDNA, DNA analogs, etc. The polymorphic loci that are screened in an association study may be in a diploid or a haploid state and, ideally, would be from sites across the genome.

[00015] The term "selectively binds", "selective binding" and the like as used herein, when referring to a binding partner (*e.g.*, protein, nucleic acid, antibody or other affinity capture agent, etc.), refers to a binding reaction of two or more binding partners with high affinity and/or complementarity to ensure selective hybridization under designated assay conditions. Typically, specific binding will be at least three times the standard deviation of the background signal. Thus, under designated conditions the binding partner binds to its particular "target" molecule and does not bind in a significant amount to other molecules present in the sample.

[00016] "Sequencing", "sequence determination" and the like means determination of information relating to the nucleotide base sequence of a nucleic acid. Such information may include the identification or determination of partial as well as full sequence information of the nucleic acid. Sequence information may be determined with varying degrees of statistical reliability or confidence. In one aspect, the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid. "High throughput digital sequencing" or "next generation sequencing" means sequence determination using methods that determine many (typically thousands to billions) of nucleic acid sequences in an intrinsically parallel manner, *i.e.* where DNA templates are prepared for sequencing not one at a time, but in a bulk process, and where many sequences are read out preferably in parallel, or alternatively using an ultra-high

throughput serial process that itself may be parallelized. Such methods include but are not limited to pyrosequencing (for example, as commercialized by 454 Life Sciences, Inc., Branford, CT); sequencing by ligation (for example, as commercialized in the SOLiD™ technology, Life Technology, Inc., Carlsbad, CA); sequencing by synthesis using modified nucleotides (such as commercialized in TruSeq™ and HiSeq™ technology by Illumina, Inc., San Diego, CA, HeliScope™ by Helicos Biosciences Corporation, Cambridge, MA, and PacBio RS by Pacific Biosciences of California, Inc., Menlo Park, CA), sequencing by ion detection technologies (Ion Torrent, Inc., South San Francisco, CA); sequencing of DNA nanoballs (Complete Genomics, Inc., Mountain View, CA); nanopore-based sequencing technologies (for example, as developed by Oxford Nanopore Technologies, LTD, Oxford, UK), and like highly parallelized sequencing methods.

[00017] The term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation, T_m=81.5+0.41 (% G+C), when a nucleic acid is in aqueous solution at 1M NaCl (see *e.g.*, Anderson and Young, Quantitative Filter Hybridization, in *Nucleic Acid Hybridization* (1985)). Other references (*e.g.*, Allawi and SantaLucia, Jr., Biochemistry, 36:10581-94 (1997)) include alternative methods of computation which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m.

DETAILED DESCRIPTION OF THE INVENTION

[00018] The practice of the techniques described herein may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology, which are within the skill of those who practice in the art. Such conventional techniques include polymer array synthesis, hybridization and ligation of polynucleotides, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the examples

herein. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Green, et al., Eds., *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV) (1999); Weiner, Gabriel, Stephens, Eds., *Genetic Variation: A Laboratory Manual* (2007); Dieffenbach, Dveksler, Eds., *PCR Primer: A Laboratory Manual* (2003); Bowtell and Sambrook, *DNA Microarrays: A Molecular Cloning Manual* (2003); Mount, *Bioinformatics: Sequence and Genome Analysis* (2004); Sambrook and Russell, *Condensed Protocols from Molecular Cloning: A Laboratory Manual* (2006); and Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (2002) (all from Cold Spring Harbor Laboratory Press); Stryer, *Biochemistry* (4th Ed.) (1995) W.H. Freeman, New York N.Y.; Gait, "*Oligonucleotide Synthesis: A Practical Approach*" (2002) IRL Press, London; Nelson and Cox, *Lehninger, Principles of Biochemistry* (2000) 3rd Ed., W. H. Freeman Pub., New York, N.Y.; and Berg, et al., *Biochemistry* (2002) 5th Ed., W.H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

[00019] Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" refers to one or more nucleic acids, and reference to "the assay" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

[00020] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing devices, formulations and methodologies that may be used in connection with the presently described invention.

[00021] Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated

range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[00022] In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

The Invention in General

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

[00024] The assay system further provides instrumentation with an ability to deliver reagents in a spatially-defined pattern. This instrumentation, together with software, reagents and protocols, provides a key component of the highly innovative assay system of the invention, allowing for measurement of numerous biological targets or activities in a meaningful spatial environment, including gene expression and peptide localization. An encoding scheme used in these assay systems allows one to determine the location of biological targets or activity (or lack thereof) in the biological samples after the products of the multiplexed assay are removed from the biological sample and pooled for analysis. Decoding of the encoding scheme can be performed by, *e.g.*, next-generation sequencing, which easily provides millions to trillions of datapoints at low cost. The assay results such as the amount or activity of biological targets can then be mapped back to specific location

Curio EX1010-p. 17
Curio v Prognosys
IPR2024-01289

in the biological sample. The assay systems open a new analytical window into the complex spatial patterns of cellular function and regulation in biological samples.

[00025] A simplified overview of the assay system 100 of the present invention is provided at Figure 1. At step 110, a biological sample affixed to a support is provided. The biological sample contains biological targets of interest. Biological targets can include any molecule of interest, such as nucleic acids (including, *e.g,* RNA transcripts, genomic DNA sequences, cDNAs, amplicons, or other nucleic acid sequences) and proteins, enzymes and the like. At step 120, encoded probes are delivered to the biological sample according to a known spatial pattern. Encoded probes comprise probes, which can interact with biological targets of interest, and coding tags, which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample. Coding tags in most embodiments are oligonucleotides. However, coding tags may also be mass tags, fluorescent labels, or other moieties.

[00026] In some embodiments, the probe and coding tag portions of the encoded probe are pre-coupled before being delivered to the biological sample, For example, in the case where the encoded probes are oligonucleotides, both the probe and coding tag sequence can be synthesized as a single oligonucleotide. Alternatively, the probe and coding tag portions of the encoding probes can be synthesized or obtained separately and combined before delivery to the biological sample (*e.g.,*two separate oligonucleotides can be synthesized and coupled by, *e.g.,* ligation; or an antibody and an oligonucleotide can be prepared separately and conjugated before delivery to the biological sample). Also, as is described in Figures 2-5, the probes and the coding tags (in encoding oligonucleotides) are synthesized separately, and are delivered to the biological sample at different steps (*e.g.,* probes first and coding tags thereafter, or vice versa) in the assay.

[00027] At step 130, the encoded probes are allowed to react or interact with the biological targets, *i.*e., conditions are provided to allow *e.g.,* oligonucleotides to hybridize to nucleic acid targets, enzymes to catalyze reactions with protein targets, antibodies to bind epitopes, etc. In the case where the biological targets are nucleic acids, the encoded probes are typically oligonucleotides and hybridize to the target nucleic acids. In the case that the biological targets are proteins, the encoded probes typically are aptamers, small

molecules, or oligonucleotide-conjugated proteins that interact with target proteins by binding to them or by reacting with them (that is, one of the proteins is a substrate for the other). Encoding oligonucleotides may be coupled to the probes (proteins) by conjugation, chemical or photo-crosslinking via suitable groups and the like.

[00028] Once encoded probes interact with the biological targets, the encoded probes that interacted with the biological targets must be separated from the encoded probes that did not interact with the biological targets at step 140. In the case where the biological targets are nucleic acids and the encoded probes are oligonucleotides, the separation can be accomplished by, *e.g.,* washing the unhybridized encoded probes from the sample. Similarly, for other assays that are based on affinity binding, including those using aptamer, small molecule, and protein probes, washing steps can be used to remove low affinity binders. In the case where the probe is transformed via interaction with the target, *e.g.*, in the case of a peptide, *e.g.*, via cleavage by a protease or phosphorylation by a kinase, it is convenient to collect, all encoded probes--both encoded probes that interacted with the biological targets and were transformed and encoded probes that were not transformed. After collection or pooling, an antibody or other affinity capture agent can be used to capture probes that were transformed by addition of a moiety (*e.g.*, a phosphate group). In cases where probes have been transformed via cleavage, the transformed probes can be separated, *e.g.,* by capturing the non-transformed probes via a tag that is removed from the transformed probes during the transformation (*e.g.*, by cleavage), or by adding a new tag at the site of cleavage.

[00029] Once the reacted (transformed) or interacted encoded probes are separated from the unreacted or un-interacted encoded probes, the sequence of the reacted and/or interacted encoded probes is determined by, preferably, sequencing. The sequence of the encoded probes allows the mapping of the assay results back to locations in the biological sample.

[00030] Figure 2 provides a simplified overview of an assay system of the present invention embodying an efficient implementation of a combinatorial coding scheme for the encoding of spatial information. For purposes of this overview, the probes are oligonucleotides, but as explained elsewhere, other types of probes can also be used. In step 210, a biological sample affixed to a support, *e.g.,* a tissue sample or other biological

Curio EX1010-p. 19
Curio v Prognosys
IPR2024-01289

structure, is provided. In step 220, one or more oligonucleotide probes are delivered to the biological sample, where the oligonucleotide probes are capable of hybridizing with biological targets in the biological sample. In step 230, the oligonucleotide probes are allowed to interact with (hybridize to) the nucleic acid targets; that is, appropriate conditions are provided where oligonucleotide probes can hybridize to the target nucleic acids.

[00031] In step 240, the oligonucleotide probes that did not hybridize to target nucleic acids are removed, and thereby separated from oligonucleotide probes that did hybridize to target nucleic acids. In this embodiment, separation can be accomplished by, *e.g.,* washing the sample to remove unhybridized oligonucleotide probes. Next, in step 250, encoding oligonucleotides (the encoding agents) are delivered to the biological sample according to a chosen spatial pattern, where the encoding oligonucleotides comprise coding tags that are used to encode the location of biological targets in the biological sample. Note that in contrast to the assay system of Figure 1, here the probes and encoding agents (encoding oligonucleotides) are delivered in separate steps. In step 260, the encoding oligonucleotides are coupled to the oligonucleotide probes to create encoded probes. In this case where the probes are oligonucleotides, the encoding oligonucleotides may be coupled to the oligonucleotides probes by, *e.g.,* ligation. Alternatively, the information in the encoding oligonucleotides can be transferred by using a DNA polymerase to extend a probe oligonucleotide that acts as a primer, and thereby copy and incorporate the sequence of the encoding oligonucleotides.

[00032] In step 270, the sequence of the coding tags in the encoded probes as well as the sequence or a portion of the sequence of the probe itself is determined, and in step 280, the target nucleic acids are mapped back to the biological sample. In some embodiments, the abundance of sequences reveals the relative quantity of biological targets at the location. Although this embodiment shows the individual steps in a particular order, so as to better explain the invention, the precise order of the steps can be varied. For example, steps 220 and 250 can be combined, so that a mixture of the probes and encoding oligonucleotides is delivered according to a chosen spatial pattern. Coupling step 260 can then be carried out immediately after the combined steps 220 and 250, or concomitantly with them. In this case, step 240 would then occur after step 260. It can therefore be appreciated that the two

Curio EX1010-p. 20
Curio v Prognosys
IPR2024-01289

key results of this series of steps, *i.e.*, the location-specific encoding of probe molecules and the separation of probe molecules based on their ability to interact with corresponding target molecules, can be accomplished with some flexibility in the implementation of the particular steps. Similarly, there is considerable flexibility in the design of the coding scheme. As described *infra,* the assays of the invention are particularly amenable to combinatorial methods.

[00033] Thus, the present invention provides an ability to look at many different biological targets in many locations, providing the resolution of in situ hybridization with the highly-parallel data analysis of sequencing. In some embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 20, in other embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 50, in other embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 100, greater than 500, 1,000, 10,000, 25,000, 100,000, 500,000, 1,000,000. It will be appreciated that, due to the spatial encoding dimension of the invention, even much larger numbers can be contemplated. For example, assaying 10,000 targets per location x 10,000 locations would generate 10^8 different assays, and even larger numbers than these can easily be contemplated, particularly if spatial locations with resolution on the order of that of single cells are utilized. Further, in embodiments where high-throughput digital sequencing is employed, the sequences of at least 1,000 encoding probes are typically determined in parallel. More typically, using a digital readout, it is desirable to obtain multiple sequence reads for each assay (defined by a probe and a spatial location code). It is desirable to obtain an average of at least 3 copies per assay, and more typically at least 10 or at least 30 copies per assay, depending on the design of the experiment and requirements of the assay. For a quantitative readout with suitable dynamic range, it may be desirable to obtain at lest 1,000 reads per assay. Therefore, if 1,000,000 assays are carried out, the number of sequence reads may be 1 billion or more. With high-throughput digital sequencing, and allowing for redundnacy, the sequence of at least 10,000 encoding probes are determined in parallel, or the sequence of at least 100,000, 500,000, 1,000,000, 10,000,000, 100,000,000, 1,000,000,000 or more encoding probes are determined in parallel.

Assays

[00034] The assay portion of the assay systems of the present invention comprise the following general steps: delivering probes and encoding agents where the encoding agents (in some embodiments pre-coupled to the probes) are delivered to the sample according to a known spatial pattern, allowing the probes to interact or react with biological targets in the sample, and, if the probes and encoding agents have not been pre-coupled, coupling the encoding agents to probes.

[00035] The 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, where the ability to tie an assayed biological target or activity back to the location within the biological sample is important. Exemplary biological samples include tissue sections (*e.g.*,including whole animal sectioning and tissue biopsies), cell populations on slides or culture dishes, and the like. The assay systems of the invention are particularly advantageous in that they are compatible with numerous biological sample types, including fresh samples, such as primary tissue sections, and preserved samples including but not limited to frozen samples and paraformalin-fixed, paraffin-embedded (FFPE) samples. An important aspect of the assay systems of the invention is that the biological samples are immobilized on a substrate surface having discrete, independently measureable areas.

[00036] The biological targets to be detected can be any biological molecules including but not limited to proteins, nucleic acids, lipids, carbohydrates, ions, or multicomponent complexes containing any of the above. Examples of subcellular targets include organelles, e.g., mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplasts, endocytic vesicles, exocytic vesicles, vacuoles, lysosomes, etc.

[00037] In some particular embodiments, the assay system is used to analyze nucleic acids, *e.g.*, by genotyping, quantitation of DNA copy number or RNA transcripts, localization of particular transcripts within samples, and the like. Figure 3 illustrates an overall scheme for an exemplary assay for, *e.g.*, detecting single nucleotide polymorphisms (SNPs) that can be used with the assay system of the invention. In Figure 3, two oligonucleotide probes are provided. Each oligonucleotide probe comprises a

target-specific region (located on either side of the SNP to be analyzed) seen at 305 and 307, and ligation regions, seen at 301 and 303. The oligonucleotide probes are allowed to hybridize to a target nucleic acid (not shown) in the biological sample. At step 302, one of the oligonucleotide probes is extended to incorporate the SNP sequence and ligated to the other probe to form an extended probe comprising target nucleic acid region 309 and ligation regions 301 and 303.

[00038] Two encoding agents, both comprising a coding tag (seen at 315 and 317), a ligation region (seen at 311 and 313), and a primer region (seen at 319 and 321) are combined with and ligated to the extended probe at step 304 to form an encoded target-specific oligonucleotide. Again, in contrast with Figure 1, the probes and encoding agents are delivered at separate steps. Doing so allows use of the combinatorial embodiments described *infra*. In preferred embodiments, the encoding oligonucleotides within a pair of encoding oligonucleotides ligate specifically to one side of the target sequence or the other (*i.e.,*5' or 3' of the target sequence). Also, typically, the ligation and primer regions of the encoding oligonucleotides and probes are universal; that is, the set of ligation and primer regions used in constructing the probes and encoding oligonucleotides are constant, and only the target-specific regions of the probes and the coding tags of the encoding oligonucleotides differ. However, again in alternative embodiments, the ligation and primer regions are not universal and differ between probes and encoding agents.

[00039] Following ligation, the encoded probes are eluted, pooled, and, optionally, sequencing adapters are added to the encoded probes via PCR. In alternative embodiments, sequencing primers may be ligated to the encoding oligonucleotides, or sequencing primer sequences can be included as part of the encoding oligonucleotide. As seen in Figure 3, each sequencing adapter comprises primer region 319 or 321, compatible with the primer regions 319 and 321 on the encoded probes. The final construct comprising first adapter 327, first primer region 319, first coding tag 315, ligation regions 311 and 301, target region 309, ligation regions 313 and 303, second coding tag 317, second primer region 325 and second adapter 329 is now ready for input into a digital high-throughput sequencing process.

[00040] A combination of extension and ligation reactions are exemplified in Figure 3, but it should be appreciated that a variety of reactions may be used to couple the encoding

oligonucleotides to the target-specific oligonucleotides, including ligation only (*e.g.*, for oligonucleotides that hybridize to contiguous portions of the target nucleic acid sequence). Alternatively, an assay utilizing an additional oligonucleotide, such as in the GOLDENGATE® assay (see Fan, et al., Cold Spring Symp. Quant. Biol., 68:69-78 (2003); (Illumina, Inc., San Diego, CA)), may be employed.

[00041] In other embodiments, the assay system of the invention also can be used to analyze peptides or proteins, the presence of antibodies, enzymatic and other protein activities, posttranslational modifications, active and non-active forms of peptides, as well as peptide isoforms in a biological sample. Accordingly, the probes may comprise an active region of an enzyme, a binding domain of an immunoglobulin, defined domains of proteins, whole proteins, synthetic peptides, peptides with introduced mutations, aptamers and the like.

[00042] In certain aspects, the probes are substrates for enzymes or proenzymes, *e.g.*, kinases, phosphatases, zymogens, proteases, or fragments thereof. In certain aspects, the probes are phosphorylation substrates used to detect proteins involved in one or more signal transduction pathways, *e.g.*, a kinase or a phosphatase. In another specific aspect of the invention, the probes are specific protease substrates that associate only with individual proteases or classes of proteases. In other aspects, the probes are different processed forms, isoforms and/or domains of an enzyme. Protein-based probes are typically conjugated or otherwise linked to oligonucleotide encoding agents. The oligonucleotide encoding agents in this case would also include a nucleotide sequence component that allows for identification of the protein probe.

[00043] In certain aspects, the present invention provides assays for evaluating differences in the amount and/or activity of biological targets between different locations in a sample and/or between samples. The method includes determining a plurality of encoded results from the biological sample and evaluating the differences in quantity of the biological targets at each location in the biological sample.

Combinatorial Embodiments

[00044] To maximize the efficiency of encoding, a combinatorial approach using pairs of coding tags in the encoding oligonucleotides can be used. By de-coupling the target-

Curio EX1010-p. 24
Curio v Prognosys
IPR2024-01289

specific information and the coding tags, the number of oligonucleotides required is dramatically reduced, with a concomitant decrease in cost.

[00045] Figure 4 illustrates a general mechanism for one embodiment of a combinatorial encoding scheme of the assay systems of the invention., where nucleic acids in a representative tissue section (shown at 416) are assayed. Figure 4 at A shows two target-specific/encoding oligonucleotide constructs 420 and 422 (*e.g.*, formed between steps 302 and 304 of Figure 3) specifically bound to a target nucleic acid 402 of interest. The first encoded probe 420 comprises coding tag 408, associated with, *e.g.*, a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 404. The second encoded probe 422 comprises coding tag 406, associated with, *e.g.*, a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410.

[00046] Figure 4 at B shows the spatial pattern that may be used for twenty different coding tags, a1 through a10 (coding tag 406 on encoded probe 420) and b1 through b10 (coding tag 408 encoded probe 422). Coding tag a1, for example, is deposited on the biological sample in ten discrete areas or spots (shown as the first horizontal line of spots in 412). Coding tag a2 is deposited on the biological sample in ten spots on the second horizontal line in 412. Coding tag a3 is deposited on the biological sample in ten spots on the third horizontal line in 412, and so on. Whereas the "a" tags are deposited in ten horizontal rows, the "b" tags are deposited in ten vertical rows as shown in 414. For example, coding tag b1 is deposited on the biological sample in ten discrete spots in the first vertical row of 414, coding tag b2 is deposited on the biological sample in ten discrete spots in the second vertical row of 414, and so on. Using such a configuration allows for twenty coding tags to uniquely define 100 different locations on the biological sample.

[00047] Figure 4 at C shows a representative tissue section 416 coincident with coding tag grid 418. The arrows show how the "a" coding tags and the "b" coding tags are deposited on grid 418 that is coincident with tissue section 416. If, once sequenced, coding tags a1 and b4, *e.g.*, are associated with a target nucleic acid sequence, then that target nucleic acid sequence (*i.e.*, biological target) was present in the tissue section at location a1, b4.

Curio EX1010-p. 25
Curio v Prognosys
IPR2024-01289

[00048] Figure 5 provides a simplified, specific example of the encoding scheme of the assay systems of the invention. Figure 5 shows encoding oligonucleotides 510, comprising a1, a2, a3, a4 and b1, b3, b3 and b4. Target-specific oligonucleotides (TSOs) (probes) 1 and 2 are shown at 520. A deposit or dispensing scheme is shown at 530. Like the grid exemplified in Figure 4, encoding oligonucleotides a1 through a4 are deposited in spots in a pattern (here, in a vertical pattern), and encoding oligonucleotides b1 through b4 are deposited in spots in a pattern (here, a horizontal pattern). The grid though shown as a square with spots is actually a deposition pattern on a biological sample (not shown) such as tissue section 416 shown in Figure 4.

[00049] The target-specific oligonucleotides are delivered to the biological sample, where the target-specific oligonucleotides hybridize to target nucleic acids in the biological sample if target nucleic acids are present. Unhybridized target-specific oligonucleotides are then removed, *e.g.,* by washing. The encoding oligonucleotides are then delivered to the biological sample according to the spatial pattern shown at 530. The encoding oligonucleotides are ligated (or, *e.g.,* extended and ligated) to any target-specific oligonucleotides that hybridized to the target nucleic acid in the biological sample, the ligated constructs are then eluted from the biological sample, pooled, and sequencing adapters are added through, *e.g.*, PCR or ligation, if the sequences were not previously included in the encoding oligonucleotides. The ligated constructs are sequenced by, *e.g.*, high throughput or "next generation" sequencing.

[00050] The pool of resulting sequences is shown at 540. A sequence readout was obtained for target-specific oligonucleotide 1 only at a4b1, a4b2, a1b3, a2b3, a3b3, a4b3 and a4b4 (positions shown with horizontal lines). A sequence readout was obtained for target-specific oligonucleotide 2 only at a1b1 (position shown with vertical lines). A sequence readout was obtained for both target-specific oligonucleotides 1 and 2 at positions a2b1, a3b1, a1b2, a2b2, and a3b2 (positions shown with cross-hatching). No sequence readout was obtained for either target-specific oligonucleotides at a1b4, a2b4 or a3b4 (positions shown without shading). Thus, in the biological sample on which the assay took place the first target nucleic acid was detected in a large portion of the left side and at the bottom of the biological sample, the second target nucleic acid was detected only in the upper left portion of the biological sample, and neither target nucleic acid was

Curio EX1010-p. 26
Curio v Prognosys
IPR2024-01289

detected in the upper right portion of the biological sample. The differential expression of the two target nucleic acids now can be mapped back to the biological sample and to the biological structures or cell types in these locations in the biological sample.

[00051] In addition to location information, information relating to relative abundance of the encoded tags can be obtained. For example, if it is found that there are ten times as many a4T1b1 sequences occurring in the data set as compared to a4T1b2 sequences, this would indicate that target nucleic acid sequence 1 is ten times more abundant at the a4T1b1 location than at the a4T1b2 location.

[00052] In the case of nucleotide analysis as shown in Figure 3, by ligating the coding tags directly to target-specific oligonucleotides, only 2n target-specific oligonucleotides are needed for n targets. For example, using the combinatorial approach outlined in Figure 2, assaying 100 different targets at 10,000 spatial locations would require 2 x 100 target-specific oligonucleotides and 2 x 100 encoding oligonucleotides. The total count of assay oligonucleotides would be only 400 (200 target-specific and 200 encoding), not counting universal primers. In contrast, if the coding oligonucleotides were not decoupled from the target-specific oligonucleotides, (n x X positional codes) + (n x Y positional codes) would be needed, or in the above example, 20,000 oligonucleotides, not counting universal primer sequences. Moreover, though the embodiments shown in Figures 2- 5 depict a combinatorial scheme using two encoding agents (coding tags), three, four or more encoding agents and coding tags may be used, and attached to the probe or one another by varying means and in varying combinations of steps.

[00053] Due to the spatial encoding aspect of the assay system of the invention, a large amount of information can be generated with even a modest number of assays. For example, five or more biological targets assayed at five or more positions in the sample generates 25 or more combinations. Using digital sequencing as a readout, the optimum number of sequence reads per combination depends on the sensitivity and dynamic range required, and can be adjusted. For example, if for each combination on average 100 reads are sampled, the total for 25 combination is 25,000 reads. If 1,000 targets are assayed at 1,000 locations with an average sampling depth of 1,000, then 10^9 reads are required. These numbers, although large, are within the capacity of intrinsically parallel digital sequencing methods, which can generate datasets of billions or even trillions of reads in a

reasonable timeframe and at a very low cost per read. Therefore, by varying the numbers of positions interrogated or biological targets assayed, or both, and using digital sequencing, large amounts of information can be obtained. In specific aspects, multiple locations are interrogated for two or more biological molecules.

Reagent Delivery Systems

[00054] The reagent delivery system of the invention includes instrumentation that allows the delivery of reagents to discrete portions of the biological sample, maintaining the integrity of the spatial patterns of the encoding scheme. Reagent delivery systems of the assay systems of the invention comprise optional imaging means, reagent delivery hardware and control software. Reagent delivery can be achieved in a number of different ways. It should be noted that reagent delivery may be to many different biological samples at one time. A single tissue section has been exemplified herein; however, multiple biological samples may be affixed and analyzed simultaneously. For example, serial sections of a tissue sample can be analyzed in parallel and the data combined to build a 3D map.

[00055] Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents onto the biological sample. Technologies for formulating and delivering both biological molecules (*e.g.* oligonucleotides or antibodies) and chemical reagents (*e.g.*, small molecules or dNTPs) are known in the art, and uses of these instrument systems are known to one skilled in the art and easily adaptable to the assay systems of the invention. One example of a suitable reagent delivery system is the Labcyte™ Echo acoustic liquid handler, which can be used to deliver nanoliter scale droplets containing biological molecules with high precision and reproducibility. One skilled in the art could incorporate this reagent delivery device into the overall system, using software to specify the locations to which reagents should be delivered.

[00056] Other instruments that can be used for the deposition of agents and/or coding identifiers onto biological samples include, but are not limited to, ink jet spotting; mechanical spotting by means of pin, pen or capillary; micro contact printing; photochemical or photolithographic methods; and the like. For several applications, it may be preferred to segment or sequester certain areas of the biological samples into one or

more assay areas for different reagent distributions and/or biological target determination. The assay areas may be physically separated using barriers or channels.

[00057] In one exemplary aspect, the reagent delivery system may be a flow-based system. The flow-based systems for reagent delivery in the present invention can include instrumentation such as one or more pumps, valves, fluid reservoirs, channels, and/or reagent storage cells. Reagent delivery systems are configured to move fluid to contact a discrete section of the biological sample. Movement of the reagents can be driven by a pump disposed, for example, downstream of the fluid reagents. The pump can drive each fluid reagent to (and past) the reaction compartment. Alternatively, reagents may be driven through the fluid by gravity. US Pub. Nos. 20070166725 and 20050239192 disclose certain general-purpose fluidics tools that can be used with the assay systems of the invention, allowing for the precise manipulation of gases, liquids and solids to accomplish very complex analytical manipulations with relatively simple hardware.

[00058] In a more specific example, one or more flow-cells can be attached to the substrate-affixed biological sample from above. The flow-cell can include inlet and outlet tubes connected thereto and optionally an external pump is used to deliver reagents to the flow-cell and across the biological sample. The flow cells are configured to deliver reagents only to certain portions of the biological sample, restricting the amount and type of reagent delivered to any specific section of the biological sample.

[00059] In another aspect, a microfluidic system can be integrated into the substrate upon which the biological sample is disposed or externally attached on top of the substrate. Microfluidic passages for holding and carrying fluid may be formed on and/or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs.

[00060] Pumps generally include any mechanism for moving fluid and/or reagents disposed in fluid. In some examples, the pump can be configured to move fluid and/or reagents through passages with small volumes (*i.e.*, microfluidic structures). The pump can operate mechanically by exerting a positive or negative pressure on fluid and/or on a structure carrying fluid, electrically by appropriate application of an electric field(s), or both, among other means. Exemplary mechanical pumps may include syringe pumps,

Curio EX1010-p. 29
Curio v Prognosys
IPR2024-01289

peristaltic pumps, rotary pumps, pressurized gas, pipettors, etc. Mechanical pumps may be micromachined, molded, etc. Exemplary electrical pumps may include electrodes and may operate by electrophoresis, electroendoosmosis, electrocapillarity, dielectrophoresis (including traveling wave forms thereof), and/or the like.

[00061] Valves generally include any mechanism for regulating the passage of fluid through a channel. Valves can include, for example, deformable members that can be selectively deformed to partially or completely close a channel, a movable projection that can be selectively extended into a channel to partially or completely block a channel, an electrocapillary structure, and/or the like.

[00062] An open gasket can be attached to the top of the biological sample and the sample and reagents can be injected into the gasket. Suitable gasket materials include, but are not limited to, neoprene, nitrile, and silicone rubber. Alternatively, a watertight reaction chamber may be formed by a gasket sandwiched between the biological sample on the substrate and a chemically inert, water resistant material such as, but not limited to, black-anodized aluminum, thermoplastics (*e.g.*, polystyrene, polycarbonate, etc), glass, etc.

[00063] In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest. The images obtained, *e.g.,* may be used to design the deposition pattern of the reagents. Imaging means are optional, as an individual can instead view the biological sample using, *e.g.,* a microscope, analyze the organization of the biological sample, and specify a spatial pattern for delivery assay reagents. If included, the delivery system can comprise a microcircuit arrangement including an imager, such as a CCD or IGFET-based (*e.g.*, CMOS-based) imager and an ultrasonic sprayer for reagent delivery such as described in US Pub. No. 20090197326, which is incorporated herein by reference. Also, it should be noted that although Figures 4 and 5 illustrate using a x,y grid configuration, other configurations can be used, such as, *e.g.,* following the topology of a tissue sample; targeting certain groups of cells, cell layers and/or cell types in a tissue, and the like.

[00064] In yet another alternative, the reagent delivery system controls the delivery of reagents to specific patterns on a biological sample surface using semiconductor techniques such as masking and spraying. Specific areas of a biological sample can be protected from exposure to reagents through use of a mask to protect specific areas from

Curio EX1010-p. 30
Curio v Prognosys
IPR2024-01289

exposure. The reagents may be introduced to the biological sample using conventional techniques such as spraying or fluid flow. The use of masked delivery results in a patterned delivery scheme on the substrate surface.

[00065] In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology. There are a variety of different ink-jetting mechanisms (*e.g.,* thermal, piezoelectric) and compatibility has been shown with aqueous and organic ink formulations. Sets of independently actuated nozzles can be used to deliver multiple reagents at the same time, and very high resolutions are be achieved.

[00066] In order to target specific sites of interest, an informative image of the biological sample to be assayed may be used to assist in the reagent delivery methods and associated encoding scheme. Sample regions of the biological sample can be identified using image processing (*e.g.,* images of cell types differentiated by immunohistochemistry or other staining chemistries) integrated with other features of the assay system. In some aspects, software is used to automatically translate image information into a reagent delivery pattern. A mechanism to register and align very precisely the biological sample for reagent delivery is thus an important component of the assay systems of the invention. Mechanisms such as the use of fiducial markers on slides and/or other very accurate physical positioning systems can be adapted to this purpose.

[00067] The invention preferably comprises a complete suite of software tailored to the assay system. Optionally, oligonucleotide design software is used to design the encoding nucleotides (and in embodiments where nucleic acids are assayed, the target-specific oligonucleotides) for the specific assay to be run, and may be integrated as a part of the system. Also optionally, algorithms and software for reagent delivery and data analysis (*i.e.,*sequence analysis) may be integrated to determine assay results. Integrated data analysis is particularly useful, as the type of dataset that is generated may be massive as a consequence of scale. Algorithms and software tools that are specifically designed for analysis of the spatially-associated data generated by the assay systems, including pattern-analysis software and visualization tools, enhance the value of the data generated by the assay systems.

[00068] In certain aspects, the assay system comprises processes for making and carrying out the quality control of reagents, *e.g.,* the integrity and sequence fidelity of

Curio EX1010-p. 31
Curio v Prognosys
IPR2024-01289

oligonucleotide pools. In particular, reagents are formulated according to factors such as volatility, stability at key temperatures, and chemical compatibility for compatibility with the reagent delivery instrumentation and may be analyzed by instrumentation integrated within the assay system.

Sequencing

[00069] Numerous methods can be used to identify the coding tags and probe sequences in the encoded probes of the assay systems of the invention. The coding tags can be detected using techniques such as mass spectroscopy (*e.g.*, Maldi-T of, LC-MS/MS), nuclear magnetic resonance imaging, or, preferably, nucleic acid sequencing. Examples of techniques for decoding the coding tags of the present invention can be found, for example, in US Pub. No. 20080220434, which is incorporated herein by reference. For example, the coding tags may be oligonucleotide mass tags (OMTs or massTags). Such tags are described, *e.g.*, in US Pub. No. 20090305237, which is incorporated by reference in its entirety. In yet another alternative, the encoded probes can be amplified and hybridized to a microarray. This would require separate amplification reactions to be carried out, in which each amplification is specific to a particular spatial code or subset of codes, accomplished by using code-specific primers. Each amplification would also incorporate a different resolvable label (e.g. fluorophor). Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels.

[00070] In one particularly preferred aspect, the resulting coding tags according to the assay system are substrates for high-throughput, next-generation sequencing, and highly parallel next-generation sequencing methods are used to confirm the sequence of the coding tags, for example, with SOLiD™ technology (Life Technologies, Inc.) or Genome Ananlyzer (Illumina, Inc.). Such next-generation sequencing methods can be carried out, for example, using a one pass sequencing method or using paired-end sequencing. Next generation sequencing methods include, but are not limited to, hybridization-based methods, such as disclosed in *e.g.*, Drmanac, U.S. Pat. Nos. 6,864,052; 6,309,824; and 6,401,267; and Drmanac et al, U.S. patent publication 2005/0191656; sequencing-by-synthesis methods, *e.g.*, U.S. Pat. Nos. 6,210,891; 6,828,100; 6,969,488; 6,897,023;

6,833,246; 6,911,345; 6,787,308; 7,297,518; 7,462,449 and 7,501,245; US Publication Application Nos. 20110059436; 20040106110; 20030064398; and 20030022207; Ronaghi, et al, Science, 281: 363-365 (1998); and Li, et al, Proc. Natl. Acad. Sci., 100: 414-419 (2003); ligation-based methods, *e.g.*, U.S. Patent Nos. 5,912,148 and 6,130,073; and U.S. Pat. Appln Nos. 20100105052, 20070207482 and 20090018024; nanopore sequencing *e.g.*, U.S. Pat. Appln Nos. 20070036511; 20080032301; 20080128627; 20090082212; and Soni and Meller, Clin Chem 53: 1996-2001 (2007)), as well as other methods, *e.g.*, U.S. Pat. Appln Nos. 20110033854; 20090264299; 20090155781; and 20090005252; also, see, McKernan, et al., Genome Res., 19:1527-41 (2009) and Bentley, et al., Nature 456:53-59 (2008), all of which are incorporated herein in their entirety for all purposes.

Applications of Assay System

[00071] It will be apparent to one skilled in the art upon reading the present disclosure that there are numerous important areas of biological research, diagnostics, and drug development that will benefit from a high throughput multiplexed assay system that can measure simultaneously the amount and spatial location of a biological target in a biological sample. For example, combining the ability to estimate the relative abundance of different RNA trancripts with the ability to reconstruct an image of spatial patterns of abundance across many locations, which may be as small as or even smaller than individual cells, in a tissue enables many different areas of basic research. The following are exemplary uses and are by no means meant to be limiting in scope.

[00072] In one example, 3-dimensional patterns of gene expression are determined by analyzing a series of tissue sections, in a manner analogous to image reconstruction in CT scanning. Such a method can be used to measure changes in gene expression in disease pathology, *e.g.,* in cancerous tissue and/or a tissue upon injury, inflammation or infection. With the assay systems of the invention, more detailed information on gene expression and protein localization in complex tissues is obtained, leading to new insights into the function and regulation both in normal and diseased states, and provides new hypotheses that can be tested. For example, an assay system of the invention may enable some of the

insights gained from many individual studies and larger programs like ENCODE (Birney, et al., Nature, 447:799-816 (2007)) and modENCODE to be integrated at the tissue level. The assay systems also aid computational efforts to model interacting networks of gene expression in the field of systems biology.

[00073] The assay systems also provide a novel approach to analysis of somatic variation, *e.g.,* somatic mutations in cancer or variability in response to infectious organisms. For example, tumors are typically highly heterogeneous, containing cancer cells as well as genetically normal cells in an abnormal local environment. Cancer cells undergo mutation and selection, and in this process it is not unusual for local clones to develop. Identifying relatively rare somatic mutations in the context of tumors may enable the study of the role of key mutations in the selection of clonal variants. Transcriptional patterns associated with angiogenesis, inflammation, or other cancer-related processes in both cancer and genetically normal cells can be analyzed for insights into cancer biology and assist in the development of new therapeutic agents for the treatment of cancers. In another example, individuals have varying susceptibility to infectious organisms, and the assay systems of the invention can be used to study the interaction between microbes and tissues or the various cell types within the tissue.

[00074] Importantly, in addition to providing spatially-associated information, the invention allows a great increase in the sensitivity of detecting rare mutations, as signal to noise can be dramatically increased since only a small location is assayed in any given reaction. In a typical assay for rare mutations in a mixed sample, the sample is treated in bulk, *i.e.,* nucleic acids are extracted from many cells into a single pool. Thus, if a mutation is present in one cell in 10,000, it must be detected against a background of normal DNA from ~10,000 cells. In contrast, with the assay systems of the invention many cells can be analyzed, but individual cells or small groups of cells would be identified by the spatial coding system. Therefore, in the assay systems of the present invention, background is reduced by orders of magnitude, greatly increasing sensitivity. Furthermore, the spatial organization of mutant cells can be observed, which may be particularly important in detecting key mutations in tissue sections in cancer. Already molecular histological analyses are yielding insights into cancer biology and may have

Curio EX1010-p. 34
Curio v Prognosys
IPR2024-01289

potential for use in diagnostics. The technology of the invention promises to greatly increase the power of such approaches.

EXAMPLES

[00075] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventor regards as his invention, nor are they intended to represent or imply that the experiments below are all of or the only experiments performed. It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[00076] Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees centigrade, and pressure is at or near atmospheric.

EXAMPLE 1: Initial Proof of Concept of Encoding Scheme

[00077] As an initial proof of concept, a model system is developed using a microarray to demonstrate a working single-plex assay. The basic design validates the concept of the assay, and establishes a working assay prior to addressing issues related to the analysis of a more complicated biological sample. Conventional sequencing is used as a readout for this proof of concept.

[00078] A microarray is used as a proxy for a tissue section. The target sequences of the microarray are fully specified, so that the composition of the targets are known and can be varied systematically. Synthetic oligonucleotide templates are attached to a glass slide via a 5' amino modification. Each slide has a single oligonucleotide template sequence, and

the assays that are carried out may employ either ligation, or extension followed by ligation as this may be useful in determining certain polymorphisms.

[00079] Once the *in situ* part of the assay is complete, the reaction products are eluted and analyzed by qPCR to determine presence or absence of a product and estimate yield, and by conventional sequencing to determine the structure of the assay products. The single-plex assays that are tested include appropriate positive and negative controls, and a single nucleotide variant (SNV) to check ability to discriminate single base variants.

EXAMPLE 2: Scalability

[00080] The complexity of the assay system is increased to establish scalability of the assay for use in high throughput studies. Scalability of both the spatial encoding and assay systems is demonstrated by carrying out a 24-plex x 24-site assay using a microarray model system.

[00081] The amount of biological target, here a DNA target sequence, at each assay location is systematically varied on microarray substrate. For example, in a microarray with 50 micron spot size (center to center), a $1mm^2$ area contains ~400 spots. The region around each site is optionally occupied by a region that is devoid of these spots to allow individual resolvability of the target sequences. Alternatively, the spots may be clustered, with two or more directly adjacent spots surrounded by or adjacent to a region that is devoid of target sequences.

[00082] In order to demonstrate that spatial encoding is accurate, the sites comprise different target compositions to show that the assay readout matches the expected composition of each site. With 24 target sequences, a simple digital pattern is made with each site having a different set of 12 targets present and 12 targets absent, to make a binary code (0 = absent, 1 = present). The assay readout is then determined to show that the detected regions match the expected signal after spatial decoding. In this particular example, the code space is large enough (2^{24}) so that even a few errors would not result in different codes being mixed up. Moreover, this design allows identification of errors and allows an estimation not only of accuracy of spatial encoding but also of accuracy calling the presence or absence of target sequences.

Curio EX1010-p. 36
Curio v Prognosys
IPR2024-01289

[00083] The ability to detect quantitative differences is evaluated by generating dose-response curves for each of the 24 assays that are carried out at each site in a 24-site assay. This allows estimation of the limit of detection, dynamic range, and power to detect a given fold-change across the range.

[00084] In one aspect, a latin square design is used to represent individual targets at different ratios by varying the number of features for each target. In other words, with multiple spots in a site, the number of spots allocated to each of the 24 target sequences can be varied and each of the 24 sites can have a different composition. A 1 x 3 inch microarray is sufficiently large to permit multiple replicates. This larger set of 24 sequences will require deconvolution, and this is accomplished using high throughput techniques such as next-generation sequencing technologies (*e.g.,* SOLiD™ technology (Life Technologies, Inc., Carlsbad, CA) or Genome Analyzer (Illumina, Inc., San Diego, CA)). The use of the 24-plex assay demonstrates both the accuracy of spatial encoding and decoding, and the quantitative response of the assay system.

EXAMPLE 3: Adaptation of the assay to preserved samples.

[00085] Genomic DNA is assayed as a proof of concept for assaying RNA, as it provides a way to establish a single-copy reference signal. Once a working assay is developed for FFPE samples, it is adapted to an RNA assay. To this end, assay oligonucleotide concentrations are assayed to ensure compatibility with high multiplexing. Assuming a cell diameter of 10 microns, and delivery of a 10 micron diameter reagent droplet to an individual cell, the volume of the droplet will be ~500 µl and can contain ~3 x 10^{11} molecules at a 1 µM concentration. To assay 1,000 target sequences in 10,000 cells, ~2,000 targeting oligonucleotides would be required in a droplet. Therefore, each droplet could contain ~160 million copies of each assay oligo, a vast excess over the few thousand target sequences in a cell.

[00086] The handling of small absolute numbers of product molecules generated from very small or compromised samples are enhanced to counter the issue of low recovery efficiency; that is, elution is efficient and losses resulting from adsorption of molecules to surfaces are prevented. An approach to addressing the latter issue is to include a carrier material, such as glycogen or carrier nucleic acids.

Example 4: Adapting the Assay to a Biological Sample.

[00087] A control RNA template is immobilized to a solid support in order to create an artificial system. The assay is performed using T4 DNA ligase, which can repair nicks in DNA/RNA hybrids. Assays are carried out on matched slides, or different sections of the same slide, where in one case gDNA is assayed and in the other RNA is assayed. When assaying gDNA the slide can be pretreated with RNase, and when assaying RNA the slide is pretreated with DNase. Results of the assay are confirmed by extracting gDNA or RNA and quantitating the relative amounts by qPCR or RT-qPCR respectively.

[00088] In order make the tissue section RNA assays as informative as possible, pre-existing information on expression levels in specific tissues to target transcripts across a range of abundances are used in the assay design. Both high abundance transcripts, as well as some medium and low abundance transcripts, are targeted to enable an initial assessment of the quantitative performance characteristics of the assay.

[00089] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims. In the claims that follow, unless

Curio EX1010-p. 38
Curio v Prognosys
IPR2024-01289

the term "means" is used, none of the features or elements recited therein should be construed as means-plus-function limitations pursuant to 35 U.S.C. §112, ¶6.

Curio EX1010-p. 39
Curio v Prognosys
IPR2024-01289

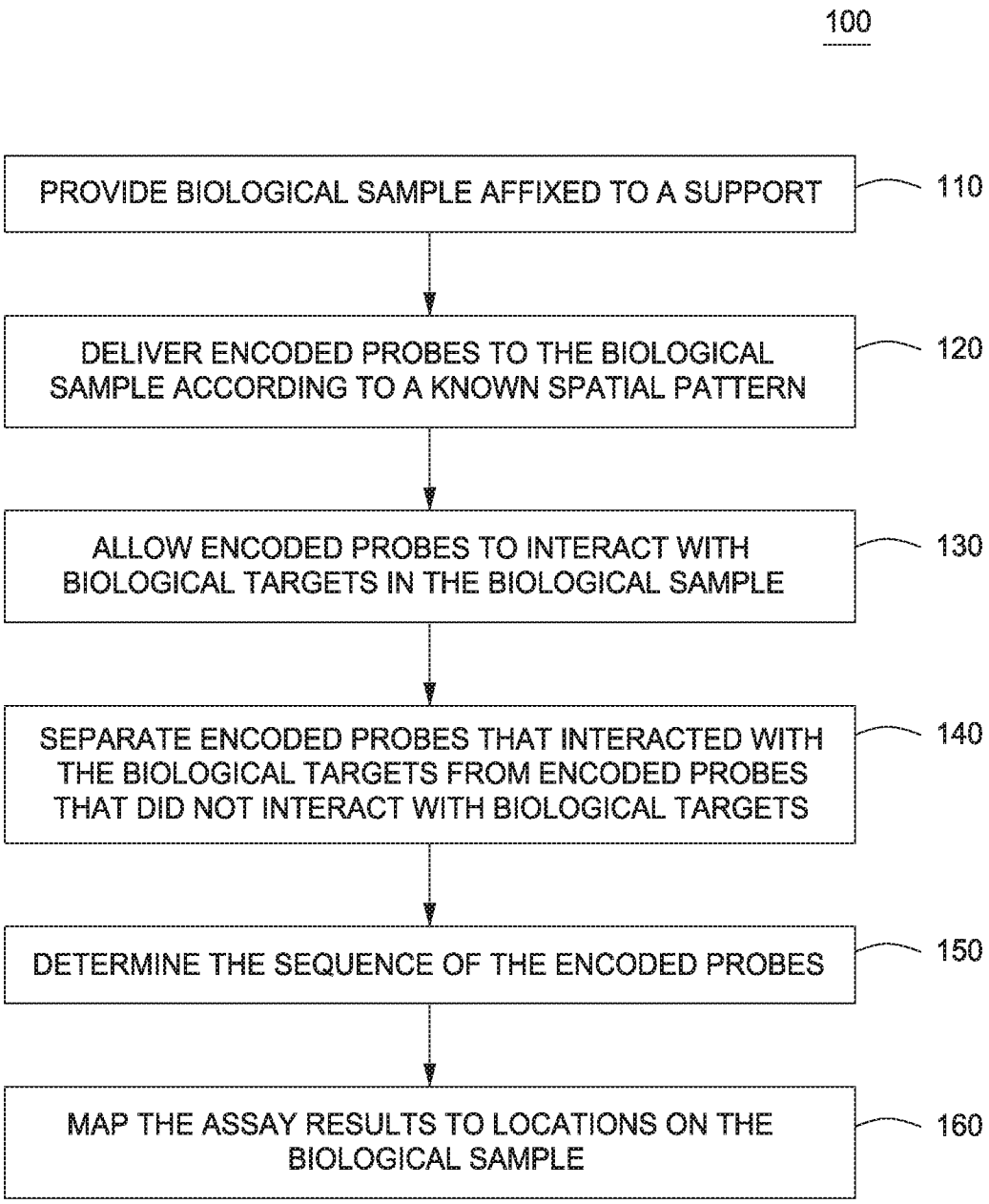
100
PROVIDE BIOLOGICAL SAMPLE AFFIXED TO A SUPPORT
110
DELIVER ENCODED PROBES TO THE BIOLOGICAL SAMPLE ACCORDING TO A KNOWN SPATIAL PATTERN
120
ALLOW ENCODED PROBES TO INTERACT WITH BIOLOGICAL TARGETS IN THE BIOLOGICAL SAMPLE
130
SEPARATE ENCODED PROBES THAT INTERACTED WITH THE BIOLOGICAL TARGETS FROM ENCODED PROBES THAT DID NOT INTERACT WITH BIOLOGICAL TARGETS
140
DETERMINE THE SEQUENCE OF THE ENCODED PROBES
150
MAP THE ASSAY RESULTS TO LOCATIONS ON THE BIOLOGICAL SAMPLE
160

FIG. 1

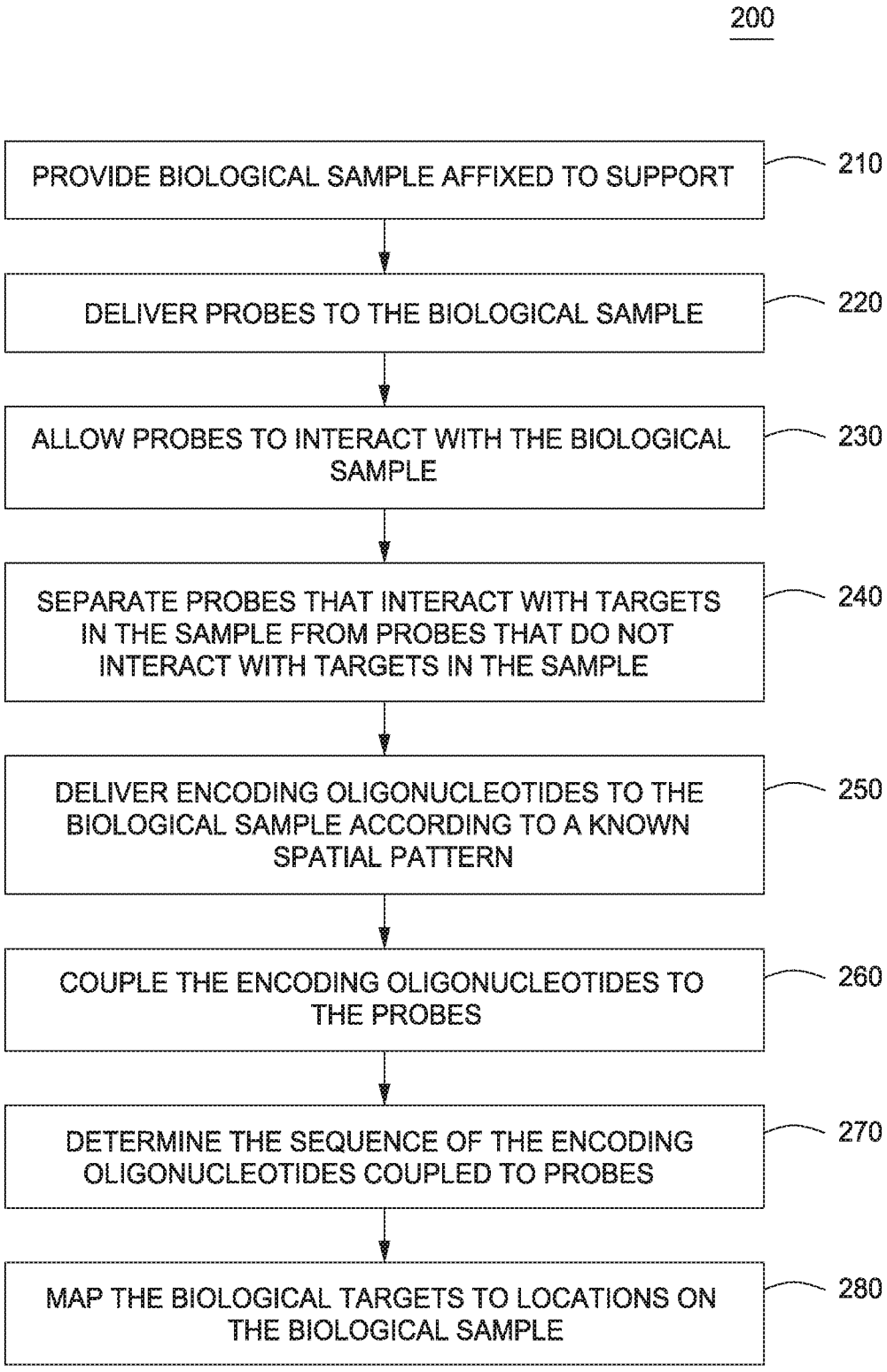
200
PROVIDE BIOLOGICAL SAMPLE AFFIXED TO SUPPORT
210
DELIVER PROBES TO THE BIOLOGICAL SAMPLE
220
ALLOW PROBES TO INTERACT WITH THE BIOLOGICAL SAMPLE
230
SEPARATE PROBES THAT INTERACT WITH TARGETS IN THE SAMPLE FROM PROBES THAT DO NOT INTERACT WITH TARGETS IN THE SAMPLE
240
DELIVER ENCODING OLIGONUCLEOTIDES TO THE BIOLOGICAL SAMPLE ACCORDING TO A KNOWN SPATIAL PATTERN
250
COUPLE THE ENCODING OLIGONUCLEOTIDES TO THE PROBES
260
DETERMINE THE SEQUENCE OF THE ENCODING OLIGONUCLEOTIDES COUPLED TO PROBES
270
MAP THE BIOLOGICAL TARGETS TO LOCATIONS ON THE BIOLOGICAL SAMPLE
280

FIG. 2

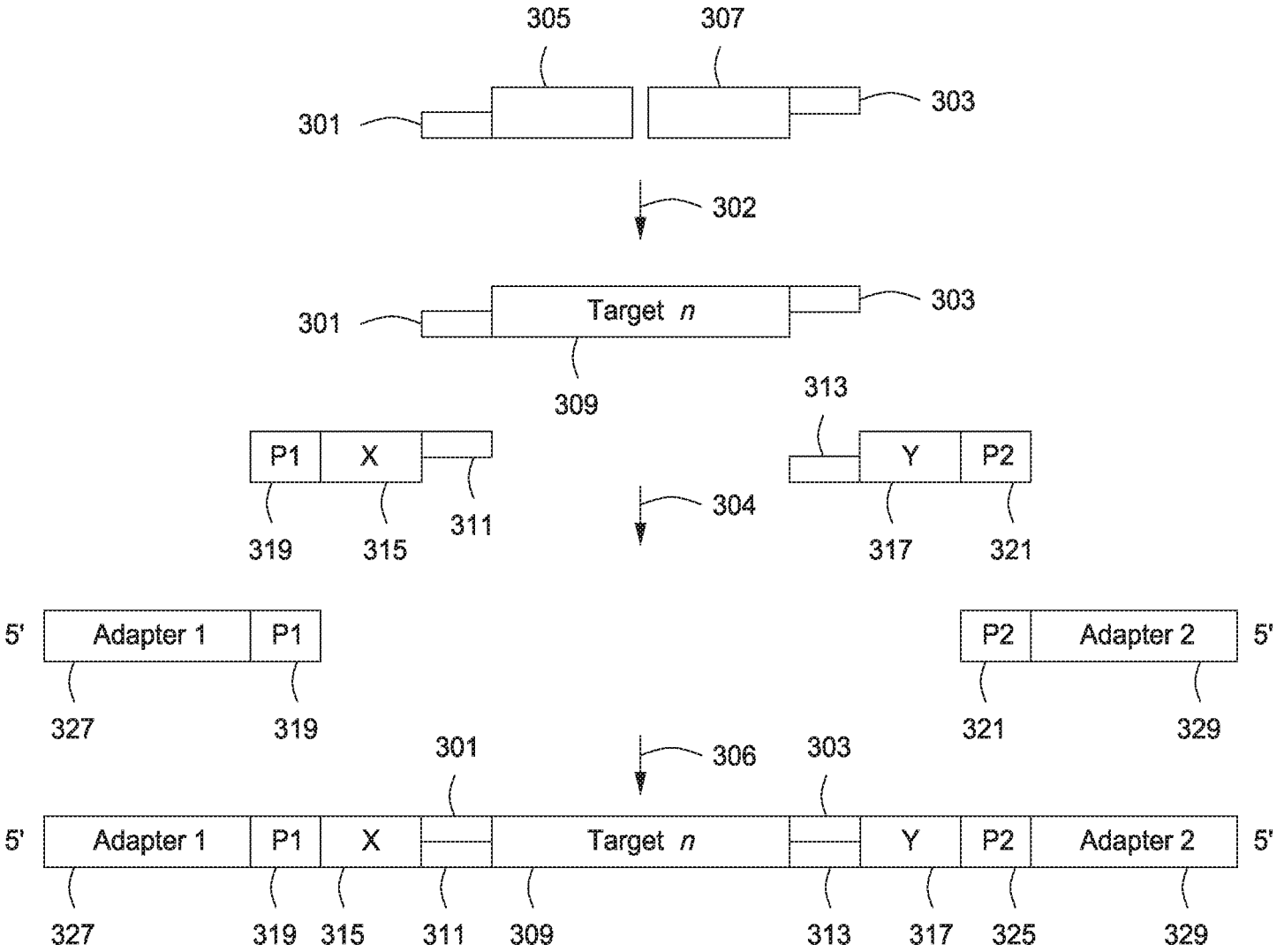
305
307
301
303
302
301
Target n
303
309
313
P1
X
Y
P2
311
304
319
315
317
321
5'
Adapter 1
P1
P2
Adapter 2
5'
327
319
321
329
301
306
303
5'
Adapter 1
P1
X
Target n
Y
P2
Adapter 2
5'
327
319
315
311
309
313
317
325
329

FIG. 3

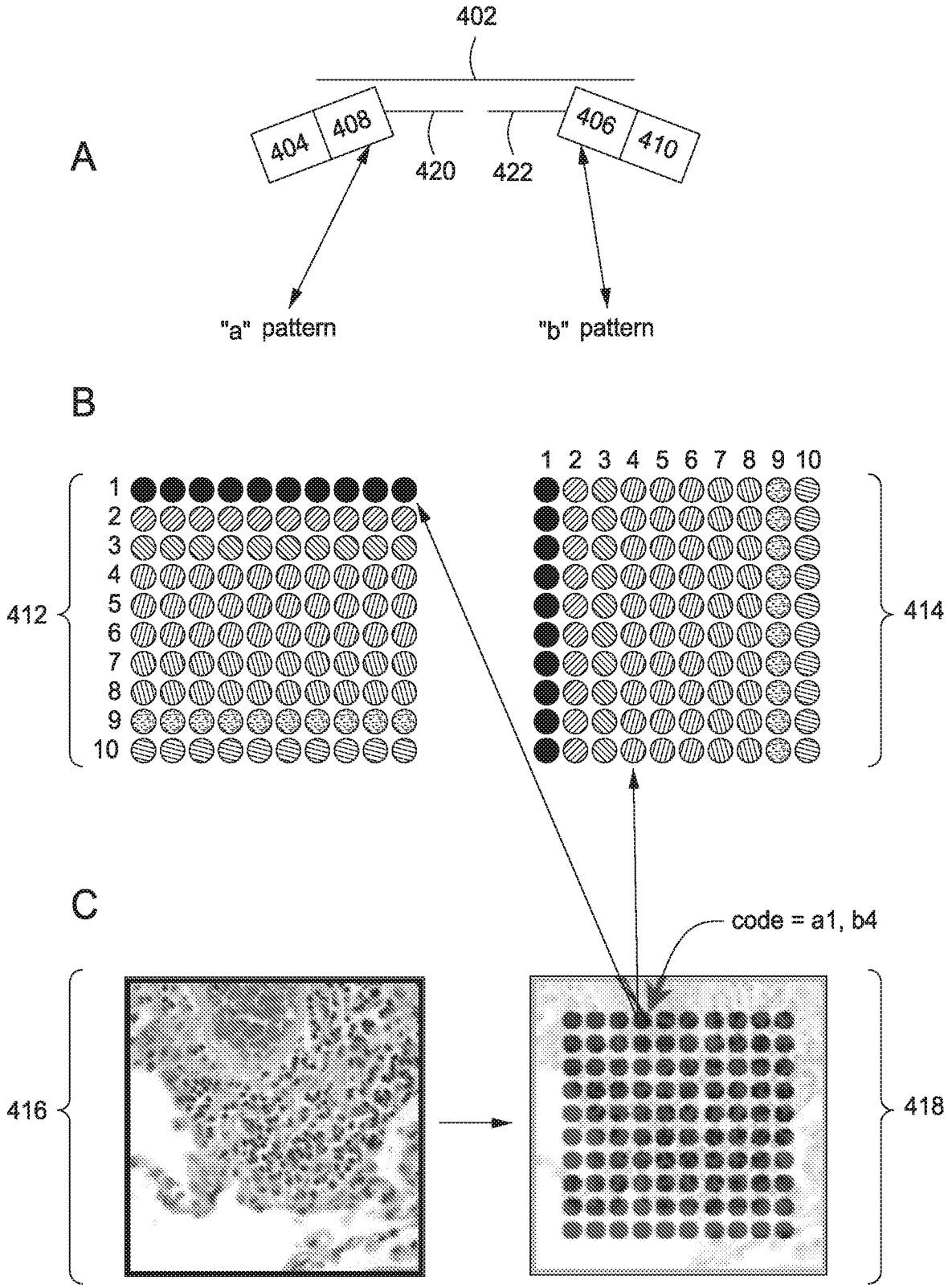
A
402
404
408
420
422
406
410
"a" pattern
"b" pattern
B
1 2 3 4 5 6 7 8 9 10
412
414
C
code = a1, b4
416
418

FIG. 4

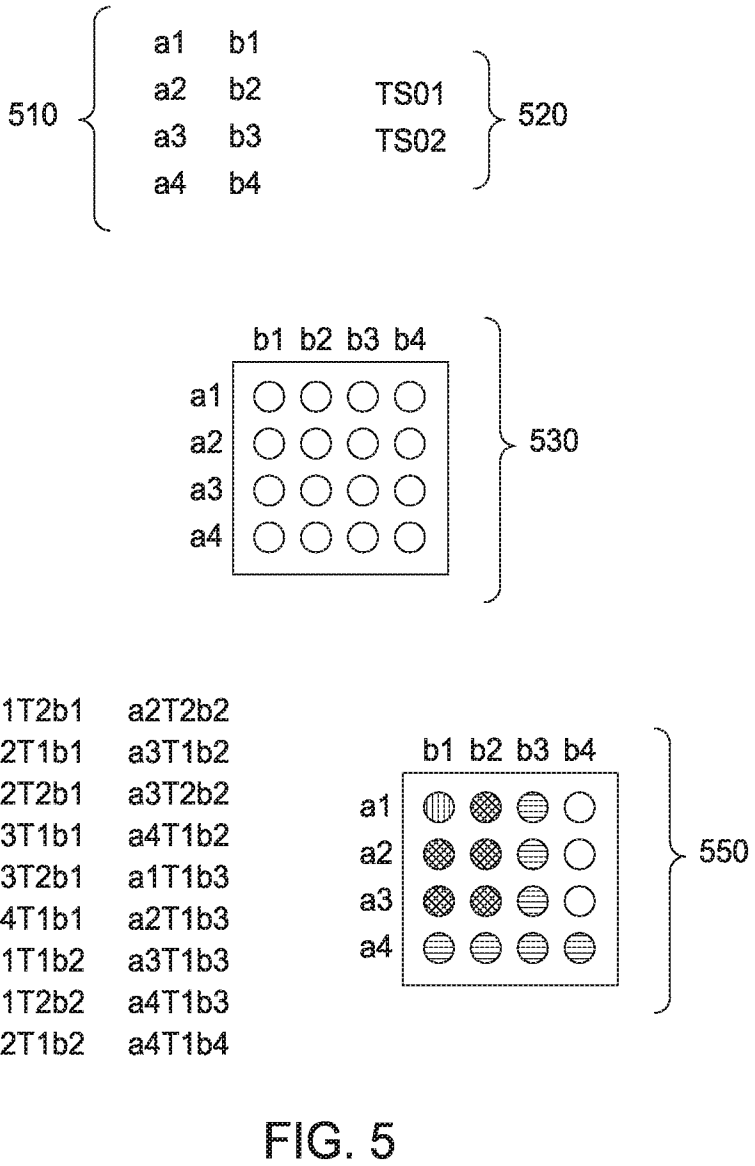
510
a1 b1
a2 b2
a3 b3
a4 b4
TS01
TS02
520
b1 b2 b3 b4
a1
a2
a3
a4
530
540
a1T2b1 a2T2b2
a2T1b1 a3T1b2
a2T2b1 a3T2b2
a3T1b1 a4T1b2
a3T2b1 a1T1b3
a4T1b1 a2T1b3
a1T1b2 a3T1b3
a1T2b2 a4T1b3
a2T1b2 a4T1b4
b1 b2 b3 b4
a1
a2
a3
a4
550

FIG. 5

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	SPATIALLY ENCODED BIOLOGICAL ASSAYS

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 15/187,661 filed on 06/20/2016 .

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Mark S. CHEE Date (Optional) : _____

Signature: Mark S. Chee

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

699932000201

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. Chee
Filer:	Peng Lin/Judith Wasilkus
Attorney Docket Number:	47706-0004003

Filed as Small Entity

Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY FILING FEE (ELECTRONIC FILING)	4011	1	75	75
UTILITY SEARCH FEE	2111	1	330	330
UTILITY EXAMINATION FEE	2311	1	380	380
REQUEST FOR PRIORITIZED EXAMINATION	2817	1	2000	2000
Pages:				
Claims:				
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	2830	1	70	70

Petition:	
Patent-Appeals-and-Interference:	
Post-Allowance-and-Post-Issuance:	
Extension-of-Time:	
Miscellaneous:	
Total in USD ($)	**2855**

Electronic Acknowledgement Receipt

EFS ID:	35155762
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. Chee
Customer Number:	159367
Filer:	Peng Lin/Alysha Claflin
Filer Authorized By:	Peng Lin
Attorney Docket Number:	47706-0004003
Receipt Date:	14-FEB-2019
Filing Date:	
Time Stamp:	17:05:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$2855
RAM confirmation Number	021519INTEFSW00006771061050
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	TrackOne Request	Request.pdf	146354 19e14b94a23346b2ad860b50c2d532f8d5c55bfe	no	2
Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	1256227 dfb88f7dd257eb82045b801295321b634f0b08e1	no	8
Warnings:					
Information:					
3	Transmittal of New Application	Transmittal.pdf	101969 579022a231a39346fb755d93531eb6cf797815d6	no	3
Warnings:					
Information:					
4		Application.pdf	3819140 92be832d8911a9713ed0b7de74a3583f98fd95b1	yes	36

Multipart Description/PDF files in .zip description

Document Description	Start	End
Abstract	36	36
Claims	35	35
Specification	1	34

Warnings:					
Information:					
5	Drawings-only black and white line drawings	Figures.pdf	824075 e243ea387014c5fc6866a44215eb6f6ca06db023	no	5

Warnings:					
Information:					
6	Oath or Declaration filed	Declaration.pdf	48408 3388bb26440da2ee37d5c40faa34f853b27bfa29	no	1
Warnings:					
Information:					
7	Fee Worksheet (SB06)	fee-info.pdf	40434 9614d94c01e54c786d38e753e17460340a666ed6	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	6236607	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (03-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Mark S. Chee	Non provisional Application Number (if known):	
Title of Invention:	Spatially Encoded Biological Assays		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims.

3. The applicable box is checked below:

I. ☒ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original non provisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original non provisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original non provisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Peng Lin/	Date February 14, 2019
Name (Print/Typed) Peng Lin	Practitioner Registration Number 74,999

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☒ *Total of 1 forms are submitted.

61846717.doc

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant *(i.e.,* GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	47706-0004003
	Application Number	
Title of Invention	Spatially Encoded Biological Assays	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Mark	S.	CHEE	

Residence Information (Select One) ● US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
San Diego	CA	US

Mailing Address of Inventor:

Address 1	11125 Flintkote Avenue
Address 2	Suite A

City	State/Province	Postal Code	Country
San Diego	CA	92121	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	159367		
Email Address	apsi@fr.com	Add Email	Remove Email

Application Information:

Title of the Invention	Spatially Encoded Biological Assays		
Attorney Docket Number	47706-0004003	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	5	**Suggested Figure for Publication (if any)**	

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	47706-0004003
	Application Number	
Title of Invention	Spatially Encoded Biological Assays	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	159367		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	15/187661	2016-06-20

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	47706-0004003
	Application Number	
Title of Invention	Spatially Encoded Biological Assays	

Prior Application Status	Patented	Remove

Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/187661	Continuation of	13/080616	2011-04-05	9371598	2016-06-21

Prior Application Status	Expired	Remove

Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13/080616	Claims benefit of provisional	61/321124	2010-04-05

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	47706-0004003
	Application Number	
Title of Invention	Spatially Encoded Biological Assays	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	47706-0004003
	Application Number	
Title of Invention	Spatially Encoded Biological Assays	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Prognosys Biosciences, Inc.		
Mailing Address Information For Applicant:			
Address 1	11125 Flintkote Avenue		
Address 2	Suite A		
City	San Diego	**State/Province**	CA
Country	US	Postal Code	92121
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	47706-0004003
	Application Number	
Title of Invention	Spatially Encoded Biological Assays	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

Organization Name	Prognosys Biosciences, Inc.

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1	11125 Flintkote Avenue		
Address 2	Suite A		
City	San Diego	**State/Province**	CA
Country i	US	Postal Code	92121
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Peng Lin/			Date (YYYY-MM-DD)	2019-02-14
First Name	Peng	Last Name	Lin	Registration Number	74999

Additional Signature may be generated within this form by selecting the Add button. Add

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	47706-0004003
	Application Number	
Title of Invention	Spatially Encoded Biological Assays	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Curio EX1010-p. 60
Curio v Prognosys
IPR2024-01289

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/276,235	02/14/2019	1636	785	47706-0004003	1	1

CONFIRMATION NO. 7944

FILING RECEIPT

159367
FISH & RICHARDSON P.C.
10x Genomics
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

OC000000106299323

Date Mailed: 03/06/2019

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Mark S. CHEE, San Diego, CA;

Applicant(s)

Prognosys Biosciences, Inc., San Diego, CA;

Assignment For Published Patent Application

Prognosys Biosciences, Inc., San Diego, CA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 15/187,661 06/20/2016
which is a CON of 13/080,616 04/05/2011 PAT 9371598
which claims benefit of 61/321,124 04/05/2010

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

Curio EX1010-p. 61
Curio v Prognosys
IPR2024-01289

If Required, Foreign Filing License Granted: 03/04/2019

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/276,235**

Projected Publication Date: 06/13/2019

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

Spatially Encoded Biological Assays

Preliminary Class

435

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

Curio EX1010-p. 62
Curio v Prognosys
IPR2024-01289

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/276,235

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	75		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	330		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	1 minus 20 =	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*	x 230 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	785		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/276,235	02/14/2019	Mark S. CHEE	47706-0004003	7944

159367 7590 03/14/2019

FISH & RICHARDSON P.C.
10x Genomics
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
MARTINELL, JAMES	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
03/14/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com
ipadmin@10xgenomics.com

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 16/276,235	**Applicant(s)** CHEE, Mark S.	
	Examiner CHERYL P GIBSON BAYLOR	**Art Unit** OPET	**AIA (First Inventor to File) Status** No

1. THE REQUEST FILED 14 February 2019 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

A. ☑ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;

C. filing a **request for continued examination** ;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to CHERYL GIBSON BAYLOR at (571)272-3213. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/CHERYL GIBSON BAYLOR/ Paralegal Specialist, OPET	

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/276,235	Filing Date 02/14/2019	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $50 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $230 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	03/15/2019	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0		x $50 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $230 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	SLIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/DEIDRE C KING-WOODWARD/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Mark S. Chee	Art Unit	:	1634
Application No.	:	16/276,235	Examiner	:	Martinell, James
Filed	:	February 14, 2019	Conf. No.	:	7944
Title	:	SPATIALLY ENCODED BIOLOGICAL ASSAYS			

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as indicated on the following pages.

Amendments to the Specification:

The present application claims priority to and incorporates by reference the entire text of U.S. Provisional Application Serial No. 61/321,124, filed April 5, 2010. The present specification has been amended herein to include text from U.S. Provisional Application Serial No. 61/321,124. A marked-up and clean copy of the substitute specification in accordance with 37 C.F.R. § 1.125 is provided herewith.

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

1. **(Canceled)**

2. **(New)** A method for determining a spatial location of a biological molecule of a tissue sample comprising

(a) providing a plurality of beads in a spatial pattern, 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 in the spatial pattern 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;

(c) using the coding identifier to identify the location in the spatial pattern where the binding agent interacted with the biological molecule, thereby determining a spatial location of the biological molecule of the tissue sample.

3. **(New)** The method of claim 2, wherein the plurality of beads is provided on a substrate surface.

4. **(New)** The method of claim 3, wherein the plurality of beads is provided in wells on the substrate surface.

5. **(New)** The method of claim 2, wherein the biological molecule is a nucleic acid.

6. (**New**) The method of claim 5, wherein the binding agent of the plurality of binding agents comprises a nucleic acid.

7. (**New**) The method of claim 6, wherein the binding agent interacts with the biological molecule via hybridization.

8. (**New**) The method of claim 2, wherein the binding agent and coding identifier are provided as a single molecule.

9. (**New**) The method of claim 8, wherein the binding agent and coding identifier are provided as a single molecule through nucleic acid synthesis.

10. (**New**) The method of claim 8, wherein the binding agent and coding identifier are provided as a ligation product.

11. (**New**) The method of claim 8, wherein the binding agent and coding identifier are provided as a single molecule through chemical coupling.

12. (**New**) The method of claim 2, 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).

13. (**New**) The method of claim 12, wherein the additional coding identifier corresponds to a location in the spatial pattern at which the additional binding agent of the plurality of binding agents interacts with said another biological molecule of the tissue sample.

14. (**New**) The method of claim 13, wherein the location in the spatial pattern at which the binding agent interacts with the biological molecule and the additional binding agent interacts with said another biological molecule is different.

15. (**New**) The method of claim 14, wherein the spatial location of the biological molecule and said another biological molecule of the tissue sample is different.

16. (**New**) The method of claim 2, wherein step (c) comprises determining the spatial locations of a plurality of biological molecules of the tissue sample simultaneously.

17. (**New**) The method of claim 2, wherein step (c) comprises determining presence or absence of the biological molecule at the location at which it is identified.

18. (**New**) The method of claim 2, wherein step (c) comprises determining the relative amount of the biological molecule at the location at which it is identified.

19. (**New**) The method of claim 2, wherein step (c) comprises determining all or a portion of the nucleic acid sequence of the coding identifier.

20. (**New**) The method of claim 2, wherein step (c) comprises sequencing all or a portion of the nucleic acid sequence of the coding identifier.

21. (**New**) The method of claim 2, wherein spatial organization of cells in the tissue sample is preserved.

22. (**New**) The method of claim 2, wherein the biological molecule of the biological sample is selected from a protein, a nucleic acid, a lipid, a carbohydrate, and an ion.

23. (**New**) The method of claim 22, wherein the biological molecule is part of a multicomponent complex.

24. (**New**) The method of claim 2, wherein the tissue sample is a fresh tissue sample.

25. (**New**) The method of claim 2, wherein the tissue sample is a preserved tissue sample.

26. (**New**) The method of claim 25, wherein the preserved tissue sample is a frozen tissue sample.

27. (**New**) The method of claim 26, wherein the preserved tissue sample is a paraformalin-fixed paraffin-embedded (FFPE) tissue sample.

28. (**New**) The method of claim 3, further comprising analyzing gene expression in the tissue sample.

29. (**New**) The method of claim 2, wherein the spatial pattern is a defined spatial pattern.

30. (**New**) The method of claim 3, wherein the location of the binding agent is known prior to step (b).

31. (**New**) The method of claim 3, wherein the binding agent is immobilized directly or indirectly onto a bead of the plurality of beads.

REMARKS

The present application claims priority to and incorporates by reference the entire text of U.S. Provisional Application Serial No. 61/321,124, filed April 5, 2010. The present specification has been amended herein to include text from U.S. Provisional Application Serial No. 61/321,124. A marked-up and clean copy of the substitute specification in accordance with 37 C.F.R. § 1.125 is provided herewith.

Claim 1 has been canceled without prejudice, and new claims 2-31 have been added herein. Applicant's specification fully supports the newly-added claims. Exemplary support is shown the table below:

Claim	Support – paragraphs of the substitute specification are indicated
2	[00101], [00102], [00111], [00112], [00123]
3	[00111]
4	[00111]
5	[00123]
6	[00112]
7	[0027]
8	[00124]
9	[00124]
10	[00124]
11	[00124]
12	[00101], [00102]
13	[00101], [00102]
14	[0050], [00101], [00102]
15	[0050], [00101], [00102]
16	[00102]
17	[00103]
18	[00104]
19	[0008]
20	[0014]
21	[00105]
22	[00122]
23	[00122]
24	[00124]
25	[00124]

26	[00124]
27	[00124]
28	[00128]
29	[00101]
30	[00111]
31	[00112]

No new matter has been added. Applicant reserves the right to pursue any canceled subject matter in future prosecution, either in this application or in one or more continuing applications.

Applicant asks that all claims be examined in view of the amendment to the claims.

Pease apply any necessary charges or credit any overpayments to Deposit Account 06-1050, referencing the above attorney docket number.

Respectfully submitted,

Date: March 15, 2019

/Cameron Luitjens/

Cameron M. Luitjens, Ph.D., J.D.
Reg. No. 58,674

Customer Number 159367
Fish & Richardson P.C.
Telephone: (612) 766-2006
Facsimile: (877) 769-7945

SPATIALLY ENCODED BIOLOGICAL ASSAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Patent Application No. 61/321,124, filed April 5, 2010 and is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention relates to assays of biological molecules, and more particularly to assays for determining spatial distributions of a large number of biological molecules in a solid sample simultaneously.

BACKGROUND OF THE INVENTION

[0003] In the following discussion certain articles and methods will be described for background and introductory purposes. Nothing contained herein is to be construed as an "admission" of prior art. Applicant expressly reserves the right to demonstrate, where appropriate, that the articles and methods referenced herein do not constitute prior art under the applicable statutory provisions.

[0004] Comprehensive gene expression analysis and protein analysis have been useful tools in understanding mechanisms of biology. Use of these tools has allowed the identification of genes and proteins involved in development and in various diseases such as cancer and autoimmune disease. Conventional 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. Other technologies that have enabled the quantitative analysis of many RNA sequences per sample include microarrays (see Shi, et al., Nature Biotechnology, 24(9):1151-61 (2006); and Slonim and Yanai, Plos Computational Biology, 5(10):e1000543 (2009)); serial analysis of gene expression (SAGE) (see Velculescu, et al, Science, 270(5235):484-87 (1995)), high-throughput implementations of qPCR (see Spurgeon, et al., Plos ONE, 3(2):e1662 (2008)) and *in situ* PCR (see Nuovo, Genome Res., 4:151-67 (1995)). As useful as these methods are, however, they do

not enable simultaneous measurement of the expression of many genes or the presence and/or activity of multiple proteins at many spatial locations in a sample. Laser capture microdissection has permitted the analysis of many genes at a small number of locations, but it is very expensive, laborious, and does not scale well. 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.

[0005] At present, no practical method exists to analyze at high resolution the spatial expression patterns of large numbers of genes, proteins, or other biologically active molecules simultaneously. There is thus a need for reproducible, high-resolution spatial maps of biological molecules in tissues. The present invention addresses this need.

SUMMARY OF THE INVENTION

[0006] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the following written Detailed Description including those aspects illustrated in the accompanying drawings and defined in the appended claims.

[0007] The invention encompasses assay systems that provide high-resolution spatial maps of biological activity in tissues. The assay system comprises an assay capable of high levels of multiplexing where encoded probes 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. In short, the present invention provides the ability to look at many biological targets in many locations, providing the resolution of in situ hybridization with the highly- parallel data analysis of sequencing.

[0008] Thus, in some embodiments, the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that

identifies a location of the site to which the encoded probe was delivered; allowing the encoded probes to interact with the biological targets; separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets; determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

[0009] In particular aspects of the invention the biological targets comprise nucleic acids and the encoded probes are oligonucleotides, and in some aspects, there are two encoded probes for each of the multiple nucleic acid targets. In some aspects, the multiple biological targets comprise proteins, the probe regions of the encoding probes are proteins and the coding tags comprise oligonucleotides. In some aspects the multiple biological targets comprise enzymes. In some aspects the probe regions of the encoded probes comprise antibodies, aptamers or small molecules.

[0010] Some aspects of the assay system further comprise an amplification step between the separating step and the determining step. In some aspects, the determining step is performed by nucleic acid sequencing, and in preferred aspects, the sequencing is high-throughput digital nucleic acid sequencing.

[0011] In some aspects of the invention, the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 20, in some aspects product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 50, in some aspects the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 75, 100, 150, 500, 750, 1,000, 5,000, 10,000, 25,000, 50,000, 100,000, 500,000, or 1,000,000 or more. In other aspects, the sequence of at least fifty thousand encoding probes are determined in parallel, in other aspects the sequence of at least one hundred thousand encoding probes are determined in parallel, in some aspects the sequence of at least five hundred thousand encoding probes are determined in parallel, and in some aspects the sequence of at least one million, ten million, one hundred million, one billion, ten billion, one hundred billion or more encoding probes are determined in parallel.

[0012] In some aspects, the known spatial pattern is determined by histological features of the sample. Also in some aspects, software programmed hardware performs at least two steps of the delivering step, the separation step, the determining step and the associating step.

[0013] In some aspects, the probe regions of the encoded probes are proteins and the

separating step is accomplished by encoded probes that interact with the biological targets being captured by an affinity capture agent. In some aspects the probe regions of the encoding probes are nucleic acids and the separating step is accomplished by a washing of the sample.

[0014] In other embodiments there is provided an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering oligonucleotide probes for multiple nucleic acid targets to the multiple sites in the sample in a known spatial pattern; allowing the oligonucleotide probes to hybridize with the nucleic acid targets; washing unhybridized encoded oligonucleotide probes from the sample; delivering one or more encoding agents to locations of the multiple sites in the sample according to a known spatial pattern, where the combination of encoding agents delivered to each site is different; coupling the encoding agents and the oligonucleotide probes to form encoded probes; determining all or a portion of a sequence of the encoded probes using high-throughput sequencing, and associating the abundance or activity or both of multiple biological targets to the locations of multiple sites in the sample.

[0015] Other embodiments of the invention provide an assay system to determine spatial patterns of abundance or activity or both of multiple protein targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple protein targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a protein probe region that may interact with the protein targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and the protein probe region of the encoding probe of which the coding tag is part; allowing the encoded probes to interact with the protein targets; separating encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets; determining all or a portion of a sequence of the encoded probes by high throughput sequencing, and associating the abundance or activity or both of the multiple protein targets to the locations of the multiple sites in the sample.

[0016] Other embodiments provide an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe

was delivered and identifies the biological target; allowing the encoded probes to interact with the biological targets; determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

[0017] The assay system of the invention can utilize various detection mechanisms, based on the molecules to be detected and the reagents needed for such detection system. Exemplary methods that can be used with the assay systems of the invention are described in more detail below.

DESCRIPTION OF THE FIGURES

[0018] Figure 1 provides a simplified overview of the assay system of the present invention.

[0019] Figure 2 provides a simplified overview of one embodiment of the assay system of the present invention for detecting nucleic acids.

[0020] Figure 3 is a representational depiction of one embodiment of the assay overviewed in Figure 2.

[0021] Figure 4 illustrates a general mechanism for one embodiment of a combinatorial encoding scheme of the assay systems of the invention.

[0022] Figure 5 provides a simplified, specific example of the embodiment of a combinatorial encoding scheme shown in Figure 4.

DEFINITIONS

[0023] The terms used herein are intended to have the plain and ordinary meaning as understood by those of ordinary skill in the art. The following definitions are intended to aid the reader in understanding the present invention, but are not intended to vary or otherwise limit the meaning of such terms unless specifically indicated.

[0024] The term "antibody" as used herein is intended to refer to an entire immunoglobulin or antibody or any functional fragment of an immunoglobulin molecule which is capable of specific binding to an antigen (antibodies and antigens are "binding partners" as defined herein). "Antibody" as used herein is meant to include the entire antibody as well as any antibody fragments capable of binding the antigen or antigenic fragment of interest. Examples of such peptides include complete antibody molecules, antibody fragments, such as Fab, F(ab')2, CDRS, VL, VH, and any other portion of an antibody which is capable of specifically binding to an antigen. Antibodies for assays of the invention are

immunoreactive or immunospecific for, and therefore specifically and selectively bind to, proteins either detected *(i.e.,* biological targets) or used for detection *(i.e.,* probes) in the assays of the invention.

.....**[0025]** The term "binding agent" as used herein refers to any agent that specifically binds

to a biological molecule of interest

[0026] "Complementary" or "substantially complementary" refers to the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, such as, for instance, between the two strands of a double-stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single-stranded nucleic acid. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single-stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the other strand, usually at least about 90% to about 95%, and even about 98% to about 100%).

[0027] "Hybridization" refers to the process in which two single-stranded polynucleotides bind non-covalently to form a stable double-stranded polynucleotide, The resulting (usually) double-stranded polynucleotide is a "hybrid" or "duplex," "Hybridization conditions" will typically include salt concentrations of approximately less than 1M, often less than about 500 mM and may be less than about 200 mM. A "hybridization buffer" is a buffered salt solution such as 5% SSPE, or other such buffers known in the art. Hybridization temperatures can be as low as 5°C, but are typically greater than 22°C, and more typically greater than about 30°C, and typically in excess of 37°C. Hybridizations are often performed under stringent conditions, *i.e.,* conditions under which a primer will hybridize to its target subsequence but will not hybridize to the other, non-complementary sequences. Stringent conditions are sequence-dependent and are different in different circumstances. For example, longer fragments may require higher hybridization temperatures for specific hybridization than short fragments. As other factors may affect the stringency of hybridization, including base composition and length of the complementary strands, presence of organic solvents, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one parameter alone. Generally stringent conditions are selected to be about 5°C lower than the T_m for the specific sequence at a defined ionic strength and pH. Exemplary stringent conditions include a salt concentration of at least 0.01 M to no more than 1M sodium ion concentration (or other salt) at a pH of about 7.0 to about 8.3 and a temperature of at least 25°C. For example, conditions of 5xSSPE (750 mM NaCl, 50 mM

sodium phosphate, 5 mM EDTA at pH 7.4) and a temperature of approximately 30°C are suitable for allele-specific hybridizations, though a suitable temperature depends on the length and/or GC content of the region hybridized.

[0028] "Ligation" means to form a covalent bond or linkage between the termini of two or more nucleic acids, *e.g.,* oligonucleotides and/or polynucleotides, in a template-driven reaction. The nature of the bond or linkage may vary widely and the ligation may be carried out enzymatically or chemically. As used herein, ligations are usually carried out enzymatically to form a phosphodiester linkage between a 5' carbon terminal nucleotide of one oligonucleotide with a 3' carbon of another nucleotide.

[0029] "Nucleic acid", "oligonucleotide", "oligo" or grammatical equivalents used herein refers generally to at least two nucleotides covalently linked together. A nucleic acid generally will contain phosphodiester bonds, although in some cases nucleic acid analogs may be included that have alternative backbones such as phosphoramidite, phosphorodithioate, or methylphophoroamidite linkages; or peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with bicyclic structures including locked nucleic acids, positive backbones, non-ionic backbones and non-ribose backbones. Modifications of the ribose-phosphate backbone may be done to increase the stability of the molecules; for example, PNA:DNA hybrids can exhibit higher stability in some environments.

[0030] "Primer" means an oligonucleotide, either natural or synthetic, that is capable, upon forming a duplex with a polynucleotide template, of acting as a point of initiation of nucleic acid synthesis and being extended from its 3' end along the template so that an extended duplex is formed. The sequence of nucleotides added during the extension process is determined by the sequence of the template polynucleotide. Primers usually are extended by a DNA polymerase.

[0031] The term "SNP" or "single nucleotide polymorphism" refers to a genetic variation between individuals; *e.g.,* a single nitrogenous base position in the DNA of organisms that is variable. SNPs are found across the genome; much of the genetic variation between individuals is due to variation at SNP loci, and often this genetic variation results in phenotypic variation between individuals. SNPs for use in the present invention and their respective alleles may be derived from any number of sources, such as public databases (U.C. Santa Cruz Human Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway) or the NCBI dbSNP website (http://www.ncbi.nlm.nih.gov/SNP/), or may be experimentally determined as described in U.S. Pat No. 6,969,589; and US Pub. No. 2006/0188875 entitled "Human Genomic Polymorphisms." Although the use of SNPs is described in some of the

embodiments presented herein, it will be understood that other biallelic or multi-allelic genetic markers may also be used. A biallelic genetic marker is one that has two polymorphic forms, or alleles. As mentioned above, for a biallelic genetic marker that is associated with a trait, the allele that is more abundant in the genetic composition of a case group as compared to a control group is termed the "associated allele," and the other allele may be referred to as the "unassociated allele." Thus, for each biallelic polymorphism that is associated with a given trait *(e.g.,* a disease or drug response), there is a corresponding associated allele. Other biallelic polymorphisms that may be used with the methods presented herein include, but are not limited to multinucleotide changes, insertions, deletions, and translocations. It will be further appreciated that references to DNA herein may include genomic DNA, mitochondrial DNA, episomal DNA, and/or derivatives of DNA such as amplicons, RNA transcripts, cDNA, DNA analogs, etc. The polymorphic loci that are screened in an association study may be in a diploid or a haploid state and, ideally, would be from sites across the genome.

[0032] The term "selectively binds", "selective binding" and the like as used herein, when referring to a binding partner *(e.g.*. protein, nucleic acid, antibody or other affinity capture agent, etc.), refers to a binding reaction of two or more binding partners with high affinity and/or complementarity to ensure selective hybridization under designated assay conditions. Typically, specific binding will be at least three times the standard deviation of the background signal. Thus, under designated conditions the binding partner binds to its particular "target" molecule and does not bind in a significant amount to other molecules present in the sample.

[0033] "Sequencing", "sequence determination" and the like means determination of information relating to the nucleotide base sequence of a nucleic acid. Such information may include the identification or determination of partial as well as full sequence information of the nucleic acid. Sequence information may be determined "with varying degrees of statistical reliability or confidence. In one aspect, the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, "High throughput digital sequencing" or "next generation sequencing" means sequence determination using methods that determine many (typically thousands to billions) of nucleic acid sequences in an intrinsically parallel manner, *i.e.* where DNA templates are prepared for sequencing not one at a time, but in a bulk process, and where many sequences are read out preferably in parallel, or alternatively using an ultra-high throughput serial process that itself may be parallelized. Such methods include but are not limited to pyrosequencing (for example, as commercialized by 454 Life Sciences, Inc., Branford, CT); sequencing by ligation (for example, as

commercialized in the SOLiD™ technology, Life Technology, Inc., Carlsbad, CA); sequencing by synthesis using modified nucleotides (such as commercialized in TruSeq™ and HiSeq™ technology by Illumina, Inc., San Diego, CA, HeliScope™ by Helicos Biosciences Corporation, Cambridge, MA, and PacBio RS by Pacific Biosciences of California, Inc., Menlo Park, CA), sequencing by ion detection technologies (Ion Torrent, Inc., South San Francisco, CA); sequencing of DNA nanoballs (Complete Genomics, Inc., Mountain View, CA); nanopore-based sequencing technologies (for example, as developed by Oxford Nanopore Technologies, LTD, Oxford, UK), and like highly parallelized sequencing methods.

[0034] The term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation, T_m=81.5+0.41 (% G+C), when a nucleic acid is in aqueous solution at 1M NaCl (see *e.g.,* Anderson and Young, Quantitative Filter Hybridization, in *Nucleic Acid Hybridization* (1985)). Other references *(e.g.,* Allawi and SantaLucia, Jr., Biochemistry, 36:10581-94 (1997)) include alternative methods of computation which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m.

DETAILED DESCRIPTION OF THE INVENTION

[0035] The practice of the techniques described herein may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology, which are within the skill of those who practice in the art. Such conventional techniques include polymer array synthesis, hybridization and ligation of polynucleotides, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the examples herein. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Green, et al., Eds., *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV) (1999); Weiner, Gabriel, Stephens, Eds., *Genetic Variation: A Laboratory Manual* (2007); Dieffenbach, Dveksler, Eds., *PCR Primer: A Laboratory Manual* (2003); Bowtell and Sambrook, *DNA Microarrays: A Molecular Cloning Manual* (2003); Mount, *Bioinformatics: Sequence and Genome Analysis* (2004); Sambrook and Russell, *Condensed Protocols from Molecular*

Cloning: A Laboratory Manual (2006); and Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (2002) (all from Cold Spring Harbor Laboratory Press); Stryer, *Biochemistry* (4th Ed.) (1995) W.H, Freeman, New York N.Y.; Gait, *"Oligonucleotide Synthesis: A Practical Approach"* (2002) IRL Press, London; Nelson and Cox, *Lehninger, Principles of Biochemistry* (2000) 3rd Ed., W. H. Freeman Pub., New York, N.Y.; and Berg, et al., *Biochemistry* (2002) 5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

[0036] Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" refers to one or more nucleic acids, and reference to "the assay" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

[0037] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing devices, formulations and methodologies that may be used in connection with the presently described invention.

[0038] Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention,

[0039] In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

The Invention in General

[0040] The assay systems of the invention provide spatially-encoded, multiplexed assays comprising 1) an assay capable of high levels of multiplexing with an efficient spatial

encoding scheme; 2) instrumentation capable of delivering reagents according to a spatial pattern; and 3) decoding determined by a readout that is digital in nature. The assay systems of the invention detect the presence or absence and relative amount of a biological target or biological activity indicative of a biological target, as well as the location of the biological target or activity in a biological sample, *e.g.,* a tissue section or other biological structure disposed upon a support such as a microscope slide or culture dish.

[0041] 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, allowing for measurement of numerous biological targets or activities in a meaningful spatial environment, including gene expression and peptide localization, An encoding scheme used in these assay systems allows one to determine the location of biological targets or activity (or lack thereof) in the biological samples after the products of the multiplexed assay are removed from the biological sample and pooled for analysis. Decoding of the encoding scheme can be performed by, *e.g.,* next-generation sequencing, which easily provides millions to trillions of data points at low cost. The assay results such as the amount or activity of biological targets can then be mapped back to specific location in the biological sample. The assay systems open a new analytical window into the complex spatial patterns of cellular function and regulation in biological samples.

[0042] A simplified overview of the assay system 100 of the present invention is provided at Figure 1, At step 110, a biological sample affixed to a support is provided. The biological sample contains biological targets of interest. Biological targets can include any molecule of interest, such as nucleic acids (including, *e.g,* RNA transcripts, genomic DNA sequences, cDNAs, amplicons, or other nucleic acid sequences) and proteins, enzymes and the like. At step 120, encoded probes are delivered to the biological sample according to a known spatial pattern. Encoded probes comprise probes, which can interact "with biological targets of interest, and coding tags, which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample. Coding tags in most embodiments are oligonucleotides. However, coding tags may also be mass tags, fluorescent labels, or other moieties.

[0043] In some embodiments, the probe and coding tag portions of the encoded probe are pre-coupled before being delivered to the biological sample, For example, in the case where the encoded probes are oligonucleotides, both the probe and coding tag sequence can be synthesized as a single oligonucleotide. Alternatively, the probe and coding tag portions of

the encoding probes can be synthesized or obtained separately and combined before delivery to the biological sample *(e.g.,* two separate oligonucleotides can be synthesized and coupled by, *e.g.,* ligation; or an antibody and an oligonucleotide can be prepared separately and conjugated before delivery to the biological sample). Also, as is described in Figures 2-5, the probes and the coding tags (in encoding oligonucleotides) are synthesized separately, and are delivered to the biological sample at different steps (*e.g.,* probes first and coding tags thereafter, or vice versa) in the assay.

[0044] At step 130, the encoded probes are allowed to react or interact with the biological targets, *i.e.,* conditions are provided to allow *e.g.,* o1igonucleotides to hybridize to nucleic acid targets, enzymes to catalyze reactions with protein targets, antibodies to bind epitopes, etc. In the case where the biological targets are nucleic acids, the encoded probes are typically oligonucleotides and hybridize to the target nucleic acids. In the case that the biological targets are proteins, the encoded probes typically are aptamers, small molecules, or oligonucleotide-conjugated proteins that interact with target proteins by binding to them or by reacting with them (that is, one of the proteins is a substrate for the other). Encoding oligonucleotides may be coupled to the probes (proteins) by conjugation, chemical or photo-crosslinking via suitable groups and the like.

[0045] Once encoded probes interact with the biological targets, the encoded probes that interacted with the biological targets must be separated from the encoded probes that did not interact with the biological targets at step 140. In the case where the biological targets are nucleic acids and the encoded probes are oligonucleotides, the separation can be accomplished by, *e.g.,* washing the unhybridized encoded probes from the sample. Similarly, for other assays that are based on affinity binding, including those using aptamer, small molecule, and protein probes, washing steps can be used to remove low affinity binders. In the case where the probe is transformed via interaction with the target, *e.g.,* in the case of a peptide, *e.g.,* via cleavage by a protease or phosphorylation by a kinase, it is convenient to collect, all encoded probes--both encoded probes that interacted with the biological targets and were transformed and encoded probes that were not transformed. After collection or pooling, an antibody or other affinity capture agent can be used to capture probes that were transformed by addition of a moiety (*e.g.,* a phosphate group). In cases where probes have been transformed via cleavage, the transformed probes can be separated, *e.g.,* by capturing the non-transformed probes via a tag that is removed from the transformed probes during the transformation *(e.g.,* by cleavage), or by adding a new tag at the site of cleavage.

[0046] Once the reacted (transformed) or interacted encoded probes are separated

from the unreacted or un-interacted encoded probes, the sequence of the reacted and/or interacted encoded probes is determined by, preferably, sequencing. The sequence of the encoded probes allows the mapping of the assay results back to locations in the biological sample.

[0047] Figure 2 provides a simplified overview of an assay system of the present invention embodying an efficient implementation of a combinatorial coding scheme for the encoding of spatial information. For purposes of this overview, the probes are oligonucleotides, but as explained elsewhere, other types of probes can also be used. In step 210, a biological sample affixed to a support, *e.g.,* a tissue sample or other biological structure, is provided. In step 220, one or more oligonucleotide probes are delivered to the biological sample, where the oligonucleotide probes are capable of hybridizing with biological targets in the biological sample. In step 230, the oligonucleotide probes are allowed to interact with (hybridize to) the nucleic acid targets; that is, appropriate conditions are provided where oligonucleotide probes can hybridize to the target nucleic acids.

[0048] In step 240, the oligonucleotide probes that did not hybridize to target nucleic acids are removed, and thereby separated from oligonucleotide probes that did hybridize to target nucleic acids. In this embodiment, separation can be accomplished by, *e.g.,* washing the sample to remove unhybridized oligonucleotide probes. Next, in step 250, encoding oligonucleotides (the encoding agents) are delivered to the biological sample according to a chosen spatial pattern, where the encoding oligonucleotides comprise coding tags that are used to encode the location of biological targets in the biological sample. Note that in contrast to the assay system of Figure 1, here the probes and encoding agents (encoding oligonucleotides) are delivered in separate steps. In step 260, the encoding oligonucleotides are coupled to the oligonucleotide probes to create encoded probes. In this case where the probes are oligonucleotides, the encoding oligonucleotides may be coupled to the oligonucleotides probes by, *e.g.,* ligation. Alternatively, the information in the encoding oligonucleotides can be transferred by using a DNA polymerase to extend a probe oligonucleotide that acts as a primer, and thereby copy and incorporate the sequence of the encoding oligonucleotides.

[0049] In step 270, the sequence of the coding tags in the encoded probes as well as the sequence or a portion of the sequence of the probe itself is determined, and in step 280, the target nucleic acids are mapped back to the biological sample. In some

embodiments, the abundance of sequences reveals the relative quantity of biological targets at the location. Although this embodiment shows the individual steps in a particular order, so as to better explain the invention, the precise order of the steps can be varied. For example, steps 220 and 250 can be combined, so that a mixture of the probes and encoding oligonucleotides is delivered according to a chosen spatial pattern. Coupling step 260 can then be carried out immediately after the combined steps 220 and 250, or concomitantly with them. In this case, step 240 would then occur after step 260. It can therefore be appreciated that the two key results of this series of steps, *i.e.,* the location-specific encoding of probe molecules and the separation of probe molecules based on their ability to interact with corresponding target molecules, can be accomplished with some flexibility in the implementation of the particular steps. Similarly, there is considerable flexibility in the design of the coding scheme. As described *infra,* the assays of the invention are particularly amenable to combinatorial methods.

[0050] Thus, the present invention provides an ability to look at many different biological targets in many locations, providing the resolution of in situ hybridization with the highly-parallel data analysis of sequencing. In some embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 20, in other embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 50, in other embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 100, greater than 500, 1,000, 10,000, 25,000, 100,000, 500,000, 1,000,000. It will be appreciated that, due to the spatial encoding dimension of the invention, even much larger numbers can be contemplated. For example, assaying l 0,000 targets per location x 10,000 locations would generate $1O^{'8}$ different assays, and even larger numbers than these can easily be contemplated, particularly if spatial locations with resolution on the order of that of single cells are utilized. Further, in embodiments where high-throughput digital sequencing is employed, the sequences of at least 1,000 encoding probes are typically determined in parallel. More typically, using a digital readout, it is desirable to obtain multiple sequence reads for each assay (defined by a probe and a spatial location code). It is desirable to obtain an average of at least 3 copies per assay, and more typically at least 10 or at least 30 copies per assay, depending on the design of the experiment and requirements of the assay. For a quantitative readout with suitable dynamic range, it may be desirable to obtain at least

1,000 reads per assay. Therefore, if 1,000,000 assays are carried out, the number of sequence reads may be 1 billion or more. With high-throughput digital sequencing, and allowing for redundancy, the sequence of at least 10,000 encoding probes are determined in parallel, or the sequence of at least 100,000, 500,000, 1,000,000, 10,000,000, 100,000,000, 1,000,000,000 or more encoding probes are determined in parallel.

Assays

[0051] The assay portion of the assay systems of the present invention comprise the following general steps: delivering probes and encoding agents where the encoding agents (in some embodiments pre-coupled to the probes) are delivered to the sample according to a known spatial pattern, allowing the probes to interact or react with biological targets in the sample, and, if the probes and encoding agents have not been pre- coupled, coupling the encoding agents to probes.

[0052] The 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, where the ability to tie an assayed biological target or activity back to the location within the biological sample is important. Exemplary biological samples include tissue sections (e.g., including whole animal sectioning and tissue biopsies), cell populations on slides or culture dishes, and the like. The assay systems of the invention are particularly advantageous in that they are compatible with numerous biological sample types, including fresh samples, such as primary tissue sections, and preserved samples including but not limited to frozen samples and paraformalin-fixed, paraffin-embedded (FFPE) samples. An important aspect of the assay systems of the invention is that the biological samples are immobilized on a substrate surface having discrete, independently measurable areas.

[0053] The biological targets to be detected can be any biological molecules including but not limited to proteins, nucleic acids, lipids, carbohydrates, ions, or multicomponent complexes containing any of the above. Examples of subcellular targets include organelles, e.g., mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplasts, endocytic vesicles, exocytic vesicles, vacuoles, lysosomes, etc.

[0054] In some particular embodiments, the assay system is used to analyze nucleic acids, *e.g.,* by genotyping, quantitation of DNA copy number or RNA transcripts, localization of particular transcripts within samples, and the like. Figure 3 illustrates an overall scheme for an exemplary assay for, *e.g.,* detecting single nucleotide polymorphisms (SNPs) that can be used with the assay system of the invention. In Figure 3, two oligonucleotide probes are provided. Each oligonucleotide probe comprises a target-specific region (located on either side of the SNP to be analyzed) seen at 305 and 307, and ligation regions, seen at 301 and 303, The oligonucleotide probes are allowed to hybridize to a target nucleic acid (not shown) in the biological sample. At step 302, one of the oligonucleotide probes is extended to incorporate the SNP sequence and ligated to the other probe to form an extended probe comprising target nucleic acid region 309 and ligation regions 301 and 303.

[0055] Two encoding agents, both comprising a coding tag (seen at 315 and 317), a ligation region (seen at 311 and 313), and a primer region (seen at 319 and 321) are combined with and ligated to the extended probe at step 304 to form an encoded target- specific oligonucleotide. Again, in contrast with Figure 1, the probes and encoding agents are delivered at separate steps. Doing so allows use of the combinatorial embodiments described *infra*. In preferred embodiments, the encoding oligonucleotides within a pair of encoding oligonucleotides ligate specifically to one side of the target sequence or the other *(i.e., 5'* or 3' of the target sequence). Also, typically, the ligation and primer regions of the encoding oligonucleotides and probes are universal; that is, the set of ligation and primer regions used in constructing the probes and encoding oligonucleotides are constant, and only the target-specific regions of the probes and the coding tags of the encoding oligonucleotides differ. However, again in alternative embodiments, the ligation and primer regions are not universal and differ between probes and encoding agents.

[0056] Following ligation, the encoded probes are eluted, pooled, and, optionally, sequencing adapters are added to the encoded probes via PCR. In alternative embodiments, sequencing primers may be ligated to the encoding oligonucleotides, or sequencing primer sequences can be included as part of the encoding oligonucleotide. As seen in Figure 3, each sequencing adapter comprises primer region 319 or 321, compatible with the primer regions 319 and 321 on the encoded probes. The final construct comprising first adapter 327, first primer region 319, first coding tag 315, ligation regions 311 and 301, target region 309, ligation regions 313 and 303, second coding tag 317, second primer region 325 and second adapter 329 is now ready for input into a digital high-throughput sequencing process.

[0057] A combination of extension and ligation reactions are exemplified in Figure 3, but it should be appreciated that a variety of reactions may be used to couple the encoding oligonucleotides to the target-specific oligonucleotides, including ligation only (e.g., for oligonucleotides that hybridize to contiguous portions of the target nucleic acid sequence). Alternatively, an assay utilizing an additional oligonucleotide, such as in the GOLDENGATE® assay (see Fan, et al., Cold Spring Symp. Quant. Biol., 68:69-78 (2003); (Ilumina, Inc., San Diego, CA)), may be employed.

[0058] In other embodiments, the assay system of the invention also can be used to analyze peptides or proteins, the presence of antibodies, enzymatic and other protein activities, posttranslational modifications, active and non-active forms of peptides, as well as peptide isoforms in a biological sample. Accordingly, the probes may comprise an active region of an enzyme, a binding domain of an immunoglobulin, defined domains of proteins, whole proteins, synthetic peptides, peptides with introduced mutations, aptamers and the like.

[0059] In certain aspects, the probes are substrates for enzymes or proenzymes, *e.g.,* kinases, phosphatases, zymogens, proteases, or fragments thereof. In certain aspects, the probes are phosphorylation substrates used to detect proteins involved in one or more signal transduction pathways, *e.g.,* a kinase or a phosphatase. In another specific aspect of the invention, the probes are specific protease substrates that associate only with individual proteases or classes of proteases. In other aspects, the probes are different processed forms, isoforms and/or domains of an enzyme. Protein-based probes are typically conjugated or otherwise linked to oligonucleotide encoding agents. The oligonucleotide encoding agents in this case would also include a nucleotide sequence component that allows for identification of the protein probe.

[0060] In certain aspects, the present invention provides assays for evaluating differences in the amount and/or activity of biological targets between different locations in a sample and/or between samples. The method includes determining a plurality of encoded results from the biological sample and evaluating the differences in quantity of the biological targets at each location in the biological sample.

Combinatorial Embodiments

[0061] To maximize the efficiency of encoding, a combinatorial approach using pairs of coding tags in the encoding oligonucleotides can be used. By de-coupling the target-specific information and the coding tags, the number of oligonucleotides required is dramatically reduced, with a concomitant decrease in cost.

[0062] Figure 4 illustrates a general mechanism for one embodiment of a combinatorial encoding scheme of the assay systems of the invention, where nucleic acids in a representative tissue section (shown at 416) are assayed. Figure 4 at A shows two target-specific/encoding oligonucleotide constructs 420 and 422 *(e.g.,* formed between steps 302 and 304 of Figure 3) specifically bound to a target nucleic acid 402 of interest, The first encoded probe 420 comprises coding tag 408, associated with, *e.g.,* a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 404. The second encoded probe 422 comprises coding tag 406, associated with, *e.g.,* a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410.

[0063] Figure 4 at B shows the spatial pattern that may be used for twenty different coding tags, al through a10 (coding tag 406 on encoded probe 420) and b1 through b10

(coding tag 408 encoded probe 422). Coding tag al., for example, is deposited on the biological sample in ten discrete areas or spots (shown as the first horizontal line of spots in 412). Coding tag a2 is deposited on the biological sample in ten spots on the second horizontal line in 412. Coding tag a3 is deposited on the biological sample in ten spots on the third horizontal line in 412, and so on. Whereas the "a" tags are deposited in ten horizontal rows, the "b" tags are deposited in ten vertical rows as shown in 414. For example, coding tag b1 is deposited on the biological sample in ten discrete spots in the first vertical row of 414, coding tag b2 is deposited on the biological sample in ten discrete spots in the second vertical row of 414, and so on. Using such a configuration allows for twenty coding tags to uniquely define 100 different locations on the biological sample,

[0064] Figure 4 at C shows a representative tissue section 416 coincident with coding tag grid 418. The arrows show how the "a" coding tags and the "b" coding tags are deposited on grid 418 that is coincident with tissue section 416. If, once sequenced, coding tags al and b4, *e.g.,* are associated with a target nucleic acid sequence, then that target nucleic acid sequence *(i.e.,* biological target) was present in the tissue section at location al, b4.

[0065] Figure 5 provides a simplified, specific example of the encoding scheme of the assay systems of the invention. Figure 5 shows encoding oligonucleotides 510, comprising al, a2, a3, a4 and b1, b3, b3 and b4. Target-specific oligonucleotides (TSOs) (probes) 1 and 2 are shown at 520. A deposit or dispensing scheme is shown at 530. Like the grid exemplified in Figure 4, encoding oligonucleotides al through a4 are deposited in spots in a pattern (here, in a vertical pattern), and encoding oligonucleotides bl through b4 are deposited in spots in a pattern (here, a horizontal pattern). The grid though shown as a square with spots is actually a deposition pattern on a biological sample (not shown) such as tissue section 416 shown in Figure 4.

[0066] The target-specific oligonucleotides are delivered to the biological sample, where the target-specific oligonucleotides hybridize to target nucleic acids in the biological sample if target nucleic acids are present. Unhybridized target-specific oligonucleotides are then removed, *e.g.,* by washing. The encoding oligonucleotides are then delivered to the biological sample according to the spatial pattern shown at 530. The encoding oligonucleotides are ligated (or, *e.g.,* extended and ligated) to any target-specific oligonucleotides that hybridized to the target nucleic acid in the biological sample, the ligated constructs are then eluted from the biological sample, pooled, and sequencing adapters are added through, *e.g.,* PCR or ligation, if the sequences were not previously included in the encoding oligonucleotides. The ligated constructs are sequenced by, *e.g.,* high throughput or

"next generation" sequencing.

[0067] The pool of resulting sequences is shown at 540. A sequence readout was obtained for target-specific oligonucleotide 1 only at a4bl, a4b2, alb3, a2b3, a3b3, a4b3 and a4b4 (positions shown with horizontal lines). A sequence readout was obtained for target-specific oligonucleotide 2 only at a1b1 (position shown with vertical lines). A sequence readout was obtained for both target-specific oligonucleotides 1 and 2 at positions a2b1, a3b1, a1b2, a2b2, and a3b2 (positions shown with cross-hatching). No sequence readout was obtained for either target-specific oligonucleotides at alb4, a2b4 or a3b4 (positions shown without shading). Thus, in the biological sample on which the assay took place the first target nucleic acid was detected in a large portion of the left side and at the bottom of the biological sample, the second target nucleic acid was detected only in the upper left portion of the biological sample, and neither target nucleic acid was detected in the upper right portion of the biological sample. The differential expression of the two target nucleic acids now can be mapped back to the biological sample and to the biological structures or cell types in these locations in the biological sample.

[0068] In addition to location information, information relating to relative abundance of the encoded tags can be obtained. For example, if it is found that there are ten times as many a4T1b1 sequences occurring in the data set as compared to a4T1b2 sequences, this would indicate that target nucleic acid sequence 1 is ten times more abundant at the a4T1b1 location than at the a4T1b2 location.

[0069] In the case of nucleotide analysis as shown in Figure 3, by ligating the coding tags directly to target-specific oligonucleotides, only 2n target-specific oligonucleotides are needed for n targets. For example, using the combinatorial approach outlined in Figure 2, assaying 100 different targets at 10,000 spatial locations would require 2 x 100 target-specific oligonucleotides and 2 x 100 encoding oligonucleotides. The total count of assay oligonucleotides would be only 400 (200 target-specific and 200 encoding), not counting universal primers. In contrast, if the coding oligonucleotides were not decoupled from the target-specific o1igonucleotides, (n x X positional codes) + (n x Y positional codes) would be needed, or in the above example, 20,000 oligonucleotides, not counting universal primer sequences. Moreover, though the embodiments shown in Figures 2- 5 depict a combinatorial scheme using two encoding agents (coding tags), three, four or more encoding agents and coding tags may be used, and attached to the probe or one another by varying means and in varying combinations of steps.

[0070] Due to the spatial encoding aspect of the assay system of the invention, a large amount of information can be generated with even a modest number of assays. For example, five or more biological targets assayed at five or more positions in the sample generates 25 or more combinations. Using digital sequencing as a readout, the optimum number of sequence reads per combination depends on the sensitivity and dynamic range required, and can be adjusted, For example, if for each combination on average 100 reads are sampled, the total for 25 combination is 25,000 reads. If 1,000 targets are assayed at 1,000 locations with an average sampling depth of 1,000, then 10^9 reads are required. These numbers, although large, are within the capacity of intrinsically parallel digital sequencing methods, which can generate datasets of billions or even trillions of reads in a reasonable timeframe and at a very low cost per read. Therefore, by varying the numbers of positions interrogated or biological targets assayed, or both, and using digital sequencing, large amounts of information can be obtained. In specific aspects, multiple locations are interrogated for two or more biological molecules.

Reagent Delivery Systems

[0071] The reagent delivery system of the invention includes instrumentation that allows the delivery of reagents to discrete portions of the biological sample, maintaining the integrity of the spatial patterns of the encoding scheme. Reagent delivery systems of the assay systems of the invention comprise optional imaging means, reagent delivery hardware and control software. Reagent delivery can be achieved in a number of different ways. It should be noted that reagent delivery may be to many different biological samples at one time. A single tissue section has been exemplified herein; however, multiple biological samples may be affixed and analyzed simultaneously. For example, serial sections of a tissue sample can be analyzed in parallel and the data combined to build a 3D map.

[0072] Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents onto the biological sample. Technologies for formulating and delivering both biological molecules *(e.g.* oligonucleotides or antibodies) and chemical reagents (e.g., small molecules or dNTPs) are known in the art, and uses of these instrument systems are known to one skilled in the art and easily adaptable to the assay systems of the invention. One example of a suitable reagent delivery system is the Labcyte™ Echo acoustic liquid handier, which can he used to deliver nanoliter scale

droplets containing biological molecules with high precision and reproducibility. One skilled in the art could incorporate this reagent delivery device into the overall system, using software to specify the locations to which reagents should be delivered.

[0073] Other instruments that can be used for the deposition of agents and/or coding identifiers onto biological samples include, but are not limited to, ink jet spotting; mechanical spotting by means of pin, pen or capillary; micro contact printing; photochemical or photolithographic methods; and the like. For several applications, it may be preferred to segment or sequester certain areas of the biological samples into one or more assay areas for different reagent distributions and/or biological target determination. The assay areas may be physically separated using barriers or channels.

[0074] In one exemplary aspect, the reagent delivery system may be a flow-based system. The flow-based systems for reagent delivery in the present invention can include instrumentation such as one or more pumps, valves, fluid reservoirs, channels, and/or reagent storage cells. Reagent delivery systems are configured to move fluid to contact a discrete section of the biological sample. Movement of the reagents can be driven by a pump disposed, for example, downstream of the fluid reagents. The pump can drive each fluid reagent to (and past) the reaction compartment. Alternatively, reagents may be driven through the fluid by gravity. US Pub. Nos. 20070166725 and 20050239192 disclose certain general-purpose fluidics tools that can be used with the assay systems of the invention, allowing for the precise manipulation of gases, liquids and solids to accomplish very complex analytical manipulations with relatively simple hardware.

[0075] In a more specific example, one or more flow-cells can be attached to the substrate-affixed biological sample from above. The flow-cell can include inlet and outlet tubes connected thereto and optionally an external pump is used to deliver reagents to the flow-cell and across the biological sample. The flow cells are configured to deliver reagents only to certain portions of the biological sample, restricting the amount and type of reagent delivered to any specific section of the biological sample.

[0076] In another aspect, a microfluidic system can be integrated into the substrate upon which the biological sample is disposed or externally attached on top of the substrate. Microfluidic passages for holding and carrying fluid may be formed on and/or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs.

[0077] Pumps generally include any mechanism for moving fluid and/or reagents

disposed in fluid. In some examples, the pump can be configured to move fluid and/or reagents through passages with small volumes *(i.e.,* microfluidic structures). The pump can operate mechanically by exerting a positive or negative pressure on fluid and/or on a structure carrying fluid, electrically by appropriate application of an electric field(s), or both, among other means. Exemplary mechanical pumps may include syringe pumps, peristaltic pumps, rotary pumps, pressurized gas, pipettors, etc. Mechanical pumps may be micromachined, molded, etc. Exemplary electrical pumps may include electrodes and may operate by electrophoresis, electroendoosmosis, electrocapillarity, dielectrophoresis (including traveling wave forms thereof), and/or the like.

[0078] Valves generally include any mechanism for regulating the passage of fluid through a channel. Valves can include, for example, deformable members that can be selectively deformed to partially or completely close a channel, a movable projection that can be selectively extended into a channel to partially or completely block a channel, an electrocapillary structure, and/or the like.

[0079] An open gasket can be attached to the top of the biological sample and the sample and reagents can be injected into the gasket. Suitable gasket materials include, but are not limited to, neoprene, nitrile, and silicone rubber. Alternatively, a watertight reaction chamber may be formed by a gasket sandwiched between the biological sample on the substrate and a chemically inert, water resistant material such as, but not limited to, black-anodized aluminum, thermoplastics *(e.g.,* polystyrene, polycarbonate, etc), glass, etc.

[0080] In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest. The images obtained, *e.g.,* may be used to design the deposition pattern of the reagents, Imaging means are optional, as an individual can instead view the biological sample using, *e.g.,* a microscope, analyze the organization of the biological sample, and specify a spatial pattern for delivery assay reagents. If included, the delivery system can comprise a microcircuit arrangement including an imager, such as a CCD or IGFET-based *(e.g.,* CMOS-based) imager and an ultrasonic sprayer for reagent delivery such as described in US Pub. No. 20090197326, which is incorporated herein by reference. Also, it should be noted that although Figures 4 and 5 illustrate using a x,y grid configuration, other configurations can be used, such as, *e.g.,* following the topology of a tissue sample; targeting certain groups of cells, cell layers and/or cell types in a tissue, and the like.

[0081] In yet another alternative, the reagent delivery system controls the delivery of reagents to specific patterns on a biological sample surface using semiconductor techniques

such as masking and spraying. Specific areas of a biological sample can be protected from exposure to reagents through use of a mask to protect specific areas from exposure. The reagents may be introduced to the biological sample using conventional techniques such as spraying or fluid flow. The use of masked delivery results in a patterned delivery scheme on the substrate surface.

[0082] In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology. There are a variety of different ink-jetting mechanisms (*e.g.,* thermal, piezoelectric) and compatibility has been shown with aqueous and organic ink formulations. Sets of independently actuated nozzles can be used to deliver multiple reagents at the same time, and very high resolutions are be achieved.

[0083] In order to target specific sites of interest, an informative image of the biological sample to be assayed may be used to assist in the reagent delivery methods and associated encoding scheme. Sample regions of the biological sample can be identified using image processing (e.g., images of cell types differentiated by immunohistochemistry or other staining chemistries) integrated with other features of the assay system. In some aspects, software is used to automatically translate image information into a reagent delivery pattern. A mechanism to register and align very precisely the biological sample for reagent delivery is thus an important component of the assay systems of the invention. Mechanisms such as the use of fiducial markers on slides and/or other very accurate physical positioning systems can be adapted to this purpose.

[0084] The invention preferably comprises a complete suite of software tailored to the assay system. Optionally, oligonucleotide design software is used to design the encoding nucleotides (and in embodiments where nucleic acids are assayed, the target-specific oligonucleotides) for the specific assay to be run, and may be integrated as a part of the system. Also optionally, algorithms and software for reagent delivery and data analysis (i.e., sequence analysis) may be integrated to determine assay results. Integrated data analysis is particularly useful, as the type of dataset that is generated may be massive as a consequence of scale. Algorithms and software tools that are specifically designed for analysis of the spatially-associated data generated by the assay systems, including pattern-analysis software and visualization tools, enhance the value of the data generated by the assay systems.

[0085] In certain aspects, the assay system comprises processes for making and carrying out the quality control of reagents, *e.g.,* the integrity and sequence fidelity of oligonucleotide pools. In particular, reagents are formulated according to factors such as volatility, stability at key temperatures, and chemical compatibility for compatibility with the

reagent delivery instrumentation and may be analyzed by instrumentation integrated within the assay system.

Sequencing

[008[[5]]6] Numerous methods can be used to identify the coding tags and probe sequences in the encoded probes of the assay systems of the invention. The coding tags can be detected using techniques such as mass spectroscopy *(e.g.,* Maldi-T of, LC-MS/MS), nuclear magnetic resonance imaging, or, preferably, nucleic acid sequencing. Examples of techniques for decoding the coding tags of the present invention can be found, for example, in US Pub. No. 20080220434, which is incorporated herein by reference. For example, the coding tags may be oligonucleotide mass tags (OMTs or massTags). Such tags are described, *e.g.,* in US Pub. No. 20090305237, which is incorporated by reference in its entirety. In yet another alternative, the encoded probes can be amplified and hybridized to a microarray. This would require separate amplification reactions to be carried out, in which each amplification is specific to a particular spatial code or subset of codes, accomplished by using code-specific primers. Each amplification would also incorporate a different resolvable label (e.g. fluorophor). Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can he determined by the relative abundances of the resolvable labels.

[008[[6]]7] In one particularly preferred aspect, the resulting coding tags according to the assay system are substrates for high-throughput, next-generation sequencing, and highly parallel next-generation sequencing methods are used to confirm the sequence of the coding tags, for example, with SOLiD™ technology (Life Technologies, Inc.) or Genome Ananlyzer (Illumina, Inc.). Such next-generation sequencing methods can be carried out, for example, using a one pass sequencing method or using paired-end sequencing. Next generation sequencing methods include, but are not limited to, hybridization-based methods, such as disclosed in *e.g.,* Drmanac, U.S. Pat. Nos. 6,864,052; 6,309,824; and 6,401,267; and Drmanac et al, U.S. patent publication 2005/0191656; sequencing-by-synthesis methods, *e.g.,* U.S. Pat Nos. 6,210,891; 6,828,100; 6,969,488; 6,897,023; 6,833,246; 6,911,345; 6,787,308; 7,297,518; 7,462,449 and 7,501,245; US Publication Application Nos. 20110059436; 20040106110; 20030064398; and 20030022207; Ronaghi, et al, Science, 281: 363-365 (1998); and Li, et al, Proc. Natl. Acad. Sci., 100: 414-419 (2003); ligation-based methods, *e.g.,* U.S, Patent Nos. 5,912,148 and 6,130,073; and U.S. Pat. Appln Nos. 20100105052, 20070207482 and 20090018024; nanopore sequencing *e.g.,* U.S. Pat. Appln Nos.

20070036511; 20080032301; 20080128627; 20090082212; and Soni and Meller, Clin Chem 53: 1996-2001 (2007)), as well as other methods, *e.g.,* U.S. Pat. Appln Nos. 20110033854; 20090264299; 20090155781; and 20090005252; also, see, McKernan, et al., Genome Res., 19:1527-41 (2009) and Bentley, et al., Nature 456:53-59 (2008), all of which are incorporated herein in their entirety for all purposes.

Applications of Assay System

[[[0087]]0088] It will be apparent to one skilled in the art upon reading the present disclosure that there are numerous important areas of biological research, diagnostics, and drug development that will benefit from a high throughput multiplexed assay system that can measure simultaneously the amount and spatial location of a biological target in a biological sample. For example, combining the ability to estimate the relative abundance of different RNA transcripts with the ability to reconstruct an image of spatial patterns of abundance across many locations, which may be as small as or even smaller than individual cells, in a tissue enables many different areas of basic research. The following are exemplary uses and are by no means meant to be limiting in scope.

[[[0088]]0089] In one example, 3-dimensional patterns of gene expression are determined by analyzing a series of tissue sections, in a manner analogous to image reconstruction in CT scanning, Such a method can be used to measure changes in gene expression in disease pathology, *e.g.,* in cancerous tissue and/or a tissue upon injury, inflammation or infection. With the assay systems of the invention, more detailed information on gene expression and protein localization in complex tissues is obtained, leading to new insights into the function and regulation both in normal and diseased states, and provides new hypotheses that can be tested. For example, an assay system of the invention may enable some of the insights gained from many individual studies and larger programs like ENCODE (Birney, et al., Nature, 447:799-816 (2007)) and modENCODE to be integrated at the tissue level. The assay systems also aid computational efforts to model interacting networks of gene expression in the field of systems biology.

[[[0089]]0090] The assay systems also provide a novel approach to analysis of somatic variation, *e.g.,* somatic mutations in cancer or variability in response to infectious organisms. For example, tumors are typically highly heterogeneous, containing cancer cells as well as genetically normal cells in an abnormal local environment. Cancer cells undergo mutation and selection, and in this process it is not unusual for local clones to develop.

Identifying relatively rare somatic mutations in the context of tumors may enable the study of the role of key mutations in the selection of clonal variants. Transcriptional patterns associated with angiogenesis, inflammation, or other cancer-related processes in both cancer and genetically normal cells can be analyzed for insights into cancer biology and assist in the development of new therapeutic agents for the treatment of cancers. In another example, individuals have varying susceptibility to infectious organisms, and the assay systems of the invention can be used to study the interaction between microbes and tissues or the various cell types within the tissue.

[[[0090]]0[0100] Importantly, in addition to providing spatially-associated information, the invention allows a great increase in the sensitivity of detecting rare mutations, as signal to noise can he dramatically increased since only a small location is assayed in any given reaction. In a typical assay for rare mutations in a mixed sample, the sample is treated in bulk, *i.e.,* nucleic acids are extracted from many cells into a single pool. Thus, if a mutation is present in one cell in 10,000, it must be detected against a background of normal DNA from ~10,000 cells. In contrast, with the assay systems of the invention many cells can be analyzed, but individual cells or small groups of cells would be identified by the spatial coding system. Therefore, in the assay systems of the present invention, background is reduced by orders of magnitude, greatly increasing sensitivity. Furthermore, the spatial organization of mutant cells can be observed, which may be particularly important in detecting key mutations in tissue sections in cancer. Already molecular histological analyses are yielding insights into cancer biology and may have potential for use in diagnostics. The technology of the invention promises to greatly increase the power of such approaches.

~~[0091]~~ [0[0101] (New) The present invention provides assays, assay systems, and methods of using such assays in spatially encoded biological assays. The invention provides an assay system comprising one or more agents provided in defined spatial patterns on a substrate surface, and a detection system for identifying the presence or absence, relative amount, and location of a biological molecule. Such biological molecules include, but are not limited to, nucleic acids, peptides, carbohydrates, cellular components, and the like. The assay system is a novel multiplexing approach, as it allows multiple molecules and their respective multiple locations to be identified in a single system using a unique encoding scheme. This encoding scheme uses both molecule-specific binding agents and coding identifiers to provide a practical and cost-effective determination of information on multiple biological molecules, including specific positional information of such molecules in a

Formatted: Indent: Left: 0.51", First line: 0.44", No bullets or numbering

biological sample, e.g., a tissue section. The single molecule detection analysis using the encoding system also allows relative amounts of biological molecules to be detected, thus providing information on expression levels, sequestering in specific locales, and the like.

~~[00104]~~ [00102] (New) The assay systems detect the presence or absence, and relative amount, of a biological molecule at more than one spatial location in a sample. In addition, the assays provide methods for doing this for multiple biological molecules simultaneously. The assay systems utilize one or more binding agents that specifically bind to the biological molecule of interest and unique coding identifiers associated with specific binding agents. The detection system utilizes a method for identifying the presence and spatial address of the agent binding based on the positive and/or negative results that are obtained using detection of the agent and identifier and the encoding scheme of the spatial patterns on the substrate surface. In a specific aspect, the encoding scheme employs limited reagent delivery to the spatial patterns on the substrate surface, and access of the coding identifiers and/or binding agents to portions of the sample is controlled through such limited delivery.

[00103] In one aspect, the assay system detects the presence or absence and spatial location of a biological molecule based on the positive and/or negative results that are obtained using limited reagent delivery and the encoding scheme of the spatial patterns on the substrate surface.

[00104] The assay system and methods of the invention are based on relational, solid-state substrates with positions that represent specific spatial locations within a biological sample, e.g., a cell, organelle or tissue. The ability to use encoding features to represent locations allows high-throughput analysis of the presence or absence, and relative amount, of a biological molecule at more than one spatial location in a sample. The encoding features also allow provide assaying of multiple biological molecules at these multiple locations simultaneously.

[00105] A primary feature of the invention is the preservation of the spatial organization of elements in a sample of interest through the use of an encoding scheme. For example, the assay may be designed to preserve the relative position of cells in a tissue, and the assay may interrogate the individual cells for genomic DNA variation (including epigenetic modifications), and RNA and protein expression.

[00106] In one specific aspect, the encoding scheme of the assay system comprises the use of two or more coding patterns, each comprising regions defined by spatial patterns on the substrate surface. For example, the assay system can utilize an

encoding scheme that comprises a 2-dimensional grid format based on the discrete positioning of the binding agents in the substrate surfaces. In another example, the spatial patterns may be based on more randomized cell locations, e.g., the patterns on the substrate surface follow an underlying biological structure rather than a strict, x,y grid pattern. This aspect includes systems with two or more substantially identical spatial patterns using different binding agents and/or coding identifiers, as well as systems having different patterns for different agents and/or coding identifiers. The encoding scheme of the systems can be controlled by delivery of different reagents to discrete regions on the substrate surfaces, which allows different reactions to take place on substantially similar agents of known location on the substrate surfaces.

[0107] In one specific aspect, the invention provides high resolution, high- throughput analysis of nucleic acids and/or expression levels that provides both detection and spatial identification of large numbers of nucleic acids, e.g., DNA or RNA.

[0108] In another specific aspect, the invention provides high resolution, high- throughput analysis of proteins that provides both detection and spatial identification of large numbers of such proteins, e.g., kinases or proteases.

[0109] Numerous reagent delivery systems can be used with the assay system of the invention. The primary criteria of such reagent delivery systems is the ability to direct delivery of specific agents based on spatial patterns on the substrate surface.

[0110] In one preferred aspect, the encoding scheme utilizes a reagent delivery system based on printing and informatics technologies to implement the spatial patterns used for identification and localization of the biological materials. For example, the patterns found in the encoding scheme may be created using ink jet printing technology to provide reagents at specific locations on one or more substrate surfaces. The desired patterns are set out in specific coding patterns on the substrate surface.

[0111] In certain aspects of the invention, the binding agents are immobilized directly to the substrate surface, and the location of the binding agents is known or determined prior to use of the substrate surface in the assay system. In another aspect, the binding agents are immobilized onto beads or other separate structural elements that are then provided in known locations on the substrate surface. In yet another aspect, the binding agents may be provided in or on features of the substrate surface, e.g., provided in wells or channels.

[0112] In specific aspects of the invention, the binding agents are

nucleic acids immobilized directly or indirectly to the substrate surface, e.g., directly through the use of amino groups on the substrate surface or indirectly through the use of a linker. The location of the nucleic acid sequences is known or determined prior to use of the substrate surface in the assay system. In another specific aspect, the nucleic acids may be immobilized directly or indirectly onto beads that are then provided in known locations on the substrate surface. In yet another aspect, the nucleic acids may be provided in or on features of the substrate surface, e.g., provided in wells.

[0113] In these aspects involving nucleic acid agents, any methods of sequence determination can be used, e.g., ~~sequencing~~ sequencing, hybridization and the like. In a preferred aspect, nucleic acid ~~sequencing~~ sequencing, and preferably next-generation sequencing, is used to decode the spatial encoding scheme in the assay system of the invention. This provides a very wide dynamic range for very large numbers of assays, allowing for efficient multiplexing.

[0114] In some aspects, the assay utilizes two or more oligonucleotides, the oligonucleotides comprising a universal primer region and a region that correlates specifically to a single spatial pattern within the spatial encoding scheme. In a specific aspect, the assay comprises two allele specific oligonucleotides and one locus specific oligonucleotides. These oligonucleotides allow the identification of specific SNPs, indels or mutations within an allele. This is useful in the identification of genetic changes in somatic cells, genotyping of tissues, and the like.

[0115] In other specific aspects of the invention, the binding agents are peptides. In one aspect, these peptides are associated directly or indirectly to known locations on a substrate surface, e.g., using binding protein pairs or through oligonucleotide linkers complementary to oligonucleotides on the substrate surface. In another aspect, the binding agents are peptides are immobilized directly or indirectly onto beads or other separate structural elements that are then provided in known locations on the substrate surface. In yet another aspect, the peptides may be provided in or on features of the substrate surface, e.g., provided in wells.

[0116] In yet other specific aspects of the invention, the binding agents are chemical entities (e.g., small molecules) that are coded, e.g. using sequence tags or mass spectroscopy tags as coding identifiers. In one aspect, these chemical entities can be are immobilized directly to the substrate surface. In another aspect, the binding agents are immobilized onto beads or other separate structural elements that are then provided in known locations on the substrate surface. In yet another aspect, the binding

Formatted: Strikethrough
Formatted: Underline
Formatted: Strikethrough
Formatted: Underline

agents may be provided in or on features of the substrate surface, e.g., provided in wells.

[0042][0117] The assay system of the invention can utilize various detection mechanisms, based on the molecules to be detected and the reagents needed for such detection system. Exemplary methods that can be used with the assay systems of the invention are described in more detail below.

[0043][0118] (New) The Invention in General

~~(New) The Invention in General~~

[0[0042]]00119] The assay system and methods of the invention are based on relational methods that allow extraction of data to detect the presence or absence and relative amount of a biological molecule, and the location of this molecule in a sample having a distinct structure, *e.g.*, a tissue section or other biological structure with distinct locations of specific biological molecules. The encoding scheme used in these systems corresponds to the structural elements of the sample, and the information obtained using a two-dimensional coding system is indicative of the spatial addresses of these molecules in a sample of interest.

[[[00043]]00120] Integral to the assay system of the invention is a method for spatial patterning of reagents. Technologies for formulating and delivering both biological molecules *(e.g.* DNA or antibodies) and chemical reagents *(e.g.*, small molecules or dNTPs) have already been demonstrated, and use of these systems will be available to one skilled in the art and easily adaptable upon reading this specification.

[[[00044]]00121] The assay design of the invention provides an accurate and easily scalable spatial encoding system. The ability to deliver reagents in a spatially defined pattern together with software, reagents and protocols comprises a novel and highly innovative assay system for spatial analysis of various biological molecules and activities. This allows the assays to measure numerous biological functions in a meaningful spatial environment, including functions such as gene expression and peptide localization. The systems provide the potential to open a new analytical window into the complex spatial patterns of cellular function and regulation in biological systems.

Formatted: Font: Bold

[[[00045]]00122] The biological molecules to be detected can be any biological molecules such as proteins, nucleic acids, lipids, carbohydrates, ions, or multicomponent complexes containing any of the above. Further examples of subcellular objects include organelles, *e.g.*, mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplast, endocytic vesicle, exocytic vesicles, vacuole, lysosome, etc.

[[[00046]]00123] Fig. 1 illustrates such a target-specific assay system for identification of nucleic acid sequences in a sample. In this system 101, two reagents 120, 122 that specifically bind to a biological molecule of interest are associated with coding identifiers 106, 108 that encode for a spatial location in the sample. These coding identifiers 106, 108 are optionally associated with sites that assist in their identification in the assay format, *e.g.*, universal priming sites 104, 110 for amplification of the assay products or adapters to enable identification of the coding identifiers and the binding agents using sequencing technologies. The sample that is tested, here shown as a tissue section 116 is encoded using the combination of the patterns 112, 114 created using the separate coding identifiers 106, 108 which provide a two dimensional code 118 that shows the location of any positive detection of the biological molecule 102 as well as quantifying the biological molecule 102 at each location assayed in the tissue.

[[[00047]]00124] The assay systems of the invention are particularly advantageous in that they are compatible with numerous samples types, such as fresh samples, such as primary tissue sections, and preserved samples including but not limited to frozen samples and paraformalin-fixed, paraffin-embedded (FFPE) samples. An important aspect of the assay systems of the invention is that the binding agents are immobilized on a substrate surface in discrete, independently measureable areas. These discrete areas can be formed by spatially selective deposition of the binding agents on the substrate surface. Numerous methods can be used for the deposition of the agent and the coding identifiers associates with the agent. For example, the coding identifiers can be delivered together or separately from the agent. If delivered together they can be attached (*e.g.*, synthesized as a

single molecule or attached through ligation or a chemical coupling mechanism) or simply mixed together to be attached after delivery to the substrate. In a preferred aspect, the agent and the coding identifier are made separately, mixed together for attachment, and delivered either attached or as a mixture to be attached on the surface. In a specific aspect the binding agents are delivered generally over the substrate surface and the coding identifiers are delivered in a pattern-specific manner.

[[[00048]]00125] Examples of methods that can be used for deposition of agents and/or coding identifiers onto the substrate surface include, but are not limited to, ink jet spotting, mechanical spotting by means of pin, pen or capillary, micro contact printing, fluidically contacting the measurement areas with the biological or biochemical or synthetic recognition elements upon their supply in parallel or crossed micro channels, upon exposure to pressure differences or to electric or electromagnetic potentials, and photochemical or photolithographic immobilization methods.

[[[0049]]00126] For several applications, it may be preferred to arrange the substrates into segments of one or more measurement areas for reagent distribution and agent determination. These regions may be physically separated using barriers or channels. They may still comprise several additional discrete measurement areas with agents that are different or in different combination from each other.

[[[00050]]00127] In certain aspects, the present invention provides a method, *e.g.*, a machine-based method, for evaluating changes in the presence and/or location of a biological molecule over time. The method includes providing a plurality of encoded array results representative of the biological molecule over time and evaluating the differences in detection and/or localization of the biological molecules.

Nucleic Acid Detection and Localization

[[[00051]]00128] In a particular aspect, the assay system is used to analyze nucleic acids, *e.g.*

genotyping, gene expression analysis, localization of particular transcripts within samples, and the like.

[[[00052]]00129] Genotyping may be performed using any technique known to those of skill in the art. Preferred techniques permit rapid, accurate determination of multiple variations with a minimum of sample handling. Some examples of suitable techniques involve but are not limited to direct DNA sequencing, capillary electrophoresis, hybridization, allele-specific probes or primers, single-strand conformation polymorphism analysis, nucleic acid arrays, bead arrays, restriction fragment length polymorphism analysis, cleavage fragment length polymorphism analysis, random amplified polymorphic DNA, ligase detection reaction, heteroduplex or fragment analysis, differential sequencing with mass spectrometry, atomic force microscopy, pyrosequencing, FRET *(e.g.,* TaqMan (Applied Biosystems, Inc., Foster City, Calif.) and Molecular Beacon (Stratagene, La Jolla, Calif.) assays), and other related techniques. Several methods for DNA sequencing are well known and generally available in the art. See, for example, Sambrook, et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, New York) (2001); Ausubel, et al., Current Protocols in Molecular Biology (John Wiley and Sons, New York) (1997), Twyman, et al. (2003) "Techniques Patents for SNP Genotyping", Pharmacogenomics 4(1):67-79; and Kristensen, et al. (2001) "High-Throughput Methods for Detection of Genetic Variation", BioTechniques 30(2):318-332. For details on the use of nucleic acid arrays (DNA chips) for the detection of, for example, SNPs, see U.S. Pat. No. 6,300,063 issued to Lipshultz, et al., and U.S. Pat. No. 5,837,832 to Chee, et al., HuSNP Mapping Assay, reagent kit and user manual, Affymetrix Part No. 90094 (Affymetrix, Santa Clara, Calif.). The molecular inversion probe (MIP) assay format (Hardenbol et al., 2003) is another example of a highly multiplexable assay that may be used with the assay systems of the invention.

[[[00053]]00130] In one exemplary and preferred method for analyzing nucleic acids using the assay system of the invention, the detection of nucleic acids uses two allele- specific oligonucleotides and a locus specific oligonucleotide. The assay methods are carried out according

to the strategy outlined in Fig. 2 using next-generation sequencing or another highly parallel nucleic acid assay technology. In this assay, a set of two oligonucleotides is designed to hybridize to each target sequence, with a common oligonucleotide and two unique coding identifiers. The allele can be determined, *e.g.* by primer extension of the locus specific oligonucleotide. Following primer extension and ligation, an amplifiable template is formed with universal primer sequences at either end. Assay oligonucleotides are annealed to a template and enzymatic reactions are used to join the two oligonucleotides only when both are correctly annealed. The detection techniques and read out parameters used in this system of the invention include a much shorter tag than the oligonucleotides used in the assays that are based on capture by hybridization. These shorter tags are designed to be read out by sequencing or, preferably, used to ligate codes onto both ends of the fragment as illustrated in Fig. 2.

[[[00054]]00140] In Figure 2, two target-specific assay oligonucleotides are ligated together 202 following *in situ* hybridization to target sequences. At the same time, encoding oligonucleotides containing tag sequence sets X and Y are ligated 204 to the target specific oligonucleotides. Oligonucleotides containing X ligate specifically to one side of the targeting construct and oligonucleotides containing Y ligate to the other. The oligonucleotides contain universal primer sites P1 and P2. Following ligation, the constructs are eluted and, optionally, sequencing adapters can be attached 206, *e.g.*, via PCR.

[[[00055]]00150] In one preferred aspect, the final construct created from the assay method is a substrate for next-generation sequencing, and highly parallel next-generation sequencing methods are used to confirm the sequence of constructs. Such sequencing methods can be carried out, for example, using a one pass sequencing method or using paired-end sequencing. Next generation sequencing methods include, but are not limited to, hybridization-based methods, such as disclosed in Drmanac, U.S. Pat. Nos. 6,864,052; 6,309,824; and 6,401,267; and Drmanac et al. [[ ]]U[[U]].S. patent publication 2005/0191656, and sequencing by synthesis methods, *e.g.*, Nyren

Formatted: Indent: Left: 0", First line: 0.44", Right: 0.08", Space Before: 0.65 pt

et al, U.S. Pat. No. 6,210,891; Ronaghi, U.S. Pat. No. 6,828,100; Ronaghi et al (1998), Science, 281: 363-365; Balasubramanian, U.S. Pat. No. 6,833,246; Quake, U.S. Pat. No. 6,911,345; Li et al, Proc. Natl. Acad. Sci., 100: 414-419 (2003); Smith et al, PCT publication WO 2006/074351; use of reversible extension terminators, *e.g.*, Turner, U.S. Pat. No. 6,833,246 and Turner, U.S. Pat. No. 6,833,246 and ligation-based methods, *e.g.*, Shendure et al (2005), Science, 309: 1728-1739, Macevicz, U.S. Pat. No. 6,306,597; which references are incorporated by reference. Soddart et al., PNAS U S A. 2009 Apr 20; Xiao et al., Nat Methods. 2009 Mar;6(3):199-201. Epub 2009 Feb 8.

[[[00056]]00151] To maximize the efficiency of encoding, a combinatorial approach using pairs of oligonucleotides can be used. For example, with only two sets of 100 codes, a substrate can theoretically encode up to 10,000 locations. The number of assay oligonucleotides required is dramatically reduced, the cost decreased, and the robustness of the approach increased by decoupling the coding sequences from the genome-specific sequences. Alternative assay formats can also be used (e.g. ligation or primer extension followed by ligation).

[[[00057]]00152] By ligating the codes on separately, only 2n target-specific assay oligonucleotides are needed for n targets. For example, assaying 100 different targets at 10,000 spatial locations would require 2 x 100 targeting oligonucleotides and 2 x 100 encoding oligonucleotides, using a combinatorial approach outlined in Fig. 2. The total count of assay oligonucleotides would be only 400 (200 target- specific and 200 encoding), not counting universal primers. In contrast, if the coding oligonucleotides were not decoupled, (n x X positional codes) + (n x Y positional codes) would be needed, or in the above example, 20,000 oligonucleotides, not counting universal primer sequences.

[[[00058]]00153] Due to the matrix system of the invention, a large amount of information can be obtained even using five or more positions interrogated for five or more biological molecules. By varying one or the other of these, large amounts of information can be obtained, both in terms of locations and/or specific biological In specific aspects, multiple

locations are interrogated for two or more biological molecules. As an example, for each datapoint ~1,000 reads may be sampled, for a total of -10E9 reads for 10E6 datapoints.

Peptide Detection Systems

[[[00059]]00154] The assay system of the invention can be used to analyze biological molecules using peptide agents that are associated with the substrate surface in a spatial pattern. Such peptides may comprise an active region of an enzyme, a binding domain of an immunoglobulin, defined domains of proteins, whole proteins, synthetic peptides, peptides with introduced mutations, etc.

[[[00060]]00155] The assay system of the invention allows the identification and spatial location of various forms of peptides, including isoforms and peptides that have undergone posttranslational modification. Importantly, certain aspects of the invention allow the identification of active versus non-active forms of such peptides in a sample. This allows the identification of the presence or absence of specific peptide isoforms, and also acts as a proxy for identification of peptide activity in a sample.

[[[00061]]00156] In certain aspects of the invention, the binding agents associated with the substrate surfaces of the assay system include substrates for enzymes or proenzymes, *e.g.*, a kinase, a phosphatase, a zymogen, a protease, or a fragment thereof. In certain aspects, the binding agents associated with the substrate surfaces are phosphorylation substrates used to detect proteins involved with one or more signal transduction pathways, *e.g.*, a kinase or a phosphatase. In another specific aspect of the invention, the binding agents are specific protease substrates that associate only with individual or classes of proteases. In other aspects, the binding agents on the substrate surface are different processed forms, isoforms and/or domains of an enzyme.

Reagent Delivery

[[[00062]]00157] The reagent delivery system of the invention can be any system that allows the delivery of reagents to discrete portions of the array in order to keep the integrity of the defined spatial patterns of the encoding scheme. Such discrete delivery can be achieved in a number of different ways.

[[[00063]]00158] In one exemplary aspect, the reagent delivery system can be a flow-based system. The flow-based systems for reagent delivery in the present invention can include one or more pumps, valves, fluid reservoirs, channels, and/or reagent storage cells. Such a reagent delivery system is configured to move fluid in contact with a discrete section of the substrate surface. Movement of the reagents can be driven through a fluid by a pump disposed, for example, downstream of the fluid reagents. The pump can drive each fluid reagent to (and past) the reaction compartment. Alternatively, the reagents may be driven through the fluid by gravity.

[[[00064]]00159] US Appln Nos. 20070166725 and 20050239192 disclose certain general-purpose fluidics tools that can be used with the assay systems of the invention. These allow the precise manipulation of gases, liquids and solids to accomplish very complex analytical manipulations with relatively simple hardware.

[[[00065]]00160] In a more specific example, one or more flow-cells can be attached to the substrate from above. The flow-cell can include inlet and outlet tubes connected thereto and optionally an external pump can be used to deliver the sample or reagents to the flow-cell and across the substrate. The flow cell is configured to deliver reagents only to certain portions of the array, restricting the amount and type of reagent delivered to any specific section of the array.

[[[00066]]00161] In another aspect, a microfluidic system can be integrated into the substrate or externally attached on top of the substrate. Microfluidic passages for holding and carrying fluid can be formed on and/or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected according to selective opening and closing of valves disposed between

reagent reservoirs.

[[[00067]]00162] Pumps generally include any mechanism for moving fluid and/or reagents disposed in fluid. In some examples, the pump can be configured to move fluid and/or reagents through passages with small volumes (i.e., microfluidic structures). The pump can operate mechanically by exerting a positive or negative pressure on fluid and/or on a structure carrying fluid, electrically by appropriate application of an electric field(s), or both, among others. Exemplary mechanical pumps may include syringe pumps, peristaltic pumps, rotary pumps, pressurized gas, pipettors, etc. The mechanical pumps may be micromachined, molded, etc. Exemplary electrical pumps can include electrodes and may operate by electrophoresis, electroendoosmosis, electrocapillarity, dielectrophoresis (including traveling wave forms thereof), and/or the like.

[[[00068]]00163] Valves generally include any mechanism for regulating the passage of fluid through a channel. The valves can include, for example, deformable members that can be selectively deformed to partially or completely close a channel, a movable projection that can be selectively extended into the channel to partially or completely block the channel, an electrocapillary structure, and/or the like.

[[[00069]]00164] In yet another aspect, an open gasket can be attached to the top of the substrate and the sample and reagents can be injected into the gasket. Suitable gasket materials include, but are not limited to, neoprene, nitrile, and silicone rubber. Alternatively, a watertight reaction chamber formed by a gasket sandwiched between the substrate and a chemically inert, water resistant material such as, but not limited to, black-anodized aluminum, thermoplastics (e.g., polystyrene, polycarbonate, etc), glass, etc.

[[[00070]]00165] In a specific aspect of the present invention, the delivery system can compnse a microcircuit arrangement including an imager, such as a CCD or IGFET-based (e.g., CMOS-based) imager and an ultrasonic sprayer for reagent delivery such as described in US Appln

No. 20090197326, which is incorporated herein by reference.

[[[00071]]00166] In yet another aspect of the invention, the reagent delivery system controls the delivery of reagents to specific patterns on a substrate surface using semiconductor techniques such as masking and spraying. Specific areas of a substrate surface can be protected from exposure to reagents through use of a mask to protect specific areas from exposure. The reagents may be introduced to the substrate using conventional techniques such as spraying or fluid flow. The use of the masked substrate delivery results in a patterned delivery scheme on the substrate surface.

[[[00072]]00167] In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology. There are a variety of different ink-jetting mechanisms *(e.g.,* thermal, piezoelectric) and compatibility has been shown with aqueous and organic ink formulations. Sets of independently actuated nozzles can be used to deliver multiple reagents at the same time, and very high resolutions can be achieved.

Software for use in the Assay System

[[[00073]]00168] In order to target specific sites of interest, an informative image of the biological section to be analyzed can be used to assist in the reagent delivery methods and associated encoding scheme. Sample regions can be identified using image processing *(e.g.,* images of cell types differentiated by immunohistochemistry or other staining chemistries) integrated with the other features of the assay system. In some aspects, software is used to automatically translate this information into a reagent delivery pattern. A mechanism to register and align very precisely the biological sample in a targeting system is thus a preferred component of the assay systems of the invention. Mechanisms such as the use of fiducial markers on slides and other very accurate physical positioning systems can be adapted to this purpose.

[[[00074]]00169] Additional software components will also be key components that will be

part of a complete assay system. The invention thus preferably comprises a complete suite of software tailored to the assay system. Optionally, oligonucleotide design software will be customized for the specific assay to be run, and may be integrated as a part of the system. Also optionally, algorithms and software for data analysis may be integrated to assist in determination of results of the assays. This can be especially useful, as the type of dataset that will be generated will be novel, particularly as a consequence of scale. The ability to provide algorithms and software tools that are specifically designed for analysis of spatially-associated data for significant patterns, including pattern-analysis software and visualization tools, is a novel feature that will enhance the value of the data generated by the assay systems.

[[[00075]]00170] In certain aspects, the assay system will comprise processes for making and carrying out quality control of reagents, *e.g.*, the integrity and sequence fidelity of oligonucleotide pools. In particular, reagents will need to be formulated for compatibility with the reagent delivery instrumentation. Factors such as volatility, stability at key temperatures, and chemical compatibility can be optimized by those skilled in the art upon reading the present disclosure.

Applications of Assay System

[[[00076]]00171] It will be apparent to one skilled in the art upon reading the present disclosure that there are numerous very important areas of biological research, diagnostics, and drug development that will benefit from a high throughput means to simultaneously measure the presence or absence and spatial location of a biological molecule in a sample. For example, this technology combining the ability to analyze semi-quantitatively the expression of many different genes with the ability to image the spatial organization of expression across many cells in a tissue is enabling for many different areas of basic research. The following are exemplary uses and are by no means meant to be limiting in scope.

[[[00077]]00172] In one example, 3-dimensional patterns of expression can be determined

by analyzing a series of tissue sections, in a manner analogous to image reconstruction in CT scanning. This can be used to measure changes in gene expression in disease pathology, *e.g.*, in cancerous tissue and/or a tissue upon injury, inflammation or infection. With the assay systems of the invention, more detailed information on gene expression and protein localization in complex tissues can be obtained. This may lead to new insights into the function and regulation both in normal and diseased states, and is likely to provide new hypotheses that can be tested. For example, a system of the invention may enable some of the insights gained from many individual studies and larger programs like ENCODE (Birney et al., 2007) and modENCODE to be integrated at the tissue level. The assay systems will also aid in computational efforts to model interacting networks of gene expression in the field of systems biology.

[[[00078]]00173] The assay systems also provide a novel approach that enables the analysis of somatic variation, *e.g.*, somatic mutations in cancer or variability in response to infectious organisms. For example, tumors are typically highly heterogeneous, containing cancer cells as well as genetically normal cells in an abnormal local environment. Cancer cells undergo mutation and selection, and in this process it is not unusual for local clones to develop. Identifying relatively rare somatic mutations in the context of tumors may enable the study of the role of key mutations in the selection of clonal variants. Transcriptional patterns associated with angiogenesis, inflammation, or other cancer related processes in both cancer and genetically normal cells can be analyzed for insights into cancer biology and assist in the development of new therapeutic agents for the treatment of cancers.

[[[00079]]00174] In another example, different people have varying susceptibility to infectious organisms, and much of this may be to underlying genetic differences in individuals and/or populations. Identifying these differences will aid in an understanding of the underlying disease pathologies and assist in the development of vaccines or therapeutics to prevent or ameliorate these disease states.

[[[00080]]]**00175]** Importantly, in addition to providing spatially associated information, the technology of the invention will allow a great increase in the sensitivity of detecting rare mutations. The reason is that signal to noise can be dramatically increased because the approach of the invention assays a small location in any given reaction. In a typical assay for rare mutations in a mixed sample, the sample is treated in bulk, i.e. nucleic acids are extracted from many cells into a single pool. Thus, if a mutation is present in 1 cell in 10,000, it must be detected against a background of normal DNA from ~10,000 cells. In contrast, with the systems of the invention many cells can be analyzed, but individual cells or small groups of cells would be identified by the spatial coding system. Therefore, the background can be reduced by orders of magnitude, greatly increasing sensitivity. Furthermore, the spatial organization of mutant cells can be observed. This may be particularly important in detecting key mutations in tissue sections in cancer. Already, molecular histological analyses are yielding insights into cancer biology and may have potential for use in diagnostics (Choe et al., 2003). The technology of the invention promises to greatly increase the power of such approaches.

Formatted: Indent: First line: 0.44", Right: 0.08", Space Before: 0.2 pt, Line spacing: Multiple 2.08 li, No bullets or numbering

EXAMPLES

[[[0091]]]00176] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventor regards as his invention, nor are they intended to represent or imply that the experiments below are all of or the only experiments performed. It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[[[0092]]]00177] Efforts have been made to ensure accuracy with respect to numbers used *(e.g.,* amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average

molecular weight, temperature is in degrees centigrade, and pressure is at or near atmospheric.

EXAMPLE 1: Initial Proof of Concept of Encoding Scheme

[[[0093]]00178] As an initial proof of concept, a model system is developed using a microarray to demonstrate a working single-plex assay. The basic design validates the concept of the assay, and establishes a working assay prior to addressing issues related to the analysis of a more complicated biological sample. Conventional sequencing is used as a readout for this proof of concept.

[[[0094]]00179] A microarray is used as a proxy for a tissue section. The target sequences of the microarray are fully specified, so that the composition of the targets are known and can be varied systematically. Synthetic oligonucleotide templates are attached to a glass slide via a 5' amino modification. Each slide has a single oligonucleotide template sequence, and the assays that are carried out may employ either ligation, or extension followed by ligation as this may be useful in determining certain polymorphisms.

[[[0095]]00180] Once the *in situ* part of the assay is complete, the reaction products are eluted and analyzed by qPCR to determined presence or absence of a product and estimate yield, and by conventional sequencing to determine the structure of the assay products. The single plex assays that are tested include appropriate positive and negative controls, and a single nucleotide variant (SNV) to check ability to discriminate single base variants.

EXAMPLE 2: Scalability

[[[0096]]00181] The complexity of the assay system is increased to establish scalability of the assay for use in high throughput studies. Scalability of both the spatial encoding and assay systems is demonstrated by carrying out a 24-plex x 24-site assay using a microarray model system.

[[[0097]]00182] The amount of biological target, here a DNA target sequence, at each assay location is systematically varied on microarray substrate. For example, in a microarray with 50 micron spot size (center to center), a 1 mm^2 area contains ~400 spots. The region around each site is optionally occupied by a region that is devoid of these spots to allow individual resolvability of the target sequences. Alternatively, the spots may be clustered, with two or more directly adjacent spots surrounded by or adjacent to a region that is devoid of target sequences.

[[[0098]]00183] In order to demonstrate that spatial encoding is accurate, the sites comprise different target compositions to show that the assay readout matches the expected composition of each site. With 24 target sequences, a simple digital pattern is made with each site having a different set of 12 targets present and 12 targets absent, to make a binary code (0= absent, 1=present). The assay readout is then determined to show that the detected regions match the expected signal after spatial

decoding. In this particular example, the code space is large enough (2^{24}) so that even a few errors would not result in different codes being mixed up. Moreover, this design allows identification of errors and allows an estimation not only of accuracy of spatial encoding but also of accuracy calling the presence or absence of target sequences.

In an exemplary aspect, a 4 x 4 arrangement of 16 sequences is used for the array configuration. A white square indicates that the sequence is absent and a black square that it is present, i.e. 8 of the 16 possible sequences are present in this sample. In a different sample, a different pattern of absent and present sequences can be constructed. In this way, unique patterns are associated with spatial locations so that the accuracy of spatial encoding can be measured.

[[[0099]]00184] The ability to detect quantitative differences is evaluated by generating dose-response curves for each of the 24 assays that are carried out at each site in a 24-site assay. This allows estimation of the limit of detection, dynamic range, and power to detect a given fold-change across the range.

[[[0100]]00185] In one aspect, a latin square design is used to represent individual targets at different ratios by varying the number of features for each target. In other words, with multiple spots in a site, the number of spots allocated to each of the 24 target sequences can be varied and each of the 24 sites can have a different composition. A 1 x 3 inch microarray is sufficiently large to permit multiple replicates. This larger set of 24 sequences will require deconvolution, and this is accomplished using high throughput techniques such as next-generation sequencing technologies *(e.g.,* SOLiD™ technology (Life Technologies, Inc., Carlsbad, CA) or Genome Analyzer (Illumina, Inc., San Diego, CA)). The use of the 24-plex assay demonstrates both the accuracy of spatial encoding and decoding, and the quantitative response of the assay system.

EXAMPLE 3: Adaptation of the assay to preserved samples.

[[[0101]]00186] Genomic DNA is assayed as a proof of concept for assaying RNA, as it provides a way to establish a single-copy reference signal. Once a working assay is developed for FFPE samples, it is adapted to an RNA assay. To this end, assay oligonucleotide concentrations are assayed to ensure compatibility with high multiplexing. Assuming a cell diameter of 10 microns, and delivery of a 10 micron diameter reagent droplet to an individual cell, the volume of the droplet will be ~500 µl and can contain ~3 x 10^{11} molecules at a 1 µM concentration. To assay 1,000 target sequences in 10,000 cells, ~2,000 targeting oligonucleotides would be required in a droplet. Therefore, each droplet could contain ~160 million copies of each assay oligo, a vast excess over the few thousand target sequences in a cell.

[[[0102]]00187] The handling of small absolute numbers of product molecules generated

from very small or compromised samples are enhanced to counter the issue of low recovery efficiency; that is, elution is efficient and losses resulting from adsorption of molecules to surfaces are prevented. An approach to addressing the latter issue is to include a carrier material, such as glycogen or carrier nucleic acids.

Example 4: Adapting the Assay to a Biological Sample.

[[[0103]]00188] A control RNA template is immobilized to a solid support in order to create an artificial system. The assay is performed using T4 DNA ligase, which can repair nicks in DNA/RNA hybrids. Assays are carried out on matched slides, or different sections of the same slide, where in one case gDNA is assayed and in the other RNA is assayed. When assaying gDNA the slide can be pretreated with RNase, and when assaying RNA the slide is pretreated with DNase. Results of the assay are confirmed by extracting gDNA or RNA and quantitating the relative amounts by qPCR or RT-qPCR respectively.

[[[0104]]00189] In order make the tissue section RNA assays as informative as possible, pre-existing information on expression levels in specific tissues to target transcripts across a range of abundances are used in the assay design. Both high abundance transcripts, as well as some medium and low abundance transcripts, are targeted to enable an initial assessment of the quantitative performance characteristics of the assay.

[[[0105]]00190] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims. In the claims that follow, unless the term "means" is used, none of the features or elements

recited therein should be construed as means-plus-function limitations pursuant to 35 U.S.C. §112, ¶6.

I claim:

1. An assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the assay system performs the following steps:

providing a sample affixed to a support;

delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered;

allowing the encoded probes to interact with the biological targets;

separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets;

determining all or a portion of a sequence of the encoded probes, and

associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

SPATIALLY ENCODED BIOLOGICAL ASSAYS

Abstract of the Disclosure

The present invention provides assays and assay systems for use in spatially encoded biological assays. The invention provides an assay system comprising an assay 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.

Electronic Acknowledgement Receipt

EFS ID:	35437379
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. CHEE
Customer Number:	159367
Filer:	Cameron Mark Luitjens/Judith Wasilkus
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	47706-0004003
Receipt Date:	15-MAR-2019
Filing Date:	14-FEB-2019
Time Stamp:	14:16:02
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		0004003PrelimAmend.pdf	90618 8e04007851728f2d842d45587478af827edc8003	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Preliminary Amendment	1	1
	Specification	2	2
	Claims	3	6
	Applicant Arguments/Remarks Made in an Amendment	7	8

Warnings:					
Information:					
2	Specification	00040033SUBSTITUTESPEC_MARKEDUP.pdf	448220 e9eb3307ccd3dba899caac2d9f1ea5a814158686	no	49
Warnings:					
Information:					
3	Specification	0040003SUBSTITUTESPEC_CLEAN.pdf	333243 5e86ac196583544d664816abc37ed3eefac6a5a2	no	44
Warnings:					
Information:					
		Total Files Size (in bytes):	872081		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

SPATIALLY ENCODED BIOLOGICAL ASSAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Patent Application No. 61/321,124, filed April 5, 2010 and is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention relates to assays of biological molecules, and more particularly to assays for determining spatial distributions of a large number of biological molecules in a solid sample simultaneously.

BACKGROUND OF THE INVENTION

[0003] In the following discussion certain articles and methods will be described for background and introductory purposes. Nothing contained herein is to be construed as an "admission" of prior art. Applicant expressly reserves the right to demonstrate, where appropriate, that the articles and methods referenced herein do not constitute prior art under the applicable statutory provisions.

[0004] Comprehensive gene expression analysis and protein analysis have been useful tools in understanding mechanisms of biology. Use of these tools has allowed the identification of genes and proteins involved in development and in various diseases such as cancer and autoimmune disease. Conventional 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. Other technologies that have enabled the quantitative analysis of many RNA sequences per sample include microarrays (see Shi, et al., Nature Biotechnology, 24(9):1151-61 (2006); and Slonim and Yanai, Plos Computational Biology, 5(10):e1000543 (2009)); serial analysis of gene expression (SAGE) (see Velculescu, et al, Science, 270(5235):484-87 (1995)), high-throughput implementations of qPCR (see Spurgeon, et al., Plos ONE, 3(2):e1662 (2008)) and *in situ* PCR (see Nuovo, Genome Res., 4:151-67 (1995)). As useful as these methods are, however, they do not enable simultaneous measurement of the expression of many genes or the presence and/or activity of multiple proteins at many spatial locations in a sample. Laser capture microdissection has permitted the analysis of many genes at a small number of locations, but it is very expensive, laborious, and does not scale well. Certain PCR assays in a 2D format preserve spatial

Curio EX1010-p. 127
Curio v Prognosys
IPR2024-01289

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.

[0005] At present, no practical method exists to analyze at high resolution the spatial expression patterns of large numbers of genes, proteins, or other biologically active molecules simultaneously. There is thus a need for reproducible, high-resolution spatial maps of biological molecules in tissues. The present invention addresses this need.

SUMMARY OF THE INVENTION

[0006] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the following written Detailed Description including those aspects illustrated in the accompanying drawings and defined in the appended claims.

[0007] The invention encompasses assay systems that provide high-resolution spatial maps of biological activity in tissues. The assay system comprises an assay capable of high levels of multiplexing where encoded probes 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. In short, the present invention provides the ability to look at many biological targets in many locations, providing the resolution of in situ hybridization with the highly- parallel data analysis of sequencing.

[0008] Thus, in some embodiments, the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered; allowing the encoded probes to interact with the biological targets; separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets; determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

[0009] In particular aspects of the invention the biological targets comprise nucleic acids and the

encoded probes are oligonucleotides, and in some aspects, there are two encoded probes for each of the multiple nucleic acid targets. In some aspects, the multiple biological targets comprise proteins, the probe regions of the encoding probes are proteins and the coding tags comprise oligonucleotides. In some aspects the multiple biological targets comprise enzymes. In some aspects the probe regions of the encoded probes comprise antibodies, aptamers or small molecules.

[0010] Some aspects of the assay system further comprise an amplification step between the separating step and the determining step. In some aspects, the determining step is performed by nucleic acid sequencing, and in preferred aspects, the sequencing is high- throughput digital nucleic acid sequencing.

[0011] In some aspects of the invention, the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 20, in some aspects product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 50, in some aspects the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 75, 100, 150, 500, 750, 1,000, 5,000, 10,000, 25,000, 50,000, 100,000, 500,000, or 1,000,000 or more. In other aspects, the sequence of at least fifty thousand encoding probes are determined in parallel, in other aspects the sequence of at least one hundred thousand encoding probes are determined in parallel, in some aspects the sequence of at least five hundred thousand encoding probes are determined in parallel, and in some aspects the sequence of at least one million, ten million, one hundred million, one billion, ten billion, one hundred billion or more encoding probes are determined in parallel.

[0012] In some aspects, the known spatial pattern is determined by histological features of the sample. Also in some aspects, software programmed hardware performs at least two steps of the delivering step, the separation step, the determining step and the associating step.

[0013] In some aspects, the probe regions of the encoded probes are proteins and the separating step is accomplished by encoded probes that interact with the biological targets being captured by an affinity capture agent. In some aspects the probe regions of the encoding probes are nucleic acids and the separating step is accomplished by a washing of the sample.

[0014] In other embodiments there is provided an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering oligonucleotide probes for multiple nucleic acid targets to the multiple sites in the sample in a known spatial pattern; allowing the oligonucleotide probes to hybridize with the nucleic acid targets; washing unhybridized encoded oligonucleotide probes from the sample; delivering one or more encoding agents to locations of the multiple sites in the sample according to a known spatial pattern, where the

combination of encoding agents delivered to each site is different; coupling the encoding agents and the oligonucleotide probes to form encoded probes; determining all or a portion of a sequence of the encoded probes using high-throughput sequencing, and associating the abundance or activity or both of multiple biological targets to the locations of multiple sites in the sample.

[0015] Other embodiments of the invention provide an assay system to determine spatial patterns of abundance or activity or both of multiple protein targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple protein targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a protein probe region that may interact with the protein targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and the protein probe region of the encoding probe of which the coding tag is part; allowing the encoded probes to interact with the protein targets; separating encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets; determining all or a portion of a sequence of the encoded probes by high throughput sequencing, and associating the abundance or activity or both of the multiple protein targets to the locations of the multiple sites in the sample.

[0016] Other embodiments provide an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, where the assay system performs the following steps: providing a sample affixed to a support; delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, where each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and identifies the biological target; allowing the encoded probes to interact with the biological targets; determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

[0017] The assay system of the invention can utilize various detection mechanisms, based on the molecules to be detected and the reagents needed for such detection system. Exemplary methods that can be used with the assay systems of the invention are described in more detail below.

DESCRIPTION OF THE FIGURES

[0018] Figure 1 provides a simplified overview of the assay system of the present invention.

[0019] Figure 2 provides a simplified overview of one embodiment of the assay system of the present invention for detecting nucleic acids.

[0020] Figure 3 is a representational depiction of one embodiment of the assay overviewed in Figure 2.

[0021] Figure 4 illustrates a general mechanism for one embodiment of a combinatorial encoding scheme of the assay systems of the invention.

[0022] Figure 5 provides a simplified, specific example of the embodiment of a combinatorial encoding scheme shown in Figure 4.

DEFINITIONS

[0023] The terms used herein are intended to have the plain and ordinary meaning as understood by those of ordinary skill in the art. The following definitions are intended to aid the reader in understanding the present invention, but are not intended to vary or otherwise limit the meaning of such terms unless specifically indicated.

[0024] The term "antibody" as used herein is intended to refer to an entire immunoglobulin or antibody or any functional fragment of an immunoglobulin molecule which is capable of specific binding to an antigen (antibodies and antigens are "binding partners" as defined herein). "Antibody" as used herein is meant to include the entire antibody as well as any antibody fragments capable of binding the antigen or antigenic fragment of interest. Examples of such peptides include complete antibody molecules, antibody fragments, such as Fab, F(ab')2, CDRS, VL, VH, and any other portion of an antibody which is capable of specifically binding to an antigen. Antibodies for assays of the invention are immunoreactive or immunospecific for, and therefore specifically and selectively bind to, proteins either detected *(i.e.,* biological targets) or used for detection *(i.e.,* probes) in the assays of the invention.

[0025] The term "binding agent" as used herein refers to any agent that specifically binds to a biological molecule of interest

[0026] "Complementary" or "substantially complementary" refers to the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, such as, for instance, between the two strands of a double-stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single-stranded nucleic acid. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single-stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the other strand, usually at least about 90% to about 95%, and even about 98% to about 100%).

[0027] "Hybridization" refers to the process in which two single-stranded polynucleotides bind non-covalently to form a stable double-stranded polynucleotide, The resulting (usually) double-stranded polynucleotide is a "hybrid" or "duplex," "Hybridization conditions" will typically include salt concentrations of approximately less than 1M, often less than about 500 mM and may be less than about

Curio EX1010-p. 131
Curio v Prognosys
IPR2024-01289

200 mM. A "hybridization buffer" is a buffered salt solution such as 5% SSPE, or other such buffers known in the art. Hybridization temperatures can be as low as 5°C, but are typically greater than 22°C, and more typically greater than about 30°C, and typically in excess of 37°C. Hybridizations are often performed under stringent conditions, *i.e.,* conditions under which a primer will hybridize to its target subsequence but will not hybridize to the other, non-complementary sequences. Stringent conditions are sequence-dependent and are different in different circumstances. For example, longer fragments may require higher hybridization temperatures for specific hybridization than short fragments. As other factors may affect the stringency of hybridization, including base composition and length of the complementary strands, presence of organic solvents, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one parameter alone. Generally stringent conditions are selected to be about 5°C lower than the T_m for the specific sequence at a defined ionic strength and pH. Exemplary stringent conditions include a salt concentration of at least 0.01 M to no more than 1M sodium ion concentration (or other salt) at a pH of about 7.0 to about 8.3 and a temperature of at least 25°C. For example, conditions of 5xSSPE (750 mM NaCl, 50 mM sodium phosphate, 5 mM EDTA at pH 7.4) and a temperature of approximately 30°C are suitable for allele-specific hybridizations, though a suitable temperature depends on the length and/or GC content of the region hybridized.

[0028] "Ligation" means to form a covalent bond or linkage between the termini of two or more nucleic acids, *e.g.,* oligonucleotides and/or polynucleotides, in a template-driven reaction. The nature of the bond or linkage may vary widely and the ligation may be carried out enzymatically or chemically. As used herein, ligations are usually carried out enzymatically to form a phosphodiester linkage between a 5' carbon terminal nucleotide of one oligonucleotide with a 3' carbon of another nucleotide.

[0029] "Nucleic acid", "oligonucleotide", "oligo" or grammatical equivalents used herein refers generally to at least two nucleotides covalently linked together. A nucleic acid generally will contain phosphodiester bonds, although in some cases nucleic acid analogs may be included that have alternative backbones such as phosphoramidite, phosphorodithioate, or methylphophoroamidite linkages; or peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with bicyclic structures including locked nucleic acids, positive backbones, non-ionic backbones and non-ribose backbones. Modifications of the ribose-phosphate backbone may be done to increase the stability of the molecules; for example, PNA:DNA hybrids can exhibit higher stability in some environments.

[0030] "Primer" means an oligonucleotide, either natural or synthetic, that is capable, upon forming a duplex with a polynucleotide template, of acting as a point of initiation of nucleic acid synthesis and being extended from its 3' end along the template so that an extended duplex is formed.

The sequence of nucleotides added during the extension process is determined by the sequence of the template polynucleotide. Primers usually are extended by a DNA polymerase.

[0031] The term "SNP" or "single nucleotide polymorphism" refers to a genetic variation between individuals; *e.g.,* a single nitrogenous base position in the DNA of organisms that is variable. SNPs are found across the genome; much of the genetic variation between individuals is due to variation at SNP loci, and often this genetic variation results in phenotypic variation between individuals. SNPs for use in the present invention and their respective alleles may be derived from any number of sources, such as public databases (U.C. Santa Cruz Human Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway) or the NCBI dbSNP website (http://www.ncbi.nlm.nih.gov/SNP/), or may be experimentally determined as described in U.S. Pat No. 6,969,589; and US Pub. No. 2006/0188875 entitled "Human Genomic Polymorphisms." Although the use of SNPs is described in some of the embodiments presented herein, it will be understood that other biallelic or multi-allelic genetic markers may also be used. A biallelic genetic marker is one that has two polymorphic forms, or alleles. As mentioned above, for a biallelic genetic marker that is associated with a trait, the allele that is more abundant in the genetic composition of a case group as compared to a control group is termed the "associated allele," and the other allele may be referred to as the "unassociated allele." Thus, for each biallelic polymorphism that is associated with a given trait *(e.g.,* a disease or drug response), there is a corresponding associated allele. Other biallelic polymorphisms that may be used with the methods presented herein include, but are not limited to multinucleotide changes, insertions, deletions, and translocations. It will be further appreciated that references to DNA herein may include genomic DNA, mitochondrial DNA, episomal DNA, and/or derivatives of DNA such as amplicons, RNA transcripts, cDNA, DNA analogs, etc. The polymorphic loci that are screened in an association study may be in a diploid or a haploid state and, ideally, would be from sites across the genome.

[0032] The term "selectively binds", "selective binding" and the like as used herein, when referring to a binding partner *(e.g.*. protein, nucleic acid, antibody or other affinity capture agent, etc.), refers to a binding reaction of two or more binding partners with high affinity and/or complementarity to ensure selective hybridization under designated assay conditions. Typically, specific binding will be at least three times the standard deviation of the background signal. Thus, under designated conditions the binding partner binds to its particular "target" molecule and does not bind in a significant amount to other molecules present in the sample.

[0033] "Sequencing", "sequence determination" and the like means determination of information relating to the nucleotide base sequence of a nucleic acid. Such information may include the identification or determination of partial as well as full sequence information of the nucleic acid.

Sequence information may be determined "with varying degrees of statistical reliability or confidence. In one aspect, the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, "High throughput digital sequencing" or "next generation sequencing" means sequence determination using methods that determine many (typically thousands to billions) of nucleic acid sequences in an intrinsically parallel manner, *i.e.* where DNA templates are prepared for sequencing not one at a time, but in a bulk process, and where many sequences are read out preferably in parallel, or alternatively using an ultra-high throughput serial process that itself may be parallelized. Such methods include but are not limited to pyrosequencing (for example, as commercialized by 454 Life Sciences, Inc., Branford, CT); sequencing by ligation (for example, as commercialized in the SOLiD™ technology, Life Technology, Inc., Carlsbad, CA); sequencing by synthesis using modified nucleotides (such as commercialized in TruSeq™ and HiSeq™ technology by Illumina, Inc., San Diego, CA, HeliScope™ by Helicos Biosciences Corporation, Cambridge, MA, and PacBio RS by Pacific Biosciences of California, Inc., Menlo Park, CA), sequencing by ion detection technologies (Ion Torrent, Inc., South San Francisco, CA); sequencing of DNA nanoballs (Complete Genomics, Inc., Mountain View, CA); nanopore-based sequencing technologies (for example, as developed by Oxford Nanopore Technologies, LTD, Oxford, UK), and like highly parallelized sequencing methods.

[0034] The term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation, T_m=81.5+0.41 (% G+C), when a nucleic acid is in aqueous solution at 1M NaCl (see *e.g.,* Anderson and Young, Quantitative Filter Hybridization, in *Nucleic Acid Hybridization* (1985)). Other references *(e.g.,* Allawi and SantaLucia, Jr., Biochemistry, 36:10581-94 (1997)) include alternative methods of computation which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m.

DETAILED DESCRIPTION OF THE INVENTION

[0035] The practice of the techniques described herein may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology, which are within the skill of those who practice in the art. Such conventional techniques include polymer array synthesis, hybridization and ligation of polynucleotides, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the examples

herein. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Green, et al., Eds., *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV) (1999); Weiner, Gabriel, Stephens, Eds., *Genetic Variation: A Laboratory Manual* (2007); Dieffenbach, Dveksler, Eds., *PCR Primer: A Laboratory Manual* (2003); Bowtell and Sambrook, *DNA Microarrays: A Molecular Cloning Manual* (2003); Mount, *Bioinformatics: Sequence and Genome Analysis* (2004); Sambrook and Russell, *Condensed Protocols from Molecular Cloning: A Laboratory Manual* (2006); and Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (2002) (all from Cold Spring Harbor Laboratory Press); Stryer, *Biochemistry* (4th Ed.) (1995) W.H, Freeman, New York N.Y.; Gait, *"Oligonucleotide Synthesis: A Practical Approach"* (2002) IRL Press, London; Nelson and Cox, *Lehninger, Principles of Biochemistry* (2000) 3rd Ed., W. H. Freeman Pub., New York, N.Y,; and Berg, et al., *Biochemistry* (2002) 5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

[0036] Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" refers to one or more nucleic acids, and reference to "the assay" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

[0037] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing devices, formulations and methodologies that may be used in connection with the presently described invention.

[0038] Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention,

[0039] In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been

Curio EX1010-p. 135
Curio v Prognosys
IPR2024-01289

described in order to avoid obscuring the invention.

The Invention in General

[0040] The assay systems of the invention provide spatially-encoded, multiplexed assays comprising 1) an assay capable of high levels of multiplexing with an efficient spatial encoding scheme; 2) instrumentation capable of delivering reagents according to a spatial pattern; and 3) decoding determined by a readout that is digital in nature. The assay systems of the invention detect the presence or absence and relative amount of a biological target or biological activity indicative of a biological target, as well as the location of the biological target or activity in a biological sample, *e.g.*, a tissue section or other biological structure disposed upon a support such as a microscope slide or culture dish.

[0041] 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, allowing for measurement of numerous biological targets or activities in a meaningful spatial environment, including gene expression and peptide localization, An encoding scheme used in these assay systems allows one to determine the location of biological targets or activity (or lack thereof) in the biological samples after the products of the multiplexed assay are removed from the biological sample and pooled for analysis. Decoding of the encoding scheme can be performed by, *e.g.*, next-generation sequencing, which easily provides millions to trillions of data points at low cost. The assay results such as the amount or activity of biological targets can then be mapped back to specific location in the biological sample. The assay systems open a new analytical window into the complex spatial patterns of cellular function and regulation in biological samples.

[0042] A simplified overview of the assay system 100 of the present invention is provided at Figure 1, At step 110, a biological sample affixed to a support is provided. The biological sample contains biological targets of interest. Biological targets can include any molecule of interest, such as nucleic acids (including, *e.g.* RNA transcripts, genomic DNA sequences, cDNAs, amplicons, or other nucleic acid sequences) and proteins, enzymes and the like. At step 120, encoded probes are delivered to the biological sample according to a known spatial pattern. Encoded probes comprise probes, which can interact "with biological targets of interest, and coding tags, which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample. Coding tags in most embodiments are oligonucleotides. However, coding tags may also be mass tags, fluorescent labels, or other moieties.

Curio EX1010-p. 136
Curio v Prognosys
IPR2024-01289

[0043] In some embodiments, the probe and coding tag portions of the encoded probe are pre-coupled before being delivered to the biological sample, For example, in the case where the encoded probes are oligonucleotides, both the probe and coding tag sequence can be synthesized as a single oligonucleotide. Alternatively, the probe and coding tag portions of the encoding probes can be synthesized or obtained separately and combined before delivery to the biological sample *(e.g.,* two separate oligonucleotides can be synthesized and coupled by, *e.g.,* ligation; or an antibody and an oligonucleotide can be prepared separately and conjugated before delivery to the biological sample). Also, as is described in Figures 2-5, the probes and the coding tags (in encoding oligonucleotides) are synthesized separately, and are delivered to the biological sample at different steps (*e.g.,* probes first and coding tags thereafter, or vice versa) in the assay.

[0044] At step 130, the encoded probes are allowed to react or interact with the biological targets, *i.e.,* conditions are provided to allow *e.g.,* oligonucleotides to hybridize to nucleic acid targets, enzymes to catalyze reactions with protein targets, antibodies to bind epitopes, etc. In the case where the biological targets are nucleic acids, the encoded probes are typically oligonucleotides and hybridize to the target nucleic acids. In the case that the biological targets are proteins, the encoded probes typically are aptamers, small molecules, or oligonucleotide-conjugated proteins that interact with target proteins by binding to them or by reacting with them (that is, one of the proteins is a substrate for the other). Encoding oligonucleotides may be coupled to the probes (proteins) by conjugation, chemical or photo-crosslinking via suitable groups and the like.

[0045] Once encoded probes interact with the biological targets, the encoded probes that interacted with the biological targets must be separated from the encoded probes that did not interact with the biological targets at step 140. In the case where the biological targets are nucleic acids and the encoded probes are oligonucleotides, the separation can be accomplished by, *e.g.,* washing the unhybridized encoded probes from the sample. Similarly, for other assays that are based on affinity binding, including those using aptamer, small molecule, and protein probes, washing steps can be used to remove low affinity binders. In the case where the probe is transformed via interaction with the target, *e.g.,* in the case of a peptide, *e.g.,* via cleavage by a protease or phosphorylation by a kinase, it is convenient to collect, all encoded probes--both encoded probes that interacted with the biological targets and were transformed and encoded probes that were not transformed. After collection or pooling, an antibody or other affinity capture agent can be used to capture probes that were transformed by addition of a moiety (*e.g.,* a phosphate group). In cases where probes have been transformed via cleavage, the transformed probes can be separated, *e.g.,* by capturing the non-transformed probes via a tag that is removed from the transformed probes during the transformation *(e.g.,* by cleavage), or by adding a new tag at the site of cleavage.

[0046] Once the reacted (transformed) or interacted encoded probes are separated from the unreacted or un-interacted encoded probes, the sequence of the reacted and/or interacted encoded probes is determined by, preferably, sequencing. The sequence of the encoded probes allows the mapping of the assay results back to locations in the biological sample.

[0047] Figure 2 provides a simplified overview of an assay system of the present invention embodying an efficient implementation of a combinatorial coding scheme for the encoding of spatial information. For purposes of this overview, the probes are oligonucleotides, but as explained elsewhere, other types of probes can also be used. In step 210, a biological sample affixed to a support, *e.g.,* a tissue sample or other biological structure, is provided. In step 220, one or more oligonucleotide probes are delivered to the biological sample, where the oligonucleotide probes are capable of hybridizing with biological targets in the biological sample. In step 230, the oligonucleotide probes are allowed to interact with (hybridize to) the nucleic acid targets; that is, appropriate conditions are provided where oligonucleotide probes can hybridize to the target nucleic acids.

[0048] In step 240, the oligonucleotide probes that did not hybridize to target nucleic acids are removed, and thereby separated from oligonucleotide probes that did hybridize to target nucleic acids. In this embodiment, separation can be accomplished by, *e.g.,* washing the sample to remove unhybridized oligonucleotide probes. Next, in step 250, encoding oligonucleotides (the encoding agents) are delivered to the biological sample according to a chosen spatial pattern, where the encoding oligonucleotides comprise coding tags that are used to encode the location of biological targets in the biological sample. Note that in contrast to the assay system of Figure 1, here the probes and encoding agents (encoding oligonucleotides) are delivered in separate steps. In step 260, the encoding oligonucleotides are coupled to the oligonucleotide probes to create encoded probes. In this case where the probes are oligonucleotides, the encoding oligonucleotides may be coupled to the oligonucleotides probes by, *e.g.,* ligation. Alternatively, the information in the encoding oligonucleotides can be transferred by using a DNA polymerase to extend a probe oligonucleotide that acts as a primer, and thereby copy and incorporate the sequence of the encoding oligonucleotides.

[0049] In step 270, the sequence of the coding tags in the encoded probes as well as the sequence or a portion of the sequence of the probe itself is determined, and in step 280, the target nucleic acids are mapped back to the biological sample. In some embodiments, the abundance of sequences reveals the relative quantity of biological targets at the location. Although this embodiment shows the individual steps in a particular order, so as to better explain the invention,

the precise order of the steps can be varied. For example, steps 220 and 250 can be combined, so that a mixture of the probes and encoding oligonucleotides is delivered according to a chosen spatial pattern. Coupling step 260 can then be carried out immediately after the combined steps 220 and 250, or concomitantly with them. In this case, step 240 would then occur after step 260. It can therefore be appreciated that the two key results of this series of steps, *i.e.,* the location-specific encoding of probe molecules and the separation of probe molecules based on their ability to interact with corresponding target molecules, can be accomplished with some flexibility in the implementation of the particular steps. Similarly, there is considerable flexibility in the design of the coding scheme. As described *infra,* the assays of the invention are particularly amenable to combinatorial methods.

[0050] Thus, the present invention provides an ability to look at many different biological targets in many locations, providing the resolution of in situ hybridization with the highly-parallel data analysis of sequencing. In some embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 20, in other embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 50, in other embodiments, the sum of the multiple biological targets being assayed and the multiple sites in the biological sample is greater than 100, greater than 500, 1,000, 10,000, 25,000, 100,000, 500,000, 1,000,000. It will be appreciated that, due to the spatial encoding dimension of the invention, even much larger numbers can be contemplated. For example, assaying l 0,000 targets per location x 10,000 locations would generate $1O^{8}$ different assays, and even larger numbers than these can easily be contemplated, particularly if spatial locations with resolution on the order of that of single cells are utilized. Further, in embodiments where high-throughput digital sequencing is employed, the sequences of at least 1,000 encoding probes are typically determined in parallel. More typically, using a digital readout, it is desirable to obtain multiple sequence reads for each assay (defined by a probe and a spatial location code). It is desirable to obtain an average of at least 3 copies per assay, and more typically at least 10 or at least 30 copies per assay, depending on the design of the experiment and requirements of the assay. For a quantitative readout with suitable dynamic range, it may be desirable to obtain at least 1,000 reads per assay. Therefore, if 1,000,000 assays are carried out, the number of sequence reads may be 1 billion or more. With high-throughput digital sequencing, and allowing for redundancy, the sequence of at least 10,000 encoding probes are determined in parallel, or the sequence of at least 100,000, 500,000, 1,000,000, 10,000,000, 100,000,000, 1,000,000,000 or more encoding probes are determined in parallel.

Curio EX1010-p. 139
Curio v Prognosys
IPR2024-01289

Assays

[0051] The assay portion of the assay systems of the present invention comprise the following general steps: delivering probes and encoding agents where the encoding agents (in some embodiments pre-coupled to the probes) are delivered to the sample according to a known spatial pattern, allowing the probes to interact or react with biological targets in the sample, and, if the probes and encoding agents have not been pre- coupled, coupling the encoding agents to probes.

[0052] The 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, where the ability to tie an assayed biological target or activity back to the location within the biological sample is important. Exemplary biological samples include tissue sections (e.g., including whole animal sectioning and tissue biopsies), cell populations on slides or culture dishes, and the like. The assay systems of the invention are particularly advantageous in that they are compatible with numerous biological sample types, including fresh samples, such as primary tissue sections, and preserved samples including but not limited to frozen samples and paraformalin-fixed, paraffin-embedded (FFPE) samples. An important aspect of the assay systems of the invention is that the biological samples are immobilized on a substrate surface having discrete, independently measurable areas.

[0053] The biological targets to be detected can be any biological molecules including but not limited to proteins, nucleic acids, lipids, carbohydrates, ions, or multicomponent complexes containing any of the above. Examples of subcellular targets include organelles, e.g., mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplasts, endocytic vesicles, exocytic vesicles, vacuoles, lysosomes, etc.

[0054] In some particular embodiments, the assay system is used to analyze nucleic acids, *e.g.,* by genotyping, quantitation of DNA copy number or RNA transcripts, localization of particular transcripts within samples, and the like. Figure 3 illustrates an overall scheme for an exemplary assay for, *e.g.,* detecting single nucleotide polymorphisms (SNPs) that can be used with the assay system of the invention. In Figure 3, two oligonucleotide probes are provided. Each oligonucleotide probe comprises a target-specific region (located on either side of the SNP to be analyzed) seen at 305 and 307, and ligation regions, seen at 301 and 303, The oligonucleotide probes are allowed to hybridize to a target nucleic acid (not shown) in the biological sample. At step 302, one of the oligonucleotide probes is extended to incorporate the SNP sequence and ligated to the other probe to form an extended probe comprising target nucleic acid region 309 and ligation regions 301 and 303.

[0055] Two encoding agents, both comprising a coding tag (seen at 315 and 317), a ligation region

Curio EX1010-p. 140
Curio v Prognosys
IPR2024-01289

(seen at 311 and 313), and a primer region (seen at 319 and 321) are combined with and ligated to the extended probe at step 304 to form an encoded target- specific oligonucleotide. Again, in contrast with Figure 1, the probes and encoding agents are delivered at separate steps. Doing so allows use of the combinatorial embodiments described *infra.* In preferred embodiments, the encoding oligonucleotides within a pair of encoding oligonucleotides ligate specifically to one side of the target sequence or the other *(i.e., 5'* or 3' of the target sequence). Also, typically, the ligation and primer regions of the encoding oligonucleotides and probes are universal; that is, the set of ligation and primer regions used in constructing the probes and encoding oligonucleotides are constant, and only the target-specific regions of the probes and the coding tags of the encoding oligonucleotides differ. However, again in alternative embodiments, the ligation and primer regions are not universal and differ between probes and encoding agents.

[0056] Following ligation, the encoded probes are eluted, pooled, and, optionally, sequencing adapters are added to the encoded probes via PCR. In alternative embodiments, sequencing primers may be ligated to the encoding oligonucleotides, or sequencing primer sequences can be included as part of the encoding oligonucleotide. As seen in Figure 3, each sequencing adapter comprises primer region 319 or 321, compatible with the primer regions 319 and 321 on the encoded probes. The final construct comprising first adapter 327, first primer region 319, first coding tag 315, ligation regions 311 and 301, target region 309, ligation regions 313 and 303, second coding tag 317, second primer region 325 and second adapter 329 is now ready for input into a digital high-throughput sequencing process.

[0057] A combination of extension and ligation reactions are exemplified in Figure 3, but it should be appreciated that a variety of reactions may be used to couple the encoding oligonucleotides to the target-specific oligonucleotides, including ligation only (e.g., for oligonucleotides that hybridize to contiguous portions of the target nucleic acid sequence). Alternatively, an assay utilizing an additional oligonucleotide, such as in the GOLDENGATE® assay (see Fan, et al., Cold Spring Symp. Quant. Biol., 68:69-78 (2003); (Ilumina, Inc., San Diego, CA)), may be employed.

[0058] In other embodiments, the assay system of the invention also can be used to analyze peptides or proteins, the presence of antibodies, enzymatic and other protein activities, posttranslational modifications, active and non-active forms of peptides, as well as peptide isoforms in a biological sample. Accordingly, the probes may comprise an active region of an enzyme, a binding domain of an immunoglobulin, defined domains of proteins, whole proteins, synthetic peptides, peptides with introduced mutations, aptamers and the like.

[0059] In certain aspects, the probes are substrates for enzymes or proenzymes, *e.g.,* kinases, phosphatases, zymogens, proteases, or fragments thereof. In certain aspects, the probes are

phosphorylation substrates used to detect proteins involved in one or more signal transduction pathways, *e.g.,* a kinase or a phosphatase. In another specific aspect of the invention, the probes are specific protease substrates that associate only with individual proteases or classes of proteases. In other aspects, the probes are different processed forms, isoforms and/or domains of an enzyme. Protein-based probes are typically conjugated or otherwise linked to oligonucleotide encoding agents. The oligonucleotide encoding agents in this case would also include a nucleotide sequence component that allows for identification of the protein probe.

[0060] In certain aspects, the present invention provides assays for evaluating differences in the amount and/or activity of biological targets between different locations in a sample and/or between samples. The method includes determining a plurality of encoded results from the biological sample and evaluating the differences in quantity of the biological targets at each location in the biological sample.

Combinatorial Embodiments

[0061] To maximize the efficiency of encoding, a combinatorial approach using pairs of coding tags in the encoding oligonucleotides can be used. By de-coupling the target-specific information and the coding tags, the number of oligonucleotides required is dramatically reduced, with a concomitant decrease in cost.

[0062] Figure 4 illustrates a general mechanism for one embodiment of a combinatorial encoding scheme of the assay systems of the invention, where nucleic acids in a representative tissue section (shown at 416) are assayed. Figure 4 at A shows two target-specific/encoding oligonucleotide constructs 420 and 422 *(e.g.,* formed between steps 302 and 304 of Figure 3) specifically bound to a target nucleic acid 402 of interest, The first encoded probe 420 comprises coding tag 408, associated with, *e.g.,* a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 404. The second encoded probe 422 comprises coding tag 406, associated with, *e.g.,* a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410.

[0063] Figure 4 at B shows the spatial pattern that may be used for twenty different coding tags, al through a10 (coding tag 406 on encoded probe 420) and b1 through b10 (coding tag 408 encoded probe 422). Coding tag al., for example, is deposited on the biological sample in ten discrete areas or spots (shown as the first horizontal line of spots in 412). Coding tag a2 is deposited on the biological sample in ten spots on the second horizontal line in 412. Coding tag a3 is deposited on the biological sample in ten spots on the third horizontal line in 412, and so on. Whereas the "a" tags are deposited

in ten horizontal rows, the "b" tags are deposited in ten vertical rows as shown in 414. For example, coding tag b1 is deposited on the biological sample in ten discrete spots in the first vertical row of 414, coding tag b2 is deposited on the biological sample in ten discrete spots in the second vertical row of 414, and so on. Using such a configuration allows for twenty coding tags to uniquely define 100 different locations on the biological sample,

[0064] Figure 4 at C shows a representative tissue section 416 coincident with coding tag grid 418. The arrows show how the "a" coding tags and the "b" coding tags are deposited on grid 418 that is coincident with tissue section 416. If, once sequenced, coding tags al and b4, *e.g.,* are associated with a target nucleic acid sequence, then that target nucleic acid sequence *(i.e.,* biological target) was present in the tissue section at location al, b4.

[0065] Figure 5 provides a simplified, specific example of the encoding scheme of the assay systems of the invention. Figure 5 shows encoding oligonucleotides 510, comprising al, a2, a3, a4 and b1, b3, b3 and b4. Target-specific oligonucleotides (TSOs) (probes) 1 and 2 are shown at 520. A deposit or dispensing scheme is shown at 530. Like the grid exemplified in Figure 4, encoding oligonucleotides al through a4 are deposited in spots in a pattern (here, in a vertical pattern), and encoding oligonucleotides bl through b4 are deposited in spots in a pattern (here, a horizontal pattern). The grid though shown as a square with spots is actually a deposition pattern on a biological sample (not shown) such as tissue section 416 shown in Figure 4.

[0066] The target-specific oligonucleotides are delivered to the biological sample, where the target-specific oligonucleotides hybridize to target nucleic acids in the biological sample if target nucleic acids are present. Unhybridized target-specific oligonucleotides are then removed, *e.g.,* by washing. The encoding oligonucleotides are then delivered to the biological sample according to the spatial pattern shown at 530. The encoding oligonucleotides are ligated (or, *e.g.,* extended and ligated) to any target-specific oligonucleotides that hybridized to the target nucleic acid in the biological sample, the ligated constructs are then eluted from the biological sample, pooled, and sequencing adapters are added through, *e.g.,* PCR or ligation, if the sequences were not previously included in the encoding oligonucleotides. The ligated constructs are sequenced by, *e.g.,* high throughput or "next generation" sequencing.

[0067] The pool of resulting sequences is shown at 540. A sequence readout was obtained for target-specific oligonucleotide 1 only at a4bl, a4b2, alb3, a2b3, a3b3, a4b3 and a4b4 (positions shown with horizontal lines). A sequence readout was obtained for target-specific oligonucleotide 2 only at a1b1 (position shown with vertical lines). A sequence readout was obtained for both target-specific oligonucleotides 1 and 2 at positions a2b1, a3b1, a1b2, a2b2, and a3b2 (positions shown with cross-hatching). No sequence readout was obtained for either target-specific oligonucleotides at alb4, a2b4

or a3b4 (positions shown without shading). Thus, in the biological sample on which the assay took place the first target nucleic acid was detected in a large portion of the left side and at the bottom of the biological sample, the second target nucleic acid was detected only in the upper left portion of the biological sample, and neither target nucleic acid was detected in the upper right portion of the biological sample. The differential expression of the two target nucleic acids now can be mapped back to the biological sample and to the biological structures or cell types in these locations in the biological sample.

[0068] In addition to location information, information relating to relative abundance of the encoded tags can be obtained. For example, if it is found that there are ten times as many a4T1b1 sequences occurring in the data set as compared to a4T1b2 sequences, this would indicate that target nucleic acid sequence 1 is ten times more abundant at the a4T1b1 location than at the a4T1b2 location.

[0069] In the case of nucleotide analysis as shown in Figure 3, by ligating the coding tags directly to target-specific oligonucleotides, only 2n target-specific oligonucleotides are needed for n targets. For example, using the combinatorial approach outlined in Figure 2, assaying 100 different targets at 10,000 spatial locations would require 2 x 100 target-specific oligonucleotides and 2 x 100 encoding oligonucleotides. The total count of assay oligonucleotides would be only 400 (200 target-specific and 200 encoding), not counting universal primers. In contrast, if the coding oligonucleotides were not decoupled from the target-specific oligonucleotides, (n x X positional codes) + (n x Y positional codes) would be needed, or in the above example, 20,000 oligonucleotides, not counting universal primer sequences. Moreover, though the embodiments shown in Figures 2- 5 depict a combinatorial scheme using two encoding agents (coding tags), three, four or more encoding agents and coding tags may be used, and attached to the probe or one another by varying means and in varying combinations of steps.

[0070] Due to the spatial encoding aspect of the assay system of the invention, a large amount of information can be generated with even a modest number of assays. For example, five or more biological targets assayed at five or more positions in the sample generates 25 or more combinations. Using digital sequencing as a readout, the optimum number of sequence reads per combination depends on the sensitivity and dynamic range required, and can be adjusted, For example, if for each combination on average 100 reads are sampled, the total for 25 combination is 25,000 reads. If 1,000 targets are assayed at 1,000 locations with an average sampling depth of 1,000, then 10^9 reads are required. These numbers, although large, are within the capacity of intrinsically parallel digital sequencing methods, which can generate datasets of billions or even trillions of reads in a reasonable timeframe and at a very low cost per read. Therefore, by varying the numbers of positions interrogated or biological targets assayed, or both, and using digital sequencing, large

amounts of information can be obtained. In specific aspects, multiple locations are interrogated for two or more biological molecules.

Reagent Delivery Systems

[0071] The reagent delivery system of the invention includes instrumentation that allows the delivery of reagents to discrete portions of the biological sample, maintaining the integrity of the spatial patterns of the encoding scheme. Reagent delivery systems of the assay systems of the invention comprise optional imaging means, reagent delivery hardware and control software. Reagent delivery can be achieved in a number of different ways. It should be noted that reagent delivery may be to many different biological samples at one time. A single tissue section has been exemplified herein; however, multiple biological samples may be affixed and analyzed simultaneously. For example, pions of a tissue sample can be analyzed in parallel and the data combined to build a 3D map.

[0072] Integral to the assay system of the invention is instrumentation that allows for spatial patterning of reagents onto the biological sample. Technologies for formulating and delivering both biological molecules *(e.g.* oligonucleotides or antibodies) and chemical reagents (e.g., small molecules or dNTPs) are known in the art, and uses of these instrument systems are known to one skilled in the art and easily adaptable to the assay systems of the invention. One example of a suitable reagent delivery system is the Labcyte™ Echo acoustic liquid handier, which can he used to deliver nanoliter scale droplets containing biological molecules with high precision and reproducibility. One skilled in the art could incorporate this reagent delivery device into the overall system, using software to specify the locations to which reagents should be delivered.

[0073] Other instruments that can be used for the deposition of agents and/or coding identifiers onto biological samples include, but are not limited to, ink jet spotting; mechanical spotting by means of pin, pen or capillary; micro contact printing; photochemical or photolithographic methods; and the like. For several applications, it may be preferred to segment or sequester certain areas of the biological samples into one or more assay areas for different reagent distributions and/or biological target determination. The assay areas may be physically separated using barriers or channels.

[0074] In one exemplary aspect, the reagent delivery system may be a flow-based system. The flow-based systems for reagent delivery in the present invention can include instrumentation such as one or more pumps, valves, fluid reservoirs, channels, and/or reagent storage cells. Reagent delivery systems are configured to move fluid to contact a discrete section of the biological sample. Movement

of the reagents can be driven by a pump disposed, for example, downstream of the fluid reagents. The pump can drive each fluid reagent to (and past) the reaction compartment. Alternatively, reagents may be driven through the fluid by gravity. US Pub. Nos. 20070166725 and 20050239192 disclose certain general-purpose fluidics tools that can be used with the assay systems of the invention, allowing for the precise manipulation of gases, liquids and solids to accomplish very complex analytical manipulations with relatively simple hardware.

[0075] In a more specific example, one or more flow-cells can be attached to the substrate-affixed biological sample from above. The flow-cell can include inlet and outlet tubes connected thereto and optionally an external pump is used to deliver reagents to the flow-cell and across the biological sample. The flow cells are configured to deliver reagents only to certain portions of the biological sample, restricting the amount and type of reagent delivered to any specific section of the biological sample.

[0076] In another aspect, a microfluidic system can be integrated into the substrate upon which the biological sample is disposed or externally attached on top of the substrate. Microfluidic passages for holding and carrying fluid may be formed on and/or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs.

[0077] Pumps generally include any mechanism for moving fluid and/or reagents disposed in fluid. In some examples, the pump can be configured to move fluid and/or reagents through passages with small volumes *(i.e.,* microfluidic structures). The pump can operate mechanically by exerting a positive or negative pressure on fluid and/or on a structure carrying fluid, electrically by appropriate application of an electric field(s), or both, among other means. Exemplary mechanical pumps may include syringe pumps, peristaltic pumps, rotary pumps, pressurized gas, pipettors, etc. Mechanical pumps may be micromachined, molded, etc. Exemplary electrical pumps may include electrodes and may operate by electrophoresis, electroendoosmosis, electrocapillarity, dielectrophoresis (including traveling wave forms thereof), and/or the like.

[0078] Valves generally include any mechanism for regulating the passage of fluid through a channel. Valves can include, for example, deformable members that can be selectively deformed to partially or completely close a channel, a movable projection that can be selectively extended into a channel to partially or completely block a channel, an electrocapillary structure, and/or the like.

[0079] An open gasket can be attached to the top of the biological sample and the sample and reagents can be injected into the gasket. Suitable gasket materials include, but are not limited to, neoprene, nitrile, and silicone rubber. Alternatively, a watertight reaction chamber may be formed by a gasket sandwiched between the biological sample on the substrate and a chemically inert, water

resistant material such as, but not limited to, black-anodized aluminum, thermoplastics *(e.g.,* polystyrene, polycarbonate, etc), glass, etc.

[0080] In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest. The images obtained, *e.g.,* may be used to design the deposition pattern of the reagents, Imaging means are optional, as an individual can instead view the biological sample using, *e.g.,* a microscope, analyze the organization of the biological sample, and specify a spatial pattern for delivery assay reagents. If included, the delivery system can comprise a microcircuit arrangement including an imager, such as a CCD or IGFET-based *(e.g.,* CMOS-based) imager and an ultrasonic sprayer for reagent delivery such as described in US Pub. No. 20090197326, which is incorporated herein by reference. Also, it should be noted that although Figures 4 and 5 illustrate using a x,y grid configuration, other configurations can be used, such as, *e.g.,* following the topology of a tissue sample; targeting certain groups of cells, cell layers and/or cell types in a tissue, and the like.

[0081] In yet another alternative, the reagent delivery system controls the delivery of reagents to specific patterns on a biological sample surface using semiconductor techniques such as masking and spraying. Specific areas of a biological sample can be protected from exposure to reagents through use of a mask to protect specific areas from exposure. The reagents may be introduced to the biological sample using conventional techniques such as spraying or fluid flow. The use of masked delivery results in a patterned delivery scheme on the substrate surface.

[0082] In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology. There are a variety of different ink-jetting mechanisms (*e.g.,* thermal, piezoelectric) and compatibility has been shown with aqueous and organic ink formulations. Sets of independently actuated nozzles can be used to deliver multiple reagents at the same time, and very high resolutions are be achieved.

[0083] In order to target specific sites of interest, an informative image of the biological sample to be assayed may be used to assist in the reagent delivery methods and associated encoding scheme. Sample regions of the biological sample can be identified using image processing (e.g., images of cell types differentiated by immunohistochemistry or other staining chemistries) integrated with other features of the assay system. In some aspects, software is used to automatically translate image information into a reagent delivery pattern. A mechanism to register and align very precisely the biological sample for reagent delivery is thus an important component of the assay systems of the invention. Mechanisms such as the use of fiducial markers on slides and/or other very accurate physical positioning systems can be adapted to this purpose.

[0084] The invention preferably comprises a complete suite of software tailored to the assay

Curio EX1010-p. 147
Curio v Prognosys
IPR2024-01289

system. Optionally, oligonucleotide design software is used to design the encoding nucleotides (and in embodiments where nucleic acids are assayed, the target-specific oligonucleotides) for the specific assay to be run, and may be integrated as a part of the system. Also optionally, algorithms and software for reagent delivery and data analysis (i.e., sequence analysis) may be integrated to determine assay results. Integrated data analysis is particularly useful, as the type of dataset that is generated may be massive as a consequence of scale. Algorithms and software tools that are specifically designed for analysis of the spatially-associated data generated by the assay systems, including pattern-analysis software and visualization tools, enhance the value of the data generated by the assay systems.

[0085] In certain aspects, the assay system comprises processes for making and carrying out the quality control of reagents, *e.g.,* the integrity and sequence fidelity of oligonucleotide pools. In particular, reagents are formulated according to factors such as volatility, stability at key temperatures, and chemical compatibility for compatibility with the reagent delivery instrumentation and may be analyzed by instrumentation integrated within the assay system.

Sequencing

[0086] Numerous methods can be used to identify the coding tags and probe sequences in the encoded probes of the assay systems of the invention. The coding tags can be detected using techniques such as mass spectroscopy *(e.g.,* Maldi-T of, LC-MS/MS), nuclear magnetic resonance imaging, or, preferably, nucleic acid sequencing. Examples of techniques for decoding the coding tags of the present invention can be found, for example, in US Pub. No. 20080220434, which is incorporated herein by reference. For example, the coding tags may be oligonucleotide mass tags (OMTs or massTags). Such tags are described, *e.g.,* in US Pub. No. 20090305237, which is incorporated by reference in its entirety. In yet another alternative, the encoded probes can be amplified and hybridized to a microarray. This would require separate amplification reactions to be carried out, in which each amplification is specific to a particular spatial code or subset of codes, accomplished by using code-specific primers. Each amplification would also incorporate a different resolvable label (e.g. fluorophor). Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can he determined by the relative abundances of the resolvable labels.

[0087] In one particularly preferred aspect, the resulting coding tags according to the assay system are substrates for high-throughput, next-generation sequencing, and highly parallel next-generation sequencing methods are used to confirm the sequence of the coding tags, for example, with SOLiD™ technology (Life Technologies, Inc.) or Genome Ananlyzer (Illumina, Inc.). Such next-

generation sequencing methods can be carried out, for example, using a one pass sequencing method or using paired-end sequencing. Next generation sequencing methods include, but are not limited to, hybridization-based methods, such as disclosed in *e.g.,* Drmanac, U.S. Pat. Nos. 6,864,052; 6,309,824; and 6,401,267; and Drmanac et al, U.S. patent publication 2005/0191656; sequencing-by-synthesis methods, *e.g.,* U.S. Pat Nos. 6,210,891; 6,828,100; 6,969,488; 6,897,023; 6,833,246; 6,911,345; 6,787,308; 7,297,518; 7,462,449 and 7,501,245; US Publication Application Nos. 20110059436; 20040106110; 20030064398; and 20030022207; Ronaghi, et al, Science, 281: 363-365 (1998); and Li, et al, Proc. Natl. Acad. Sci., 100: 414-419 (2003); ligation-based methods, *e.g.,* U.S, Patent Nos. 5,912,148 and 6,130,073; and U.S. Pat. Appln Nos. 20100105052, 20070207482 and 20090018024; nanopore sequencing *e.g.,* U.S. Pat. Appln Nos. 20070036511; 20080032301; 20080128627; 20090082212; and Soni and Meller, Clin Chem 53: 1996-2001 (2007)), as well as other methods, *e.g.,* U.S. Pat. Appln Nos. 20110033854; 20090264299; 20090155781; and 20090005252; also, see, McKernan, et al., Genome Res., 19:1527-41 (2009) and Bentley, et al., Nature 456:53-59 (2008), all of which are incorporated herein in their entirety for all purposes.

Applications of Assay System

[0088] It will be apparent to one skilled in the art upon reading the present disclosure that there are numerous important areas of biological research, diagnostics, and drug development that will benefit from a high throughput multiplexed assay system that can measure simultaneously the amount and spatial location of a biological target in a biological sample. For example, combining the ability to estimate the relative abundance of different RNA transcripts with the ability to reconstruct an image of spatial patterns of abundance across many locations, which may be as small as or even smaller than individual cells, in a tissue enables many different areas of basic research. The following are exemplary uses and are by no means meant to be limiting in scope.

[0089] In one example, 3-dimensional patterns of gene expression are determined by analyzing a series of tissue sections, in a manner analogous to image reconstruction in CT scanning, Such a method can be used to measure changes in gene expression in disease pathology, *e.g.,* in cancerous tissue and/or a tissue upon injury, inflammation or infection. With the assay systems of the invention, more detailed information on gene expression and protein localization in complex tissues is obtained, leading to new insights into the function and regulation both in normal and diseased states, and provides new hypotheses that can be tested. For example, an assay system of the invention may enable some of the insights gained from many individual studies and larger programs like ENCODE (Birney, et al., Nature, 447:799-816 (2007)) and modENCODE to be integrated at the tissue level. The assay

systems also aid computational efforts to model interacting networks of gene expression in the field of systems biology.

[0090] The assay systems also provide a novel approach to analysis of somatic variation, *e.g.*, somatic mutations in cancer or variability in response to infectious organisms. For example, tumors are typically highly heterogeneous, containing cancer cells as well as genetically normal cells in an abnormal local environment. Cancer cells undergo mutation and selection, and in this process it is not unusual for local clones to develop. Identifying relatively rare somatic mutations in the context of tumors may enable the study of the role of key mutations in the selection of clonal variants. Transcriptional patterns associated with angiogenesis, inflammation, or other cancer-related processes in both cancer and genetically normal cells can be analyzed for insights into cancer biology and assist in the development of new therapeutic agents for the treatment of cancers. In another example, individuals have varying susceptibility to infectious organisms, and the assay systems of the invention can be used to study the interaction between microbes and tissues or the various cell types within the tissue.

[00100] Importantly, in addition to providing spatially-associated information, the invention allows a great increase in the sensitivity of detecting rare mutations, as signal to noise can he dramatically increased since only a small location is assayed in any given reaction. In a typical assay for rare mutations in a mixed sample, the sample is treated in bulk, *i.e.*, nucleic acids are extracted from many cells into a single pool. Thus, if a mutation is present in one cell in 10,000, it must be detected against a background of normal DNA from ~10,000 cells. In contrast, with the assay systems of the invention many cells can be analyzed, but individual cells or small groups of cells would be identified by the spatial coding system. Therefore, in the assay systems of the present invention, background is reduced by orders of magnitude, greatly increasing sensitivity. Furthermore, the spatial organization of mutant cells can be observed, which may be particularly important in detecting key mutations in tissue sections in cancer. Already molecular histological analyses are yielding insights into cancer biology and may have potential for use in diagnostics. The technology of the invention promises to greatly increase the power of such approaches.

[00101] The present invention provides assays, assay systems, and methods of using such assays in spatially encoded biological assays. The invention provides an assay system comprising one or more agents provided in defined spatial patterns on a substrate surface, and a detection system for identifying the presence or absence, relative amount, and location of a biological molecule. Such biological molecules include, but are not limited to, nucleic acids, peptides, carbohydrates, cellular components, and the like. The assay system is a novel multiplexing approach, as it allows multiple molecules and their respective multiple locations to be identified in a single system using a unique

encoding scheme. This encoding scheme uses both molecule-specific binding agents and coding identifiers to provide a practical and cost-effective determination of information on multiple biological molecules, including specific positional information of such molecules in a biological sample, e.g., a tissue section. The single molecule detection analysis using the encoding system also allows relative amounts of biological molecules to be detected, thus providing information on expression levels, sequestering in specific locales, and the like.

[00102] The assay systems detect the presence or absence, and relative amount, of a biological molecule at more than one spatial location in a sample. In addition, the assays provide methods for doing this for multiple biological molecules simultaneously. The assay systems utilize one or more binding agents that specifically bind to the biological molecule of interest and unique coding identifiers associated with specific binding agents. The detection system utilizes a method for identifying the presence and spatial address of the agent binding based on the positive and/or negative results that are obtained using detection of the agent and identifier and the encoding scheme of the spatial patterns on the substrate surface. In a specific aspect, the encoding scheme employs limited reagent delivery to the spatial patterns on the substrate surface, and access of the coding identifiers and/or binding agents to portions of the sample is controlled through such limited delivery.

[00103] In one aspect, the assay system detects the presence or absence and spatial location of a biological molecule based on the positive and/or negative results that are obtained using limited reagent delivery and the encoding scheme of the spatial patterns on the substrate surface.

[00104] The assay system and methods of the invention are based on relational, solid-state substrates with positions that represent specific spatial locations within a biological sample, e.g., a cell, organelle or tissue. The ability to use encoding features to represent locations allows high-throughput analysis of the presence or absence, and relative amount, of a biological molecule at more than one spatial location in a sample. The encoding features also allow provide assaying of multiple biological molecules at these multiple locations simultaneously.

[00105] A primary feature of the invention is the preservation of the spatial organization of elements in a sample of interest through the use of an encoding scheme. For example, the assay may be designed to preserve the relative position of cells in a tissue, and the assay may interrogate the individual cells for genomic DNA variation (including epigenetic modifications), and RNA and protein expression.

[00106] In one specific aspect, the encoding scheme of the assay system comprises the use of two or more coding patterns, each comprising regions defined by spatial patterns on the substrate surface. For example, the assay system can utilize an encoding scheme that comprises a 2-dimensional grid format based on the discrete positioning of the binding agents in the substrate surfaces. In another

Curio EX1010-p. 151
Curio v Prognosys
IPR2024-01289

example, the spatial patterns may be based on more randomized cell locations, e.g., the patterns on the substrate surface follow an underlying biological structure rather than a strict, x,y grid pattern. This aspect includes systems with two or more substantially identical spatial patterns using different binding agents and/or coding identifiers, as well as systems having different patterns for different agents and/or coding identifiers. The encoding scheme of the systems can be controlled by delivery of different reagents to discrete regions on the substrate surfaces, which allows different reactions to take place on substantially similar agents of known location on the substrate surfaces.

[00107] In one specific aspect, the invention provides high resolution, high- throughput analysis of nucleic acids and/or expression levels that provides both detection and spatial identification of large numbers of nucleic acids, e.g., DNA or RNA.

[00108] In another specific aspect, the invention provides high resolution, high- throughput analysis of proteins that provides both detection and spatial identification of large numbers of such proteins, e.g., kinases or proteases.

[00109] Numerous reagent delivery systems can be used with the assay system of the invention. The primary criteria of such reagent delivery systems is the ability to direct delivery of specific agents based on spatial patterns on the substrate surface.

[00110] In one preferred aspect, the encoding scheme utilizes a reagent delivery system based on printing and informatics technologies to implement the spatial patterns used for identification and localization of the biological materials. For example, the patterns found in the encoding scheme may be created using ink jet printing technology to provide reagents at specific locations on one or more substrate surfaces. The desired patterns are set out in specific coding patterns on the substrate surface.

[00111] In certain aspects of the invention, the binding agents are immobilized directly to the substrate surface, and the location of the binding agents is known or determined prior to use of the substrate surface in the assay system. In another aspect, the binding agents are immobilized onto beads or other separate structural elements that are then provided in known locations on the substrate surface. In yet another aspect, the binding agents may be provided in or on features of the substrate surface, e.g., provided in wells or channels.

[00112] In specific aspects of the invention, the binding agents are nucleic acids immobilized directly or indirectly to the substrate surface, e.g., directly through the use of amino groups on the substrate surface or indirectly through the use of a linker. The location of the nucleic acid sequences is known or determined prior to use of the substrate surface in the assay system. In another specific aspect, the nucleic acids may be immobilized directly or indirectly onto beads that are then provided in known locations on the substrate surface. In yet another aspect, the nucleic acids may be provided in or on features of the substrate surface, e.g., provided in wells.

[00113] In these aspects involving nucleic acid agents, any methods of sequence determination can be used, e.g., sequencing, hybridization and the like. In a preferred aspect, nucleic acid sequencing, and preferably next-generation sequencing, is used to decode the spatial encoding scheme in the assay system of the invention. This provides a very wide dynamic range for very large numbers of assays, allowing for efficient multiplexing.

[00114] In some aspects, the assay utilizes two or more oligonucleotides, the oligonucleotides comprising a universal primer region and a region that correlates specifically to a single spatial pattern within the spatial encoding scheme. In a specific aspect, the assay comprises two allele specific oligonucleotides and one locus specific oligonucleotides. These oligonucleotides allow the identification of specific SNPs, indels or mutations within an allele. This is useful in the identification of genetic changes in somatic cells, genotyping of tissues, and the like.

[00115] In other specific aspects of the invention, the binding agents are peptides. In one aspect, these peptides are associated directly or indirectly to known locations on a substrate surface, e.g., using binding protein pairs or through oligonucleotide linkers complementary to oligonucleotides on the substrate surface. In another aspect, the binding agents are peptides are immobilized directly or indirectly onto beads or other separate structural elements that are then provided in known locations on the substrate surface. In yet another aspect, the peptides may be provided in or on features of the substrate surface, e.g., provided in wells.

[00116] In yet other specific aspects of the invention, the binding agents are chemical entities (e.g., small molecules) that are coded, e.g. using sequence tags or mass spectroscopy tags as coding identifiers. In one aspect, these chemical entities can be are immobilized directly to the substrate surface. In another aspect, the binding agents are immobilized onto beads or other separate structural elements that are then provided in known locations on the substrate surface. In yet another aspect, the binding agents may be provided in or on features of the substrate surface, e.g., provided in wells.

[00117] The assay system of the invention can utilize various detection mechanisms, based on the molecules to be detected and the reagents needed for such detection system. Exemplary methods that can be used with the assay systems of the invention are described in more detail below.

[00118] **The Invention in General**

[000119] The assay system and methods of the invention are based on relational methods that allow extraction of data to detect the presence or absence and relative amount of a biological molecule, and the location of this molecule in a sample having a distinct structure, *e.g.,* a tissue section or other biological structure with distinct locations of specific biological molecules. The encoding scheme

Curio EX1010-p. 153
Curio v Prognosys
IPR2024-01289

used in these systems corresponds to the structural elements of the sample, and the information obtained using a two-dimensional coding system is indicative of the spatial addresses of these molecules in a sample of interest.

[00120] Integral to the assay system of the invention is a method for spatial patterning of reagents. Technologies for formulating and delivering both biological molecules *(e.g.* DNA or antibodies) and chemical reagents *(e.g.,* small molecules or dNTPs) have already been demonstrated, and use of these systems will be available to one skilled in the art and easily adaptable upon reading this specification.

[00121] The assay design of the invention provides an accurate and easily scalable spatial encoding system. The ability to deliver reagents in a spatially defined pattern together with software, reagents and protocols comprises a novel and highly innovative assay system for spatial analysis of various biological molecules and activities. This allows the assays to measure numerous biological functions in a meaningful spatial environment, including functions such as gene expression and peptide localization. The systems provide the potential to open a new analytical window into the complex spatial patterns of cellular function and regulation in biological systems.

[00122] The biological molecules to be detected can be any biological molecules such as proteins, nucleic acids, lipids, carbohydrates, ions, or multicomponent complexes containing any of the above. Further examples of subcellular objects include organelles, *e.g.,* mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplast, endocytic vesicle, exocytic vesicles, vacuole, lysosome, etc.

[00123] Fig. 1 illustrates such a target-specific assay system for identification of nucleic acid sequences in a sample. In this system 101, two reagents 120, 122 that specifically bind to a biological molecule of interest are associated with coding identifiers 106, 108 that encode for a spatial location in the sample. These coding identifiers 106, 108 are optionally associated with sites that assist in their identification in the assay format, *e.g.,* universal priming sites 104, 110 for amplification of the assay products or adapters to enable identification of the coding identifiers and the binding agents using

sequencing technologies. The sample that is tested, here shown as a tissue section 116 is encoded using the combination of the patterns 112, 114 created using the separate coding identifiers 106, 108 which provide a two dimensional code 118 that shows the location of any positive detection of the biological molecule 102 as well as quantifying the biological molecule 102 at each location assayed in the tissue.

[00124] The assay systems of the invention are particularly advantageous in that they are compatible with numerous samples types, such as fresh samples, such as primary tissue sections, and preserved samples including but not limited to frozen samples and paraformalin-fixed, paraffin-embedded (FFPE) samples. An important aspect of the assay systems of the invention is that the binding agents are immobilized on a substrate surface in discrete, independently measureable areas. These discrete areas can be formed by spatially selective deposition of the binding agents on the substrate surface. Numerous methods can be used for the deposition of the agent and the coding identifiers associates with the agent. For example, the coding identifiers can be delivered together or separately from the agent. If delivered together they can be attached *(e.g.,* synthesized as a single molecule or attached through ligation or a chemical coupling mechanism) or simply mixed together to be attached after delivery to the substrate. In a preferred aspect, the agent and the coding identifier are made separately, mixed together for attachment, and delivered either attached or as a mixture to be attached on the surface. In a specific aspect the binding agents are delivered generally over the substrate surface and the coding identifiers are delivered in a pattern-specific manner.

[00125] Examples of methods that can be used for deposition of agents and/or coding identifiers onto the substrate surface include, but are not limited to, ink jet spotting, mechanical spotting by means of pin, pen or capillary, micro contact printing, fluidically contacting the measurement areas with the biological or biochemical or synthetic recognition elements upon their supply in parallel or crossed micro channels, upon exposure to pressure differences or to electric or electromagnetic potentials, and photochemical or photolithographic immobilization methods.

Curio EX1010-p. 155
Curio v Prognosys
IPR2024-01289

[00126] For several applications, it may be preferred to arrange the substrates into segments of one or more measurement areas for reagent distribution and agent determination. These regions may be physically separated using barriers or channels. They may still comprise several additional discrete measurement areas with agents that are different or in different combination from each other.

[00127] In certain aspects, the present invention provides a method, *e.g.,* a machine-based method, for evaluating changes in the presence and/or location of a biological molecule over time. The method includes providing a plurality of encoded array results representative of the biological molecule over time and evaluating the differences in detection and/or localization of the biological molecules.

Nucleic Acid Detection and Localization

[00128] In a particular aspect, the assay system is used to analyze nucleic acids, *e.g* genotyping, gene expression analysis, localization of particular transcripts within samples, and the like.

[00129] Genotyping may be performed using any technique known to those of skill in the art. Preferred techniques permit rapid, accurate determination of multiple variations with a minimum of sample handling. Some examples of suitable techniques involve but are not limited to direct DNA sequencing, capillary electrophoresis, hybridization, allele-specific probes or primers, single-strand conformation polymorphism analysis, nucleic acid arrays, bead arrays, restriction fragment length polymorphism analysis, cleavage fragment length polymorphism analysis, random amplified polymorphic DNA, ligase detection reaction, heteroduplex or fragment analysis, differential sequencing with mass spectrometry, atomic force microscopy, pyrosequencing, FRET *(e.g.,* TaqMan (Applied Biosystems, Inc., Foster City, Calif.) and Molecular Beacon (Stratagene, La Jolla, Calif.) assays), and other related techniques. Several methods for DNA sequencing are well known and generally available in the art. See, for example, Sambrook, et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, New York) (2001); Ausubel, et al., Current Protocols in

Curio EX1010-p. 156
Curio v Prognosys
IPR2024-01289

Molecular Biology (John Wiley and Sons, New York) (1997), Twyman, et al. (2003) "Techniques Patents for SNP Genotyping", Pharmacogenomics 4(1):67-79; and Kristensen, et al. (2001) "High-Throughput Methods for Detection of Genetic Variation", BioTechniques 30(2):318-332. For details on the use of nucleic acid arrays (DNA chips) for the detection of, for example, SNPs, see U.S. Pat. No. 6,300,063 issued to Lipshultz, et al., and U.S. Pat. No. 5,837,832 to Chee, et al., HuSNP Mapping Assay, reagent kit and user manual, Affymetrix Part No. 90094 (Affymetrix, Santa Clara, Calif.). The molecular inversion probe (MIP) assay format (Hardenbol et al., 2003) is another example of a highly multiplexable assay that may be used with the assay systems of the invention.

[00130] In one exemplary and preferred method for analyzing nucleic acids using the assay system of the invention, the detection of nucleic acids uses two allele- specific oligonucleotides and a locus specific oligonucleotide. The assay methods are carried out according to the strategy outlined in Fig. 2 using next-generation sequencing or another highly parallel nucleic acid assay technology. In this assay, a set of two oligonucleotides is designed to hybridize to each target sequence, with a common oligonucleotide and two unique coding identifiers. The allele can be determined, *e,g,* by primer extension of the locus specific oligonucleotide. Following primer extension and ligation, an amplifiable template is formed with universal primer sequences at either end. Assay oligonucleotides are annealed to a template and enzymatic reactions are used to join the two oligonucleotides only when both are correctly annealed. The detection techniques and read out parameters used in this system of the invention include a much shorter tag than the oligonucleotides used in the assays that are based on capture by hybridization. These shorter tags are designed to be read out by sequencing or, preferably, used to ligate codes onto both ends of the fragment as illustrated in Fig. 2.

[00140] In Figure 2, two target-specific assay oligonucleotides are ligated together 202 following *in situ* hybridization to target sequences. At the same time, encoding oligonucleotides containing tag sequence sets X and Y are ligated 204 to the target specific oligonucleotides. Oligonucleotides

Curio EX1010-p. 157
Curio v Prognosys
IPR2024-01289

containing X ligate specifically to one side of the targeting construct and oligonucleotides containing Y ligate to the other. The oligonucleotides contain universal primer sites Pl and P2. Following ligation, the constructs are eluted and, optionally, sequencing adapters can be attached 206, *e.g.,* via PCR.

[00150] In one preferred aspect, the final construct created from the assay method is a substrate for next-generation sequencing, and highly parallel next-generation sequencing methods are used to confirm the sequence of constructs. Such sequencing methods can be carried out, for example, using a one pass sequencing method or using paired-end sequencing. Next generation sequencing methods include, but are not limited to, hybridization-based methods, such as disclosed in Drmanac, U.S. Pat. Nos. 6,864,052; 6,309,824; and 6,401,267; and Drmanac et al, U.S. patent publication 2005/0191656, and sequencing by synthesis methods, *e.g.,* Nyren et al, U.S. Pat. No. 6,210,891; Ronaghi, U.S. Pat. No. 6,828,100; Ronaghi et al (1998), Science, 281: 363-365; Balasubramanian, U.S. Pat. No. 6,833,246; Quake, U.S. Pat. No. 6,911,345; Li et al, Proc. Natl. Acad. Sci., 100: 414-419 (2003); Smith et al, PCT publication WO 2006/074351; use of reversible extension terminators, *e.g.,* Turner, U.S. Pat. No. 6,833,246 and Turner, U.S. Pat. No. 6,833,246 and ligation-based methods, *e.g.,* Shendure et al (2005), Science, 309: 1728-1739, Macevicz, U.S. Pat. No. 6,306,597; which references are incorporated by reference. Soddart et al., PNAS U S A. 2009 Apr 20; Xiao et al., Nat Methods. 2009 Mar;6(3):199-201. Epub 2009 Feb 8.

[00151] To maximize the efficiency of encoding, a combinatorial approach using pairs of oligonucleotides can be used. For example, with only two sets of 100 codes, a substrate can theoretically encode up to 10,000 locations. The number of assay oligonucleotides required is dramatically reduced, the cost decreased, and the robustness of the approach increased by decoupling the coding sequences from the genome-specific sequences. Alternative assay formats can also be used (e.g. ligation or primer extension followed by ligation).

[00152] By ligating the codes on separately, only 2n target-specific assay oligonucleotides are needed

for n targets. For example, assaying 100 different targets at 10,000 spatial locations would require 2 x 100 targeting oligonucleotides and 2 x 100 encoding oligonucleotides, using a combinatorial approach outlined in Fig. 2. The total count of assay oligonucleotides would be only 400 (200 target- specific and 200 encoding), not counting universal primers. In contrast, if the coding oligonucleotides were not decoupled, (n x X positional codes) + (n x Y positional codes) would be needed, or in the above example, 20,000 oligonucleotides, not counting universal primer sequences.

[00153] Due to the matrix system of the invention, a large amount of information can be obtained even using five or more positions interrogated for five or more biological molecules. By varying one or the other of these, large amounts of information can be obtained, both in terms of locations and/or specific biological In specific aspects, multiple locations are interrogated for two or more biological molecules. As an example, for each datapoint ~1,000 reads may be sampled, for a total of -10E9 reads for 10E6 datapoints.

Peptide Detection Systems

[00154] The assay system of the invention can be used to analyze biological molecules using peptide agents that are associated with the substrate surface in a spatial pattern. Such peptides may comprise an active region of an enzyme, a binding domain of an immunoglobulin, defined domains of proteins, whole proteins, synthetic peptides, peptides with introduced mutations, etc.

[00155] The assay system of the invention allows the identification and spatial location of various forms of peptides, including isoforms and peptides that have undergone posttranslational modification. Importantly, certain aspects of the invention allow the identification of active versus non-active forms of such peptides in a sample. This allows the identification of the presence or absence of specific peptide isoforms, and also acts as a proxy for identification of peptide activity in a sample.

[00156] In certain aspects of the invention, the binding agents associated with the substrate surfaces of the assay system include substrates for enzymes or proenzymes, *e.g.,* a kinase, a phosphatase, a

zymogen, a protease, or a fragment thereof. In certain aspects, the binding agents associated with the substrate surfaces are phosphorylation substrates used to detect proteins involved with one or more signal transduction pathways, *e.g.,* a kinase or a phosphatase. In another specific aspect of the invention, the binding agents are specific protease substrates that associate only with individual or classes of proteases. In other aspects, the binding agents on the substrate surface are different processed forms, isoforms and/or domains of an enzyme.

Reagent Delivery

[00157] The reagent delivery system of the invention can be any system that allows the delivery of reagents to discrete portions of the array in order to keep the integrity of the defined spatial patterns of the encoding scheme. Such discrete delivery can be achieved in a number of different ways.

[00158] In one exemplary aspect, the reagent delivery system can be a flow-based system. The flow-based systems for reagent delivery in the present invention can include one or more pumps, valves, fluid reservoirs, channels, and/or reagent storage cells. Such a reagent delivery system is configured to move fluid in contact with a discrete section of the substrate surface. Movement of the reagents can be driven through a fluid by a pump disposed, for example, downstream of the fluid reagents. The pump can drive each fluid reagent to (and past) the reaction compartment. Alternatively, the reagents may be driven through the fluid by gravity.

[00159] US Appln Nos. 20070166725 and 20050239192 disclose certain general- purpose fluidics tools that can be used with the assay systems of the invention. These allow the precise manipulation of gases, liquids and solids to accomplish very complex analytical manipulations with relatively simple hardware.

[00160] In a more specific example, one or more flow-cells can be attached to the substrate from above. The flow-cell can include inlet and outlet tubes connected thereto and optionally an external pump can be used to deliver the sample or reagents to the flow-cell and across the substrate. The flow

cell is configured to deliver reagents only to certain portions of the array, restricting the amount and type of reagent delivered to any specific section of the array.

[00161] In another aspect, a microfluidic system can be integrated into the substrate or externally attached on top of the substrate. Microfluidic passages for holding and carrying fluid can be formed on and/or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected according to selective opening and closing of valves disposed between reagent reservoirs.

[00162] Pumps generally include any mechanism for moving fluid and/or reagents disposed in fluid. In some examples, the pump can be configured to move fluid and/or reagents through passages with small volumes (i.e., microfluidic structures). The pump can operate mechanically by exerting a positive or negative pressure on fluid and/or on a structure carrying fluid, electrically by appropriate application of an electric field(s), or both, among others. Exemplary mechanical pumps may include syringe pumps, peristaltic pumps, rotary pumps, pressurized gas, pipettors, etc. The mechanical pumps may be micromachined, molded, etc. Exemplary electrical pumps can include electrodes and may operate by electrophoresis, electroendoosmosis, electrocapillarity, dielectrophoresis (including traveling wave forms thereof), and/or the like.

[00163] Valves generally include any mechanism for regulating the passage of fluid through a channel. The valves can include, for example, deformable members that can be selectively deformed to partially or completely close a channel, a movable projection that can be selectively extended into the channel to partially or completely block the channel, an electrocapillary structure, and/or the like.

[00164] In yet another aspect, an open gasket can be attached to the top of the substrate and the sample and reagents can be injected into the gasket. Suitable gasket materials include, but are not limited to, neoprene, nitrile, and silicone rubber. Alternatively, a watertight reaction chamber formed by a gasket sandwiched between the substrate and a chemically inert, water resistant material such as, but not limited to, black-anodized aluminum, thermoplastics (e.g., polystyrene, polycarbonate, etc),

glass, etc.

[00165] In a specific aspect of the present invention, the delivery system can compnse a microcircuit arrangement including an imager, such as a CCD or IGFET-based (e.g., CMOS-based) imager and an ultrasonic sprayer for reagent delivery such as described in US Appln No. 20090197326, which is incorporated herein by reference.

[00166] In yet another aspect of the invention, the reagent delivery system controls the delivery of reagents to specific patterns on a substrate surface using semiconductor techniques such as masking and spraying. Specific areas of a substrate surface can be protected from exposure to reagents through use of a mask to protect specific areas from exposure. The reagents may be introduced to the substrate using conventional techniques such as spraying or fluid flow. The use of the masked substrate delivery results in a patterned delivery scheme on the substrate surface.

[00167] In a preferred aspect of the invention, the reagent delivery instrumentation is based on inkjet printing technology. There are a variety of different ink-jetting mechanisms *(e.g.,* thermal, piezoelectric) and compatibility has been shown with aqueous and organic ink formulations. Sets of independently actuated nozzles can be used to deliver multiple reagents at the same time, and very high resolutions can be achieved.

Software for use in the Assay System

[00168] In order to target specific sites of interest, an informative image of the biological section to be analyzed can be used to assist in the reagent delivery methods and associated encoding scheme. Sample regions can be identified using image processing *(e.g.,* images of cell types differentiated by immunohistochemistry or other staining chemistries) integrated with the other features of the assay system. In some aspects, software is used to automatically translate this information into a reagent delivery pattern. A mechanism to register and align very precisely the biological sample in a targeting

system is thus a preferred component of the assay systems of the invention. Mechanisms such as the use of fiducial markers on slides and other very accurate physical positioning systems can be adapted to this purpose.

[00169] Additional software components will also be key components that will be part of a complete assay system. The invention thus preferably comprises a complete suite of software tailored to the assay system. Optionally, oligonucleotide design software will be customized for the specific assay to be run, and may be integrated as a part of the system. Also optionally, algorithms and software for data analysis may be integrated to assist in determination of results of the assays. This can be especially useful, as the type of dataset that will be generated will be novel, particularly as a consequence of scale. The ability to provide algorithms and software tools that are specifically designed for analysis of spatially-associated data for significant patterns, including pattern-analysis software and visualization tools, is a novel feature that will enhance the value of the data generated by the assay systems.

[00170] In certain aspects, the assay system will comprise processes for making and carrying out quality control of reagents, *e.g.,* the integrity and sequence fidelity of oligonucleotide pools. In particular, reagents will need to be formulated for compatibility with the reagent delivery instrumentation. Factors such as volatility, stability at key temperatures, and chemical compatibility can be optimized by those skilled in the art upon reading the present disclosure.

Applications of Assay System

[00171] It will be apparent to one skilled in the art upon reading the present disclosure that there are numerous very important areas of biological research, diagnostics, and drug development that will benefit from a high throughput means to simultaneously measure the presence or absence and spatial location of a biological molecule in a sample. For example, this technology combining the ability to analyze semi-quantitatively the expression of many different genes with the ability to image the

spatial organization of expression across many cells in a tissue is enabling for many different areas of basic research. The following are exemplary uses and are by no means meant to be limiting in scope.

[00172] In one example, 3-dimensional patterns of expression can be determined by analyzing a series of tissue sections, in a manner analogous to image reconstruction in CT scanning. This can be used to measure changes in gene expression in disease pathology, *e.g.,* in cancerous tissue and/or a tissue upon injury, inflammation or infection. With the assay systems of the invention, more detailed information on gene expression and protein localization in complex tissues can be obtained. This may lead to new insights into the function and regulation both in normal and diseased states, and is likely to provide new hypotheses that can be tested. For example, a system of the invention may enable some of the insights gained from many individual studies and larger programs like ENCODE (Birney et al., 2007) and modENCODE to be integrated at the tissue level. The assay systems will also aid in computational efforts to model interacting networks of gene expression in the field of systems biology.

[00173] The assay systems also provide a novel approach that enables the analysis of somatic variation, *e.g.,* somatic mutations in cancer or variability in response to infectious organisms. For example, tumors are typically highly heterogeneous, containing cancer cells as well as genetically normal cells in an abnormal local environment. Cancer cells undergo mutation and selection, and in this process it is not unusual for local clones to develop. Identifying relatively rare somatic mutations in the context of tumors may enable the study of the role of key mutations in the selection of clonal variants. Transcriptional patterns associated with angiogenesis, inflammation, or other cancer related processes in both cancer and genetically normal cells can be analyzed for insights into cancer biology and assist in the development of new therapeutic agents for the treatment of cancers.

[00174] In another example, different people have varying susceptibility to infectious organisms, and much of this may be to underlying genetic differences in individuals and/or populations. Identifying these differences will aid in an understanding of the underlying disease pathologies and assist in the development of vaccines or therapeutics to prevent or ameliorate these disease states.

Curio EX1010-p. 164
Curio v Prognosys
IPR2024-01289

[00175] Importantly, in addition to providing spatially associated information, the technology of the invention will allow a great increase in the sensitivity of detecting rare mutations. The reason is that signal to noise can be dramatically increased because the approach of the invention assays a small location in any given reaction. In a typical assay for rare mutations in a mixed sample, the sample is treated in bulk, i.e. nucleic acids are extracted from many cells into a single pool. Thus, if a mutation is present in 1 cell in 10,000, it must be detected against a background of normal DNA from ~10,000 cells. In contrast, with the systems of the invention many cells can be analyzed, but individual cells or small groups of cells would be identified by the spatial coding system. Therefore, the background can be reduced by orders of magnitude, greatly increasing sensitivity. Furthermore, the spatial organization of mutant cells can be observed. This may be particularly important in detecting key mutations in tissue sections in cancer. Already, molecular histological analyses are yielding insights into cancer biology and may have potential for use in diagnostics (Choe et al., 2003). The technology of the invention promises to greatly increase the power of such approaches.

EXAMPLES

[00176] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventor regards as his invention, nor are they intended to represent or imply that the experiments below are all of or the only experiments performed. It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[00177] Efforts have been made to ensure accuracy with respect to numbers used *(e.g.,* amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees centigrade, and pressure is at or near atmospheric.

Curio EX1010-p. 165
Curio v Prognosys
IPR2024-01289

EXAMPLE 1: Initial Proof of Concept of Encoding Scheme

[00178] As an initial proof of concept, a model system is developed using a microarray to demonstrate a working single-plex assay. The basic design validates the concept of the assay, and establishes a working assay prior to addressing issues related to the analysis of a more complicated biological sample. Conventional sequencing is used as a readout for this proof of concept.

[00179] A microarray is used as a proxy for a tissue section. The target sequences of the microarray are fully specified, so that the composition of the targets are known and can be varied systematically. Synthetic oligonucleotide templates are attached to a glass slide via a 5' amino modification. Each slide has a single oligonucleotide template sequence, and the assays that are carried out may employ either ligation, or extension followed by ligation as this may be useful in determining certain polymorphisms.

[00180] Once the *in situ* part of the assay is complete, the reaction products are eluted and analyzed by qPCR to determined presence or absence of a product and estimate yield, and by conventional sequencing to determine the structure of the assay products. The single plex assays that are tested include appropriate positive and negative controls, and a single nucleotide variant (SNV) to check ability to discriminate single base variants.

EXAMPLE 2: Scalability

[00181] The complexity of the assay system is increased to establish scalability of the assay for use in high throughput studies. Scalability of both the spatial encoding and assay systems is demonstrated by carrying out a 24-plex x 24-site assay using a microarray model system.

[00182] The amount of biological target, here a DNA target sequence, at each assay location is systematically varied on microarray substrate. For example, in a microarray with 50 micron spot size (center to center), a 1 mm^2 area contains ~400 spots. The region around each site is optionally occupied by a region that is devoid of these spots to allow individual resolvability of the target sequences. Alternatively, the spots may be clustered, with two or more directly adjacent spots surrounded by or adjacent to a region that is devoid of target sequences.

[00183] In order to demonstrate that spatial encoding is accurate, the sites comprise different target compositions to show that the assay readout matches the expected composition of each site. With 24 target sequences, a simple digital pattern is made with each site having a different set of 12 targets present and 12 targets absent, to make a binary code (0= absent, 1=present). The assay readout is then determined to show that the detected regions match the expected signal after spatial decoding. In this particular example, the code space is large enough (2^{24}) so that even a few errors would not result in different codes being mixed up. Moreover, this design allows identification of errors and allows an

estimation not only of accuracy of spatial encoding but also of accuracy calling the presence or absence of target sequences.

In an exemplary aspect, a 4 x 4 arrangement of 16 sequences is used for the array configuration. A white square indicates that the sequence is absent and a black square that it is present, i.e. 8 of the 16 possible sequences are present in this sample. In a different sample, a different pattern of absent and present sequences can be constructed. In this way, unique patterns are associated with spatial locations so that the accuracy of spatial encoding can be measured.

[00184] The ability to detect quantitative differences is evaluated by generating dose-response curves for each of the 24 assays that are carried out at each site in a 24-site assay. This allows estimation of the limit of detection, dynamic range, and power to detect a given fold-change across the range.

[00185] In one aspect, a latin square design is used to represent individual targets at different ratios by varying the number of features for each target. In other words, with multiple spots in a site, the number of spots allocated to each of the 24 target sequences can be varied and each of the 24 sites can have a different composition. A 1 x 3 inch microarray is sufficiently large to permit multiple replicates. This larger set of 24 sequences will require deconvolution, and this is accomplished using high throughput techniques such as next-generation sequencing technologies *(e.g.,* SOLiD™ technology (Life Technologies, Inc., Carlsbad, CA) or Genome Analyzer (Illumina, Inc., San Diego, CA)). The use of the 24-plex assay demonstrates both the accuracy of spatial encoding and decoding, and the quantitative response of the assay system.

EXAMPLE 3: Adaptation of the assay to preserved samples.

[00186] Genomic DNA is assayed as a proof of concept for assaying RNA, as it provides a way to establish a single-copy reference signal. Once a working assay is developed for FFPE samples, it is adapted to an RNA assay. To this end, assay oligonucleotide concentrations are assayed to ensure compatibility with high multiplexing. Assuming a cell diameter of 10 microns, and delivery of a 10 micron diameter reagent droplet to an individual cell, the volume of the droplet will be ~500 µl and can contain ~3 x 10^{11} molecules at a 1 µM concentration. To assay 1,000 target sequences in 10,000 cells, ~2,000 targeting oligonucleotides would be required in a droplet. Therefore, each droplet could contain ~160 million copies of each assay oligo, a vast excess over the few thousand target sequences in a cell.

[00187] The handling of small absolute numbers of product molecules generated from very small or compromised samples are enhanced to counter the issue of low recovery efficiency; that is, elution is efficient and losses resulting from adsorption of molecules to surfaces are prevented. An approach

Curio EX1010-p. 167
Curio v Prognosys
IPR2024-01289

to addressing the latter issue is to include a carrier material, such as glycogen or carrier nucleic acids.

Example 4: Adapting the Assay to a Biological Sample.

[00188] A control RNA template is immobilized to a solid support in order to create an artificial system. The assay is performed using T4 DNA ligase, which can repair nicks in DNA/RNA hybrids. Assays are carried out on matched slides, or different sections of the same slide, where in one case gDNA is assayed and in the other RNA is assayed. When assaying gDNA the slide can be pretreated with RNase, and when assaying RNA the slide is pretreated with DNase. Results of the assay are confirmed by extracting gDNA or RNA and quantitating the relative amounts by qPCR or RT-qPCR respectively.

[00189] In order make the tissue section RNA assays as informative as possible, pre-existing information on expression levels in specific tissues to target transcripts across a range of abundances are used in the assay design. Both high abundance transcripts, as well as some medium and low abundance transcripts, are targeted to enable an initial assessment of the quantitative performance characteristics of the assay.

[00190] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims. In the claims that follow, unless the term "means" is used, none of the features or elements recited therein should be construed as means-plus-function limitations pursuant to 35 U.S.C. §112, ¶6.

I claim:

l. An assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the assay system performs the following steps:
providing a sample affixed to a support;
delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered;
allowing the encoded probes to interact with the biological targets;
separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets;
determining all or a portion of a sequence of the encoded probes, and
associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample.

Curio EX1010-p. 169
Curio v Prognosys
IPR2024-01289

SPATIALLY ENCODED BIOLOGICAL ASSAYS

Abstract of the Disclosure

The present invention provides assays and assay systems for use in spatially encoded biological assays. The invention provides an assay system comprising an assay 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.

Curio EX1010-p. 170
Curio v Prognosys
IPR2024-01289

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Mark S. Chee	Art Unit	:	Unknown
Application No.	:	16/276,235	Examiner	:	Unknown
Filed	:	February 14, 2019	Conf. No.	:	7944
Title	:	SPATIALLY ENCODED BIOLOGICAL ASSAYS			

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

The applicant wishes to make the examiner aware of the following related applications. The prosecution histories for these applications are available on PAIR, and are not provided with this statement.

Application No.	Filing Date	First Named Inventor	Attorney Docket No.
13/080,616	4/5/2011	Mark S. Chee	47706-0004001
15/187,661	6/20/2016	Mark S. Chee	47706-0004002
16/276,260	2/14/2019	Mark S. Chee	47706-0004004
14/900,602	12/21/2015	Mark S. Chee	47706-0005US1
14/900,604	12/21/2015	Mark S. Chee	47706-0006US1
15/831,158	12/4/2017	Mark S. Chee	47706-0006002
15/565,637	10/10/2017	Jonas Frisen	47706-0009US1
14/111,482	10/11/2013	Jonas Frisen	47706-0011US1
16/013,654	6/20/2018	Jonas Frisen	47706-0011002
16/042,950	7/23/2018	Jonas Frisen	47706-0011003
16/043,038	7/23/2018	Jonas Frisen	47706-0011004
16/254,443	1/22/2019	Jonas Frisen	47706-0011005
14/434,274	4/8/2015	Jonas Frisen	47706-0012US1

This statement is being filed within three months of the filing date of the application.

Apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) to deposit account 06-1050, referencing the above attorney docket number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: March 15, 2019

/Cameron Luitjens/

Cameron M. Luitjens, Ph.D., J.D.
Reg. No. 58,674

Customer Number 159367
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

61870675.doc

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	1.	4,683,195	7/28/1987	Mullis			
	2.	4,683,202	7/28/1987	Mullis			
	3.	4,800,159	1/24/1989	Mullis			
	4.	4,883,867	11/28/1989	Lee			
	5.	4,965,188	10/23/1990	Mullis			
	6.	5,002,882	3/26/1991	Lunnen			
	7.	5,130,238	7/14/1992	Malek			
	8.	5,308,751	5/3/1994	Ohkawa			
	9.	5,321,130	6/14/1994	Yue			
	10.	5,410,030	4/25/1995	Yue			
	11.	5,436,134	7/25/1995	Haugland			
	12.	5,455,166	10/3/1995	Walker			
	13.	5,512,439	4/30/1996	Hornes			
	14.	5,512,462	4/30/1996	Cheng			
	15.	5,582,977	12/10/1996	Yue			
	16.	5,599,675	2/4/1997	Brenner			
	17.	5,641,658	6/24/1997	Adams			
	18.	5,658,751	8/19/1997	Yue			
	19.	5,750,341	5/12/1998	Macevicz			
	20.	5,763,175	6/1/1998	Brenner			
	21.	5,863,753	1/26/1999	Haugland			
	22.	5,912,148	6/15/1999	Eggerding			
	23.	6,013,440	1/11/2000	Lipshutz			
	24.	6,130,073	10/10/2000	Eggerding			
	25.	6,143,496	11/7/2000	Brown			
	26.	6,153,389	11/28/2000	Haarer			

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	27.	6,210,891	4/3/2001	Nyren			
	28.	6,210,894	4/3/2001	Brennan			
	29.	6,214,587	4/10/2001	Dattagupta			
	30.	6,258,558	7/10/2001	Szostak			
	31.	6,258,568	7/10/2001	Nyren			
	32.	6,261,804	7/17/2001	Szostak			
	33.	6,266,459	7/24/2001	Walt			
	34.	6,274,320	8/14/2001	Rothberg			
	35.	6,281,804	8/28/2001	Haller			
	36.	6,309,824	10/30/2001	Drmanac			
	37.	6,344,316	2/5/2002	Lockhart			
	38.	6,355,431	3/12/2002	Chee			
	39.	6,401,267	6/11/2002	Drmanac			
	40.	6,404,907	6/11/2002	Gilchrist			
	41.	6,416,950	7/9/2002	Lohse			
	42.	6,485,982	11/26/2002	Charlton			
	43.	6,518,018	2/11/2003	Szostak			
	44.	6,565,727	5/20/2003	Shenderov			
	45.	6,579,695	6/17/2003	Lambalot			
	46.	6,632,641	10/14/2003	Brennan			
	47.	6,770,441	8/3/2004	Dickinson			
	48.	6,773,566	8/10/2004	Shenderov			
	49.	6,773,886	8/10/2004	Kaufman			
	50.	6,787,308	9/7/2004	Balasubramanian			
	51.	6,800,453	10/5/2004	Labaer			
	52.	6,828,100	12/7/2004	Ronaghi			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	53.	6,833,246	12/21/2004	Balasubramanian			
	54.	6,859,570	2/22/2005	Walt			
	55.	6,864,052	3/8/2005	Drmanac			
	56.	6,867,028	3/15/2005	Janulaitis			
	57.	6,878,515	4/12/2005	Landegren			
	58.	6,897,023	5/24/2005	Fu			
	59.	6,911,132	6/28/2005	Pamula			
	60.	6,911,345	6/28/2005	Quake			
	61.	6,969,488	11/29/2005	Bridgham			
	62.	6,969,589	11/29/2005	Patil			
	63.	6,977,033	12/20/2005	Becker			
	64.	7,052,244	5/30/2006	Fouillet			
	65.	7,057,026	6/6/2006	Barnes			
	66.	7,115,400	10/3/2006	Adessi			
	67.	7,118,883	10/10/2006	Inoue			
	68.	7,163,612	1/16/2007	Sterling			
	69.	7,192,735	3/20/2007	Lambalot			
	70.	7,211,414	5/1/2007	Hardin			
	71.	7,229,769	6/12/2007	Kozlov			
	72.	7,244,559	7/17/2007	Rothberg			
	73.	7,264,929	9/4/2007	Rothberg			
	74.	7,270,950	9/18/2007	Szostak			
	75.	7,297,518	11/20/2007	Quake			
	76.	7,315,019	1/1/2008	Turner			
	77.	7,328,979	2/12/2008	Decre			
	78.	7,329,492	2/12/2008	Hardin			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	79.	7,378,242	5/27/2008	Hurt			
	80.	7,393,665	7/1/2008	Brenner			
	81.	7,405,281	7/29/2008	Xu			
	82.	7,407,757	8/5/2008	Brenner			
	83.	7,462,449	12/9/2008	Quake			
	84.	7,501,245	3/10/2009	Quake			
	85.	7,537,897	5/26/2009	Brenner			
	86.	7,544,473	6/9/2009	Brennan			
	87.	7,547,380	6/16/2009	Velev			
	88.	7,579,153	8/25/2009	Brenner			
	89.	7,595,883	9/29/2009	Gamal			
	90.	7,601,498	10/13/2009	Mao			
	91.	7,635,566	12/22/2009	Brenner			
	92.	7,641,779	1/5/2010	Becker			
	93.	7,666,612	2/23/2010	Johnsson			
	94.	7,674,752	3/9/2010	He			
	95.	7,754,429	7/13/2010	Rigatti			
	96.	7,776,547	8/17/2010	Roth			
	97.	7,776,567	8/17/2010	Mao			
	98.	7,803,943	9/28/2010	Mao			
	99.	7,858,321	12/28/2010	Glezer			
	100.	7,960,120	6/14/2011	Rigatti			
	101.	8,092,784	1/10/2012	Mao			
	102.	8,148,068	4/3/2012	Brenner			
	103.	8,148,518	4/3/2012	Buchanan			
	104.	8,207,093	6/26/2012	Szostak			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	105.	8,288,103	10/16/2012	Oliphant			
	106.	8,337,851	12/25/2012	Aukerman			
	107.	8,343,500	1/1/2013	Wraith			
	108.	8,383,338	2/26/2013	Kitzman			
	109.	8,460,865	6/11/2013	Chee			
	110.	8,481,257	7/9/2013	Van Eijk			
	111.	8,481,292	7/9/2013	Casbon			
	112.	8,486,625	7/16/2013	Gunderson			
	113.	8,586,310	11/19/2013	Mitra			
	114.	8,614,073	12/24/2013	Van Eijk			
	115.	8,637,242	1/28/2014	Shen			
	116.	8,685,889	4/1/2014	Van Eijk			
	117.	8,741,606	6/3/2014	Casbon			
	118.	8,778,849	7/15/2014	Bowen			
	119.	8,785,353	7/22/2014	Van Eijk			
	120.	8,815,512	8/26/2014	Van Eijk			
	121.	8,835,358	9/16/2014	Fodor			
	122.	8,865,410	10/21/2014	Shendure			
	123.	8,895,249	11/25/2014	Shen			
	124.	8,911,945	12/16/2014	Van Eijk			
	125.	8,936,912	1/20/2015	Mitra			
	126.	8,951,781	2/10/2015	Reed			
	127.	9,023,768	5/5/2015	Van Eijk			
	128.	9,062,348	6/23/2015	Van Eijk			
	129.	9,080,210	7/14/2015	Van Eijk			
	130.	9,085,798	7/21/2015	Chee			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	131.	9,121,069	9/1/2015	Lo			
	132.	9,194,001	11/24/2015	Brenner			
	133.	9,290,808	3/22/2016	Fodor			
	134.	9,290,809	3/22/2016	Fodor			
	135.	9,328,383	5/3/2016	Van Eijk			
	136.	9,334,536	5/10/2016	Van Eijk			
	137.	9,340,830	5/17/2016	Lipson			
	138.	9,371,598	6/21/2016	Chee			
	139.	9,376,716	6/28/2016	Van Eijk			
	140.	9,376,719	6/28/2016	Van Eijk			
	141.	9,404,156	8/2/2016	Hicks			
	142.	9,447,459	9/20/2016	Van Eijk			
	143.	9,453,256	9/27/2016	Van Eijk			
	144.	9,493,820	11/15/2016	Van Eijk			
	145.	9,506,061	11/29/2016	Brown			
	146.	9,574,230	2/21/2017	Van Eijk			
	147.	9,593,365	3/14/2017	Frisen et al.			
	148.	9,657,335	5/23/2017	Van Eijk			
	149.	9,670,542	6/6/2017	Van Eijk			
	150.	9,702,004	7/11/2017	Van Eijk			
	151.	9,727,810	8/8/2017	Fodor et al.			
	152.	9,745,627	8/29/2017	Van Eijk			
	153.	9,777,324	10/3/2017	Van Eijk			
	154.	9,868,979	1/16/2018	Chee et al.			
	155.	9,879,313	1/30/2018	Chee et al.			
	156.	9,896,721	2/20/2018	Van Eijk			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	157.	9,898,576	2/20/2018	Van Eijk			
	158.	9,898,577	2/20/2018	Van Eijk			
	159.	10,002,316	6/19/2018	Fodor et al.			
	160.	10,023,907	7/17/2018	Van Eijk			
	161.	10,030,261	7/24/2018	Frisen et al.			
	162.	10,095,832	10/9/2018	Van Eijk			
	163.	10,138,509	11/27/2018	Church et al.			
	164.	10,179,932	1/15/2019	Church et al.			
	165.	13/080,616	10/6/2011	Chee			
	166.	2002/0055100	5/9/2002	Kawashima			
	167.	2002/0064779	5/30/2002	Landegren			
	168.	2002/0150909	10/17/2002	Stuelpnagel			
	169.	2002/0168645	11/14/2002	Taylor			
	170.	2003/0022207	1/30/2003	Balasubramanian			
	171.	2003/0040035	2/27/2003	Slamon			
	172.	2003/0064398	4/3/2003	Barnes			
	173.	2003/0087232	5/8/2003	Christians			
	174.	2003/0096323	5/22/2003	James			
	175.	2003/0113713	6/19/2003	Glezer			
	176.	2003/0138879	7/24/2003	Lambalot			
	177.	2003/0148335	8/7/2003	Shen et al.			
	178.	2003/0162216	8/28/2003	Gold			
	179.	2003/0170637	9/11/2003	Pirrung et al.			
	180.	2003/0205632	11/6/2003	Kim et al.			
	181.	2003/0224419	12/4/2003	Corcoran			
	182.	2003/0232382	12/18/2003	Brennan			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	183.	2003/0235852	12/25/2003	Roberts			
	184.	2004/0002090	1/1/2004	Mayer et al.			
	185.	2004/0067492	4/8/2004	Peng et al.			
	186.	2004/0096853	5/20/2004	Mayer			
	187.	2004/0106110	6/3/2004	Balasubramanian			
	188.	2004/0112442	6/17/2004	Maerkl			
	189.	2005/0003431	1/6/2005	Wucherpfennig			
	190.	2005/0019776	1/27/2005	Callow et al.			
	191.	2005/0026188	2/3/2005	Van Kessel			
	192.	2005/0037362	2/17/2005	Remacle et al.			
	193.	2005/0037393	2/17/2005	Gunderson et al.			
	194.	2005/0048580	3/3/2005	Labaer			
	195.	2005/0064460	3/24/2005	Holliger et al.			
	196.	2005/0130173	6/16/2005	Leamon et al.			
	197.	2005/0164292	7/28/2005	Farooqui			
	198.	2005/0179746	8/18/2005	Roux et al.			
	199.	2005/0191656	9/1/2005	Drmanac et al.			
	200.	2005/0191698	9/1/2005	Chee et al.			
	201.	2005/0239192	10/27/2005	Nasarabadi et al.			
	202.	2005/0255548	11/17/2005	Lipovsek et al.			
	203.	2005/0260653	11/24/2005	LaBaer			
	204.	2006/0003394	1/5/2006	Song			
	205.	2006/0039823	2/23/2006	Yamakawa et al.			
	206.	2006/0046313	3/2/2006	Roth			
	207.	2006/0079453	4/13/2006	Sidney et al.			
	208.	2006/0134669	6/22/2006	Casasanta			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	209.	2006/0164490	7/27/2006	Kim et al.			
	210.	2006/0188875	8/24/2006	Cox et al.			
	211.	2006/0194331	8/31/2006	Pamula et al.			
	212.	2006/0199207	9/7/2006	Matysiak			
	213.	2006/0211001	9/21/2006	Yu et al.			
	214.	2006/0216721	9/28/2006	Kozlov et al.			
	215.	2006/0216775	9/28/2006	Burkart et al.			
	216.	2006/0228758	10/12/2006	Muchhal et al.			
	217.	2006/0263789	11/23/2006	Kincaid			
	218.	2006/0275799	12/7/2006	Banerjee et al.			
	219.	2007/0014810	1/18/2007	Baker et al.			
	220.	2007/0020625	1/25/2007	Duchaud et al.			
	221.	2007/0020640	1/25/2007	McCloskey et al.			
	222.	2007/0020669	1/25/2007	Ericsson			
	223.	2007/0023292	2/1/2007	Kim et al.			
	224.	2007/0026430	2/1/2007	Andersen et al.			
	225.	2007/0036511	2/15/2007	Lundquist et al.			
	226.	2007/0054288	3/8/2007	Su et al.			
	227.	2007/0099208	5/3/2007	Drmanac et al.			
	228.	2007/0128624	6/7/2007	Gormley et al.			
	229.	2007/0166725	7/19/2007	McBride et al.			
	230.	2007/0172873	7/26/2007	Brenner et al.			
	231.	2007/0178503	8/2/2007	Jiang			
	232.	2007/0207482	9/6/2007	Church et al.			
	233.	2007/0254305	11/1/2007	Paik, et al.			
	234.	2007/0269805	11/22/2007	Hogers			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	235.	2008/0009420	1/10/2008	Schroth et al.			
	236.	2008/0032301	2/7/2008	Rank et al.			
	237.	2008/0071071	3/20/2008	LaBaer et al.			
	238.	2008/0108082	5/8/2005	Rank et al.			
	239.	2008/0108804	5/8/2008	Hayashizaki et al.			
	240.	2008/0124252	5/29/2008	Marchand et al.			
	241.	2008/0128627	6/5/2008	Lundquist et al.			
	242.	2008/0220434	9/11/2008	Thomas			
	243.	2008/0220981	9/11/2008	McGregor			
	244.	2008/0261204	10/23/2008	Lexow			
	245.	2008/0293591	11/27/2008	Taussig et al.			
	246.	2008/0312103	12/18/2008	Nemoto et al.			
	247.	2009/0005252	1/1/2009	Drmanac et al.			
	248.	2009/0006002	1/1/2009	Honisch et al.			
	249.	2009/0018024	1/15/2009	Church et al.			
	250.	2009/0026082	1/29/2009	Rothberg et al.			
	251.	2009/0036323	2/5/2009	van Eijk et al.			
	252.	2009/0082212	3/26/2009	Williams			
	253.	2009/0099041	4/16/2009	Church et al.			
	254.	2009/0105959	4/23/2009	Braverman et al.			
	255.	2009/0117573	5/7/2009	Fu et al.			
	256.	2009/0127589	5/21/2009	Rothberg et al.			
	257.	2009/0155781	6/18/2008	Drmanac et al.			
	258.	2009/0170713	7/2/2009	van Eijk et al.			
	259.	2009/0192044	7/30/2009	Fouillet			
	260.	2009/0197326	8/6/2009	El Gamal et al.			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	261.	2009/0215633	8/27/2009	van Eijk et al.			
	262.	2009/0233802	9/17/2009	Bignell et al.			
	263.	2009/0253581	10/8/2009	van Eijk et al.			
	264.	2009/0264299	10/22/2009	Drmanac et al.			
	265.	2009/0280487	11/12/2009	Hung et al.			
	266.	2009/0283407	11/19/2009	Shah et al.			
	267.	2009/0291854	11/26/2009	Weisinger-Mayr et al.			
	268.	2009/0305237	12/10/2009	Cantor et al.			
	269.	2009/0321262	12/31/2009	Adachi et al.			
	270.	2010/0035249	2/11/2010	Hayashizaki et al.			
	271.	2010/0069263	3/18/2010	Shendure et al.			
	272.	2010/0096266	4/22/2010	Kim et al.			
	273.	2010/0099103	4/22/2010	Hsieh et al.			
	274.	2010/0105052	4/29/2010	Drmanac et al.			
	275.	2010/0111768	5/6/2010	Banerjee et al.			
	276.	2010/0113302	5/6/2010	Williams			
	277.	2010/0120098	5/13/2010	Grunenwald et al.			
	278.	2010/0129874	5/27/2010	Mitra et al.			
	279.	2010/0137143	6/3/2010	Rothberg et al.			
	280.	2010/0151464	6/17/2010	Stuelpnagel et al.			
	281.	2010/0159446	6/24/2010	Haff et al.			
	282.	2010/0173384	7/8/2010	Johnsson et al.			
	283.	2010/0184614	7/22/2010	Ye et al.			
	284.	2010/0227329	9/9/2010	Cuppens			
	285.	2010/0282617	11/11/2010	Rothberg et al.			
	286.	2011/0033854	2/10/2011	Drmanac et al.			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents							
Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	287.	2011/0048951	3/3/2011	Wu			
	288.	2011/0059436	3/10/2011	Hardin et al.			
	289.	2011/0059865	3/10/2011	Smith et al.			
	290.	2011/0244448	10/6/2011	Shirai et al.			
	291.	2011/0245101	10/6/2011	Chee et al.			
	292.	2011/0245111	10/6/2011	Chee			
	293.	2012/0046175	2/23/2012	Rodesch et al.			
	294.	2012/0065081	3/15/2012	Chee			
	295.	2012/0129248	5/24/2012	Chee et al.			
	296.	2012/0135871	5/31/2012	van Eijk et al.			
	297.	2012/0195810	8/2/2012	Cohen et al.			
	298.	2012/0202698	8/9/2012	van Eijk et al.			
	299.	2012/0245053	9/27/2012	Shirai et al.			
	300.	2012/0258871	10/11/2012	Kozlov et al.			
	301.	2012/0270748	10/25/2012	Chee et al.			
	302.	2012/0289414	11/15/2012	Mitra et al.			
	303.	2013/0096033	4/18/2013	Routenberg			
	304.	2013/0109595	5/2/2013	Routenberg			
	305.	2013/0261019	10/3/2013	Lin et al.			
	306.	2013/0296174	11/7/2013	Peumans			
	307.	2014/0065609	3/6/2014	Hicks et al.			
	308.	2014/0066318	3/6/2014	Frisen et al.			
	309.	2014/0079923	3/20/2014	George et al.			
	310.	2014/0121118	5/1/2014	Warner			
	311.	2014/0155274	6/5/2014	Xie et al.			
	312.	2014/0227705	8/14/2014	Vogelstein et al.			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	313.	2014/0243224	8/28/2014	Barnard et al.			
	314.	2014/0274731	9/18/2014	Raymond et al.			
	315.	2014/0296081	10/2/2014	Diehn et al.			
	316.	2014/0342921	11/20/2014	Weiner			
	317.	2014/0378345	12/25/2014	Hindson et al.			
	318.	2015/0005447	1/1/2015	Berti et al.			
	319.	2015/0051085	2/19/2015	Vogelstein et al.			
	320.	2015/0087027	3/26/2015	Makarov et al.			
	321.	2015/0344942	12/3/2015	Frisen et al.			
	322.	2015/0376609	12/31/2015	Hindson et al.			
	323.	2016/0003812	1/7/2016	Porreca et al.			
	324.	2016/0024576	1/28/2016	Chee			
	325.	2016/0138091	5/19/2016	Chee et al.			
	326.	2016/0145677	5/26/2016	Chee et al.			
	327.	2016/0298180	10/13/2016	Chee			
	328.	2016/0333403	11/17/2016	Chee			
	329.	2017/0058339	3/2/2017	Chee			
	330.	2017/0058340	3/2/2017	Chee			
	331.	2017/0058345	3/2/2017	Chee			
	332.	2017/0088881	3/30/2017	Chee			
	333.	2017/0166962	6/15/2017	van Eijk et al.			
	334.	2017/0242020	8/24/2017	Yamauchi et al.			
	335.	2017/0343545	11/30/2017	HADRUP et al.			
	336.	2018/0179591	6/28/2018	Belgrader et al.			
	337.	2018/0180601	6/28/2018	Pedersen et al.			
	338.	2018/0201980	7/19/2018	Chee et al.			

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	339.	2018/0217094	8/2/2018	Herr et al.			
	340.	2018/0247017	8/30/2017	van Eijk et al.			
	341.	2018/0291439	10/11/2018	van Eijk et al.			
	342.	2019/0017106	1/17/2019	Frisen et al.			
	343.	2019/0024153	1/24/2019	Frisen et al.			
	344.	2019/0024154	1/24/2019	Frisen et al.			
	345.	61/839,313	6/25/2013	Chee et al.			
	346.	61/839,320	6/25/2013	Chee et al.			
	347.	61/902,105	11/8/2013	Kozlov et al.			
	348.	RE44596	11/12/2013	Stroun et al.			

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	349.	DE 102008025656	12/3/2009	DE			Machine Translation	
	350.	EP 1712623	10/18/2006	EP				
	351.	EP 1910562	4/16/2008	EP			Corresponds to WO2006/137733	
	352.	EP 1929039	6/11/2008	EP			Corresponds to WO2007/037678	
	353.	EP 1966393	9/10/2008	EP			Corresponds to WO2007/073165	
	354.	EP 2002017	12/17/2008	EP			Corresponds to WO2007/114693	
	355.	EP 2292788	3/9/2011	EP				
	356.	EP 2302070	3/30/2011	EP				
	357.	EP 2363504	9/7/2011	EP				
	358.	EP 2580351	4/17/2013	EP			Corresponds to WO2007/155833	
	359.	EP 2789696	10/15/2014	EP				
	360.	EP 2963127	1/6/2016	EP				

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	361.	EP 3045544	7/20/2016	EP				
	362.	EP 3239304	11/1/2017	EP				
	363.	JP 2011-182702	9/22/2011	JP			English Abstract	
	364.	JP 2014-217381	11/20/2014	JP			English Abstract	
	365.	RU 2145635	2/20/2000	RU			English Translation	
	366.	RU 2270254	2/20/2006	RU			English Abstract	
	367.	WO 1989/010977	11/16/1989	WO				
	368.	WO 1991/006678	5/16/1991	WO				
	369.	WO 1995/025116	9/21/1995	WO				
	370.	WO 1995/035505	12/28/1995	WO				
	371.	WO 1998/044151	10/8/1998	WO				
	372.	WO 1999/032654	7/1/1999	WO				
	373.	WO 2000/018957	4/6/2000	WO				
	374.	WO 2000/024940	5/4/2000	WO				
	375.	WO 2000/063437	10/26/2000	WO				
	376.	WO 2001/007915	2/1/2001	WO				
	377.	WO 2001/042796	6/14/2001	WO				
	378.	WO 2002/024952	3/28/2002	WO				
	379.	WO 2002/059355	8/1/2002	WO				
	380.	WO 2002/077283	10/3/2002	WO				
	381.	WO 2002/088396	11/7/2002	WO				
	382.	WO 2003/010176	2/6/2003	WO				
	383.	WO 2004/018497	3/4/2004	WO				
	384.	WO 2004/028955	4/8/2004	WO				
	385.	WO 2005/010145	2/3/2005	WO				
	386.	WO 2005/026387	3/24/2005	WO				

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	387.	WO 2005/042759	5/12/2005	WO				
	388.	WO 2006/074351	7/13/2006	WO				
	389.	WO 2006/084130	8/10/2006	WO				
	390.	WO 2006/117541	11/9/2006	WO				
	391.	WO 2006/137733	12/28/2006	WO				
	392.	WO 2007/030373	3/15/2007	WO				
	393.	WO 2007/037678	4/5/2007	WO				
	394.	WO 2007/041689	4/12/2007	WO				
	395.	WO 2007/060599	5/31/2007	WO				
	396.	WO 2007/073165	6/28/2007	WO				
	397.	WO 2007/073171	6/28/2007	WO				
	398.	WO 2007/076726	7/12/2007	WO				
	399.	WO 2007/114693	10/11/2007	WO				
	400.	WO 2007/120241	10/25/2007	WO				
	401.	WO 2007/123744	11/1/2007	WO				
	402.	WO 2007/145612	12/21/2007	WO				
	403.	WO 2008/093098	8/7/2008	WO				
	404.	WO 2009/032167	3/12/2009	WO				
	405.	WO 2009/036525	3/26/2009	WO				
	406.	WO 2009/152928	12/23/2009	WO			English Abstract	
	407.	WO 2010/019826	2/18/2010	WO				
	408.	WO 2010/027870	3/11/2010	WO				
	409.	WO 2010/126614	11/4/2010	WO				
	410.	WO 2010/127186	11/4/2010	WO				
	411.	WO 2011/014879	2/3/2011	WO				
	412.	WO 2011/071943	6/16/2011	WO				

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	413.	WO 2011/127006	10/13/2011	WO				
	414.	WO 2011/127099	10/13/2011	WO				
	415.	WO 2011/155833	12/15/2011	WO				
	416.	WO 2012/022975	2/13/2012	WO				
	417.	WO 2012/058096	5/3/2012	WO				
	418.	WO 2012/071428	5/31/2012	WO				
	419.	WO 2012/139110	10/11/2012	WO				
	420.	WO 2012/140224	10/18/2012	WO				
	421.	WO 2012/142213	10/18/2012	WO				
	422.	WO 2012/148477	11/1/2012	WO				
	423.	WO 2013/123442	8/22/2013	WO				
	424.	WO 2013/138510	9/19/2013	WO				
	425.	WO 2013/142389	9/26/2013	WO				
	426.	WO 2013/150083	10/10/2013	WO				
	427.	WO 2014/060483	4/24/2014	WO				
	428.	WO 2014/142841	9/18/2014	WO				
	429.	WO 2014/163886	10/9/2014	WO				
	430.	WO 2014/210223	12/31/2014	WO				
	431.	WO 2014/210225	12/31/2014	WO				
	432.	WO 2016/162309	10/13/2016	WO				
	433.	WO 2016/007839	1/14/2016	WO				
	434.	WO 2018/045186	3/5/2018	WO				
	435.	WO 2018/045181	3/5/2018	WO				

Other Documents (include Author, Title, Date, and Place of Publication)

Examiner Initial	Desig. ID	Document

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	436.	Affymetrix, "GeneChip Human Genome U133 Set," retrieved from the Internet: on the World Wide Web at affymetrix.com/support/technical/datasheets/hgu133_datasheet.pdf, retrieved on February 26, 2003.
	437.	Affymetrix, "Human Genome U95Av2," INTERNET CITATION, retrieved from the internet: on the World Wide Web affymetrix.com, retrieved on October 2, 2002
	438.	Albretsen et al., "Applications of magnetic beads with covalently attached oligonucleotides in hybridization: Isolation and detection of specific measles virus mRNA from a crude cell lysate", Anal. Biochem. 189: 40-50, 1990
	439.	ALEXANDER VALENCIA et al., "mRNA-display-based selections for proteins with desired functions: A protease-substrate case study," Biotechnology Progress, 2008, 24(3): 561-569
	440.	Allawi and SantaLucia, "Thermodynamics and NMR of Internal GâT Mismatches in DNA," Biochemistry, 36:10581-94
	441.	ANDERSON et al., "Microarrayed Compound Screening to Identify Activators and Inhibitors of AMP-Activated Protein Kinase," J. of Biomolecular Screening, 2004, 9:112
	442.	Andersson et al., "Analysis of protein expression in cell microarrays: a tool for antibody-based proteomics.," J Histochem Cytochem., 4(12): 1413-1423, 2006
	443.	Andresen et al., "Deciphering the Antibodyome - Peptide Arrays for Serum Antibody Biomarker Diagnostics," Current Proteomics, 6(1), 1-12, 2009
	444.	ANGENENDT et al., "Cell-free expression and functional assay in a nanowell chip format," Analytical Chemistry, 2004, 76(7):1844-49
	445.	ANGENENDT et al.,"Generation of High Density Protein Microarrays by Cell-free in Situ Expression of Unpurified PCR Products," Molecular and Cellular Proteomics, (2006) Ch. 5.9, pp. 1658-66
	446.	Armani et al, "2D-PCR: a method of mapping DNA in tissue sections," Lab on a Chip, 2009, 9(24):3526-34
	447.	Asp et al., "Spatial detection of fetal marker genes expressed at low level in adult human heart tissue", Scientific Reports, 7: 12941, 10 pages, 2017
	448.	Atkinson and Wells, "An Updated Protocol for High Throughput Plant Tissue Sectioning.", Front Plant Sci, 8: 1721, 2017
	449.	ATKINSON, Overview of Translation: Lecture Manuscript, U of Texas (2000) DD. 6.1-6.8
	450.	Bains et al, "A Novel Method for Nucleic Acid Sequence Determination ," Journal of Theoretical Biology, 1988, 135(3), 303-7
	451.	BAIRD et al., "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD markers," PLOS One, 2008, 3(1 0):e3376
	452.	Barnes, "PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates.," Proc. Natl. Acad. Sci USA, 91: 2216-2220, 1994
	453.	Baugh et al, "Quantitative analysis of mRNA amplification by in vitro transcription," Nucleic Acids Res., 2001, 29:5:e29
	454.	BELL, "A Simple Way to Treat PCR Products Prior to Sequencing Using ExoSAP-IT," Biotechniques, 2008, Vol. 44, No. 6

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	455.	Bentley et al, "Accurate whole human genome sequencing using reversible terminator chemistry", Nature, 2008, 456:53-59
	456.	BIELAS et al., "Human cancers express a mutator phenotype," Proc. Natl. Acad. Sci. USA, 2006, 103(48): 18238-18242
	457.	BIELAS et al., "Quantification of random genomic mutations," Nat. Methods, 2005, 2(4):285-290
	458.	Birney, et al, "Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project," Nature, 2007, 447:799-816
	459.	BLOKZIJL et al., "Profilinq protein expression and interactions: proximity liqation as a tool for personalized medicine," J Intern. Med., 2010, 268:232-245
	460.	Blow, "Tissue Issues," Nature, 448(23), 959-962, 2007
	461.	BONFIELD et al., "The application of numerical estimates of base calling accuracy to DNA sequencing projects," Nucleic Acids Research, 1995, 23(8):1406-1410
	462.	Boulgakov et al., "From Space to Sequence and Back Again: Iterative DNA Proximity Ligation and its Applications to DNA-Based Imaging", bioRxiv, 24 pages, 2018
	463.	BOWTELL, "The genesis and evolution of high-grade serous ovarian cancer," Nat. Rev. Cancer, 2010, (11):803-808 ABSTRACT
	464.	BRANDON et al., "Mitochondrial mutations in cancer," Oncogene, 2006, 25(34):4647-4662
	465.	BRENNER et al, "In vitro cloning of complex mixtures of DNA on microbeads: physical separation of differentially expressed cDNAs," Proc. Natl. Acad. Sci. USA, 2000, 97, 1665-1670
	466.	Brenner et al., "Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays", Nat. Biotech. 18: 630-634, 2000
	467.	BROCKMAN et al., "Quality scores and SNP detection in sequencing-by-synthesis systems," Methods, 2008, 18:763-770
	468.	Burgess, "A space for transcriptomics", Nature Reviews Genetics | Published online 15 Jul 2016; doi:10.1038/nrg.2016.94
	469.	Burgess, "Finding structure in gene expression", Nature Reviews Genetics | Published online 13 Apr 2018; doi:10.1038/nrg.2018.19
	470.	BURNS et al., "Well-less, gel-permeation formats for ultra-HTS," DDT, 2001, 6(12):S40-S47
	471.	Burton et al., "Coverslip Mounted-Immersion Cycled in Situ RT-PCR for the Localization of mRNA in Tissue Sections," Biotechniques, 1998, 24; pages 92-100
	472.	CARLSON et al., "Formylglycine-generating Enzyme," J. of Biological Chemistry, 2008, 283(29):20117-125
	473.	Carter et al., "Stabilization of an optical microscope to 0.1 nm in three dimensions," Applied Optics, 2007, 46:421-427
	474.	CERUTTI et al., "Generation of sequence-specific, high affinity anti-DNA antibodies," Journal of Biological Chemistry, 2001, 276(16):12769-12773
	475.	CHA et al., "Specificity, Efficiency and Fidelity of PCR," Genome Res., 1993, 3:518-29
	476.	CHANDRA et al., "Cell-free synthesis-based protein microarrays and their applications," Proteomics, 2009, 5(6):717-30.

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	477.	CHATTERJEE et al., "Protein Microarray On-Demand: A Novel Protein Microarray System," PLos One, 2008, 3(9):e3265
	478.	CHATTERJEE, et al., "Mitochondrial DNA mutations in human cancer. Oncogene," 2006, 25(34):4663-4674
	479.	CHEN et al., "DNA hybridization detection in a microfluidic Channel using two fluorescently labelled nucleic acid probes," Biosensors and Bioelectronics, 2008, 23:1878-1882
	480.	Chen et al., "Expansion Microscopy," 2015, Science. 347(6221):543-548
	481.	Chen et al., "Spatially resolved, highly multiplexed RNA profiling in single cells", Science 348(6233), 36 pages, 2015
	482.	CHENG et al., "Sensitive Detection of Small Molecules by Competitive lmmunomagnetic-Proximity Ligation Assay," Anal Chem, 2012, 84:2129-2132
	483.	CONDINA et al., "A sensitive magnetic bead method for the detection and identification of tyrosine phosphorylation in proteins by MALDI-TOF/TOF MS," Proteomics, 2009, 9:3047-3057
	484.	Constantine et al.,"Use of genechip high-density oligonucleotide arrays for gene expression monitoring," LIFE SCEIENCE NEWS, AMERSHAM LIFE SCIENCE; 11-14, 1998
	485.	COPELAND et al., "Mitochondrial DNA Alterations in Cancer," Cancer Invest., 2002, 557-569
	486.	Corces et al., "An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues", Nat. Methods. vol. 14(10): pp.959–962, 2017
	487.	Crosetto et al, "Spatially resolved transcriptomics and beyond," Nature Review Genetics, 2015, 57-66
	488.	CUJEC et al. "Selection of v-abl tyrosine kinase substate sequences from randomnized peptide and cellular proteomic libraries using mRNA display," Chemistry and Biology, 2002, 9:253-264
	489.	Dahl et al., "Circle-to-circle amplification for precise and sensitive DNA analysis," Proc. Natl. Acad. Sci., 2004, 101:4548-4553
	490.	DARMANIS, et al.,"ProteinSeq: High-Performance Proteomic Analyses by Proximity, Ligation and Next Generation Sequencing," PLos One, 2011, 6(9):e25583
	491.	Daubendiek et al., "Rolling-Circle RNA Synthesis: Circular Oligonucleotides as Efficient Substrates for T7 RNA Polymerase," J. Am. Chem. Soc., 1995, 117:77818-7819
	492.	Davies et al., "How best to identify chromosomal interactions: a comparison of approaches.", Nat. Methods 14, 125–134, 2017
	493.	Dean et al., "Comprehensive human genome amplification using multiple displacement amplification," Proc Natl. Acad. Sci. USA 99:5261-66 (2002
	494.	Dhindsa et al., "Virtual Electrowetting Channels: Electronic Liquid Transport with Continuous Channel Functionality," Lab Chip, 2010, 10:832-836
	495.	Dressman et al., "Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations," Proc. Natl. Acad. Sci. USA, 2003, 100:8817-8822
	496.	Drmanac et al., "Accurate sequencing by hybridization for DNA diagnostics and individual genomics," Nature Biotechnology, 16:54-58, 1998
	497.	Druley et al., "Quantification of rare allelic variants from pooled genomic DNA," Nat. Methods 6: 263-65, 2009

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	498.	Duncan et al., "Affinity chromatography of a sequence-specific DNA binding protein using teflon-linked oligonucleotides", Anal. Biochem. 169: 104-108, 1988
	499.	Eagen, "Principles of Chromosome Architecture Revealed by Hi-C", Trends in Biochemical Sciences, Vol. 43, No. 6, 10 pages, 2018
	500.	Eberwine et al., "Analysis of gene expression in single live neurons," Proc. Natl. Acad. Sci., USA 89, 3010-3014, 1992
	501.	Eberwine, "Amplification of mRNA Populations Using aRNA Generated from Immobi- lized Oligo(dT)-T7 Primed cDNA," BioTechniques 20 (4), 584, 1996
	502.	Eguiluz et al., "Multitissue array review: A chronological description of tissue array techniques, applications and procedures," Pathology Research and Practice, : 8: 561-568, 2002
	503.	Eldridge et al. "An in vitro selection strategy for conferring protease resistance to ligand binding peptides," Protein Eng Des Sel., 22(11): 691-698, 2009
	504.	Ellington et al., "Antibody-based protein multiplex platforms: technical and operational challenges," Clin Chem 56(2): 186-193, 2010
	505.	Eng et al., "Profiling the transcriptome by RNA SPOTs", Nat Methods., 14(12): 1153–1155, 2017
	506.	Examination Search Report for Canadian Appln. No. 2,794,522, dated 4/28/2015, 3 pages
	507.	Examination Search Report issued in Australian Appln. No. 2010278710, dated 2/11/2014, 4 pages
	508.	Examination Search Report issued in Australian Appln. No. 2010328226, dated 5/9/2013, 4 pages
	509.	Examination Search Report issued in Australian Appln. No. 2011237729, dated 7/9/2013, 3 pages
	510.	Examination Search Report issued in Australian Appln. No. 2014203638, dated 10/1/2015, 2 pages
	511.	Examination Search Report issued in Australian Appln. No. 2014203638, dated 10/1/2015, 2 pages
	512.	Extended European Search Report issued in European Appln. No. 10836568.5, dated 2/13/2013, 6 pages
	513.	Extended European Search Report issued in European Appln. No. 11766613.1, dated 1/15/2014, 4 pages
	514.	Extended European Search Report issued in European Appln. No. 12767937, dated 11/18/2014, 5 pages
	515.	Extended European Search Report issued in European Appln. No. 14765026.1, dated 9/26/2016, 9 pages
	516.	Extended European Search Report issued in European Appln. No. 14816674.7, dated 2/3/2017, 8 pages
	517.	Extended European Search Report issued in European Appln. No. 16183356.1, dated 4/24/2017, 12 pages
	518.	Fan et al., " Highly parallel SNP genotyping," Cold Spring Symp. Quant. Biol., 68: 69-78, 2003
	519.	Fire and Xu, "Rolling replication of short DNA circles," Proc. Natl. Acad. Sci., 92: 4641-4645, 1995

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	520.	Flanigon et al., "Multiplex protein detection with DNA readout via mass spectrometry," N. Biotechnol., 30(2): 153-158, 2013
	521.	Fodor et al., "Light-Directed, Spatially Addressable Parallel Chemical Synthesis," Science, 251(4995), 767-773, 1995
	522.	Forcucci et al., "All-plastic miniature fluorescence microscope for point-of-care readout of bead-based bioassays.," J. Biomed Opt. 20 (10): 105010, 15 pages, 2015
	523.	Fredriksson et al., "Multiplexed protein detection by proximity ligation for cancer detection," Nature Methods, 4(4): 327-29, 2007
	524.	Fredriksson et al., "Multiplexed proximity ligation assays to profile putative plasma biomarkers relevant to pancreatic and ovarian cancer," Clin. Chem., 5(3): 582-89, 2008
	525.	Fredriksson et al., "Protein detection using proximity-dependent DNA ligation assays," Nature Biotech., 20: 473-77, 2002
	526.	Frese et al., "Formylglycine Aldehyde Tag-Protein Engineering through a Novel Posttranslational Modification," ChemBioChem., 10: 425-27, 2009
	527.	Fu et al., "Counting individual DNA molecules by the stochastic attachment of diverse labels," PNAS, 108: 9026-9031, 2011
	528.	Fullwood et al., "Next-generation DNA sequencing of paired-end tags (PET) for transcriptome and genome analyses," Genome Res., 19: 521-532, 2009
	529.	Gao et al., "High density peptide microarrays. In situ synthesis and applications," Molecular Diversity, 8, 177-187, 2004
	530.	Gao et al., "Q&A: Expansion microscopy", BMC Biology, 15: 50, 9 pages, 2017
	531.	Gerdtsson et al., "Evaluation of Solid Supports for Slide- and Well-Based Recombinant Antibody Microarrays", Microarrays (2016) 5:16, 2016
	532.	Giacomello et al., "Spatially resolved transcriptome profiling in model plant species", NATURE PLANTS 3, 17061, 11 pages, 2017
	533.	Gnanapragasam, "Unlocking the molecular archive: the emerging use of formalin-fixed paraffin-embedded tissue for biomarker research in urological cancer," BJU International,105, 274-278, 2009
	534.	Goldkom and Prockop, "A simple and efficient enzymatic method for covalent attachment of DNA to cellulose. Application for hybridization-restriction analysis and for in vitro synthesis of DNA probes.", Nucleic Acids Res. 14: 9171-9191, 1986
	535.	Gow et al., "Amplifications of mRNA populations using aRNA generated from immobilized oligo(dT)-T7 primed cDNA," BioTech., vol. 20, no. 4, 584-591, 1996
	536.	Gu et al., "Protein tag-mediated conjugation of oligonucleotides to recombinant affinity binders for proximity ligation," N Biotechnol., 30(2): 144-152, 2013
	537.	Gunderson et al., "Decoding Randomly Ordered DNA Arrays," Genome Research 14: 870-877, 2004
	538.	Hammond et al., "Profiling cellular protein complexes by proximity ligation with dual tag microarray readout," 7(7): e40405, 2012
	539.	Han et al., "3C and 3C-based techniques: the powerful tools for spatial genome organization deciphering. Mol. Cytogenet. 11, 21, 10 pages, 2018

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	540.	He et al., "In situ synthesis of protein arrays," Current Opinion in Biotechnology, 19: 4-9, 2008
	541.	He et al., "Printing protein arrays from DNA arrays," Nature Methods, 5: 175-77, 2008
	542.	He, "Cell-free protein synthesis: applications in proteomics and biotechnology," New Biotechnology 25: 126-132, 2008
	543.	Hedskog et al., "Dynamics of HIV-1 Quasispecies during Antiviral Treatment Dissected usinq Ultra-Deep Pyrosequencinq," PLoS One, 5(7): e11345, 2010
	544.	Hein et al., "Click Chemistry, A Powerful Tool for Pharmaceutical Sciences", Pharm Res., 25(10): 2216–2230, 2008
	545.	Hiatt et al., "Parallel, tag-directed assembly of locally-derived short sequence reads," Nature Methods, 7(2): 119-25, 2010
	546.	Inoue and Wittbrodt, "One for All—A Highly Efficient and Versatile Method for Fluorescent Immunostaining in Fish Embryos," PLoS One 6, e19713, 2011
	547.	Jabara et al., "Accurate sampling and deep sequencing of the HIV-1 protease gene using a Primer ID. PNAS 108(50); 20166-20171, 2011
	548.	Jamur and Oliver, "Permeabilization of cell membranes.," Method Mal. Biol., 588: 63-66, 2010
	549.	Jemt et al., "An automated approach to prepare tissue-derived spatially barcoded RNA-sequencing libraries", Scientific Reports, 6: 37137, 10 pages, 2016
	550.	Jones et al., Comparative lesion sequencing provides insights into tumor evolution. Proc. Natl. Acad. Sci. USA 105(11): 4283-4288, 2008
	551.	Kanehisa, "Use of sttistical cteria for sreening potential homologies in nudeic add sequences," Nucleic Acids Res. 12: 203-213, 1984
	552.	Kap et al., "Histological Assessment of PAXgene Tissue Fixation and Stabilization Reagents," PLoS One 6, e27704, 10 pages, 2011
	553.	Kolb et al., "Click Chemistry: Diverse Chemical Function from a Few Good Reactions.", Angew. Chem. Int. Ed., 40(11): 2004-2021, 2001
	554.	Kolovos et al., "Investigation of the spatial structure and interactions of the genome at sub-kilobasepair resolution using T2C.", Nat. Protoc. 13, 459–477, 2018
	555.	Korbel et al., "Paired-End Mapping Reveals Extensive Structural Variation in the Human Genome," Science, 318(5849): 420-426, 2007
	556.	Korlach et al., "Selective aluminum passivation for targeted immobilization of single DNA polymerase molecules in zero-mode waveguide nanostructures," Proc. Natl. Acad. Sci. USA 105, 1176-1181, 2008
	557.	Kozlov et al., "A High-Complexity Multiplexed Solution-Phase Assay for Profiling Protease Activity on Microarrays," Comb. Chem. And Hiah Throuahout, 11: 24-35, 2008
	558.	Kozlov et al., "A Highly Scalable Peptide-Based Assay System for Proteomics," PLoS ONE, 7(6): e37441, 2012
	559.	Kozlov et al., "A Method for Rapid Protease Substrate Evaluation and Optimization," Comb. Chem. and Hiah Throuahout, 9: 481-87, 2006
	560.	Kurz et al., "eDNA-Protein Fusions: Covalent Protein-Gene Conjugates for the In Vitro Selection of Peptides and Proteins," ChemBioChem., 2: 666-72, 2001

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	561.	Lage et al., "Whole Genome Analysis of Genetic Alterations in Small DNA Samples Using Hyperbranched Strand Displacement Amplification and Array–CGH," Genome Research 13: 294-307, 2003
	562.	Langdale et al., "A rapid method of gene detection using DNA bound to Sephacryl", Gene 36: 201-210, 1985
	563.	Larman et al., "Autoantigen discovery with a synthetic human peptidome," Nature Biotechnology, doi:1 0.1038/nbt.1856, 2011
	564.	Lassmann et al., A Novel Approach For Reliable Microarray Analysis of Microdissected Tumor Cells From Formalin-Fixed and Paraffin-Embedded Colorectal Cancer Resection Specimens, J Mol Med, 87, 211-224, 2009
	565.	Lee et al., "Fluorescent in situ sequencing (FISSEQ) of RNA for gene expression profiling in intact cells and tissues", Nature Protec, 10(3): 442-458, 2015
	566.	Lee et al., "Highly multiplexed subcellular RNA sequencing in situ", Science, 343(6177): 1360–1363, 2014
	567.	Lein et al., "The promise of spatial transcriptomics for neuroscience in the era of molecular cell typing", Science 358, 64–69, 2017
	568.	Leriche et al., "Cleavable linkers in chemical biology.", Bioorganic & Medicinal Chemistry, 20: 571–582, 2012
	569.	Levene et al., "Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations," Science 299, 682-686, 2003
	570.	Li et al., "A photocleavable fluorescent nucleotide for DNA sequencing and analysis," Proc. Natl. Acad. Sci., 100: 414-419, 2003
	571.	Linnarsson, "Recent advances in DNA sequencing methods - general principles of sample preparation," Experimental Cell Research, 316: 1339-1343, 2010
	572.	Lizardi et al., "Mutation detection and single-molecule counting using isothermal rolling-circle amplification," Nat. Genet. 19: 225-232, 1998
	573.	Lovatt et al., "Transcriptome in vivo analysis (TIVA) of spatially defined single cells in live tissue.", Nature Methods 11, 190–196, 2014
	574.	Lund et al., "Assessment of methods for covalent binding of nucleic acids to magnetic beads, Dynabeads, and the characteristics of the bound nucleic acids in hybridization reactions.", Nucleic Acids Res., 16: 10861-80, 1988
	575.	Lundberg et al., "High-fidelity amplification using a thermostable DNA polymerase isolated from Pyrococcus furiosus.," Gene., 108(1): 1-6, 1991
	576.	Lundberg et al., "Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood," Nucleic Acids Res., 39(15): e102, 2011
	577.	Lundberg et al., "Multiplexed homogeneous proximity ligation assays for high-throughput protein biomarker research in serological material," Mol Cell Proteomics, 10(4): M110.004978, 2011
	578.	Lundquist et al., "Parallel confocal detection of single molecules in real time," Opt. Lett. 33, 1026-1028, 2008
	579.	Lyck, et al., "Immunohistochemical Markers for Quantitative Studies of Neurons and Glia in Human Neocortex," J Histochem Cytochem 56, 201-21, 2008

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	580.	MacIntyre, "Unmasking antigens for immunohistochemistry.," Br J Biomed Sci. 58, 190-6, 2001
	581.	Macosko et al., "Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets," Cell 161, 1202-1214, 2015
	582.	Maniatis et al., "Spatiotemporal Dynamics of Molecular Pathology in Amyotrophic Lateral Sclerosis", 54 pages, 2018
	583.	McCloskey et al., "Encoding PCR Products with Batch-stamps and Barcodes.," Biochem. Genet. 45: 761-767, 2007
	584.	Mcgee, "Structure and Analysis of Affymetrix Arrays," UTSW Microarray Analysis Course, October 28, 2005
	585.	McKernan et al., "Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding," Genome Res., 19: 1527-41, 2009
	586.	Metzker "Sequencing technologies - the next generation," Nature Reviews Genetics, 11: 31-46, 2010
	587.	Miller et al., "Basic Concepts of Microarrays and Potential Applications in Clinical Microbiology," Clinical Microbiology Reviews, Vol. 22, No. 4, pp. 611-633, 2009
	588.	Mir et al., "Sequencing by cyclic ligation and cleavage (CycliC) directly on a microarray captured template," Nucleic Acids Research, 37(1): e5-1, 2009
	589.	Mishra and Hawkins, "Three-dimensional genome architecture and emerging technologies: looping in disease. Genome Med. 9, 87, 14 pages, 2017
	590.	Mishra, "Three-dimensional genome architecture and emerging technologies: looping in disease", Genome Medicine, 9: 87, 14 pages, 2017
	591.	Mitsuhashi et al., "Gene manipulation on plastic plates," Nature 357: 519-520, 1992
	592.	Mizusawa et al., "A bacteriophage lambda vector for cloning with BamHI and Sau3A," Gene, 20: 317-322, 1982
	593.	Moncada et al., "Building a tumor atlas: integrating single-cell RNA-Seq data with spatial transcriptomics in pancreatic ductal adenocarcinoma", Institute for Computational Medicine, bioRxiv, 28 pages, 2018
	594.	Moor et al., "Spatial transcriptomics: paving the way for tissue-level systems biology", Science Direct, Current Opinion in Biotechnology, 46: 126–133, 2017
	595.	Mortazavi et al., "Mapping and quantifying mammalian transcriptomes by RNA-Seq," Nature Methods, 5(7): 621-8, 2008
	596.	Nawy, "Spatial transcriptomics", Nature Methods, Vol. 15, No. 1, 2018
	597.	Ng et al., "Gene identification signature (GIS) analysis for transcriptome characterization and genome annotation," Nature Methods, 2(2): 105-111, 2005
	598.	Ng et al., "Massively parallel sequencing and rare disease," Human Malec. Genetics, 19(2): R119-R124, 2010
	599.	NG et al., "Multiplex sequencing of paired-end ditags (MS-PET): a strategy for the ultra-high-throughput analysis of transcriptomes and genomes," Nucleic Acids Research, July 2006, 34(12): e84, 10 pages
	600.	Niemeyer, "The developments of semisynthetic DNA-protein conjugates," TrendsBiotechnol, September 2002, 20(9): 395-401

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	601.	Nuovo, "In situ PCR: protocols and applications.," Genome Res, February 1995, 4 (4):151-167
	602.	Oleinikov et al., "Self-assembling protein arrays using electronic semiconductor microchips and in vitro translation," J Proteome Res, May-June 2003, 2(3): 313-319
	603.	Olink Proteomics AB, Proseek® Multiplex 96x96 User Manual, 2016, 12 pages
	604.	Osada et al., "Epitope mapping using ribosome display in a resconstituted cell-free protein synthesis system," J Biochem, May 2009, 145(5): 693-700
	605.	O-Shannessy et al., "Detection and quantitation of hexa-histidine-tagged recombinant proteins on western blots and by a surface plasmon resonance biosensor technique," Anal Biochem, 229(1): 119-124, 1995
	606.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2010/033064, dated 11/1/2011, 6 pages
	607.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2010/044134, dated 1/31/2012, 20 pages
	608.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2010/059327, dated 6/12/2012, 12 pages
	609.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2011/031163, dated 10/9/2012, 7 pages
	610.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2011/031308, dated 10/9/2012, 7 pages
	611.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2012/032759, dated 10/8/2013, 17 pages
	612.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US14/29691, dated 8/19/2014, 10 pages
	613.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US14/44196, dated 11/7/2014, 15 pages
	614.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US14/64588, dated 3/11/2015, 20 pages
	615.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2010/033064, dated 7/30/2010, 8 pages
	616.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2010/044134, dated 3/18/2011, 16 pages
	617.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2010/059327, dated 3/29/2011, 12 pages
	618.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2011/031163, dated 5/23/2011, 8 pages
	619.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2011/031308, dated 6/7/2011, 8 pages
	620.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2012/032759, dated 9/28/2012, 14 pages
	621.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2014/044191, dated 11/7/2014, 10 pages

Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	622.	Perler et al., "Intervening sequences in an Archaea DNA polymerase gene," PNAS USA, June 1992, 89(12): 5577-5581
	623.	Petterson et al., "Generations of sequencing technologies," Genomics, 2009, 105-111
	624.	Polsky-Cynkin et al., "Use of DNA Immobilizedon Plastic and Agarose Supports to DetectDNA by SandwichHybridization", Clin. Chem. 31: 1438-1443, 1985
	625.	Rajeswari et al., "Multiple pathogen biomarker detection using an encoded bead array in droplet PCR," J. Microbial Methods, August 2017, 139:22-28
	626.	Ramachandran et al., "Next-generation high-density self-assembling functional protein arrays," Nature Methods, June 2008, 5(6):535-538
	627.	Ranki et al., "Sandwich hybridization as a convenient method for the detection of nucleic acids in crude samples", Gene 21: 77-85, cellulose, 1983
	628.	ROBERTS et al., "RNA-peptide fusions for the in vitro selection of peptides and proteins," PNAS USA, November 1997, 94: 12297-122302
	629.	Ronaghi et al., "A sequencing method based on real-time pyrophosphate," Science, July 1998, 281(5375): 363-365
	630.	Ronaghi et al., "Real-time DNA sequencing using detection of pyrophosphate release," Analytical Biochemistry, November 1996, 242(1): 84-89
	631.	Ronaghi, "Pyrosequencing sheds light on DNA sequencing," Genome Res, January 2001, 11(1):3-11
	632.	ROUILLARD et al., "OligoArray 2.0: design of oligonucleotide probes for DNA microarrays usina a thermodynamic approach," Nuc. Acid Research, June 2003, 31(12): 3057-3062
	633.	ROUNTENBERG et al., "Microfluidic probe: a new tool for integrating microfluidic environments and electronic wafer-orobina," Lab Chip, October 2009, 10: 123-127
	634.	Rubin et al., "Whole-genome resequencing reveals loci under selection during chicken domestication.," Nature, March 2010, 464: 587-591
	635.	RUSH et al., "New Aldehyde Tag Sequences Identified by Screening Formylglycine Generating Enzymes in Vitro and in Vivo," J. of American Chemical Society, August 2008, 130(37): 12240-12241
	636.	Rusk, "Spatial transcriptomics", Nature Methods, vol. 13, no. 9, 2016
	637.	Salem et al., "Multidimensional transcriptomics provides detailed information about immune cell distribution and identity in HER2+ breast tumors", bioRxiv, 41 pages, 2018
	638.	Schaus et al., "A DNA nanoscope via auto-cycling proximity recording.", Nat. Commun. 8, 696, 10 pages, 2017
	639.	Schaus et al., "A DNA nanoscope via auto-cycling proximity recording", NATURE COMMUNICATIONS, 8: 696, 9 pages, 2017
	640.	Schena et al., "Quantitative monitoring of gene expression patterns with a complementary DNA microarray," Science, October 1995, 270(5235): 467-470
	641.	Schena et al., "Entering the Postgenome Era," Science, 1995, 270:368-9, 371
	642.	Schlapak et al., "Glass surfaces grafted with high-density poly (ethylene glycol) as substrates for DNA oligonucleotide microarrays," Langinuir, January 2006, 22: 277-285

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	643.	Schmitt et al., "Detection of ultra-rare mutations by next-generation sequencing," PNAS (2012) 109:14508-14523
	644.	Science Tools: results, reports and refinement for life science researchers, Amersham Pharmacia Biotech, 1998, Vol. 3(4): 16 pages
	645.	SERGEEVA et al., "Display technologies: Application for the discovery of drug and gene delivery aaents," Advanced Drua Delivery Reviews (2006) 58(15):1622-1654
	646.	Seurynck-Servoss et al., "Evaluation of Surface Chemistries for Antibody Microarrays", Anal Biochem., 371(1): 105–115, 2007
	647.	Shelbourne et al., "Fast copper-free click DNA ligation by the ring-strain promoted alkyne-azide cycloaddition reaction.", Chem. Commun., 47: 6257-6259, 2011
	648.	Shendure et al., "Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome," Science, 2005, 309:1728-1732
	649.	Shi et al., "The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements," Nature Biotechnology, 2006, 24(9):1151-61
	650.	Shirai et al., "Novel Tools for Analyzing Gene Expressions in Single Cells," The 5th International Workshop on Approaches to Single-Cell Analysis, The University of Tokyo, March 3-4th 2011, 1 page
	651.	SHOEMAKER et al., "Quantitative phenotypic analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy," Nature genetics (1996) 14:450-456
	652.	SHULTS et al., "A multiplexed protein kinase assay," Chem Bio Chem (2007) 8:933-942
	653.	Sievertzon et al., "Transcriptome analysis in primary neural stem cells using a tag cDNA amplification method," BMC Neuroscience, December 2005, 6: 28
	654.	Slonim and Yanai, "Getting started in gene expression microarray analysis," Plos Computational Biology, 2009, 5(10):e1000543
	655.	Soni and Meller, "Progress toward ultrafast DNA sequencing using solid-state nanopores," Clin Chem., 2007, 53: 1996-2001
	656.	Spitale et al., "Structural imprints in vivo decode RNA regulatory mechanisms", Nature, 519(7544): 486-90, 2015
	657.	Spurgeon et al., "High Throughput Gene Expression Measurement with Real Time PCR in a Microfluidic Dynamic Array," PlosONE, 2008, 3(2):e1662
	658.	Stahl et al., "Visualization and analysis of gene expression in tissue sections by spatial transcriptomics.", Science 353, 78-82, 2016
	659.	Strell et al., "Placing RNA in context and space – methods for spatially resolved transcriptomics", The FEBS Journal, 14 pages, 2017
	660.	Summersgill et al., "Fluorescence In Situ Hybridization Analysis of Formalin Fixed Paraffin Embedded Tissues, Including Tissue Microarrays," Chapter 4, Bridger, J. Ed., Methods in Molecular Biology 659, 2010, 51-70, 2010
	661.	Sun et al., "Direct immobilization of DNA probes on non-modified plastics by UV irradiation and integration in microfluidic devices for rapid bioassay," Anal. Bio. Chem., 402: 741-748, 2012
	662.	TAKAHASHI et al., "In Vitro Selection of Protein and Peptide Libraries Using mRNA Display," Nucleic Acid and Peptide Aptamers: Methods and Protocols (2009) 535:293-314 (Ch.17).

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	663.	Tang et al., "RNA-Seq analysis to capture the transcriptome landscape of a single cell.," Nat Protoc., 5: 516-35, 2010
	664.	Taniguchi et al., ""Quantitative analysis of gene expression in a single cell by qPCR,"" Nature Methods, 6, pp 503-506, 2009"
	665.	Taylor et al., "Mitochondrial DNA mutations in human disease." Nature Reviews Genetics. May 2005, 6(5):389-402
	666.	Tentori et al., "Detection of Isoforms Differing by a Single Charge Unit in Individual Cells.", Chem. Int. Ed., 55, 12431, 2016
	667.	Thornton, "High rate thick film growth." Annual review of materials science, August 1977, 7(1):239-60
	668.	Tian et al., "Antigen peptide-based immunosensors for rapid detection of antibodies and antigens," Anal Chem May 26, 2009, 81 (13):5218-5225
	669.	Tijssen, "Overview of principles of hybridization and the strategy of nucleic acid assays" in Techniques in 5 Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes 1993
	670.	Tolbert et al., "New Methods for Proteomic Research: Preparation of Proteins with N-Terminal Cysteines for Labeling and Conjugation" Angewandte Chemie International Edition, June 17, 2002, 41(12):2171-4
	671.	Valencia et al., "mRNA-Display-Based Selections for Proteins with Desired Functions: A Protease-Substrate Case Study." Biotechnology progress, May 2008, 24(3):561-9
	672.	Van Gelder et al., "Amplified RNA synthesized from limited quantities of heterogeneous cDNA," Proc. Natl. Acad. Sci. USA 87, 1663-1667, 1990
	673.	Van Ness et al., "A versatile solid support system for oligodeoxynucleotide probe-based hybridization assays", Nucleic Acids Res. 19: 3345-3350, 1991
	674.	Velculescu et al., "Serial analysis of gene expression." Science, October 20, 1995, 270(5235):484-7
	675.	Vickovic et al., "Massive and parallel expression profiling using microarrayed single-cell sequencing," Nature Communications, 2016 Oct 14; 7:13182. doi: 10.1038/ncomms13182
	676.	Vogelstein et al., "Digital PCR," Proceedings of the National Academy of Sciences, August 3, 1999, 96:9236-9241
	677.	Wade et al., "Genome sequence, comparative analysis, and population genetics of the domestic horse.," Science., 326: 865-7, 2009
	678.	Waichman et al., "Functional immobilization and patterning of proteins by an enzymatic transfer reaction." Analytical chemistry, January 21, 2010, 82(4):1478-85
	679.	Walker et al., "Strand displacement amplification—an isothermal, in vitro DNA amplification technique." Nucleic acids research. 1992 April 11, 1992, 20(7):1691-1696
	680.	Wang et al., "Single cell analysis: the new frontier in 'omics'.,"Trends Biotechnol., 28: 281-90, 2010
	681.	Wang et al., "Three-dimensional intact-tissue sequencing of single-cell transcriptional states", Science, 361(6400), 22 pages, 2018
	682.	Wang et al., "High-fidelity mRNA amplification for gene profiling." Nature biotechnology. April 2000, 18(4):457-459

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	683.	WEICHHART et al., "Functional selection of vaccine candidate peptides from Staphylococcus aureus whole-genome expression libraries in vitro," Infection and Immunity, 2003, 71 (8):4333-4641
	684.	Weinstein et al., "DNA microscopy: Optics-free spatio-genetic imaging by a stand-alone chemical reaction", bioRxiv, 41 pages, 2018
	685.	Willi-Monnerat et al., "Comprehensive spatiotemporal transcriptomic analyses of the ganglionic eminences demonstrate the uniqueness of its caudal subdivision," MOLECULAR AND CELLULAR NUEORSCIENCES 37: 845-856, 2008
	686.	Wolf et al., "Rapid hybridization kinetics of DNA attached to submicron latex particles", Nucleic Acids Res. 15: 2911-2926, 1987
	687.	WONG et al., "Direct Site-Selective Covalent Protein Immobilization Catalyzed by a Phosphopantetheinyl Transferase," J. Am. Chem Soc., 2008, 130:12456-64
	688.	Woo et al., "A Comparison of cDNA, Oligonucleotide, and Affymetrix GeneChip Gene Expression Microarray Platforms," Journal of Biomolecular Techniques, 2004, 15(4), 276-284
	689.	WORTHINGTON et al., "Cloning of random oligonucleotides to create single-insert plasmid libraries," Analyt. Biochem, 2001, 294:169-175
	690.	XIAO et al., "Direct determination of haplotypes from single DNA molecules," Nature Methods, 2009, 6(3):199-01
	691.	YIN et al., "Genetically encoded short peptide tag for versatile protein labeling by Sfp phosphopantetheinyl transferase," PNAS, 2005, 102(44):15815-20
	692.	YONEZAWA et al., "DNA display for in vitro selection of diverse peptide libraries," Nucleic Acids Research , 2003, 31 (19):e118
	693.	ZHANG et al., "Assembling DNA through Affinity Binding to Achieve Ultrasensitive Protein Detection," Anaew Chem Int Ed (2013) 52:2-10
	694.	ZHANG et al., "Binding-induced DNA assembly and its application to yoctomole detection of proteins," Anal Chem (2012) 84(2):877-884
	695.	ZHENG et al., Origins of human mitochondrial point mutations as DNA polymerase mediated errors. Mutat. Res. 599(1-2): 11-20 (2006
	696.	ZHOU et al., "Genetically Encoded Short Peptide Tags for Orthogonal Protein Labeling by Sfp and Ac S Phos ho entethein I Transferases," ACS Chemical Biolo 2007 2 5 : 337-346
	697.	ZILBERMAN et al., "Genome-wide analysis of DNA methylation patterns," Development (2007) 134: 3959-3965
	698.	ZLOBEC et al., "Next-generation tissue microarray (ngTMA) increases the quality of biomarker studies: an example using CD3, CD8, and CD45RO in the tumor microenvironment of six different solid tumor t es," Journal of Translational Medicine 2013 11:104

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Electronic Acknowledgement Receipt

EFS ID:	35427699
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. CHEE
Customer Number:	159367
Filer:	Cameron Mark Luitjens/Kirsten Neuman
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	47706-0004003
Receipt Date:	15-MAR-2019
Filing Date:	14-FEB-2019
Time Stamp:	14:29:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Affymetrix_2003.pdf	231827 d30d8c4f1a52ef5a5741ce38feda59596505c94b	no	2

Warnings:

Information:					
2	Non Patent Literature	Affymetrix_humangenome_2002.pdf	95938 50bf1f77bfb3b6fc570b4070b9496e84ae41a2f4	no	1
Warnings:					
Information:					
3	Non Patent Literature	Valencia_mRNADisplayBased_2008.pdf	5760101 80c2890bb9572bef3245716414c6111e0969f32f	no	9
Warnings:					
Information:					
4	Non Patent Literature	ALLAWI_ThermodynamicsAndNMR.pdf	1422982 437953498e189d3028c1d6de3684dbb3e54f8b6c	no	14
Warnings:					
Information:					
5	Non Patent Literature	ANDERSON_MicroarrayedCompoundScreening.pdf	7274311 1a5bd992190fbea6d79daf95d356dbfde4d4d7c6	no	10
Warnings:					
Information:					
6	Non Patent Literature	Andersson_2006.pdf	9284499 ea6252d95b8b779bf9217113680c8fbadf3e9804	no	11
Warnings:					
Information:					
7	Non Patent Literature	Andresen_2009.pdf	1444672 ef472bf5858fdf296dcb61185e422f4decdab800	no	12
Warnings:					
Information:					
8	Non Patent Literature	ANGENENDT_CellFreeProtein.pdf	7173415 70777aa81be576205f0ca75b8b443e96d26101ba	no	6
Warnings:					
Information:					

9	Non Patent Literature	ANGENENDT_GenerationOfHIgh.pdf	1031779 094ebdab3763ab02beecf1f62d3517358d9fb957	no	9
Warnings:					
Information:					
10	Non Patent Literature	Armani_MethodOfMapping_2009.pdf	11823902 9c3caf65fbe03bad4c345ec26649d732a2d5e16b	no	19
Warnings:					
Information:					
11	Non Patent Literature	Asp_SpatialDetection_2017.pdf	10571468 f67b066de257fa47a99555011b1fba570ff43cd2	no	10
Warnings:					
Information:					
12	Non Patent Literature	Atkinson_Wells_AnUpdatedProtocol_2017.pdf	13787052 213da5214636a5ca9e3b856a07a7453b15cf4252	no	8
Warnings:					
Information:					
13	Non Patent Literature	Bains_NovelMethodFor_1988.pdf	608022 e0c290f5cb61ec1b3d43a4ea48be2553086847cb	no	5
Warnings:					
Information:					
14	Non Patent Literature	Baird_RapidSNPDiscovery_2008.pdf	5640233 12142e22b1ba831cbe02a8cd1b32c22c997ca692	no	7
Warnings:					
Information:					
15	Non Patent Literature	Barnes_1994.pdf	10615466 92367973b568dbb8ad66272669589f864500fe93	no	5
Warnings:					
Information:					

16	Non Patent Literature	Baugh_QuantitativeAnalysisOf_2001.pdf	6335269 b507d638de091eb5f6eb5df744b5e94ab406cbf0	no	9
Warnings:					
Information:					
17	Non Patent Literature	Bell_SimpleWayTo_2008.pdf	523583 6dbacec9474598c0af2eacbb83a6eec9c9d4a6e1	no	1
Warnings:					
Information:					
18	Non Patent Literature	Bentley_AccurateWholeHuman_2008.pdf	6088623 3cc4b78dbe00794380c831e7c261c29d2bc66836	no	7
Warnings:					
Information:					
19	Non Patent Literature	Bielas_HumanCancersExpress_2006.pdf	4649956 07db4721dfc539eac7529c142f0737394a538b3f	no	5
Warnings:					
Information:					
20	Non Patent Literature	Bielas_QuantificationOfRandom_2005.pdf	4588365 fabe90ffd6bcdd795a86ae67499285feef6f7504	no	6
Warnings:					
Information:					
21	Non Patent Literature	Birney_IdentificationAndAnalysis_2007.pdf	11593390 82163fa0573ee9ed6ea8ee2fe754d2651def0b3d	no	50
Warnings:					
Information:					
22	Non Patent Literature	Blokzijl_ProfilingProteinExpression_2010.pdf	3569809 82fd80bc9650b89de644431533 2fdcd05f21bc8d	no	14
Warnings:					
Information:					

23	Non Patent Literature	Blow_2007.pdf	4674931 4a2a251269021c329c0dac464c07b374aa8be7b6	no	4
Warnings:					
Information:					
24	Non Patent Literature	Bonfiel_TheApplicationOf_1995.pdf	6997747 4acabb69155ce81e1499c7216dabba1523badbef	no	5
Warnings:					
Information:					
25	Non Patent Literature	Boulgakov_FromSpace_2018.pdf	5890809 501a1768b080545bd3b24b00b82c84f6937b3768	no	24
Warnings:					
Information:					
26	Non Patent Literature	Bowtell_TheGenesisAnd_2010.pdf	7612821 9b09146927241453 1e94477c21f3767e54acccb0	no	6
Warnings:					
Information:					
27	Non Patent Literature	Brandon_MitochondrialMutationsl_2006.pdf	3025998 d7d17acb4658f1e44d707780fd6342a4e392c231	no	16
Warnings:					
Information:					
28	Non Patent Literature	Brenner_InVitroCloning_1999.pdf	4819554 83257dbb48b84e8394ee872e96fb4e7aa9f81724	no	6
Warnings:					
Information:					
29	Non Patent Literature	Brenner_GeneExpressionAnalysis_2000.pdf	5480733 633ba8a7134be5c4ca619fc60c390f311ea82f08	no	5
Warnings:					
Information:					

30	Non Patent Literature	Burns_WellLessGel_2001.pdf	8186403 bbb12ac5052a0489294df6df0ed5bf6e3d325f49	no	8
Warnings:					
Information:					
31	Non Patent Literature	Burton_CoverslipMountedImmersion_1998.pdf	6690451 b3289da982903272a72aebf6d5222efccb27ab6c	no	6
Warnings:					
Information:					
32	Non Patent Literature	Carlson_FunctionAndStructure_2008.pdf	5372972 fd5c6fe83bc4bc1c6708ea1a2abb5e84da3a31ef	no	9
Warnings:					
Information:					
33	Non Patent Literature	Carter_StabilizationOfAn_2007.pdf	6965619 32b03b5fbd32e786029f41739f0c26841bfd55af	no	7
Warnings:					
Information:					
34	Non Patent Literature	Cerutti_GenerationOfSequence_2001.pdf	6546076 2590ff49382670b5e776149697865133d8bfe011	no	6
Warnings:					
Information:					
35	Non Patent Literature	Cha_SpecificityEfficiencyAnd_2016.pdf	5019186 72996535a064fc2387a967811f49fb6e746d6b10	no	13
Warnings:					
Information:					
36	Non Patent Literature	Chandra_CellFreeSynthesis_2010.pdf	6534481 4c0541bac1cd0169040331e492ffd53a85bebdd2	no	14
Warnings:					
Information:					

37	Non Patent Literature	Chatterjee_ProteinMicroarrayOn_2008.pdf	5336621 dd7427a320987ca916c26c8bfda34b0e7a5a7194	no	5
Warnings:					
Information:					
38	Non Patent Literature	Burgess_Aspace_2016_flat.pdf	1026782 c05b2dab414e74f3c1dab812ae8fd8c1f64f493b	no	1
Warnings:					
Information:					
39	Non Patent Literature	Burgess_FindingStructure_2018_flat.pdf	1538855 a79178dfb669fb6f967f765b320de4282cb564c8	no	1
Warnings:					
Information:					
40	Non Patent Literature	Chatterjee_MitochondrialDNAMutations2006_flat.pdf	8494708 ab69ed732fc42fe980418761ad20bdca1d4e03e8	no	13
Warnings:					
Information:					
41	Non Patent Literature	Chen_DNAHybridizationDetection_2008.pdf	5773029 cebd684c41d55b2aaa50a9494c66215880af46cb	no	5
Warnings:					
Information:					
42	Non Patent Literature	Chen_ExpansionMicroscopy_2015_Duplicate.pdf	3905155 6aae33a1521fccb3ba7aaee84908a4f5c3007e27	no	13
Warnings:					
Information:					
43	Non Patent Literature	Chen_SpatiallyResolved_2015.pdf	4978329 e23992dee1bf0cc4d9949c0b56985afd533972ed	no	36
Warnings:					
Information:					

44	Non Patent Literature	CHENG_SensitiveDetectionOf.pdf	3978228 85d4a8202c3b35791802f53e234b8598863fef3e	no	4
Warnings:					
Information:					
45	Non Patent Literature	Condina_SensitiveMagneticBead_2009.pdf	5174917 e2a66b7cf58310bf7c0f536e0995f2003dd8e51	no	11
Warnings:					
Information:					
46	Non Patent Literature	Constantine_1998.pdf	1328671 9f6cd9b34edf49c97591c89ebc82e448efbb9d5c	no	5
Warnings:					
Information:					
47	Non Patent Literature	Copeland_MitochondrialDNAAlterations_2002.pdf	5818209 0c3a00ede3c207a3229aadbeec0a2557f5c74a72	no	13
Warnings:					
Information:					
48	Non Patent Literature	Corces_AnImprovedATAC_2017.pdf	1591900 a17bea4579fe5b6b3fa69e4c2d04a8e37a798014	no	15
Warnings:					
Information:					
49	Non Patent Literature	CROSETTO_SpatiallyResolvedTranscriptomics.pdf	7420181 0e96481db2b7d03407be4f3a5983595c5d53b214	no	10
Warnings:					
Information:					
50	Non Patent Literature	Cujec_SelectionOfTyrosine_2002.pdf	6180803 bc3028560a12c89af03506cd21e86de2a00c5f51	no	12
Warnings:					
Information:					

51	Non Patent Literature	DAHL_CircleToCircle.pdf	8239270 c1e41620faf77b5f78a06d0a3b1aa33327892841	no	6
Warnings:					
Information:					
52	Non Patent Literature	Darmanis_ProteinSeqHigh_2011.pdf	4939237 cee57e0b343a9f02d4e899594f7361bca3b536cc	no	10
Warnings:					
Information:					
53	Non Patent Literature	DAUBENDIEK_RollingCircleRNA.pdf	4314025 b7a0e85ea92148178556ab35d87f3cd5453999f1	no	7
Warnings:					
Information:					
54	Non Patent Literature	Davies_HowBestTo_2017.pdf	19739137 3fa8d45cfab11f6214a68f0884158bce7bdb4178	no	29
Warnings:					
Information:					
55	Information Disclosure Statement (IDS) Form (SB08)	Transmittal.pdf	74056 1f50cebbb89e7fac0d9de60c1b9fa1b55aed02aa	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
56	Information Disclosure Statement (IDS) Form (SB08)	PTO_1449.pdf	320689 1200c5cbda1867283fecdf750bcafaea89ec6829	no	30
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
			Total Files Size (in bytes):	308105245	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	35427994
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. CHEE
Customer Number:	159367
Filer:	Cameron Mark Luitjens/Kirsten Neuman
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	47706-0004003
Receipt Date:	15-MAR-2019
Filing Date:	14-FEB-2019
Time Stamp:	14:38:42
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Hein_ClickChemistry_2008.pdf	20017175 649aa098f5d3841078bf33d41f599dbbb0c0da02	no	15

Warnings:

Information:					
2	Non Patent Literature	Hiatt_ParallelTagDirected_2010.pdf	8722023 41cd821153573ef108530b0f60300bfeb8609d83	no	13
Warnings:					
Information:					
3	Non Patent Literature	Inoue_Wittbrodt_OneForAll_2011.pdf	5415087 eeb2cccf1a35bc5cf8bdf3f59748ac06e773451c	no	7
Warnings:					
Information:					
4	Non Patent Literature	Jabara_AccurateSampling_2011.pdf	2388260 a8910191520318265551ded334d096523c9417171	no	6
Warnings:					
Information:					
5	Non Patent Literature	Jemt_AnAutomatedApproach_2016.pdf	3066119 87727057d5d30c3a1f4e75041182a1f2a0aeebc5	no	10
Warnings:					
Information:					
6	Non Patent Literature	Jones_ComparativeLesionSequencing_2008.pdf	7176750 583787e79a26a5f21eaeb8baa72905488727c421	no	6
Warnings:					
Information:					
7	Non Patent Literature	Kanehisa_UseOfSttistical_1984.pdf	4852068 79632c694477c145548d55bb5e49237410cf573f	no	11
Warnings:					
Information:					
8	Non Patent Literature	Kap_HistologicalAssessment_2011.pdf	3685251 f509d9cc64376665dc20481e1a1dd9b353cfee14	no	10
Warnings:					
Information:					

9	Non Patent Literature	Korbel_PairedEndMapping_2007.pdf	2310368 9045586cffa6437d5d79307309406028206 04b16	no	13
Warnings:					
Information:					
10	Non Patent Literature	Korlach_SelectiveAluminumPassivation_2008.pdf	8083989 ceedc2b8257abad41a7b0ad6f33d3be6afd ea859	no	6
Warnings:					
Information:					
11	Non Patent Literature	Jamur_Oliver_PermeabilizationOfCell_2010r.pdf	1643126 4419378fecc96989d36ce023fa9bf00e475d 72fb	no	4
Warnings:					
Information:					
12	Non Patent Literature	Kozlov_AHighComplexity_2008.pdf	11415331 6b62f2ac544b3cfa6fa218e659d7161af289 b308	no	12
Warnings:					
Information:					
13	Non Patent Literature	Kozlov_AHighlyScalable_2012.pdf	3034663 457c501b9b9936b4cae77c05edd329f3482 f5a33	no	10
Warnings:					
Information:					
14	Non Patent Literature	Kozlov_AMethodFor_2006.pdf	6360146 e95d7f50ea13bad3deea3cb5b7c1b4a78a5 30462	no	7
Warnings:					
Information:					
15	Non Patent Literature	Kurz_eDNAProteinFusion_2001.pdf	241735 041a81c4d240ca2ece3c95b3410b1f8c2455 ea9f	no	7
Warnings:					
Information:					

16	Non Patent Literature	Lage_WholeGenomeAnalysis_2003.pdf	3619800 07f5620213f21065f006b04e09b30d2f8e306ef5	no	14
Warnings:					
Information:					
17	Non Patent Literature	Lee_Fluorescent_2015.pdf	2743383 263e87a162416bf35f0d957575227c9e855ec9d1	no	37
Warnings:					
Information:					
18	Non Patent Literature	Lee_HighlyMultiplexed_2014.pdf	3291681 13aff21761dc53f7bd237df83936a5977d7facee	no	12
Warnings:					
Information:					
19	Non Patent Literature	Lein_ThePromise_2017.pdf	443591 f35d7ce7cb40ab51f613c17d376fba95c524983a	no	7
Warnings:					
Information:					
20	Non Patent Literature	Levene_ZeroModeWaveguides_2003.pdf	6520832 497fa1f2b206a099a34f101c35c902ff74c14a68	no	6
Warnings:					
Information:					
21	Non Patent Literature	Li_AphotocleavableFluorescent_2003.pdf	4518422 c79eeedf6cdcfa376eae533398fc93e010f6da17	no	6
Warnings:					
Information:					
22	Non Patent Literature	Linnarsson_RecentAdvancesIn_2010.pdf	159323 21918f5bee270d5562319c4b50d726a3ecc2efd0	no	5
Warnings:					
Information:					

23	Non Patent Literature	Lovatt_TranscriptomeInVivo_2014.pdf	1741245 c41c214b766da1307c9d77a4bf8cf62648c4ff07	no	22
Warnings:					
Information:					
24	Non Patent Literature	Lund_AssessmentOfMethod_1988.pdf	10770287 8bc96d868f76c60295dd682290dcb881d4c94668	no	20
Warnings:					
Information:					
25	Non Patent Literature	Lundberg_HomogeneousAntibodyBased_2011.pdf	2215998 a7a5d47159d8dbcb9022d32c306b3f9a58122177	no	8
Warnings:					
Information:					
26	Non Patent Literature	Lundberg_MultiplexedHomogeneousProximity_2011.pdf	3137838 8404ca1bc55a5979a84f051f646b847eee56c04c	no	11
Warnings:					
Information:					
27	Non Patent Literature	Lundquist_ParallelConfocalDetection_2008.pdf	5503954 7ea5331e1dd4084682ba7b6bdf0fa6d8578469f3	no	3
Warnings:					
Information:					
28	Non Patent Literature	Lyck_ImmunohistochemicalMarkers_2008.pdf	6888942 d2c87ce7c885ba86e97d882ba1d2cd8768d909c9	no	21
Warnings:					
Information:					
29	Non Patent Literature	MacIntyre_UnmaskingAntigens_2001.pdf	617351 d683a7bdf10ca42e1e0db06559d753103da7fba9	no	7
Warnings:					
Information:					

30	Non Patent Literature	Larman_AutoantigenDiscovery_2011r.pdf	8273046 73b46ce028253a4c4f891379ce7713a9c960cb66	no	9
Warnings:					
Information:					
31	Non Patent Literature	Lizardi_MutationDetection_1998r.pdf	8288775 5db43569b2e6630ca2a3375f7fa6538d6196ebf6	no	8
Warnings:					
Information:					
32	Non Patent Literature	Macosko_HighlyParallelGenomeWide_2015.pdf	3670574 6bc9d6595af1cc63de3241f7baaa28d5a36e1efe	no	14
Warnings:					
Information:					
33	Non Patent Literature	Maniatis_SpatiotemporalDynamic_2018.pdf	10349867 cc4cac7c7f9c217e8170e43fa866fa34bddee4d8	no	54
Warnings:					
Information:					
34	Non Patent Literature	McCloskey_EncodingPCR_2007.pdf	187960 b6574678daea0b0aef9e322786e465d10bc740e6	no	7
Warnings:					
Information:					
35	Non Patent Literature	McKernan_SequenceAndStructural_2009.pdf	5312290 7d7ac0a5daaedeee860d003a61ed314570740045	no	16
Warnings:					
Information:					
36	Non Patent Literature	Metzker_SequencingTechnologies_2010.pdf	14811951 356c0d0fe1ec86d776ea4abdeef403035f8851ca	no	16
Warnings:					
Information:					

37	Non Patent Literature	Mir_SequencingByCyclic_2009.pdf	2836207 28640e41e2b5db23a836e70166a27e158c5845b3	no	8
Warnings:					
Information:					
38	Non Patent Literature	Mishra_Hawkins_ThreeDimensional_2017.pdf	17795626 64b7a1d2ad07923fed3f66d8ed41c22dea64a4c0	no	14
Warnings:					
Information:					
39	Non Patent Literature	Mishra_ThreeDimensional_2017.pdf	376270 5f479fbb52e91405d6759c1923a685f2ec5dd73c	no	14
Warnings:					
Information:					
40	Non Patent Literature	Mitsuhashi_GeneManipulation_1992.pdf	2964919 d7e312386df859a3603ee26bbad9ec99c3923abc	no	2
Warnings:					
Information:					
41	Non Patent Literature	Mizusawa_Abacteriophage_1982.pdf	1151029 d2c152798f27ee1ff3d16a9cb15d1027e34a5596	no	6
Warnings:					
Information:					
42	Non Patent Literature	Moncada_BuildingaTumor_2018.pdf	5440015 be589681371c22386fc257614488dc5558b22aac	no	28
Warnings:					
Information:					
43	Non Patent Literature	Moor_SpatialTranscriptomics_2017.pdf	2651425 f0d4e195b3f1bdb184f56e2132479f2f78299ee3	no	8
Warnings:					
Information:					

44	Non Patent Literature	Mortazavi_MappingAndQuantifying_2008.pdf	4150032 d2de74208f3ab03c088e05656502783f388141bd	no	23
Warnings:					
Information:					
45	Non Patent Literature	Nawy_SpatialTranscriptomic_2018.pdf	1016874 62c1f3df8cec16d3e02d4c0ee12ce5f38d16ff50	no	1
Warnings:					
Information:					
46	Non Patent Literature	Ng_GeneIdentificationSignature_2005.pdf	5757383 68e3d75f6bf841618096fa1af0a637f182171c27	no	8
Warnings:					
Information:					
47	Non Patent Literature	Ng_MassivelyParallelSequencing_2010.pdf	1959154 7e71f5162a0d765d9e1e0fd14e97f192725e5ac2	no	6
Warnings:					
Information:					
48	Non Patent Literature	Ng_MultiplexSequencingofPaired-end_2006.pdf	8111648 57f590fe4b6a81d5e60ce45aa48d52107acb1379	no	10
Warnings:					
Information:					
49	Non Patent Literature	Niemeyer_TheDevelopments_2002r.pdf	6624557 d4a346a1274db4df1d07baaa8bb3f8dd7f6d1b9f	no	7
Warnings:					
Information:					
50	Non Patent Literature	Nuovo_InSituPCRProtocols_1995.pdf	5353103 d8159daf0d35fbc12ab488bf5b72067903915d23	no	18
Warnings:					
Information:					

51	Non Patent Literature	OLINKPROTEOMICS_ProseekMultiplex.pdf	4141786 c1ac2d71b75ac8bc14567f2b8ed986df89056b2d	no	12
Warnings:					
Information:					
52	Non Patent Literature	Osada_EpitopeMapping_2009.pdf	185428 4442f40950a04843ab14155e735f8a2b4ee7dd2f	no	8
Warnings:					
Information:					
53	Non Patent Literature	Oleinikov_SelfAssemblingProte_2003r.pdf	5235482 0feea1ebbf498e2eac0f8278277d8e2e9d3bfc37	no	7
Warnings:					
Information:					
		Total Files Size (in bytes):	267230139		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT COOPERATION TREATY

ADVANCE E-MAIL

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)
(PCT Rule 44bis.1(c))

To:

DEVORE, Dianna, L.
Convergent Law Group LLP
475 N. Whisman Rd., # 400
Mountain View, CA 94043
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 10 November 2011 (10.11.2011)	
Applicant's or agent's file reference PGNS002PCT	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/033064	29 April 2010 (29.04.2010)	30 April 2009 (30.04.2009)

Applicant
PROGNOSYS BIOSCIENCES, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer
Simin Baharlou

Curio EX1010-p. 222
Curio v Prognosys
IPR2024-01289

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference PGNS002PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2010/033064	International filing date *(day/month/year)* 29 April 2010 (29.04.2010)	Priority date *(day/month/year)* 30 April 2009 (30.04.2009)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant PROGNOSYS BIOSCIENCES, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 01 November 2011 (01.11.2011)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Simin Baharlou e-mail: pt09.pct@wipo.int

Curio EX1010-p. 223
Curio v Prognosys
IPR2024-01289

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/033064	29.04.2010	30.04.2009

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68

Applicant
PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0	see form PCT/ISA/210	Bassias, Ioannis Telephone No. +49 89 2399-8196

Curio EX1010, p. 22
Curio v Prognosys
IPR2024-01289

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-29
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V:

1 The present application relates to nucleic acid contructs which comprise a region of interest (ROI), at least of unique identifier and at least one amplification site. Said contructs are used in methods for determining the sequence of nucleic acids, for identifying nucleic acids having a desired sequence and in methods for selecting nucleic acids having a desired sequence.

2 Reference is made to the following documents:

D1 WO 2007/073171 A2 (KEYGENE NV [NL]; VAN EIJK MICHAEL JOSEPHUS THER [NL]) 28 June 2007 (2007-06-28)

D2 WO 2007/076726 A1 (LOK SI [CN]) 12 July 2007 (2007-07-12)

3 The application is drafted in a very broad manner. The terms used, "region of interest", "unique identifier", etc. are very undefined and open for interpretation in various directions. Consequently, the claims need to be interpreted in the broadest possible sense. This approach leads to objections under Articles 33(2) and (3) in view of the teaching of D1 and/or D2.

3.1 D1 describes methods for transcript profiling using high throughput sequencing techniques where adaptors are used that can be used as a template for PCR oligonucleotide primers and for sequencing. The constructs can be immobilized on a solid support such as beads. The adaptors may comprise identifier sequences that can be unique and can vary in the length. Any nucleic acid to be analyzed in D1 represents the region of interest (ROI). The unique identifiers are not further characterized in the application. Hence, the unique identifiers of D1 fully fall into the scope of this expression. These unique identifiers can vary in the length. Consequently, any ROI can be attached to different identifiers and thus the concept of having the same ROI with different unique identifiers is fulfilled. The adaptors represent the amplification site since they are used as a template for PCR oligonucleotide primers and for sequencing. Restrictions sites within the constructs are mentioned. Sequencing of the constructs anticipates the method as described in claims 11-17. The determination of the sequence and further analysis for transcript profiling requires the identification of nucleic acids with desired sequences and also the selection thereof. Consequently, the subject-matter of all claims (1-29) is anticipated by the teaching of D1 (Article 33(2) PCT).

3.2 In D2 methods are described for nucleic acid mapping and identification of fine-structural variations in nucleic acids wherein so called "Genomic Variations Tags" (GVTs) are produced that bind to target nucleic acids. Said GVTs comprise one or more restriction endonucleases sites. They can be used as unique genomic positional identifiers. Different restriction endonucleases sites can be used in the GVTs which are tagged to the ROIs. Hence, the same ROI can have different unique identifiers. Amplification and sequencing via these tags is described. Immobilisation on solid support is not specifically mentioned in this prior art document. However, this is not considered something special which would not be within the ordinary skills of a person in the art.

Consequently, claims 1-6, 9-25, 28, and 29 are not in accordance with Article 33(2) PCT in view of D2. Claims 7, 8, 26, and 27 contravene the requirements of Article 33(3) PCT in view of the teaching of D2 combined with general knowledge.

4 Claim 28 lacks clarity (Article 6 PCT) apparently due to a wrong syntax.

5 Expressions like "incorporated herein/hereby by reference" used in the description (e.g. on pages 12 and 24) render the true scope of the application unclear and are thus not in accordance with Article 6 PCT.

The same applies for the use of expressions like "spirit of the invention" (see e.g. on pages 30, 42, 43).

PATENT COOPERATION TREATY

ADVANCE E-MAIL

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)
(PCT Rule 44bis.1(c))

To:

DEVORE, Dianna, L.
Convergent Law Group LLP
475 N. Whisman Road, Suite 400
Mountain View, CA 94043
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 09 February 2012 (09.02.2012)	
Applicant's or agent's file reference PGNS003PCT	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/044134	02 August 2010 (02.08.2010)	31 July 2009 (31.07.2009)

Applicant
PROGNOSYS BIOSCIENCES, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer
Agnès Wittmann-Regis

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference PGNS003PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2010/044134	International filing date *(day/month/year)* 02 August 2010 (02.08.2010)	Priority date *(day/month/year)* 31 July 2009 (31.07.2009)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant PROGNOSYS BIOSCIENCES, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 31 January 2012 (31.01.2012)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Agnès Wittmann-Regis e-mail: pt06.pct@wipo.int

Curio EX1010-p. 229
Curio v Prognosys
IPR2024-01289

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/044134	02.08.2010	31.07.2009

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/573 C12Q1/34 C12Q1/37 G01N33/543

Applicant
PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Tuynman, Antonin Telephone No. +31 70 340-3741

Curio EX1010-p. 28
Curio v Prognosys
IPR2024-01289

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 7-47

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 7-47

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-6

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-6
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6
Industrial applicability (IA)	Yes:	Claims	1-6
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item IV

Lack of unity of invention

The problem to be solved in the present application (PA) relates to the detection of biological and/or chemical activity in a sample. The single general concept which can be identified a priori as linking the various claimed inventions and which forms a solution to the above mentioned problem relates to "the notion that an assay tool comprising a set of two or more immobilised constructs comprising a releasable component and a capture surface, wherein release of the component allows for the detection of a positive signal"

However, US2006/003394 A (hereinafter referred to as D1, relevant passages as cited in the search report) and WO2007/060599 A (hereinafter referred to as D2, relevant passages as cited in the search report) disclose such a notion.

In the light of D1 and D2, each document if taken alone, the above identified single general concept is not novel and inventive and can thus not be the single general inventive concept as required by Rule 13.1 PCT. The present application is therefore considered not to fulfill the requirement of unity as laid down in Rule 13.1 PCT.

Consequently the groups of inventions are split up as follows:

1) An assay tool for detecting biological or chemical activity in a sample (claims 1-6)

2) An assay tool for detecting activity in a sample involving constructs with cleavage agent substrate region, affinity region and detectable marker (claims 7-9,19)

3) An assay tool for detecting cleavage agent activity in a sample involving constructs with cleavage agent substrate and detectable marker (claims 10-18,34)

4) An assay tool and method for detecting activity of a cleavage agent in a sample wherein the proximity of first and second surface allow for the detection of the positive signal (claims 20-33 and 41)

5) An assay tool for detecting activity of a cleavage agent in a sample, wherein association of a first released component of a combined detectable marker with a second component thereof on the capture surface creates a positive signal though interaction of first and second components of the combined detectable marker (claim 35)

6) An assay tool and methods for detecting competitive binding of a molecule in a sample (claims 36,37,42 and 43)

7) An assay tool for detecting activity of an enzyme (claims 38 and 39)

8) A method for detecting cleavage agent activity in a sample involving a surface with releasable component constructs and capture agents (claim 40)

9) A method for detecting a modulator of enzyme activity (claims 44-47)

No other technical features could be identified that form a technical relationship among each of the separate inventions claimed and which could be considered as special technical feature within the meaning of Rule 13.2 PCT. As no additional search fees have been paid upon invitation, the present opinion is limited to group of inventions 1 (claims 1-6).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 US 2006/003394 A1 (SONG XUEDONG [US]) 5 January 2006 (2006-01-05)

D2 WO 2007/060599 A1 (KONINKL PHILIPS ELECTRONICS NV [NL]; VOSSENAAR ERIK R [NL]; PRINS MENN) 31 May 2007 (2007-05-31)

1 The present application does not meet the criteria of Article 33(2) PCT, because the subject-matter of independent claim 1 is not new.

D1 discloses : An assay tool (Figure 3, claim 24, pars. 4-6,15,18,20,30,60 and 61) for detecting biological or chemical activity in a sample, comprising: a set of at least two different immobilized constructs (e.g. Figure 3 items 47 and 49: immobilised on particle 43), wherein the constructs comprises a releasable component (item 45, figure 3); and a capture surface (Figure 3, item 31); wherein the exposure of the immobilized constructs to a biological or chemical activity may result in release of a releasable component from one or more of the immobilized constructs, and wherein the interaction of a releasable component with the capture surface directly or indirectly enables the generation of a positive signal (claim 24).

2 The present application does not meet the criteria of Article 33(3) PCT, because the subject-matter of independent claim 1 does not involve an inventive step.

2.1 As claim 1 is not novel (see point 1) it cannot be inventive either.

2.2 Also in view of D2 alone claim 1 is not inventive: D2 discloses a similar assay tool for detecting biological or chemical activity in a sample as described in detail under point 1 (see D2, figures 5-10) which differs from presently claimed assay tool in that there is only one type of immobilised construct. The effect thereof is that the tool does not allow for multiplexing assays.

The problem to be solved by the present invention may therefore be regarded as how to provide an assay tool so as to allow multiplexing assays.

The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons: D2 already suggests that different labels can be used (par. 89) in the methods, systems and devices disclosed therein. If this solution is applied to the embodiments involving the label as part of the substrate to be cleaved as well as part of the product (pars. 60-67; figures 5-10), a tool is provided which falls within the scope of present claim 1.

3 Dependent claims 2-6 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, see D1 and/or D2 (relevant passages as cited in the search report). The incorporation of the features of claims 2-6 into independent claim 1 is either known or obvious from D1-D2 or falls within the knowledge and ability of a person skilled in the art.

Re Item VII

Certain defects in the international application

4 The phrase " incorporated by reference" on several pages of the description should be deleted. According to the PCT Guidelines, II, 4.26, the patent specification should be self contained i.e. capable of being understood without reference to any other document.

5 The statement which refers to the extent of protection being expanded to cover the spirit of the invention on several pages of the description should be deleted (PCT Guidelines II, 5.30).

Re Item VIII

Certain observations on the international application

6 The application does not meet the requirements of Article 6 PCT, because claim 1 is not clear.

6.1 The phrase "constructs comprises" is unclear as it is not clear whether it was intended that only one of the constructs comprises a releasable component or whether both comprise a releasable component.

6.2 Claim 2 is unclear as only the releasable component can bind to the capture surface and not the complete immobilised constructs comprising a releasable component as the claim presently suggests.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/044134	02.08.2010	31.07.2009

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/573 C12Q1/34 C12Q1/37 G01N33/543

Applicant
PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Tuynman, Antonin Telephone No. +31 70 340-3741

Curio EX1010-p. 28
Curio v Prognosys
IPR2024-01289

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 7-47

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 7-47

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-6

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-6
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6
Industrial applicability (IA)	Yes:	Claims	1-6
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item IV

Lack of unity of invention

The problem to be solved in the present application (PA) relates to the detection of biological and/or chemical activity in a sample. The single general concept which can be identified a priori as linking the various claimed inventions and which forms a solution to the above mentioned problem relates to "the notion that an assay tool comprising a set of two or more immobilised constructs comprising a releasable component and a capture surface, wherein release of the component allows for the detection of a positive signal"

However, US2006/003394 A (hereinafter referred to as D1, relevant passages as cited in the search report) and WO2007/060599 A (hereinafter referred to as D2, relevant passages as cited in the search report) disclose such a notion.

In the light of D1 and D2, each document if taken alone, the above identified single general concept is not novel and inventive and can thus not be the single general inventive concept as required by Rule 13.1 PCT. The present application is therefore considered not to fulfill the requirement of unity as laid down in Rule 13.1 PCT.

Consequently the groups of inventions are split up as follows:

1) An assay tool for detecting biological or chemical activity in a sample (claims 1-6)

2) An assay tool for detecting activity in a sample involving constructs with cleavage agent substrate region, affinity region and detectable marker (claims 7-9,19)

3) An assay tool for detecting cleavage agent activity in a sample involving constructs with cleavage agent substrate and detectable marker (claims 10-18,34)

4) An assay tool and method for detecting activity of a cleavage agent in a sample wherein the proximity of first and second surface allow for the detection of the positive signal (claims 20-33 and 41)

5) An assay tool for detecting activity of a cleavage agent in a sample, wherein association of a first released component of a combined detectable marker with a second component thereof on the capture surface creates a positive signal though interaction of first and second components of the combined detectable marker (claim 35)

6) An assay tool and methods for detecting competitive binding of a molecule in a sample (claims 36,37,42 and 43)

7) An assay tool for detecting activity of an enzyme (claims 38 and 39)

8) A method for detecting cleavage agent activity in a sample involving a surface with releasable component constructs and capture agents (claim 40)

9) A method for detecting a modulator of enzyme activity (claims 44-47)

No other technical features could be identified that form a technical relationship among each of the separate inventions claimed and which could be considered as special technical feature within the meaning of Rule 13.2 PCT. As no additional search fees have been paid upon invitation, the present opinion is limited to group of inventions 1 (claims 1-6).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 US 2006/003394 A1 (SONG XUEDONG [US]) 5 January 2006 (2006-01-05)

D2 WO 2007/060599 A1 (KONINKL PHILIPS ELECTRONICS NV [NL]; VOSSENAAR ERIK R [NL]; PRINS MENN) 31 May 2007 (2007-05-31)

1 The present application does not meet the criteria of Article 33(2) PCT, because the subject-matter of independent claim 1 is not new.

D1 discloses : An assay tool (Figure 3, claim 24, pars. 4-6,15,18,20,30,60 and 61) for detecting biological or chemical activity in a sample, comprising: a set of at least two different immobilized constructs (e.g. Figure 3 items 47 and 49: immobilised on particle 43), wherein the constructs comprises a releasable component (item 45, figure 3); and a capture surface (Figure 3, item 31); wherein the exposure of the immobilized constructs to a biological or chemical activity may result in release of a releasable component from one or more of the immobilized constructs, and wherein the interaction of a releasable component with the capture surface directly or indirectly enables the generation of a positive signal (claim 24).

2 The present application does not meet the criteria of Article 33(3) PCT, because the subject-matter of independent claim 1 does not involve an inventive step.

2.1 As claim 1 is not novel (see point 1) it cannot be inventive either.

2.2 Also in view of D2 alone claim 1 is not inventive: D2 discloses a similar assay tool for detecting biological or chemical activity in a sample as described in detail under point 1 (see D2, figures 5-10) which differs from presently claimed assay tool in that there is only one type of immobilised construct. The effect thereof is that the tool does not allow for multiplexing assays.

The problem to be solved by the present invention may therefore be regarded as how to provide an assay tool so as to allow multiplexing assays.

The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons: D2 already suggests that different labels can be used (par. 89) in the methods, systems and devices disclosed therein. If this solution is applied to the embodiments involving the label as part of the substrate to be cleaved as well as part of the product (pars. 60-67; figures 5-10), a tool is provided which falls within the scope of present claim 1.

3 Dependent claims 2-6 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, see D1 and/or D2 (relevant passages as cited in the search report). The incorporation of the features of claims 2-6 into independent claim 1 is either known or obvious from D1-D2 or falls within the knowledge and ability of a person skilled in the art.

Re Item VII

Certain defects in the international application

4 The phrase " incorporated by reference" on several pages of the description should be deleted. According to the PCT Guidelines, II, 4.26, the patent specification should be self contained i.e. capable of being understood without reference to any other document.

5 The statement which refers to the extent of protection being expanded to cover the spirit of the invention on several pages of the description should be deleted (PCT Guidelines II, 5.30).

Re Item VIII

Certain observations on the international application

6 The application does not meet the requirements of Article 6 PCT, because claim 1 is not clear.

6.1 The phrase "constructs comprises" is unclear as it is not clear whether it was intended that only one of the constructs comprises a releasable component or whether both comprise a releasable component.

6.2 Claim 2 is unclear as only the releasable component can bind to the capture surface and not the complete immobilised constructs comprising a releasable component as the claim presently suggests.

PATENT COOPERATION TREATY

ADVANCE E-MAIL

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)
(PCT Rule 44bis.1(c))

To:

DEVORE, Dianna, L.
Convergent Law Group LLP
475 N. Whisman Road, Suite 400
Mountain View, CA 94043
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 21 June 2012 (21.06.2012)	
Applicant's or agent's file reference PGNS005PCT	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/059327	07 December 2010 (07.12.2010)	07 December 2009 (07.12.2009)

Applicant
PROGNOSYS BIOSCIENCES, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer
Yolaine Cussac

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference PGNS005PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2010/059327	International filing date *(day/month/year)* 07 December 2010 (07.12.2010)	Priority date *(day/month/year)* 07 December 2009 (07.12.2009)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant PROGNOSYS BIOSCIENCES, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 12 June 2012 (12.06.2012)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Yolaine Cussac e-mail: pt05.pct@wipo.int

Curio EX1010-p. 249
Curio v Prognosys
IPR2024-01289

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DIANNA L. DEVORE
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	29 MAR 2011
Applicant's or agent's file reference PGNS005PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 10/59327	07 December 2010 (07.12.2010)	07 December 2009 (07.12.2009)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68; G01N 33/50 (2011.01)
USPC - 435/6, 435/7.1

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [X] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	14 March 2011 (14.03.2011)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-21,23,30	YES
	Claims	1,22,24-29,31-35	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-35	NO
Industrial applicability (IA)	Claims	1-35	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by US 2008/0312103 A1 to NEMOTO et al. (hereinafter "Nemoto").

As to claim 1, Nemoto teaches an array comprising a substrate having a surface;
two or more individual constructs associated on the surface of the substrate (para [0020]; "An mRNA chip comprising a plurality of the immobilized mRNA-puromycin conjugates described"), wherein the individual constructs comprise: an oligonucleotide encoding a peptide of interest (para [0020] mRNA); and the peptide of interest (para [0039]; "synthesizing a protein by adding the mRNA-PM conjugate immobilized on a solid phase to a translation system"); wherein the oligonucleotide is associated with the substrate surface (para [0008]; "a solid phase binding site for binding an mRNA-puromycin conjugate"; sheet 1 fig 1a, b), and the peptide is associated with the oligonucleotide (para [0039]; "synthesizing a protein by adding the mRNA-PM conjugate immobilized on a solid phase to a translation system. Namely, when a conjugate in which puromycin is ligated to mRNA through the linker is contacted with a translation system, an in vitro virus virion is known to be formed in which the mRNA is ligated with a translated protein through the puromycin"; sheet 1 fig 1a, b).

Claims 22, 24-29, 31-35 lack novelty under PCT Article 33(2) as being anticipated by US 2005/0260653 A1 (LABAER et al.) (hereinafter "LaBaer").

As to claim 22, LaBaer teaches a method for constructing an array (abstract; "Arrays of polypeptides can be generated by translation of nucleic acid sequences encoding the polypeptides at individual addresses on the array") comprising:
a) synthesizing a set of nucleic acid sequences of known sequence on a solid substrate (para [0005]; " In one aspect, the invention features a method that includes: disposing, on a substrate, one or more nucleic acids that include a coding region and an anchoring agent, maintaining the substrate under conditions which enable the anchoring agent of each disposed nucleic acid to stably attached to the substrate"; para [0015]; " In one embodiment, the coding region encodes a polypeptide that includes a first amino acid sequence, e.g., an amino acid sequence of interest").
b) attaching one or more additional nucleic acid regions to the nucleic acids on the solid substrate to provide extended nucleic acids on the substrate (para [0445-0446]; "[0445] Capture Probes. The substrate can include an attached nucleic acid capture probe at each address. In one aspect, capture probes can be used to create a self-assembling array. A unique capture probe at each address selectively hybridizes to a nucleic acid encoding a test amino acid sequence, thereby organizing each encoding nucleic acid to a unique address. The capture nucleic acid can be covalently attached or bound, e.g., to a polycationic surface on the substrate. [0446] The capture probe can itself be synthesized in situ, e.g. by a light-directed method ..., or by being spotted or disposed at the addresses. The capture probe can hybridize to the nucleic acid encoding the test polypeptide. In a preferred embodiment, the capture probe anneals to the T7 promoter region of a single stranded nucleic acid encoding the test amino acid sequence. In another embodiment, the capture probe is ligated to the encoding nucleic acid sequence")
c) performing in vitro transcription and translation utilizing the extended nucleic acids as a template for production of peptides (para [0458]; " The fusion proteins will be generated with commercially available in vitro transcription/translation kits such as the Promega TNT Coupled Reticulocyte Lysate Systems or TNT Coupled Wheat Germ Extract Systems"; para [0022]); and
d) attaching the produced peptides on known locations on the solid substrate (para [0015]; " In one embodiment, the coding region encodes a polypeptide that includes a first amino acid sequence, e.g., an amino acid sequence of interest, and an affinity tag. The affinity tag binds to a binding agent. The method can also include disposing the binding agent on the substrate. In some cases, it is useful to prepare a solution that includes the nucleic acid and the binding agent, and to dispose the solution onto the substrate"; sheet 1 fig 1).

----------continued in Supplemental Box----------

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
No valid CRF was filed in response to the ISA/225 of 10 December 2010, therefore no sequence information was available for the ISR.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V-2 (Citations and Explanations)

As to claim 24, LaBaer further teaches that the nucleic acids are chemically synthesized directly on the solid substrate (para [0446]; "The capture probe can itself be synthesized in situ, e.g., by a light-directed method").

As to claim 25, LaBaer further teaches that the additional nucleic acids in step b) are attached via enzymatic ligation (para [0446]; "In another embodiment, the capture probe is ligated to the encoding nucleic acid sequence").

As to claim 26, LaBaer further teaches that the set of nucleic acids comprises at least 1,000 nucleic acid sequences on the substrate (para [0367]; For example, a single array containing 10,000 arrayed elements, for example, can be sufficient to yield 10,000 results (e.g., quantitative results)").

As to claim 27, LaBaer further teaches that the set of nucleic acids comprises at least 5,000 nucleic acid sequences on the substrate (para [0367]).

As to claim 28, LaBaer further teaches that the set of nucleic acids comprises at least 10,000 nucleic acid sequences on the substrate (para [0367]).

As to claim 29, LaBaer teaches a method for constructing a peptide array (abstract), comprising:
a) manufacturing a nucleic acid microarray comprising a set of chemicall synthesized oligonucleotides encoding amino acid sequences (para [0005], [0015]);
b) attaching one or more additional nucleic acid regions to the nucleic acids on the solid substrate to provide extended nucleic acids on the substrate (para [0446]);
c) performing in vitro transcription and translation utilizing the extended nucleic acids as a template for production of peptides (para [0458], [0022]) ; and
d) attaching the produced peptides on the solid substrate (para [0015]; sheet 1 fig 1).

As to claim 31, LaBaer further teaches that the nucleic acids are chemically synthesized directly on the solid substrate (para [0446]; "The capture probe can itself be synthesized in situ, e.g., by a light-directed method").

As to claim 32, LaBaer further teaches that the additional nucleic acids in step b) are attached via enzymatic ligation (para [0446]; "In another embodiment, the capture probe is ligated to the encoding nucleic acid sequence").

As to claim 33, LaBaer further teaches that the set of nucleic acids comprises at least 1,000 nucleic acid sequences on the substrate (para [0367]; For example, a single array containing 10,000 arrayed elements, for example, can be sufficient to yield 10,000 results (e.g., quantitative results)").

As to claim 34, LaBaer further teaches that the set of nucleic acids comprises at least 5,000 nucleic acid sequences on the substrate (para [0367]).

As to claim 35, LaBaer further teaches that the set of nucleic acids comprises at least 10,000 nucleic acid sequences on the substrate (para [0367]).

Claims 2-10, 12-15, 23,30 lack an inventive step under PCT Article 33(3) as being obvious over US 2005/0260653 A1 (LABAER et al.) (hereinafter "LaBaer") in view of the publication titled " The developments of semisynthetic DNA.protein conjugates" By NIEMEYER et al. (hereinafter "Niemeyer").

As to claim 2, LaBaer teaches a substrate having a surface; two or more individual constructs associated on the surface of the substrate, wherein the individual constructs comprise: an oligonucleotide comprising a region encoding a peptide of interest, a region encoding an affinity tag and an untranslated region (para [0005]; " In one aspect, the invention features a method that includes: disposing, on a substrate, one or more nucleic acids that include a coding region and an anchoring agent, maintaining the substrate under conditions which enable the anchoring agent of each disposed nucleic acid to stably attached to the substrate"; para [0015]; " In one embodiment, the coding region encodes a polypeptide that includes a first amino acid sequence, e.g., an amino acid sequence of interest, and an affinity tag. The affinity tag binds to a binding agent"; para [0056]; "The nucleic acid can further include one or more of: a transcription promoter; a transcription regulatory sequence; a untranslated leader sequence; a sequence encoding a cleavage site; a recombination site; a 3' untranslated sequence") the peptide of interest and an affinity tag (para [0022]; " In one embodiment, the step of contacting the substrate with a translation effector includes disposing or flowing the translation effector onto the surface, for example, using a single dispensing action or multiple dispensing actions. In one embodiment, the substrate is also contacted with a transcription effector"); and a capture agent that selectively binds to the affinity tag (para [0046]; " In another aspect, the invention features a method that includes: contemporaneously providing (e.g., depositing)----------continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
previous Supplemental Box

(claim 2 continued): (i) a binding agent that can interact with a tag and (ii) a nucleic acid that can be stably attached to a substrate and that includes a sequence encoding a first amino acid sequence and the tag onto a substrate"; sheet 1 fig 1) wherein the oligonucleotide is associated with the substrate surface (para [0005]). LaBaer does not teach that the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Consequently it would have been obvious to one of ordinary skill in the art to combine an oligonucleotide comprising a region encoding a peptide of interest, a region encoding an affinity tag and an untranslated region, as taught by LaBaer, with the peptide of interest and an affinity tag, again as taught by LaBaer, with a capture agent that selectively binds to the affinity tag, again as taught by LaBaer, with the oligonucleotide is associated with the substrate surface, again as taught by LaBaer, with the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent, as taught by Niemeyer, because it would have enabled construction of a oligonucleotide-peptide microarray.

As to claim 3, LaBaer further teaches that the untranslated region comprises a transcriptional start site and a ribosomal binding site (para [0115]; " The nucleic acid can further include one or more of: a transcription promoter; a transcription regulatory sequence; a untranslated leader sequence; a transcriptional terminator; and an internal ribosome entry site").

As to claim 4, Niemeyer further teaches that the capture agent is associated with the oligonucleotide (pg 400 fig 6).

As to claim 5, LaBaer teaches that the oligonucleotide is associated with the substrate surface via a linker (para [0040]; " In another aspect, the invention features a method that includes: providing a substrate that includes a plurality of addresses, each addresses including a nucleic acid that includes a coding region and an anchoring agent that stably attaches the nucleic acid to the substrate").

As to claim 6, LaBaer further teaches that the capture agent is associated with the linker (para [0129]; " "The binding agent can be attached to the substrate. For example, the substrate can be derivatized and the binding agent covalent attached thereto").

As to claim 7, LaBaer further teaches that the substrate is a planar support (para [0008]; " The substrate can be planar, e.g., have a horizontal plane in which the addresses are located at different discrete locations. The surface of the substrate can be flat (e.g., a glass slide) ").

As to claim 8, LaBaer further teaches that the substrate is a bead (para [0088]; " In yet another embodiment, an insoluble substrate (e.g., a bead or particle), is disposed at each address of the plurality, and the binding agent is attached to the insoluble substrate").

As to claim 9, LaBaer further teaches that the oligonucleotide is double-stranded (para [0055]; "The nucleic acid can be a RNA, or a DNA (e.g., a single-stranded DNA, or a double stranded DNA").

As to claim 10, Niemeyer further teaches that the oligonucleotide further comprises a region used for introduction of the capture agent to the oligonucleotide (pg 400 fig 6; A microarray of capture oligonucleotides is used as the immobilization matrix. Note that owing to the specificity of Watson.Crick base pairing, many different compounds can be sites pecifically immobilized simultaneously in a single step").

As to claim 12, LaBaer teaches a method of constructing an array (abstract), comprising the steps of:
(a) providing a surface comprising two or more constructs, the constructs
comprising a single-stranded oligonucleotide region encoding a peptide of interest
and an affinity tag (para [0005], [0015]; [0055]-" (para [0055]; "The nucleic acid can be a RNA, or a DNA (e.g., a single-stranded DNA, or a double stranded DNA");
(d) subjecting the double-stranded oligonucleotide region to a transcription
and translation event to produce a peptide comprising the peptide of interest and
the affinity tag (para [0458]; [0022]) wherein the protein produced in step (d) is captured by the capture agent of the construct following translation, thereby forming individual constructs
comprising the peptide associated with a substrate encoding the peptide (sheet 1 fig 1; para [0015]). LaBaer does not teach that the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). In addition, LaBaer does not teach (b) converting the single-stranded oligonucleotide region into a double stranded oligonucleotide region.----------claim continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
previous Supplemental Box

(continuation of claim 12)----However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation. Furthermore, LaBaer does not teach (c); introducing an untranslated region comprising a ribosomal start site to the 5' end of the double-stranded oligonucleotide region, wherein the untranslated region is associated with a capture agent with the ability to form a binding pair with the affinity tag. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (b) and (d), as taught by LaBaer, with claim limitation (c), as taught by LaBaer in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a microarray by way of a two oligonucleotide ligation at each addressable position of the microarray, with oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent, as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 13, LaBaer teaches a method of constructing an array (abstract), comprising the steps of:
(a) providing a substrate comprising a surface with two or more constructs associated on the surface , the constructs comprising a single-stranded oligonucleotide encoding a peptide of interest and an affinity tag (para [0005], [0015], [0055]);
(e) subjecting the double-stranded oligonucleotide to a transcription and translation event to produce a peptide from the double-stranded oligonucleotide; wherein the protein produced in step (e) is captured by the capture agent of the construct; and wherein the resulting array comprises discrete units comprising a nucleic acid associated with substrate and the peptide encoded by the nucleotide also associated with the substrate through a capture agent (sheet 1 fig 1; para [0015]). LaBaer does not specifically teach the peptide encoded by the nucleic acid is associated by the nucleic acid. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). In addition, LaBaer does not teach (d) converting the single-stranded oligonucleotide region into a double stranded oligonucleotide region. However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation (i.e. claim limitation (e)). Furthermore, LaBaer does not teach (b) ligating a second, universal oligonucleotide to the oligonucleotides of the constructs, wherein the universal oligonucleotide comprises an untranslated region comprising a ribosomal binding site. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6), thus fulfilling claim limitation (c). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (d) and (e), as taught by LaBaer, with claim limitation (b), as taught by LaBaer, in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a two oligonucleotide ligation of a microarray, with claim limitation (c), as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 14, LaBaer teaches method of constructing an array (abstract), comprising the steps of:
(a) providing a surface comprising two or more constructs, the constructs comprising a single-stranded oligonucleotide region (para [0055]) having a sequence that is the sequence encoding a peptide of interest and an affinity tag (para [0005], [0015]); LaBaer does not teach the single stranded nucleotide is a reverse complement sequence encoding the peptide of interest. However, an artisan of ordinary skill in the art would have readily appreciated that a reverse complement sequence could have readily hybridized with a mRNA molecule and could have utilized it to hybridize with mRNA introduced to the microRNA. Furthermore, LaBaer does not teach (b) converting the single-stranded oligonucleotide region into a double stranded oligonucleotide region. However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation (i.e. claim limitation (d)). LaBaer further teaches (d) subjecting the double-stranded oligonucleotide region to a transcription and translation event to produce a peptide comprising the peptide of interest and the affinity tag wherein the protein produced in step (d) is captured by the capture agent of the construct following translation, thereby forming individual constructs comprising the peptide associated with the substrate and an oligonucleotide encoding the peptide associated with the substrate. LaBaer does not specifically teach the peptide encoded by the nucleic acid is associated with the nucleic acid. ----------claim continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
previous Supplemental Box

(continuation of claim 14)----------However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1).
LaBaer does not teach (c); introducing an untranslated region comprising a ribosomal start site to the 5' end of the double-stranded oligonucleotide region, wherein the untranslated region is associated with a capture agent with the ability to form a binding pair with the affinity tag. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (b) and (d), as taught by LaBaer, in view of the observations of an artisan of ordinary skill, with claim limitation (c), as taught by LaBaer, in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a microarray by way of a two oligonucleotide ligation step of the microarray, with attachment of a peptide of interest to an oligonucleotide encoding the peptide via a capture agent, as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 15, LaBaer teaches a method of constructing an array (abstract), comprising the steps of: (a) providing a surface comprising two or more constructs, the constructs
comprising a single-stranded oligonucleotide region (para [0055]) having a sequence that is the
sequence encoding a peptide of interest and an affinity tag (para [0005], [0015]); LaBaer does not teach the single stranded nucleotide is a reverse complement sequence encoding the peptide of interest. However, an artisan of ordinary skill in the art would have readily appreciated that a reverse complement sequence could have readily hybridized with a mRNA molecule and could have utilized it to hybridize with mRNA introduced to the microRNA. LaBaer further does not teach (d) converting the single-stranded oligonucleotide regions of the constructs into double-stranded oligonucleotide regions. However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation (i.e. claim limitation (e)). LaBaer further teaches (e) subjecting the double-stranded oligonucleotide region to a transcription and translation event to produce a peptide comprising the peptide of interest and the affinity tag wherein the protein produced in step (e) is captured by the capture agent of the construct following translation, thereby forming individual constructs comprising the peptide associated with the substrate and an oligonucleotide encoding the peptide associated with the substrate. LaBaer does not specifically teach the peptide encoded by the nucleic acid is associated by the nucleic acid. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Furthermore, LaBaer does not teach (b) ligating a second, universal oligonucleotide to the oligonucleotides of the constructs, wherein the universal oligonucleotide comprises an untranslated region comprising a ribosomal binding site. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6), thus fulfilling claim limitation (c). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (d) and (e), as taught by LaBaer, with claim limitation (b), as taught by LaBaer, in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a two oligonucleotide ligation of a microarray, with claim limitation (c), as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 23 and 30, LaBaer teaches that the produced peptides are bound to the substrate (para [0015], sheet 1 fig 1). LaBaer does not teach that the produced peptides are attached to their respective nucleic acid substrates. However, Niemeyer teaches that the produced peptides are attached to their respective nucleic acid substrates (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Consequently it would have been obvious to one of ordinary skill in the art to combine peptides produced by in vitro transcription/translation of an array, as taught by LaBaer, with the produced peptides are attached to their respective nucleic acid substrates, as taught by Niemeyer, because it would have enabled construction of a protein array where the produced peptides are at discrete spatially addressable locations on the array.
----------continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
previous Supplemental Box

Claim 11 lacks an inventive step under PCT Article 33(3) as being obvious over LaBaer, in view of Niemeyer, in further view of the publication titled " High sensitivity multianalyte immunoassay using covalent DNA-labeled antibodies and polymerase chain reaction" by HENDERSON et al. (hereinafter "Henderson").

As to claim 11, Niemeyer teaches association of capture agent with substrate attached oligonucleotide by DNA annealing (pg 400 fig 6). Neither Niemeyer nor LaBaer teaches that the region is associated to the oligonucleotide via a linker molecule. However, Henderson teaches a region is associated to the oligonucleotide via a linker molecule (pg 523 fig 2; "Design of DNA-antibody reporter conjugates--chemical crosslinker). An artisan of ordinary skill in the art would have readily appreciated that a linker region was well known in the art for covalently linking DNA directly to a protein molecule such as an antibody (i.e. capture agent). Consequently, it would have been obvious to one of ordinary skill in the art to combine association of capture agent with substrate attached oligonucleotide, as taught by Niemeyer, with region of association is a linker, as taught by Henderson, because it would have enabled attaching the capture agent directly to the oligonucleotide.

Claims 16-21 lack an inventive step under PCT Article 33(3) as being obvious over LaBaer, in view of Niemeyer, in further view of the publication titled " Next-generation high-density self-assembling functional protein arrays" by RAMACHANDRAN et al. (hereinafter "Ramachandran").

As to claim 16, LaBaer teaches a method of identifying an agent that binds to a peptide of interest, comprising: providing an array (abstract) comprising:
a substrate having a surface (para [0005]);
two or more individual constructs associated on the surface of the substrate, wherein the individual constructs comprise:
(i) an oligonucleotide encoding a peptide of interest (para [0005], [0015]) and
(ii) the peptide of interest (para [0022]; wherein the oligonucleotide is associated with the substrate surface (para [0005]). LaBaer does not teach that the peptide is associated with the oligonucleotide. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Furthermore, LaBaer does not specifically teach exposing the array to an agent; and detecting the presence or absence of binding of the agent to the peptide of interest. However, Ramachandran teaches exposing the array to an agent; and detecting the presence or absence of binding of the agent to the peptide of interest (pg 537 left col para 1; " To confirm protein function on high-density arrays, we printed an array expressing 647 unique genes in duplicate, including 449 genes that we had not previously tested (Fig. 3a and Supplementary Table 3 online). We tested for binary interactions between several well characterized interacting pairs including Jun-Fos and p53-MDM2 (Fig. 3b). We co-expressed the query protein along with the arrayed proteins by adding the appropriate cDNA clone (without a GST tag) to the cell-free expression lysate. After protein expression and washing, we treated the arrays with protein-specific antibodies to detect the query protein, revealing the positions where it bound. Using Jun, Fos and MDM2 as queries, we detected selective binding to their appropriate interacting partners"; pg 538 fig 3B). An artisan of ordinary skill in the art would have readily appreciated that binding of a protein through protein-protein interaction was well known in the art at the time of the invention. Consequently, it would have been obvious to one of ordinary skill in the art to combine an oligonucleotide bound to a substrate encoding a protein of interest, as taught by LaBaer, with a peptide of interest associated with an array, also taught by LaBaer, with peptide is associated with the oligonucleotide, as taught by Niemeyer, with detecting the presence or absence of binding of an agent to the peptide of interest, as taught by Ramachandran, because it would have enabled a functional protein biochip that identifies an agent that binds to a peptide of interest.

As to claim 17, Ramachandran further teaches the agent is provided in a sample, and wherein presence of binding to the peptide of interest indicates the presence of the agent in a sample (pg 537 left col para 1; pg 538 fig 3B).

As to claim 18, Ramachandran further teaches that the agent is provided in a sample, and wherein absence of binding to the peptide of interest indicates an absence of the agent in a sample (pg 537 left col para 1; pg 538 fig 3B).
----------continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
previous Supplemental Box

As to claim 19, LaBaer teaches a method of identifying an agent that binds to a peptide of interest, comprising:
providing an array (abstract) comprising:
a substrate having a surface (para [0005]); two or more individual constructs associated on the surface of the substrate, wherein the individual constructs comprise:
(i) an oligonucleotide comprising a region encoding a peptide of interest, a region encoding an affinity tag, and an untranslated region (para [0005], [0015], [0056])
(ii) a peptide comprising the peptide of interest and an affinity tag (para [0022]); and
(iii) a capture agent that selectively binds to the affinity tag (para [0046]); wherein the oligonucleotide is associated with the substrate surface (para [0005]). LaBaer does not teach that the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Furthermore, LaBaer does not teach exposing the array to an agent; and detecting the presence or absence of binding of the agent to the peptide of interest. However, Ramachandran teaches exposing the array to an agent (pg 537 left col para 1); and detecting the presence or absence of binding of the agent to the peptide of interest (pg 537 left col para 1, pg 538 fig 3B). An artisan of ordinary skill in the art would have readily appreciated that binding of a protein through protein-protein interaction was well known in the art at the time of the invention. Consequently, it would have been obvious to one of ordinary skill in the art to combine an oligonucleotide bound to a substrate encoding a protein of interest, as taught by LaBaer, with a peptide of interest associated with an array, also taught by LaBaer, with capture agent that selectively binds to affinity tag, also as taught by LaBaer, with peptide is associated with the oligonucleotide, as taught by Niemeyer, with detecting the presence or absence of binding of an agent to the peptide of interest, as taught by Ramachandran, because it would have enabled a functional protein biochip that identifies an agent that binds to a peptide of interest.

As to claim 20, Ramachandran further teaches that the agent is provided in a sample (pg 537 left col para 1), and wherein the presence of binding to the peptide of interest indicates the presence of the agent in a sample (pg 538 fig 3B).

As to claim 21, Ramachandran the agent is provided in a sample (pg 537 left col para 1), and wherein absence of binding to the peptide of interest indicates an absence of the agent in a sample (pg 538 fig 3B).

Claims 1-35 have industrial applicability as defined in PCT Article 33(4) because the subject matter can be made or used by industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference PGNS008PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2011/031163	International filing date *(day/month/year)* 05 April 2011 (05.04.2011)	Priority date *(day/month/year)* 05 April 2010 (05.04.2010)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant PROGNOSYS BIOSCIENCES, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 09 October 2012 (09.10.2012)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Simin Baharlou e-mail: pt09.pct@wipo.int

Curio EX1010-p. 260
Curio v Prognosys
IPR2024-01289

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SARAH BRASHEARS
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	23 MAY 2011
Applicant's or agent's file reference PGNS008PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/31163	05 April 2011 (05.04.2011)	05 April 2010 (05.04.2010)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6, 287.2, 7.1, 91.2

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	16 May 2011 (16.05.2011)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-37	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-37	NO
Industrial applicability (IA)	Claims	1-37	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-12 and 16-29 lack an inventive step under PCT Article 33(3) as being obvious over US 2006/0134669 A1 (Casasanta), in view of US 2003/0224419 A1 to Corcoran et al. (hereinafter "Corcoran").

As to claim 1, Casasanta teaches a method for identifying binding agents that form a binding pair, comprising:
a) providing a first set of binding constructs immobilized on a support surface, wherein each binding construct of the first set of binding constructs comprises a first binding agent and a first nucleic acid tag unique to the first binding agent (para [0014]...first component of the binding pair is immobilized on a spot of the microarray and the second component is bound to a protein...binding pairs can include...complementary strands of DNA or RNA);
b) providing a second set of binding constructs in solution, wherein each binding construct of the second set of binding constructs comprises a second binding agent and a second nucleic acid tag unique to the second binding agent, and wherein either or both of the first and second sets of binding constructs comprises at least ten different binding agents (para [0014]...second component of the binding pair is introduced to the microarray by...solution flooding);
c) combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, thereby co-locating the first nucleic acid tags and the second nucleic acid tags (para [0014]...complementary strands of DNA or RNA are bound together, therefore are co-located);
d) creating binding event identifiers from the co-located first and second nucleic acid tags (para [0012]...spectral shifts can be measured for respective molecular binding events).

Casasanta does not specify what Corcoran does in an alternative ligation-based sequencing scheme (para [0022]), namely:
e) determining a sequence of each binding event identifier, wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair (para [0002]...massively parallel signature sequencing).

Neither Casasanta nor Corcoran specify at least ten different binding agents. Casasanta does teach testing a variety of binding pair types (para [0014]...antigen/antibody, peptide/peptide, complementary DNA/RNA), discriminating between different spots (para [0018]) and testing up to 1000 spots (para [0013]). It would have been obvious to one skilled in the art to use at least ten different binding agents, for greater economy of testing scale. Further, it would have been obvious to one skilled in the art to combine the binding pair sequence of Corcoran with the method of Casasanta to enable more specific detection of binding events between a variety of biological molecules.

As to claim 2, Corcoran further teaches wherein the sequence of the binding event identifier is determined by digital readout 36, 38 (Fig 3...display controller, display device).

As to claim 3, Corcoran further teaches wherein the sequence of the binding event identifier is determined by high throughput digital sequencing (para [0022]...massively parallel signature sequencing...identify simultaneously signatures of very large numbers of DNAs attached to microbeads disposed in closely packed arrays).

As to claims 4-7, Casasanta and Corcoran make obvious the method of claim 1. Neither specifies wherein either or both of the first and second sets of binding constructs comprises at least twenty-five, one hundred, one thousand or at least five thousand different binding agents, respectively. Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches each binding pair has a unique signature (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound.

As to claims 8, Corcoran further teaches wherein the sequences of the binding event identifiers are determined in parallel (para [0002]...massively parallel signature sequencing)

As to claims 9-10, Casasanta and Corcoran make obvious the method of claim 8. Casasanta teaches microarrays having 1008 spots (para [0013]). Corcoran teaches identifying in parallel (para [0002]) sequences of binding pairs on a microarray (para [0002]; [0083]...microbead array display). Neither specifies the sequence of 100,000 binding events determined in parallel. However, it would have been obvious to one skilled in the art that the technique of Corcoran could be applied to microarrays of any dimension.

Please continue to the supplemental page

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Citations and Explanations, Box V(2):

As to claim 11, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches wherein the binding event identifier is created by coupling the first and second nucleic acid tags (Fig 5...note the sticky ends and the two ligation steps).

As to claim 12, Casasanta and Corcoran make obvious the method of claim 10. Corcoran further teaches wherein the coupling of the first and second tags is accomplished by ligation (Fig 5...note the sticky ends and the two ligation steps).

As to claim 16, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches wherein at least one of the first and second binding agents is a peptide (para [0006]...peptide-peptide pair).

As to claim 17, Casasanta and Corcoran make obvious the method of claim 16. Casasanta further teaches wherein the first and second binding agents are peptides (para [0006]...peptide-peptide pair).

As to claim 18, Casasanta and Corcoran make obvious the method of claim 16. Casasanta further teaches wherein the second binding agent is an antibody (para [0006]...hapten-antibody pair, antigen-antibody pair).

As to claim 19, Casasanta and Corcoran make obvious the method of claim 16, wherein the second binding agent is a small molecule (para [0006]...peptides can be small molecules; avidin and biotin are small molecules).

As to claim 20, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches wherein the first or second binding agent is an aptamer (para [0014]...biotin, antigen, antibody, oligonucleotides are all aptamers).

As to claim 21, Casasanta and Corcoran make obvious the method of claim 20. Casasanta further teaches wherein the first binding agent is a peptide and the second binding agent is an aptamer (para [0014]...first component immobilized on a spot in the microarray...second component introduced by solution flooding...combinations can include antigen/antibody, peptide/peptide, or complementary strands of DNA or RNA...all of these potential components of the binding pair can function as aptamers).

As to claim 22, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches wherein the support is a microarray 8 (para [0012]).

As to claim 23, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches wherein the support is a bead (Fig 5...see 'image microbeads').

As to claim 24, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches the step of adding a third binding agent in the combining step (para [0014]...third chemical component can bind the complex of the first component and the second component).

As to claim 25, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches the step of identifying binding agents that bind promiscuously (para [0096]...optical measurements were adjusted for system background noise, which can result from nonspecific binding of probes...or incomplete ligation).

As to claim 26, Casasanta and Corcoran make obvious the method of claim 25. Corcoran further teaches wherein data from promiscuous binding agents is subtracted from binder identifier results of the determining step (para [0096]...optical measurements were adjusted for system background noise, which can result from nonspecific binding of probes...or incomplete ligation).

As to claim 27, Casasanta and Corcoran make obvious the method of claim 25. Corcoran further teaches wherein a quantitative metric can be derived for the extent of promiscuity of promiscuous binding agents (para [0096]...computing the background noise for each signal set...and subtracting that computed value from each of the four fluorescence values...).

As to claim 28. Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches wherein false positives are identified within the binding event identifiers and data from the false positives subtracted from binder identifier results of the determining step (para [0096]...computing the background noise for each signal set...and subtracting that computed value from each of the four fluorescence values...).

As to claim 29, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches the step of determining the number of each binding event identifier sequenced (para [0021]...counting of tags or signatures of DNA fragments).

Claims 13-15 and 30-37 lack an inventive step under PCT Article 33(3) as being obvious over Casasanta, in view of Corcoran and further in view of US 7,378,242 B2 (Hurt).

As to claim 13, Casasanta and Corcoran make obvious the method of claim 10. Neither specifies wherein the coupling of the first and second tags is accomplished by primer extension. Hurt teaches coupling strands of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control the nucleotide sequence.

Please continue

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Citations and Explanations, Box V(2):

As to claim 14, Casasanta and Corcoran make obvious the method of claim 1. Neither teaches wherein one or both of the first and second binding constructs comprise a primer sequence. Hurt teaches binding two pieces of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control the nucleotide sequence.

As to claim 15, Casasanta, Corcoran and Hurt make obvious the method of claim 13. Hurt further teaches the step of amplifying the binding event identifier after the creating step and before the determining step (col 6, ln 55-60...multiple temperature cycles can be used to amplify the extension product...different temperatures can be used for the three steps: association of the primer to the template, extension, and separation of the LPE product from the template).

As to claim 30, Casasanta teaches a method for identifying binding agents that form a binding pair, comprising:

a) providing a first set of binding constructs immobilized on a support surface, wherein each binding construct of the first set of binding constructs comprises a first binding agent, and a first nucleic acid tag unique to the first binding agent (para [0014]...first component of the binding pair is immobilized on a spot of the microarray and the second component is bound to a protein...binding pairs can include...complementary strands of DNA or RNA);
b) providing a second set of binding constructs in solution, wherein each binding construct of the second set of binding constructs comprises a second binding agent, and a second nucleic acid tag unique to the second binding agent, and wherein either or both of the first and second sets of binding constructs comprises at least ten different binding agents (para [0014]...second component of the binding pair is introduced to the microarray by...solution flooding);
c) combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, thereby co-locating the first nucleic acid tags and the second nucleic acid tags (para [0014]...complementary strands of DNA or RNA are bound together, therefore are co-located);
d) creating binding event identifiers from the co-located first and second nucleic acid tags (para [0012]...spectral shifts can be measured for respective molecular binding events).

Casasanta does not specify:
wherein the first set of binding constructs also comprises a first primer region,
wherein the second set of binding constructs comprises a second primer region, or
e) determining a sequence of at least one thousand binding event identifiers, wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair; and
f) determining the frequency of each binding event identifier sequenced.

Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches each binding pair has a unique signature (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound. Corcoran further teaches the step of determining the number of each binding event identifier sequenced (para [0021]...counting of tags or signatures of DNA fragments). It would have been obvious to one skilled in the art to determine the frequency of each binding event identifier sequences, to enable analysis of broader trends in binding events.

Hurt teaches coupling strands of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control the nucleotide sequence.

As to claim 31, Casasanta, Corcoran and Hurt make obvious the method of claim 30. Corcoran further teaches wherein the sequences of the binding event identifiers are determined in parallel (para [0002]...massively parallel signature sequencing).

As to claims 32-33, Casasanta, Corcoran, and Hurt make obvious the method of claim 31. None specifies wherein the sequence of at least 1,000 or 100,000 binding event identifiers, respectively, are determined in parallel. Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches determining the sequences of the binding event identifiers in parallel (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound.

Please continue

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Citations and Explanations, Box V(2):

As to claim 34, Casasanta teaches a method for characterizing the specificity of binding between binding agents that form a binding pair, comprising:
a) providing a first set of binding constructs immobilized on a support surface, wherein each binding construct of the first set of binding constructs comprises a first binding agent, and a first nucleic acid tag unique to the first binding agent (para [0014]...first component of the binding pair is immobilized on a spot of the microarray and the second component is bound to a protein...binding pairs can include...complementary strands of DNA or RNA);
b) providing a second set of binding constructs in solution, wherein each binding construct of the second set of binding constructs comprises a second binding agent, and a second nucleic acid tag unique to the second binding agent, and wherein either or both of the first and second sets of binding constructs comprises at least ten different binding agents (para [0014]...second component of the binding pair is introduced to the microarray by...solution flooding);
c) combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, thereby co-locating the first nucleic acid tags and the second nucleic acid tags (para [0014]...complementary strands of DNA or RNA are bound together, therefore are co-located);
d) creating binding event identifiers from the co-located first and second nucleic acid tags (para [0012]...spectral shifts can be measured for respective molecular binding events).

Casasanta does not specify what Corcoran does in an alternative ligation-based sequencing scheme (para [0022]), namely:
e) determining a sequence of each binding event identifier, wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair (para [0002]...massively parallel signature sequencing). It would have been obvious to one skilled in the art to determine the frequency of each binding event identifier sequences, to enable analysis of broader trends in binding events.

Neither Casasanta nor Corcoran specifies:
wherein the first set of binding constructs also comprises a first primer region,
wherein the second set of binding constructs comprises a second primer region. Hurt teaches coupling strands of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control and track the nucleotide sequence.

As to claims 35-36, Casasanta, Corcoran and Hurt make obvious the method of claim 34, but do not specify wherein the sequence of at least 1,000 or 100,000 binding event identifiers, respectively, are determined in parallel. Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches determining the sequences of the binding event identifiers in parallel (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound.

As to claim 37, Casasanta, Corcoran and Hurt make obvious the method of claim 34. Hurt further teaches the step of amplifying the binding event identifier after the creating step and before the determining step (col 6, ln 55-60...multiple temperature cycles can be used to amplify the extension product...different temperatures can be used for the three steps: association of the primer to the template, extension, and separation of the LPE product from the template).

Claims 1-37 have industrial applicability as defined by PCT Article 33(4) because the subject can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference PGNS007PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2011/031308	International filing date *(day/month/year)* 05 April 2011 (05.04.2011)	Priority date *(day/month/year)* 05 April 2010 (05.04.2010)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant PROGNOSYS BIOSCIENCES, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 09 October 2012 (09.10.2012)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Lingfei Bai e-mail: pt02.pct@wipo.int

Curio EX1010-p. 267
Curio v Prognosys
IPR2024-01289

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SARAH BRASHEARS
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	07 JUN 2011
Applicant's or agent's file reference: PGNS007PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/31308	05 April 2011 (05.04.2011)	05 April 2010 (05.04.2010)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	25 May 2011 (25.05.2011)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Curio EX1010-p. 268
Curio v Prognosys
IPR2024-01289

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	4, 5, 38, 56	YES
	Claims	1-3, 6-37, 39-55, 57	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-57	NO
Industrial applicability (IA)	Claims	1-57	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-3, 6-37, 39-55 and 57 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0046313 A1, Roth et al., (hereinafter Roth).

Regarding claim 1, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033] - 'The invention reverse phase protein assay includes forming one or more arrays of cell lysates obtained from one or more tissue samples from a patient. For example, individual cells of a cell population obtained from a patient tissue sample may be lysed and extracted 310 to form an array. If cell lysates are obtained from more than one tissue sample, two or more arrays may be formed, with an array containing the cell lysates from a particular tissue sample of the patient In an array of defined locations 320 on a substrate, a mixture of extracted cell proteins 330, 331, 332 is immobilized at (for example, spotted onto) individual defined locations,');
delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered (para [0033] - 'Then the cell lysate spots at the defined locations are contacted with a mixture of COIN-labeled probes 330, 332 under conditions suitable to form analyte-COIN complexes 341, 342, 343 as the probes bind individual protein in the spots through both specific and degenerate binding.', and para [0097]);
allowing the encoded probes to interact with the biological targets (para [0033]);
separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets (para [0033] - 'After removal of unbound COIN-labeled probes from the array');
determining all or a portion of a sequence of the encoded probes (para [0104] and [0122]), and
associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample (para [0033] - 'the array 350 may be scanned 340 to detect SERS signals at the defined locations 320 so as to identify and compile data regarding the presence of the known analytes in the tissue sample.').

Regarding claim 27, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033]);
delivering oligonucleotide probes for multiple nucleic acid targets to the multiple sites in the sample in a known spatial pattern (para [0033] and [0097]);
allowing the oligonucleotide probes to hybridize with the nucleic acid targets (para [0033] and [0102]-[0103]);
washing unhybridized encoded oligonucleotide probes from the sample (para [0033] and [0102]-[0103]);
delivering one or more encoding agents to locations of the multiple sites in the sample according to a known spatial pattern, wherein the combination of encoding agents delivered to each site is different (para [0086] and [0097] - encoding agents are 'Raman-active organic compounds');
coupling the encoding agents and the oligonucleotide probes to form encoded probes (para [0086]);
determining all or a portion of a sequence of the encoded probes using high throughput sequencing (para [0104] and [0122]), and
associating the abundance or activity or both of multiple biological targets to the locations of multiple sites in the sample (para [0033], [0104] and [0122]).

----------continued in next supplemental box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
citations and explanations from box V.2

Regarding claim 40, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple protein targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033]);
delivering encoded probes for the multiple protein targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a protein probe region that may interact with the protein targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and the protein probe region of the encoding probe of which the coding tag is part (para [0033], [0092] and [0097]);
allowing the encoded probes to interact with the protein targets (para [0033]);
separating encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets (para [0033]);
determining all or a portion of a sequence of the encoded probes by high throughput sequencing (para [0104] and [0122]), and
associating the abundance or activity or both of the multiple protein targets to the locations of the multiple sites in the sample (para [0033]).

Regarding claim 2, Roth teaches the biological targets are nucleic acids and the encoded probes are oligonucleotides (para [0102]-[0103]).

Regarding claim 3, Roth teaches there are two encoded probes for each of the multiple nucleic acid targets (para [0047] and [0091]).

Regarding claim 6, Roth teaches probe regions of the encoded probes comprise antibodies (para [0069]).

Regarding claim 7, Roth teaches the probe regions of the encoded probes are aptamers (para [0069]).

Regarding claim 8, Roth teaches the probe regions of the encoded probes are small molecules (para [0069]).

Regarding claims 9, 39 and 50, Roth teaches an amplification step between the separating step and the determining step (para [0105]).

Regarding claim 10, Roth teaches the determining step is performed by nucleic acid sequencing (para [0104] and [0122]).

Regarding claim 11, Roth teaches the sequencing is high-throughput digital nucleic acid sequencing (para [0039], [0122]-[0124]).

Regarding claims 12-19, 28-33, 41-46, Roth teaches the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 1,000,000 (para [0097]-[0098] and [0138]).

Regarding claims 20-22, 34, 35, 47-49, Roth teaches the sequence of at least one million encoding probes are determined in parallel (para [0097]-[0098], [0104] and [0138]).

Regarding claim 23, Roth teaches the known spatial pattern is determined by histological features of the sample (para [0036] and [0038]).

Regarding claim 24, Roth teaches software programmed hardware performs at least two steps of the delivering step, the separation step, the determining step and the associating step (para [0123]-[0124]).

Regarding claim 25, Roth teaches the probe regions of the encoded probes are proteins (para [0092]), and the separating step is accomplished by encoded probes that interact with the biological targets being captured by an affinity capture agent (para [0027] - 'contacting a biological sample 110 with a substrate 100 having a set of capture antibodies 120 that bind specifically to different proteins attached at defined locations on the substrate 100 to allow formation of antibody-protein complexes 130. The antibody-protein complexes are then contacted with a set of COIN-labeled probes 140 comprising a probe moiety 150 that binds specifically to a known protein analyte').

Regarding claim 26, Roth teaches the probe regions of the encoding probes are nucleic acids and the separating step is accomplished by a washing of the sample (para [0084]-[0085]).

Regarding claim 36, Roth teaches two oligonucleotide probes are delivered for each of the multiple nucleic acid targets (para [0083] - 'the term "oligonucleotide" is used herein to refer to a polynucleotide that is used as a primer or a probe. Generally, an oligonucleotide useful as a probe or primer that selectively hybridizes to a selected nucleotide sequence is at least 6 nucleotides to about 9 nucleotides in length.' --oligonucleotide probes are used as primers; later amplification would require the primers are in pairs).

----------continued in next supplemental box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 11/31308

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
previous supplemental box

Regarding claim 37, Roth teaches the coupling step is performed by ligation (para [0083] - 'Polynucleotide probes used in the invention methods are useful for detecting and hybridizing under suitable conditions to complementary polynucleotides in a biological sample by pairing a known polynucleotide probe with a known Raman-active COIN comprising one or more Raman-active organic compounds, as described herein. A covalent phosphodiester bond generally ligates the nucleotides of a polynucleotide sequence. However, the covalent bond also may be any of numerous other bonds, including a thiodiester bond, a phosphorothioate bond, a peptide-like amide bond or any other bond known to those in the art as useful for linking nucleotides to produce synthetic polynucleotides').

Regarding claim 51, Roth teaches the protein targets are enzymes (para [0090]), and the probe regions of the encoded probes are substrates, putative substrates of both for the enzymes (para [0069] and [0090]).

Regarding claim 52, Roth teaches the probe regions of the encoded probes are affinity capture agents (para [0043]).

Regarding claim 53, Roth teaches the probe regions of the encoded probes are antibodies (para [0069]).

Regarding claim 54, Roth teaches the probe regions of the encoded probes are aptamers (para [0069]).

Regarding claim 55, Roth teaches separating encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets is accomplished by an affinity capture agent that distinguishes encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets (para [0027] and [0042]-[0043]).

Regarding claim 57, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033]);
delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and identifies the biological target (para [0033], [0092] and [0097]);
allowing the encoded probes to interact with the biological targets (para [0033]);
determining all or a portion of a sequence of the encoded probes (para [0104] and [0122]), and
associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample (para [0033]).

Claims 4, 5, 38 and 56 lack an inventive step under PCT Article 33(3) as being obvious over Roth in view of US 6,344,316 B1, Lockhart et al., (hereinafter Lockhart).

Regarding claim 4, Roth teaches the multiple biological targets are proteins (para [0069]), the probe regions of the encoding probes are proteins (para [0073]). Roth does not specifically teach the coding tags comprise oligonucleotides. However, Roth does teach the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]). Lockhart teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (abstract), comprising encoded probes for the multiple biological targets (abstract), wherein the coding tags comprise oligonucleotides (abstract and col. 24, ln 8-30). It would have been obvious to one of ordinary skill in the art to have prepared an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the coding tags comprise oligonucleotides, in view of Roth teaching the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]), and in light of Lockhart teaching unique and florescent coding tags comprising oligonucleotides (col. 24, ln 37-61), since a skilled artisan would have readily appreciated use of the labeling methods taught by Lockhart applied to the probes taught by Roth, since they can be detected in a microarray.

Regarding claim 5, Roth teaches the multiple biological targets comprise enzymes (para [0090]).

Regarding claim 38, Roth does not specifically teach the coupling step is performed by extension followed by ligation. Lockhart teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (abstract), comprising coupling the one or more encoding agents and one or more oligonucleotide probes to form encoded probes (abstract and col. 24, ln 8-30), wherein the coupling step is performed by extension followed by ligation (col. 24, ln 8-30). It would have been obvious to one of ordinary skill in the art to have prepared an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the coupling step is performed by extension followed by ligation, in view of Roth teaching the probe is coupled to the encoding agents by various methods including ligation (para [0083]), and in light of Lockhart teaching coupling of one or more encoding agents and one or more oligonucleotide probes to form encoded probes (col. 24, ln 8-30), wherein the coupling step is performed by extension followed by ligation (col. 24, ln 8-30), since a skilled artisan would have readily appreciated use of the coupling methods taught by Lockhart applied to the probes taught by Roth, since they are both polynucleotide based probes, and could therefore be coupled via extension.

----------continued in next supplemental box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
previous supplemental box

Regarding claim 56, Roth does not specifically teach the coding tags are oligonucleotides. However, Roth does teach the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]). Lockhart teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (abstract), comprising encoded probes for the multiple biological targets (abstract), wherein the coding tags comprise oligonucleotides (abstract and col. 24, ln 8-30). It would have been obvious to one of ordinary skill in the art to have prepared an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the coding tags comprise oligonucleotides, in view of Roth teaching the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]), and in light of Lockhart teaching unique and florescent coding tags comprising oligonucleotides (col. 24, ln 37-61), since a skilled artisan would have readily appreciated use of the labeling methods taught by Lockhart applied to the probes taught by Roth, since they can be detected in a microarray.

Claims 1-57 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in the industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference PGNS009PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2012/032759	International filing date *(day/month/year)* 09 April 2012 (09.04.2012)	Priority date *(day/month/year)* 08 April 2011 (08.04.2011)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant PROGNOSYS BIOSCIENCES, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 08 October 2013 (08.10.2013)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Mineko Mohri e-mail: pt08.pct@wipo.int

Curio EX1010-p. 274
Curio v Prognosys
IPR2024-01289

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SARAH J. BRASHEARS
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 SEP 2012
Applicant's or agent's file reference PGNS009PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/32759	09 April 2012 (09.04.2012)	08 April 2011 (08.04.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 40/08; C40B 40/10; C40B 50/06 (2012.01)
USPC - 506/17; 506/18; 506/26

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	12 September 2012 (12.09.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-8, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. The first named invention (claims 1-3, 6-8), is limited to wherein the species are eukaryotic species. Applicant is invited to elect an additional limitations including: wherein the species are bacterial species or human pathogens (claims 4-5), to be searched by paying additional fee for each election.

Group II, claims 9-14, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed such that the peptide sequence diversity is no less than 1 % of the encoding oligonucleotide sequence diversity.

Group III, claims 15-20 drawn to a method for analyzing a sample using a set of peptide constructs, each peptide construct comprising a custom-designed peptide portion and an identifying nucleic acid portion, comprising the steps of: a. performing an assay on the set of peptide constructs, where some of the peptide constructs are acted upon by assay agents, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs of either the peptide constructs that were acted upon by the assay agents or the peptide constructs that were not acted upon by the assay agents by analyzing the nucleic acid portion of the peptide constructs.

The inventions listed as Groups I+-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+- II do not include the inventive concept of analyzing a sample using a set of peptide constructs, comprising the steps of: a. performing an assay on the set of peptide constructs, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs, as required by Group III.

Groups II-III do not include the inventive concept of a set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, as required by Groups I+.

Furthermore, among Group I+, identifying oligonucleotide sequence from eukaryotic species (claims 2-3) are structurally different from identifying oligonucleotide sequence from bacterial species or human pathogens (claims 4-5) and are collected in different databases.

The inventions of Groups I+ and III share the technical feature of a set of peptide constructs, each peptide construct comprising a custom -designed peptide portion and an identifying nucleic acid portion; and Group I+ and II further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2003/0235852 A1 to ROBERTS et al. (hereinafter 'Roberts') as follows:

**********Continued in the supplmental box**********

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-3, 6-8

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-3, 6-8	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-3, 6-8	NO
Industrial applicability (IA)	Claims	1-3, 6-8	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-3 and 7-8 lack an inventive step under PCT Article 33(3) as being obvious over US 2003/0235852 A1 to ROBERTS et al. (hereinafter 'Roberts'), in view of US 2009/0280487 A1 to HUNG et al. (hereinafter 'Hung').

Regarding claim 1, Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules', wherein '10.sup.9 different molecules' comprising at least 5,000 distinct peptide constructs; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
— each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,... prepare peptide libraries, wherein the encoding nucleic acid molecule remains physically associated with the peptide. Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide')
—that are custom-designed (para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed')
— so that the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids (para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues').

Although Roberts does not specifically teach wherein the peptide sequences of at least 12 amino acids are at least 10% of the set of peptide that have more than 80% amino acid identity to the protein sequences of interest, one of ordinary skill in the art at the time the invention was made would have known to do so, because Roberts teaches detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'), which requires the peptide having enough amino acids and requires enough percentage of peptides to have enough amino acids, including to have at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids to include a functional group that have more than 80% amino acid identity to the protein sequences of interest. And furthermore, it is a common practice to include peptides with known sequences in consensus regions that have at least 80% amino acid identity to the same proteins from different species when preparing a library (please see US 2007/0020625 A1 to DUCHAUD et al.: para [0253] - 'a DNA library prepared from the genome of a bacterium belonging to... species different from the Photorhabdus strain used to construct the DNA library'; para [0093] - "The expression "polypeptide exhibiting a certain percentage identity with another", ... "homologous polypeptide" will also be used... the amino acid sequence exhibits at least 80%, preferably 85%, 90%, 95%, 98% or 99%, identity'; please also see US 2005/0026188 A1 to van KESSEL et al.: para [0029] - 'developing a library of nucleotide sequences ... corresponding to variable regions of the cpn60 gene'; para [0060] - 'a "cnp60-like" sequence ... encoding a protein ... has a biological function similar to that of cpn60, ... homology of at least 70%, 80% or 90% may be used'; para [0062]- 'performs substantially the same role ... the homology will be at least about 80%, 85%, 90% or 95%').

Roberts does not specifically teach wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism. Hung discloses a peptide library comprising G-protein coupled receptor polypeptides from up to 100 different species (para [0062] - 'a library contains a plurality of members, ...up to about 1000 or more olfactory GPCR polypeptides.... derived from a plurality of species'; para [0019] - '"G-protein coupled receptors", or "GPCRs" are polypeptides'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal', wherein 'up to about 100... species of animal' is 'up to 100 different species of organism'; para [0129] - 'producing a library ... up to about 1000 or more...different olfactory GPCRs'; para [0056]; para [0058]) for activity determination and modulator identification (para [0096] - 'screening for olfactory GPCR modulators'; para [0103] - 'GPCR activity may be measured by assessing a reporter signal').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences of interest, based on the teaching of Roberts and common practice, wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism, based on the combination of Hung and Roberts, in order to include protein of interests in the same family from different species for facilitating identifying the peptide sequence associated with activity for characterization of protein of interest.

**********Continued in the supplemental box**********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fu lly supported by the description, are made:

For dependent claims 2-6, 10-14, 16, 19-20, the word 'where' in each claim is assumed to read as 'wherein'.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No IV (Lack of unity of invention)

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules', wherein '10.sup.9 different molecules' comprising at least 5,000 distinct peptide constructs; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,... prepare peptide libraries, wherein the encoding nucleic acid molecule remains physically associated with the peptide. Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide')
---that are custom-designed (para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed'). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups I+ further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set
of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. However, this shared technical feature does not represent a contribution over prior art as being obvious over Roberts, in view of US 2009/0280487 A1 to HUNG et al. (hereinafter 'Hung') as follow:

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090]; para [0008]; para [0127], as discussed above)
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009]; para [0005], as discussed above)
---that are custom-designed (para [0004],as discussed above)
--- so that the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids (para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues').

Although Roberts does not specifically teach wherein the peptide sequences of at least 12 amino acids are at least 10% of the set of peptide that have more than 80% amino acid identity to the protein sequences of interest, one of ordinary skill in the art at the time the invention was made would have known to do so, because Roberts teaches detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'), which requires the peptide having enough amino acids and requires enough percentage of peptides to have enough amino acids, including to have at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids to include a functional group that have more than 80% amino acid identity to the protein sequences of interest. And furthermore, it is a common practice to include peptides with known sequences in consensus regions that have at least 80% amino acid identity to the same proteins from different species when preparing a library (please see US 2007/0020625 A1 to DUCHAUD et al.: para [0253] - 'a DNA library prepared from the genome of a bacterium belonging to... species different from the Photorhabdus strain used to construct the DNA library'; para [0093] - 'The expression "polypeptide exhibiting a certain percentage identity with another", ... "homologous polypeptide" will also be used... the amino acid sequence exhibits at least 80%, preferably 85%, 90%, 95%, 98% or 99%, identity'; please also see US 2005/0026188 A1 to van KESSEL et al.: para [0029] - 'developing a library of nucleotide sequences ... corresponding to variable regions of the cpn60 gene'; para [0060] - 'a "cnp60-like" sequence ... encoding a protein ... has a biological function similar to that of cpn60, ... homology of at least 70%, 80% or 90% may be used'; para [0062]- 'performs substantially the same role ... the homology will be at least about 80%, 85%, 90% or 95%').

Roberts does not specifically teach wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism. Hung discloses a peptide library comprising G-protein coupled receptor polypeptides from up to 100 different species (para [0062] - 'a library contains a plurality of members, ...up to about 1000 or more olfactory GPCR polypeptides.... derived from a plurality of species'; para para [0019] - '"G-protein coupled receptors", or "GPCRs" are polypeptides'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal', wherein 'up to about 100...species of animal' is 'up to 100 different species of organism'; para [0129] - 'producing a library ... up to about 1000 or more...different olfactory GPCRs'; para [0056]; para [0058]) for activity determination and modulator identification (para [0096] - 'screening for olfactory GPCR modulators'; para [0103] - 'GPCR activity may be measured by assessing a reporter signal').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences of interest, based on the teaching of Roberts and the common practice, wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism, based on the combination of Hung and Roberts, in order to include protein of interests in the same family from different species for facilitating identifying the peptide sequence associated with activity for characterization of protein of interest. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V No 2 (citations and explanations)

Regarding claim 2, the combination of Roberts and Hung discloses the set of peptide constructs from claim 1, and the said combination further discloses wherein the species are eukaryotic species (Roberts: para [0066] - 'nucleic acid-peptide fusion molecules, ...the initiator AUG codon in eukaryotic systems', wherein 'the initiator AUG codon in eukaryotic systems' indicating the peptide constructs are for eukaryotic species; para [0083] - 'RNA-peptide fusions then are generated using an in vitro or in situ translation system. ... eukaryotic systems are preferred'; Hung: para [0056] - 'the olfactory GPCR may be of human origin or of non-human animal origin... may be a mouse, a rat, a dog, or any other non-human animal'; para [0066] - 'a particular species, particularly a mammalian, e.g., human or mouse species'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal').

Regarding claim 3, the combination of Roberts and Hung discloses the set of peptide constructs from claim 2, and Hung further discloses wherein the species are mammalian species (para [0056] - 'the olfactory GPCR may be of human origin or of non-human animal origin... may be a mouse, a rat, a dog, or any other non-human animal'; para [0066] - 'a particular species, particularly a mammalian, e.g., human or mouse species'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal').

Regarding claim 7, the combination of Roberts and Hung discloses the set of peptides from claim 1, and Roberts further discloses wherein the peptide portion is dissociated from the ribosome before being linked to the identifying nucleic acid (para [0009] - 'The encoding nucleic acid is translationally linked at or near its 3' terminus to the C-terminus of its encoded peptide, ... via a peptide acceptor such as puromycin'; para [0057]; para [0059] - 'puromycin acts as a universal inhibitor of protein synthesis by binding the translation complex A site, accepting the growing peptide chain, and falling off the ribosome ...methods for translationally linking an encoding nucleic acid and encoded peptide ... effecting the joining using a peptide acceptor such as puromycin, which can be linked at or near the 3' terminus of the encoding nucleic acid such that it can enter the ribosome complex during translation and be incorporated at the C-terminus of the growing (nascent) peptide, thereby terminating translation and linking the encoding nucleic acid and encoded peptide', wherein ' puromycin ... falling off the ribosome... terminating translation and linking the encoding nucleic acid and encoded peptide' indicates 'wherein the peptide portion is dissociated from the ribosome before being linked to the identifying nucleic acid'; Please see Alkinson for the translation steps: Entire documentation, especially pg 6.5, Elongation; and pg 6.6, Translocation and Termination).

Regarding claim 8, the combination of Roberts and Hung discloses the set of peptide constructs from claim 1, and the said combination further obviates wherein the set of peptide constructs comprises at least 100,000 distinct peptide constructs (Roberts: para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules'; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
---each comprising a peptide portion of at least 10 amino acids and an identifying oligonucleotide portion (Roberts: para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,...Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'; para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues')
---wherein the peptide portions are encoded within oligonucleotide sequences that are custom designed (Roberts: para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed'; para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide').
----such that at least 90% of the sequences contain no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs (Roberts: para [0092] - 'the orthogonal aminoacylated tRNA, which is charged with an unnatural amino acid residue, recognizes a stop codon... a start codon operably linked to a protein coding sequence, and each of which is linked to a peptide acceptor at the 3'-end of the peptide coding sequence, which includes or can include one or a series of stop codons, ... to produce a population of RNA-peptide fusion molecules comprising peptides', wherein additional stop codons are used for translating unatural amino acids, one of ordinary skill in the art at the time the invention was made would have known to include no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs because is the basic principle for successful protein translation; para [0094] - 'an amber suppressor tRNA, which recognizes a UAG stop codon. ...other unnatural residues such as N-methyl glycine and N--(S)-phenylethyl glycine similarly can be used'; Hung: para [0073] - 'a fusion protein may contain a heterologous N-terminal domain (e.g. an epitope tag) fused in-frame with a GPCR that has had its N-terminal methionine residue either deleted or substituted with an alternative amino acid', wherein 'fused in-frame' is the basic practice including 'in-frame codon that can function as a stop codon' for protein translation; para [0039] - 'The boundaries of the coding sequence are typically determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus', wherein 'a translation stop codon at the 3' (carboxy) terminus' indicates each conding sequence having 'one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs', one of ordinary skill in the art at the time the invention was made would have known to have ' at least 90% of the sequences contain no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs' for producing sufficient amount of distinct peptides, but not high percentage of premature peptides due to the presence of undesired stop codon).

**********Continued in the next supplemental box**********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
The previous supplemental box - Box V No 2 (citations and explanations)

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Roberts, in view of Hung, as above, and further in view of US 2007/0014810 A1 to BAKER et al. (hereinafter 'Baker').

Regarding claim 6, the combination of Roberts and Hung discloses a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, as discussed in the discussion of claim 1. Roberts further discloses using the set of peptide constructs for detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide').

Said combination does not specifically teach wherein the peptide sequences are grouped into one or more sets of partially overlapping sequences. Baker discloses a method for identifying ligand binding domains of a sequence of interest, the method comprising producing a set of peptide sequences that are partially overlapping sequences that cover the entire sequence of the sequence of interest for identifying ligand binding domains (para [0359] - 'The identification of motifs and/or super motifs that correlate with high and intermediate affinity binding ... identification of immunogenic peptide epitopes ... all possible peptides of 9 amino acids in length and overlapping by eight amino acids ... which cover the entire sequence of the ... proteins ...were evaluated for binding to five allele-specific HLA molecules'; para [0361] - 'a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets'). Although Baker does not specifically teach the peptide sequences are grouped into one or more sets of partially overlapping sequences, one of ordinary skill in the art at the time the invention was made would have known to do so by grouping partially overlapping sequences generated from the same protein in the same group for facilitating characterization of the protein function associated with the peptide fragments in the protein.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung with that of Baker, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, based on the combination of Roberts and Hung, and further wherein the peptide sequences are grouped into one or more sets of partially overlapping sequences, based on the combination of Baker, Roberts and Hung, in order to obtain a set of distinct peptide constructs that include all factional fragments of the targeted protein sequences for facilitating characterization of the functional domain of the targeted protein sequences in different species of organism.

Claims 1-3 and 6-8 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SARAH J. BRASHEARS
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 SEP 2012
Applicant's or agent's file reference PGNS009PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/32759	09 April 2012 (09.04.2012)	08 April 2011 (08.04.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 40/08; C40B 40/10; C40B 50/06 (2012.01)
USPC - 506/17; 506/18; 506/26

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	12 September 2012 (12.09.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-8, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. The first named invention (claims 1-3, 6-8), is limited to wherein the species are eukaryotic species. Applicant is invited to elect an additional limitations including: wherein the species are bacterial species or human pathogens (claims 4-5), to be searched by paying additional fee for each election.

Group II, claims 9-14, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed such that the peptide sequence diversity is no less than 1 % of the encoding oligonucleotide sequence diversity.

Group III, claims 15-20 drawn to a method for analyzing a sample using a set of peptide constructs, each peptide construct comprising a custom-designed peptide portion and an identifying nucleic acid portion, comprising the steps of: a. performing an assay on the set of peptide constructs, where some of the peptide constructs are acted upon by assay agents, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs of either the peptide constructs that were acted upon by the assay agents or the peptide constructs that were not acted upon by the assay agents by analyzing the nucleic acid portion of the peptide constructs.

The inventions listed as Groups I+-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+- II do not include the inventive concept of analyzing a sample using a set of peptide constructs, comprising the steps of: a. performing an assay on the set of peptide constructs, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs, as required by Group III.

Groups II-III do not include the inventive concept of a set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, as required by Groups I+.

Furthermore, among Group I+, identifying oligonucleotide sequence from eukaryotic species (claims 2-3) are structurally different from identifying oligonucleotide sequence from bacterial species or human pathogens (claims 4-5) and are collected in different databases.

The inventions of Groups I+ and III share the technical feature of a set of peptide constructs, each peptide construct comprising a custom -designed peptide portion and an identifying nucleic acid portion; and Group I+ and II further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2003/0235852 A1 to ROBERTS et al. (hereinafter 'Roberts') as follows:

**********Continued in the supplmental box**********

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-3, 6-8

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-3, 6-8	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-3, 6-8	NO
Industrial applicability (IA)	Claims	1-3, 6-8	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-3 and 7-8 lack an inventive step under PCT Article 33(3) as being obvious over US 2003/0235852 A1 to ROBERTS et al. (hereinafter 'Roberts'), in view of US 2009/0280487 A1 to HUNG et al. (hereinafter 'Hung').

Regarding claim 1, Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules', wherein '10.sup.9 different molecules' comprising at least 5,000 distinct peptide constructs; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
— each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,... prepare peptide libraries, wherein the encoding nucleic acid molecule remains physically associated with the peptide. Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide')
—that are custom-designed (para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed')
— so that the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids (para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues').

Although Roberts does not specifically teach wherein the peptide sequences of at least 12 amino acids are at least 10% of the set of peptide that have more than 80% amino acid identity to the protein sequences of interest, one of ordinary skill in the art at the time the invention was made would have known to do so, because Roberts teaches detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'), which requires the peptide having enough amino acids and requires enough percentage of peptides to have enough amino acids, including to have at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids to include a functional group that have more than 80% amino acid identity to the protein sequences of interest. And furthermore, it is a common practice to include peptides with known sequences in consensus regions that have at least 80% amino acid identity to the same proteins from different species when preparing a library (please see US 2007/0020625 A1 to DUCHAUD et al.: para [0253] - 'a DNA library prepared from the genome of a bacterium belonging to... species different from the Photorhabdus strain used to construct the DNA library'; para [0093] - "The expression "polypeptide exhibiting a certain percentage identity with another", ... "homologous polypeptide" will also be used... the amino acid sequence exhibits at least 80%, preferably 85%, 90%, 95%, 98% or 99%, identity'; please also see US 2005/0026188 A1 to van KESSEL et al.: para [0029] - 'developing a library of nucleotide sequences ... corresponding to variable regions of the cpn60 gene'; para [0060] - 'a "cnp60-like" sequence ... encoding a protein ... has a biological function similar to that of cpn60, ... homology of at least 70%, 80% or 90% may be used'; para [0062]- 'performs substantially the same role ... the homology will be at least about 80%, 85%, 90% or 95%').

Roberts does not specifically teach wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism. Hung discloses a peptide library comprising G-protein coupled receptor polypeptides from up to 100 different species (para [0062] - 'a library contains a plurality of members, ...up to about 1000 or more olfactory GPCR polypeptides.... derived from a plurality of species'; para [0019] - '"G-protein coupled receptors", or "GPCRs" are polypeptides'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal', wherein 'up to about 100... species of animal' is 'up to 100 different species of organism'; para [0129] - 'producing a library ... up to about 1000 or more...different olfactory GPCRs'; para [0056]; para [0058]) for activity determination and modulator identification (para [0096] - 'screening for olfactory GPCR modulators'; para [0103] - 'GPCR activity may be measured by assessing a reporter signal').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences of interest, based on the teaching of Roberts and common practice, wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism, based on the combination of Hung and Roberts, in order to include protein of interests in the same family from different species for facilitating identifying the peptide sequence associated with activity for characterization of protein of interest.

**********Continued in the supplemental box**********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fu lly supported by the description, are made:

For dependent claims 2-6, 10-14, 16, 19-20, the word 'where' in each claim is assumed to read as 'wherein'.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No IV (Lack of unity of invention)

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules', wherein '10.sup.9 different molecules' comprising at least 5,000 distinct peptide constructs; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,... prepare peptide libraries, wherein the encoding nucleic acid molecule remains physically associated with the peptide. Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide')
---that are custom-designed (para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed'). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups I+ further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set
of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. However, this shared technical feature does not represent a contribution over prior art as being obvious over Roberts, in view of US 2009/0280487 A1 to HUNG et al. (hereinafter 'Hung') as follow:

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090]; para [0008]; para [0127], as discussed above)
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009]; para [0005], as discussed above)
---that are custom-designed (para [0004],as discussed above)
--- so that the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids (para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues').
Although Roberts does not specifically teach wherein the peptide sequences of at least 12 amino acids are at least 10% of the set of peptide that have more than 80% amino acid identity to the protein sequences of interest, one of ordinary skill in the art at the time the invention was made would have known to do so, because Roberts teaches detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'), which requires the peptide having enough amino acids and requires enough percentage of peptides to have enough amino acids, including to have at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids to include a functional group that have more than 80% amino acid identity to the protein sequences of interest. And furthermore, it is a common practice to include peptides with known sequences in consensus regions that have at least 80% amino acid identity to the same proteins from different species when preparing a library (please see US 2007/0020625 A1 to DUCHAUD et al.: para [0253] - 'a DNA library prepared from the genome of a bacterium belonging to... species different from the Photorhabdus strain used to construct the DNA library'; para [0093] - 'The expression "polypeptide exhibiting a certain percentage identity with another", ... "homologous polypeptide" will also be used... the amino acid sequence exhibits at least 80%, preferably 85%, 90%, 95%, 98% or 99%, identity'; please also see US 2005/0026188 A1 to van KESSEL et al.: para [0029] - 'developing a library of nucleotide sequences ... corresponding to variable regions of the cpn60 gene'; para [0060] - 'a "cnp60-like" sequence ... encoding a protein ... has a biological function similar to that of cpn60, ... homology of at least 70%, 80% or 90% may be used'; para [0062]- 'performs substantially the same role ... the homology will be at least about 80%, 85%, 90% or 95%').
Roberts does not specifically teach wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism. Hung discloses a peptide library comprising G-protein coupled receptor polypeptides from up to 100 different species (para [0062] - 'a library contains a plurality of members, ...up to about 1000 or more olfactory GPCR polypeptides.... derived from a plurality of species'; para para [0019] - '"G-protein coupled receptors", or "GPCRs" are polypeptides'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal', wherein 'up to about 100...species of animal' is 'up to 100 different species of organism'; para [0129] - 'producing a library ... up to about 1000 or more...different olfactory GPCRs'; para [0056]; para [0058]) for activity determination and modulator identification (para [0096] - 'screening for olfactory GPCR modulators'; para [0103] - 'GPCR activity may be measured by assessing a reporter signal').
It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences of interest, based on the teaching of Roberts and the common practice, wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism, based on the combination of Hung and Roberts, in order to include protein of interests in the same family from different species for facilitating identifying the peptide sequence associated with activity for characterization of protein of interest. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V No 2 (citations and explanations)

Regarding claim 2, the combination of Roberts and Hung discloses the set of peptide constructs from claim 1, and the said combination further discloses wherein the species are eukaryotic species (Roberts: para [0066] - 'nucleic acid-peptide fusion molecules, ...the initiator AUG codon in eukaryotic systems', wherein 'the initiator AUG codon in eukaryotic systems' indicating the peptide constructs are for eukaryotic species; para [0083] - 'RNA-peptide fusions then are generated using an in vitro or in situ translation system. ... eukaryotic systems are preferred'; Hung: para [0056] - 'the olfactory GPCR may be of human origin or of non-human animal origin... may be a mouse, a rat, a dog, or any other non-human animal'; para [0066] - 'a particular species, particularly a mammalian, e.g., human or mouse species'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal').

Regarding claim 3, the combination of Roberts and Hung discloses the set of peptide constructs from claim 2, and Hung further discloses wherein the species are mammalian species (para [0056] - 'the olfactory GPCR may be of human origin or of non-human animal origin... may be a mouse, a rat, a dog, or any other non-human animal'; para [0066] - 'a particular species, particularly a mammalian, e.g., human or mouse species'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal').

Regarding claim 7, the combination of Roberts and Hung discloses the set of peptides from claim 1, and Roberts further discloses wherein the peptide portion is dissociated from the ribosome before being linked to the identifying nucleic acid (para [0009] - 'The encoding nucleic acid is translationally linked at or near its 3' terminus to the C-terminus of its encoded peptide, ... via a peptide acceptor such as puromycin'; para [0057]; para [0059] - 'puromycin acts as a universal inhibitor of protein synthesis by binding the translation complex A site, accepting the growing peptide chain, and falling off the ribosome ...methods for translationally linking an encoding nucleic acid and encoded peptide ... effecting the joining using a peptide acceptor such as puromycin, which can be linked at or near the 3' terminus of the encoding nucleic acid such that it can enter the ribosome complex during translation and be incorporated at the C-terminus of the growing (nascent) peptide, thereby terminating translation and linking the encoding nucleic acid and encoded peptide', wherein ' puromycin ... falling off the ribosome... terminating translation and linking the encoding nucleic acid and encoded peptide' indicates 'wherein the peptide portion is dissociated from the ribosome before being linked to the identifying nucleic acid'; Please see Alkinson for the translation steps: Entire documentation, especially pg 6.5, Elongation; and pg 6.6, Translocation and Termination).

Regarding claim 8, the combination of Roberts and Hung discloses the set of peptide constructs from claim 1, and the said combination further obviates wherein the set of peptide constructs comprises at least 100,000 distinct peptide constructs (Roberts: para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules'; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
---each comprising a peptide portion of at least 10 amino acids and an identifying oligonucleotide portion (Roberts: para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,...Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'; para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues')
---wherein the peptide portions are encoded within oligonucleotide sequences that are custom designed (Roberts: para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed'; para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide').
----such that at least 90% of the sequences contain no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs (Roberts: para [0092] - 'the orthogonal aminoacylated tRNA, which is charged with an unnatural amino acid residue, recognizes a stop codon... a start codon operably linked to a protein coding sequence, and each of which is linked to a peptide acceptor at the 3'-end of the peptide coding sequence, which includes or can include one or a series of stop codons, ... to produce a population of RNA-peptide fusion molecules comprising peptides', wherein additional stop codons are used for translating unatural amino acids, one of ordinary skill in the art at the time the invention was made would have known to include no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs because is the basic principle for successful protein translation; para [0094] - 'an amber suppressor tRNA, which recognizes a UAG stop codon. ...other unnatural residues such as N-methyl glycine and N--(S)-phenylethyl glycine similarly can be used'; Hung: para [0073] - 'a fusion protein may contain a heterologous N-terminal domain (e.g. an epitope tag) fused in-frame with a GPCR that has had its N-terminal methionine residue either deleted or substituted with an alternative amino acid', wherein 'fused in-frame' is the basic practice including 'in-frame codon that can function as a stop codon' for protein translation; para [0039] - 'The boundaries of the coding sequence are typically determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus', wherein 'a translation stop codon at the 3' (carboxy) terminus' indicates each conding sequence having 'one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs', one of ordinary skill in the art at the time the invention was made would have known to have ' at least 90% of the sequences contain no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs' for producing sufficient amount of distinct peptides, but not high percentage of premature peptides due to the presence of undesired stop codon).

**********Continued in the next supplemental box**********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
The previous supplemental box - Box V No 2 (citations and explanations)

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Roberts, in view of Hung, as above, and further in view of US 2007/0014810 A1 to BAKER et al. (hereinafter 'Baker').

Regarding claim 6, the combination of Roberts and Hung discloses a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, as discussed in the discussion of claim 1. Roberts further discloses using the set of peptide constructs for detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide').

Said combination does not specifically teach wherein the peptide sequences are grouped into one or more sets of partially overlapping sequences. Baker discloses a method for identifying ligand binding domains of a sequence of interest, the method comprising producing a set of peptide sequences that are partially overlapping sequences that cover the entire sequence of the sequence of interest for identifying ligand binding domains (para [0359] - 'The identification of motifs and/or super motifs that correlate with high and intermediate affinity binding ... identification of immunogenic peptide epitopes ... all possible peptides of 9 amino acids in length and overlapping by eight amino acids ... which cover the entire sequence of the ... proteins ...were evaluated for binding to five allele-specific HLA molecules'; para [0361] - 'a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets'). Although Baker does not specifically teach the peptide sequences are grouped into one or more sets of partially overlapping sequences, one of ordinary skill in the art at the time the invention was made would have known to do so by grouping partially overlapping sequences generated from the same protein in the same group for facilitating characterization of the protein function associated with the peptide fragments in the protein.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung with that of Baker, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, based on the combination of Roberts and Hung, and further wherein the peptide sequences are grouped into one or more sets of partially overlapping sequences, based on the combination of Baker, Roberts and Hung, in order to obtain a set of distinct peptide constructs that include all factional fragments of the targeted protein sequences for facilitating characterization of the functional domain of the targeted protein sequences in different species of organism.

Claims 1-3 and 6-8 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 699932000140	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US14/29691	International filing date *(day/month/year)* 14 March 2014 (14.03.2014)	(Earliest) Priority Date *(day/month/year)* 15 March 2013 (15.03.2013)
Applicant Prognosys Biosciences, Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 8A

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/29691

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/29691

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 15-21
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/29691

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/567, 33/53; C12Q 1/00 (2014.01)
USPC - 435/7.21, 7.2, 7.1, 4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): G01N 33/567, 33/53, 33/574; C12Q 1/00, 1/68; C07H 21/04; C12N 15/11, 15/62, 15/09; A61K 39/02, 38/17, 39/395 (2014.01)
USPC: 435/7.21, 7.2, 7.1, 4, 6, 69.1, 7.23; 424/185.1, 192.1, 144.1, 93.21, 88, 243.1; 514/44, 12; 530/403, 402

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; ProQuest; peptide, 'MHC,' conjugated

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 8343500 B2 (WRAITH, DC et al.) 01 January 2013; abstract; column 31, line 47; column 9, line 46; column 4, line 15; column 31, line 31; column 1, line 19; column 17, line 64; column 29, lines 9-10; column 6, lines 8-10; column 4, line 41; column 5, line 36; column 5, line 52; column 2, line 51; column 1, line 43; column 9, line 47; column 11, line 13; column 31, line 65; column 4, line 24; column 33, line 8; column 14, line 23; column 28, lines 15-16; column 29, line 9; column 8, lines 36-38; column 5, line 48; column 1, lines 32-38; column 1, line 37; column 1, lines 36-37; column 7, lines 44-45	1-5, 7-9, 10/8, 10/9, 11-13, 14/11-14/13 ----- 6
Y	US 8337851 B2 (AUKERMAN, SL et al.) 25 December 2012; column 24, lines 27-31	6

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2014 (25.07.2014)	19 AUG 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Bu Yin
Morrison & Foerster LLP
12531 High Bluff Drive, Suite 100
San Diego, CA 92130-2040
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	19 AUG 2014
Applicant's or agent's file reference 699932000140	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US14/29691	14 March 2014 (14.03.2014)	15 March 2013 (15.03.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 33/567, 33/53; C12Q 1/00 (2014.01)
USPC - 435/7.21, 7.2, 7.1, 4

Applicant Prognosys Biosciences, Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	25 July 2014 (25.07.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/29691

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☒ subsequently to this Authority for the purposes of search

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/29691

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 15-21

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 15-21 are so unclear that no meaningful opinion could be formed *(specify)*:

Claim Nos. 15-21 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 15-21

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/29691

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	6	YES
	Claims	1-5, 7-14	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-14	NO
Industrial applicability (IA)	Claims	1-14	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-5, 7-9, 10/8, 10/9, 11-13 and 14/11-14/13 lack novelty under PCT Article 33(2) as being anticipated by US 8,343,500 B2 to Wraith, et al. (hereinafter 'Wraith').

Regarding claim 1, Wraith discloses an MHC-binding peptide (MHC-binding peptide; abstract) conjugated to (binding to (conjugated to); abstract) a polynucleotide (DNA (polynucleotide); column 31, line 47).

Regarding claim 2, Wraith discloses a library of at least two MHC-binding peptides (library of peptides (library of at least two MHC-binding peptides), column 9, line 48; MHC-binding peptide (MHC-binding peptides), abstract) each (each; column 4, line 15) conjugated to (binding to (conjugated to); abstract) a polynucleotide (DNA (polynucleotide); column 31, line 47), wherein each said polynucleotide (DNA (each said polynucleotide); column 31, line 47) is identified by (identified by; column 1, line 19) a probe (probe; column 31, line 31) that specifically (specific (specifically); column 17, line 64) binds to (binds to; column 29, lines 9-10) said polynucleotide (DNA (said polynucleotide); column 31, line 47).

Regarding claim 3, Wraith discloses a composition (composition; abstract) comprising at least two MHC-binding peptides (library of peptides (at least two MHC-binding peptides), column 9, line 48; MHC-binding peptide (MHC-binding peptides), abstract) each (each; column 4, line 15) conjugated to (binding to (conjugated to); abstract) a polynucleotide (DNA (polynucleotide); column 31, line 47), wherein the at least two MHC-binding peptides (library of peptides (at least two MHC-binding peptides), column 9, line 48; MHC-binding peptide (MHC-binding peptides), abstract) are oligomerized (peptides 15-mer, 10-mer and 17-mer in length (oligomerized); column 6, lines 8-10).

Regarding claim 4, Wraith discloses the composition of claim 3, and Wraith further discloses wherein the at least two MHC-binding peptides (library of peptides (at least two MHC-binding peptides), column 9, line 48; MHC-binding peptide (MHC-binding peptides), abstract) are conjugated to (binding to (conjugated to), abstract) the same (same; column 4, line 41) polynucleotide (DNA (polynucleotide); column 31, line 47) and are thus oligomerized (peptides 15-mer, 10-mer and 17-mer in length (oligomerized); column 6, lines 8-10).

Regarding claim 5, Wraith discloses the composition of claim 3, and Wraith further discloses wherein the at least two MHC-binding peptides (library of peptides (at least two MHC-binding peptides), column 9, line 48; MHC-binding peptide (MHC-binding peptides), abstract) are each (each; column 4, line 15) conjugated to (binding to (conjugated to), abstract) a separate (separate; column 5, line 36; column 5, line 52) polynucleotide (DNA (polynucleotide); column 31, line 47), wherein the polynucleotides (DNA (polynucleotides); column 31, line 47) mediate (mediated (mediate); column 2, line 51) the oligomerization (peptides 15-mer, 10-mer and 17-mer in length (oligomerization); column 6, lines 8-10) of the at least two MHC-binding peptides (library of peptides (at least two MHC-binding peptides), column 9, line 48; MHC-binding peptide (MHC-binding peptides), abstract).

Regarding claim 7, Wraith discloses a method (method, abstract) for detecting (identifying (detecting); column 9, line 47) binding of a peptide to an MHC molecule (peptide binding to an MHC molecule (binding of a peptide to an MHC molecule), abstract), comprising: contacting (contacts (contacting),; olumn 11, line 13) said MHC molecule (said MHC molecule, abstract) with a polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate column 31, line 65), said polynucleotide peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) comprising said peptide (peptide (said peptide); abstract) and a polynucleotide (DNA (polynucleotide); column 31, line 47); contacting (contacts (contacting); column 11, line 13) said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) with a probe (probe; column 31, line 31) that specifically (specific (specifically); column 17, line 64) binds to (binds to; column 29, lines 9-10) said polynucleotide (DNA (said polynucleotide), column 31, line 47; detecting (identifying (detecting), column 9, line 47) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to said polynucleotide (DNA (said polynucleotide); column 31, line 47); and correlating (correlating; column 33, line 8) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to said polynucleotide (DNA (said polynucleotide); column 31, line 47) with binding of said peptide to said MHC molecule (peptide binding to an MHC molecule (binding of said peptide to said MHC molecule); abstract).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/29691

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. V:
-Continued from Citations and Explanations:

Regarding claim 8, Wraith discloses a method (method; abstract) for simultaneously (simultaneously; column 14 , line 23) detecting (identifying (detecting); column 9, line 47) binding of (binding of; column 4, line 24) a library of peptides (library of peptides; column 9, line 48) to an MHC molecule (abstract), comprising: providing (provided (providing); abstract) a polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) for each said peptide (peptide; abstract), said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) comprising said peptide (peptide (said peptide), abstract) and a polynucleotide (DNA (a polynucleotide); column 31, line 47); contacting (contacts (contacting); column 11, line 13) said MHC molecule (MHC molecule (said MHC molecule), abstract) with a pool (pool; column 9, line 39) of said polynucleotide-peptide conjugates (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugates, column 31, line 65); contacting (contacts (contacting); column 11, line 13) each of (each of; column 13, line 56) said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) with a probe (probe; column 31, line 31) that specifically (specific (specifically); column 17, line 64) binds to (binds to; column 29, lines 9-10) each said polynucleotide (DNA (each said polynucleotide), column 31, line 47); detecting (identifying (detecting); column 9, line 47) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) that each said probe (probe (each said probe); column 31, line 31) specifically (specific (specifically); column 17, line 64) binds (binds; column 29, line 9); and correlating (correlating; column 33, line 8) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) with binding of each corresponding peptide to said MHC molecule (peptide binding to an MHC molecule (binding of each corresponding peptide to said MHC molecule); abstract).

Regarding claim 9, Wraith discloses a method (method, abstract) for detecting (identifying (detecting); column 9, line 47) in a library of peptides (library of peptides; column 9, line 48) competitive binding of each said peptide to an MHC molecule (dominance of a particular peptide depends on relative affinity for binding the MHC molecule (competitive binding of each said peptide to an MHC molecule); column 8, lines 36-38) comprising: providing (provided (providing), abstract) a polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) for each said peptide (peptide (each said peptide); abstract), said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) comprising said peptide (peptide (said peptide); abstract) and a polynucleotide (DNA (a polynucleotide); column 31, line 47); contacting (contacts (contacting); column 11, line 13) said MHC molecule (MHC molecule (said MHC molecule); abstract) with a pool (pool; column 9, line 39) of said polynucleotide-peptide conjugates (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugates (conjugate; column 31, line 65); contacting (contacts (contacting); column 11, line 13) each of (each of; column 13, line 56) said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) with a probe (probe; column 31, line 31) that specifically (specific (specifically); column 17, line 64) binds to (binds to; column 29, lines 9-10) each said polynucleotide (DNA (each said polynucleotide); column 31, line 47); detecting (identifying (detecting); column 9, line 47) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) that each said probe (probe (each said probe); column 31, line 31) specifically (specific (specifically); column 17, line 64) binds (binds; column 29, line 9); and correlating (correlating; column 33, line 8) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) with binding of each corresponding peptide to said MHC molecule (peptide binding to an MHC molecule (binding of each corresponding peptide to said MHC molecule); abstract), wherein said peptides compete for binding of said MHC molecule (dominance of a particular peptide depends on relative affinity for binding the MHC molecule (said peptides compete for binding of said MHC molecule); column 8, lines 36-38).

Regarding claims 10/8 and 10/9, Wraith discloses a method of claims 8 and 9, and Wraith discloses further comprising comparing (compared (comparing); column 5, line 48) binding of each said peptide to said MHC molecule (peptide binding to an MHC molecule (binding of each said peptide to said MHC molecule); abstract), among the peptides in said library (library of peptides (peptides in said library); column 9, line 48).

Regarding claim 11, Wraith discloses a method (method, abstract) of detecting (identifying (detecting); column 9, line 47) binding of a peptide to a TCR (peptide-MHC complex carried to cell surface and peptide is recognized by a T cell via the TCR (binding of a peptide to a TCR); column 1, lines 32-38), comprising: contacting (contacts (contacting); column 11, line 13) said TCR (TCR (said TCR); column 1, line 37) with an MHC molecule (MHC molecule; abstract) and a polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65), said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65); comprising said peptide (peptide, abstract) and a polynucleotide (DNA (polynucleotide), column 31, line 47); contacting (contacts (contacting), column 11, line 13) said polynucleotide-peptide conjugate (DNA (polynucleotide); column 31, line 47; peptide, abstract; conjugate, column 31, line 65) with a probe (probe; column 31, line 31) that specifically (specific (specifically); column 17, line 64) binds to (binds to; column 29, lines 9-10) said polynucleotide (DNA (said polynucleotide); column 31, line 47); detecting (identifying (detecting); column 9, line 47) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to said polynucleotide (DNA (said polynucleotide); column 31, line 47); and correlating (correlating; column 33, line 8) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to said polynucleotide (DNA (said polynucleotide); column 31, line 47) with binding of said peptide to said TCR (peptide-MHC complex carried to cell surface and peptide is recognized by a T cell via the TCR (binding of said peptide to said TCR); column 1, lines 32-38).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/29691

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. V:
-Continued from Citations and Explanations:

Regarding claim 12, Wraith discloses a method (method; abstract) for simultaneously (simultaneously; column 14 , line 23) detecting (identifying (detecting); column 9, line 47) binding of (binding of; column 4, line 24) a library of peptides (library of peptides; column 9, line 48) to a TCR (TCR; column 1, line 37), comprising: providing (provided (providing); abstract) a polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, Aabstract; conjugate, column 31, line 65) for each said peptide (peptide (each said peptide); abstract), said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) comprising said peptide (peptide (said peptide); abstract) and a polynucleotide (DNA (polynucleotide), column 31, line 47); contacting (contacts (contacting); column 11, line 13) said TCR (TCR (said TCR); column 1, line 37) with a pool (pool; column 9, line 39) of said polynucleotide-peptide conjugates (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugates (conjugate (conjugates), column 31, line 65) and an MHC molecule (MHC molecule, abstract); contacting (contacts (contacting), column 11, line 13) each of (each of; column 13, line 56) said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) with a probe (probe; column 31, line 31) that specifically (specific (specifically); column 17, line 64) binds to (binds to; column 29, lines 9-10) each said polynucleotide (DNA (each said polynucleotide); column 31, line 47); detecting (identifying (detecting); column 9, line 47) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) that each said probe (probe (each said probe); column 31, line 31) specifically (specific (specifically); column 17, line 64) binds (binds; column 29, line 9); and correlating (correlating; column 33, line 8) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) with binding of each corresponding peptide to said TCR (peptide-MHC complex carried to cell surface and peptide is recognized by a T cell via the TCR (binding of each corresponding peptide to said TCR); column 1, lines 32-38).

Regarding claim 13, Wraith discloses a method (method; abstract) for detecting (identifying (detecting); column 9, line 47) in a library of peptides (library of peptides; column 9, line 48) competitive binding of each said peptide (dominance of a particular peptide depends on relative affinity for binding (competitive binding of each said peptide); column 8, lines 36-38) to a TCR (TCR; column 1, line 37), comprising: providing (provided (providing); abstract) a polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) for each said peptide (peptide (each said peptide), abstract), said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugate, column 31, line 65) comprising said peptide (peptide (said peptide), abstract) and a polynucleotide (DNA (polynucleotide); column 31, line 47); contacting (contacts (contacting); column 11, line 13) said TCR (TCR (said TCR); column 1, line 37) with a pool (pool; column 9, line 39) of said polynucleotide-peptide conjugates (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugates, column 31, line 65) and an MHC molecule (MHC molecule; abstract); contacting (contacts (contacting); column 11, line 13) each of (each of; column 13, line 56) said polynucleotide-peptide conjugate (DNA (polynucleotide), column 31, line 47; peptide, abstract; conjugates (conjugate (conjugates); column 31, line 65) with a probe (probe; column 31, line 31) that specifically (specific (specifically); column 17, line 64) binds to (binds to; column 29, lines 9-10) each said polynucleotide (DNA (each said polynucleotide); column 31, line 47); detecting (identifying (detecting); column 9, line 47) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) that each said probe (probe (each said probe); column 31, line 31) specifically (specific (specifically); column 17, line 64) binds (binds; column 29, line 9); and correlating (correlating; column 33, line 8) binding of (binding of; column 4, line 24) said probe (probe (said probe); column 31, line 31) to each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) polynucleotide (DNA (polynucleotide); column 31, line 47) with binding of (binding of; column 4, line 24) each (each; column 4, line 15) corresponding (corresponding; column 28, lines 15-16) peptide (peptide, abstract) to said TCR (TCR (said TCR); column 1, line 37), wherein said peptides compete for binding of said MHC molecule (dominance of a particular peptide depends on relative affinity for binding the MHC molecule (wherein said peptides compete for binding of said MHC molecule); column 8, lines 36-38) and said TCR (TCR (said TCR); column 1, line 37).

Regarding claims 14/11-14/13, Wraith discloses the method of claims 11-13, and Wraith further discloses wherein said TCR (TCR (said TCR)p; column 1, line 37) is a TCR on a T cell (TCR on a T cell; column 1, lines 36-37; column 7, lines 44-45).

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Wraith in view of US 8,337,851 B2 to Aukerman, et al. (hereinafter 'Aukerman').

Regarding claim 6, Wraith discloses the composition of claim 5, and Wraith further discloses wherein the mediation (mediated (mediation); column 2, line 51) is through (through; column 1, line 43). Wraith does not disclose wherein the mediation is through nucleotide sequence complementarity. Aukerman discloses nucleotide sequence complementarity (complement of DNA (nucleotide sequence complementarity), column 24, lines 27-31), in methods of monitoring the efficacy of anti-CD40 antibodies in treating a subject for a CD40-expressing cancer (abstract). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified a composition comprising at least two MHC-binding peptides, each conjugated to a polynucleotide, as previously disclosed by Wraith, for integrating nucleotide sequence complementarity, since the combination would have produced a therapeutic composition containing multiple peptide-DNA conjugates hybridized to one another, in order to form a multivalent conjugate.

Claims 1-14 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 699932000440	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2014/044196	International filing date *(day/month/year)* 25 June 2014	(Earliest) Priority Date *(day/month/year)* 25 June 2013
Applicant PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 6A

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/044196

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-11, 18-51, 64
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/044196

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2014.01)
CPC - C12Q 1/6809 (2014.09)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12N 15/11, 15/115; C12Q 1/68; C40B 30/04, 60/04; G01N 33/50, 33/53, 33/543, 37/00 (2014.01)
USPC - 506/8, 9, 15, 24, 32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12Q 1/6809, 1/6834, 1/6837, 1/6841, 1/6869; C40B 60/04, 30/04; G01N 33/6845, 2458/10 (2014.09)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Orbit, Google Patents, Google Scholar, Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0245111 A1 (CHEE) 06 October 2011 (06.10.2011) entire document	1-5, 12-17, 52-63
A	ROUTENBERG et al. "Microfluidic probe: a new tool for integrating microfluidic environments and electronic wafer-probing," Lab Chip, 29 October 2009 (29.10.2009), Vol. 10, Pgs. 123-127. entire document	1-5, 12-17, 52-63
A	US 2012/0270748 A1 (CHEE) 25 October 2012 (25.10.2012) entire document	1-5, 12-17, 52-63
A	US 2012/0195810 A1 (COHEN et al) 02 August 2012 902.08.2012) entire document	1-5, 12-17, 52-63
A	US 2003/0162216 A1 (GOLD et al) 28 August 2003 (28.08.2003) entire document	1-5, 12-17, 52-63

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2014	07 NOV 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Curio EX1010-p. 303
Curio v Prognosys
IPR2024-01289

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: BU YIN
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	07 NOV 2014
Applicant's or agent's file reference 699932000440	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2014/044196	25 June 2014	25 June 2013

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2014.01)
CPC - C12Q 1/6809 (2014.09)

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	10 October 2014	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 6-11, 18-51, 64

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 6-11, 18-51, 64 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 6-11, 18-51, 64 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 6-11, 18-51, 64

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	None	YES
	Claims	1-5, 12-17, 52-63	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-5, 12-17, 52-63	NO
Industrial applicability (IA)	Claims	1-5, 12-17, 52-63	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-5, 12-17, and 52-63 lack novelty under PCT Article 33(2) as being anticipated by Chee.

Regarding claim 1, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises: (1) a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); (2) an address tag that identifies each of the multiple sites to which the probe is delivered (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); and (3) an identity tag that identifies the probe's corresponding biological target or target-binding moiety (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); analyzing the probe bound to the one or more biological targets, the analysis comprising: (1) determining abundance, expression, and/or activity of each of the one or more biological targets by assessing the amount of the probe bound to the biological target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (2) determining the identities of the identity tag and the address tag of the probe (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]); and determining a spatial pattern of abundance and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), wherein the method does not depend on an imaging technique for determining spatial information of the one or more biological targets in the sample (in an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest, Para. [0080]; US 2011/024511 Claims: 1-57, do not explicitly cite an imagining technique, and because alternative elements are positively recited in the specification, they may be explicitly excluded in the claims, therefore the US 2011/024511 patent possessed the invention disclosed in the instant application's claims which include the negative limitation "the method does not depend on an imaging technique").

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 2, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises: (1) a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); (2) an address tag having a sequence that identifies each of the multiple sites to which the probe is delivered (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); and (3) an identity tag having a sequence that identifies the probe's corresponding biological target or target-binding moiety (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); analyzing the probe bound to the one or more biological targets by sequencing (determining all or a portion of a sequence of the encoded probes, Para. [0008]), wherein the amount of a sequencing product indicates abundance, expression, and/or activity of each of the one or more biological targets (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), the sequencing product comprising all or a portion of the address tag sequence and all or a portion of the identity tag sequence (In step 270, the sequence of the coding tags in the encoded probes as Well as the sequence or a portion of the sequence of the probe itself is determined, Para. [0049]); and determining a spatial pattern of abundance, expression, and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]).

Regarding claim 3, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises: (1) a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); and (2) an identity tag that identifies the probe's corresponding biological target or target-binding moiety (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering an address tag to each of the multiple sites in the sample, wherein the address tag is to be coupled to the probe bound to the biological target and identifies the site to which the address tag is delivered (delivering probes and encoding agents Where the encoding agents (in some embodiments pre-coupled to the probes) are delivered to the sample according to a known spatial pattern, Para. [0051]; a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); analyzing the probe/address tag conjugate bound to the one or more biological targets (determining all or a portion of a sequence of the encoded probes, Para. [0008]), the analysis comprising: (1) determining abundance, expression, and/or activity of each of the one or more biological targets by assessing the amount of the probe/address tag conjugate bound to the biological target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and (2) determining the identities of the identity tag and the address tag of the probe/address tag conjugate (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and determining a spatial pattern of abundance, expression, and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), wherein the method does not depend on an imaging technique for determining spatial information of the one or more biological targets in the sample (In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest, Para. [0080]; US 2011/024511 Claims: 1-57, do not explicitly cite an imagining technique, and because alternative elements are positively recited in the specification, they may be explicitly excluded in the claims, therefore the US 2011/024511 patent possessed the invention disclosed in the instant application's claims which include the negative limitation "the method does not depend on an imaging technique").

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 4, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises: (1) a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); and (2) an identity tag having a sequence that identifies the probe's corresponding biological target or target-binding moiety (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering an address tag to each of the multiple sites in the sample, wherein the address tag is to be coupled to the probe bound to the biological target and identifies the site to which the address tag is delivered (delivering probes and encoding agents Where the encoding agents (in some embodiments pre-coupled to the probes) are delivered to the sample according to a known spatial pattern, Para. [0051]; a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); analyzing the probe/address tag conjugate bound to the one or more biological targets by sequencing (determining all or a portion of a sequence of the encoded probes, Para. [0008]), wherein the amount of a sequencing product indicates abundance, expression, and/or activity of each of the one or more biological targets, the sequencing product comprising all or a portion of the address tag sequence and all or a portion of the identity tag sequence (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and determining a spatial pattern of abundance, expression, and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]).

Regarding claim 5, Chee discloses the method of claim 1, wherein the one or more biological targets are non-nucleic acid molecules (Biological targets can include any molecule of interest, such as...proteins, enzymes and the like, Para. [0042]).

Regarding claim 12, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of a target protein across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for a target protein to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; Biological tar gets can include...proteins, Para. [0042]), wherein the probe comprises: (1) a target-binding moiety capable of binding to the target protein (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); (2) a first address tag that identifies each of the multiple sites to which the probe is delivered (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); and (3) an identity tag that identifies the target protein or the target-binding moiety (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); allowing the probe to bind to the target protein in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]);
analyzing the probe bound to the target protein, the analysis comprising: (1) determining abundance, expression, and/or activity of the target protein by assessing the amount of the probe bound to the target protein (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (2) determining the identities of the identity tag and the first address tag of the probe for the target protein (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]); and determining a spatial pattern of abundance, expression, and/or activity of the target protein across the multiple sites in the sample based on the analysis (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 13, Chee discloses the method of claim 12, further comprising: delivering a probe for a target polynucleotide to each of the multiple sites in the sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; oligonucleotides to hybridize to nucleic acid targets, Para. [0044]), wherein the probe for the target polynucleotide comprises: (1) a sequence that hybridizes to and identifies the target polynucleotide; (2) a second address tag that identifies each of the multiple sites to which the probe for the target polynucleotide is delivered (the oligonucleotide probes are allowed to interact with (hybridize to) the nucleic acid targets, Para. [0047]; coding tags, which identify the positions in the sample of the biological targets being assayed, Para. [0042]); allowing the probe for the target polynucleotide to bind to the target polynucleotide in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); analyzing the probe bound to the target polynucleotide, the analysis comprising: (1) determining abundance, expression, and/or activity of the target polynucleotide by assessing the amount of the probe bound to the target polynucleotide (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (2) determining the identities of the sequence that hybridizes to and identifies the target polynucleotide and the second address tag of the probe for the target polynucleotide (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and determining a spatial pattern of abundance, expression, and/or activity of the target polynucleotide across the multiple sites in the sample based on the analysis of the probe bound to the target polynucleotide at each of the multiple sites in the sample (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]).

Regarding claim 14, Chee discloses method of determining a spatial pattern of abundance, expression, and/or activity of a target protein across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for a target protein to multiple sites in the sample, wherein the probe comprises: (1) a target-binding moiety capable of binding to the target protein (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; Biological tar gets can include…proteins, Para. [0042]); and (2) an identity tag that identifies the target protein or the protein-binding moiety (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]) allowing the probe to bind to the target protein in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering a first address tag to each of the multiple sites in the sample, wherein the first address tag is to be coupled to the probe bound to the target protein and identifies the site to which it is delivered (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; Biological tar gets can include…proteins, Para. [0042]); analyzing the probe/first address tag conjugate bound to the target protein, the analysis comprising: (1) determining abundance, expression, and/or activity of the target protein by assessing the amount of the probe/first address tag conjugate bound to the target protein (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (2) determining the identities of the identity tag and the first address tag of the probe/first address tag conjugate; and determining a spatial pattern of abundance, expression, and/or activity of the target protein across the multiple sites in the sample based on the analysis (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 15, Chee discloses the method of claim 14, further comprising: delivering a probe for a target polynucleotide to each of the multiple sites in the sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; oligonucleotides to hybridize to nucleic acid targets, Para. [0044]), wherein the probe for the target polynucleotide comprises a sequence that hybridizes to and identifies the target polynucleotide (the oligonucleotide probes are allowed to interact with (hybridize to) the nucleic acid targets, Para. [0047]; coding tags, which identify the positions in the sample of the biological targets being assayed, Para. [0042]); allowing the probe for the target polynucleotide to bind to the target polynucleotide in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering a second address tag to each of the multiple sites in the sample, wherein the second address tag is to be coupled to the probe bound to the target polynucleotide and identifies the site to which it is delivered a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); analyzing the probe/second address tag conjugate bound to the target polynucleotide (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), the analysis comprising: (1) determining abundance, expression, and/or activity of the target polynucleotide by assessing the amount of the probe/second address tag conjugate bound to the target polynucleotide (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and (2) determining the identities of the sequence that hybridizes to and identifies the target polynucleotide and the second address tag of the probe/second address tag conjugate (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and determining a spatial pattern of abundance, expression, and/or activity of the target polynucleotide across the multiple sites in the sample based on the analysis of the probe/second address tag conjugate bound to the target polynucleotide at each of the multiple sites in the sample (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]).

Regarding claim 16, Chee discloses the method of claim 15, wherein the target polynucleotide or the complement thereof encodes all or a portion of the target protein (Protein-based probes are typically conjugated or otherwise linked to oligonucleotide encoding agents. The oligonucleotide encoding agents in this case would also include a nucleotide sequence component that allows for identification of the protein probe, Para. [0059]).

Regarding claim 17, Chee discloses the method of claim 15, wherein the step of analyzing the probe or probe/first address tag conjugate bound to the target protein and the step of analyzing the probe or probe/second address tag conjugate bound to the target polynucleotide are performed in parallel in the same reaction run (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application; In other aspects, the sequence of at least fifty thousand encoding probes are determined in parallel, Para. [0011]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 52, Chee discloses a system for determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (The invention encompasses assay systems that provide high-resolution spatial maps of biological activity in tissues, Para. [0007]; the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: a first module for delivering a probe for each of one or more biological targets to multiple sites in a sample target (delivering encoded probes for the multiple biological targets to the multiple sites in the sample..Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]), wherein each probe comprises: (1) a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); and (2) an identity tag that identifies the probe's corresponding biological target or target-binding moiety (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]);
a second module for delivering an address tag to each of the multiple sites in the sample, wherein the address tag is to be coupled to the probe bound to the biological target and identifies the site to which the address tag is delivered (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); a third module for analyzing the probe/address tag conjugate bound to the one or more biological targets (assay system to determine spatial patterns of abundance or activity or both of multiple protein targets at multiple sites in a sample, Where the assay system performs the following steps, Para. [0015]), the analysis comprising: (1) determining abundance, expression, and/or activity of the one or more biological targets by assessing the amount of the probe/address tag conjugate bound to the biological target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (2) determining the identities of the identity tag and the address tag of the probe/address tag conjugate (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]); and a fourth module for determining a spatial pattern of abundance, expression (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis, wherein the system does not depend on an imaging technique for determining spatial information of the one or more biological targets in the sample (In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest, Para. [0080]; US 2011/024511 Claims: 1-57, do not explicitly cite an imagining technique, and because alternative elements are positively recited in the specification, they may be explicitly excluded in the claims, therefore the US 2011/024511 patent possessed the invention disclosed in the instant application's claims which include the negative limitation "the method does not depend on an imaging technique").

Regarding claim 53, Chee discloses the system of claim 52, wherein the second module comprises one or more microfluidic devices for delivering the address tags (Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]).

Regarding claim 54, Chee discloses the system of claim 53, wherein the one or more microfluidic devices comprise a first set of multiple addressing channels, each delivering a different first address tag to the sample (Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; delivering one or more encoding agents to locations of the multiple sites in the sample according to a known spatial pattern, Where the combination of encoding agents delivered to each site is different, Para. [0014]).

Regarding claim 55, Chee discloses the system of claim 54, wherein the one or more microfluidic devices further comprise a second set of multiple addressing channels, each delivering a different second address tag to the sample (Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; delivering one or more encoding agents to locations of the multiple sites in the sample according to a known spatial pattern, Where the combination of encoding agents delivered to each site is different, Para. [0014]).

Regarding claim 56, Chee discloses the method of claim 55, wherein the multiple sites in the sample are chosen by the first and second set of multiple addressing channels cooperatively delivering the first address tags and the second address tags, respectively, to each of the multiple sites, each site identified by a different combination of first and second address tags (Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; independently actuated nozzles can be used to deliver multiple reagents at the same time, Para. [0082]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 57, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering at least one adaptor to the multiple sites in the sample (Following ligation, the encoded probes are eluted, pooled, and, optionally, sequencing adapters are added to the encoded probes, Para. [0056]), wherein the at least one adaptor specifically binds to the probe and comprises an address tag that identifies each of the multiple sites to which the at least one adaptor is delivered (The first encoded probe 420 comprises coding tag 408, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies, Para. [0062]), wherein the probe and/or the adaptor comprises an identity tag that identifies the probe's and/or adaptor's corresponding biological target or target-binding moiety (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); analyzing the at least one adaptor and the probe bound to the one or more biological targets, the analysis comprising: (1) determining abundance, expression, and/or activity of each of the one or more biological targets by assessing the amount of at least one adaptor bound to the probe bound to the biological target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (2) determining the identities of the identity tag, and the address tag of the at least one adaptor (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]); and determining a spatial pattern of abundance and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), wherein the method does not depend on an imaging technique for determining spatial information of the one or more biological targets in the sample (In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest, Para. [0080]; US 2011/024511 Claims: 1-57, do not explicitly cite an imagining technique, and because alternative elements are positively recited in the specification, they may be explicitly excluded in the claims, therefore the US 2011/024511 patent possessed the invention disclosed in the instant application's claims which include the negative limitation "the method does not depend on an imaging technique").

Regarding claim 58, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering at least one adaptor to the multiple sites in the sample (Following ligation, the encoded probes are eluted, pooled, and, optionally, sequencing adapters are added to the encoded probes, Para. [0056]), wherein the at least one adaptor specifically binds to the probe and comprises an address tag that identifies each of the multiple sites to which the at least one adaptor is delivered, wherein the probe and/or the adaptor comprises an identity tag that identifies the probe's and/or adaptor's corresponding biological target or target-binding moiety (The first encoded probe 420 comprises coding tag 408, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies, Para. [0062]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; independently actuated nozzles can be used to deliver multiple reagents at the same time, Para. [0082]); analyzing the at least one adaptor and the probe bound to the one or more biological targets by sequencing, wherein the amount of a sequencing product indicates abundance, expression, and/or activity of each of the one or more biological targets, the sequencing product comprising all or a portion of the address tag sequence and all or a portion of the identity tag sequence (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and determining a spatial pattern of abundance, expression, and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); wherein the method does not depend on an imaging technique for determining spatial information of the one or more biological targets in the sample (In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest, Para. [0080]; US 2011/024511 Claims: 1-57, do not explicitly cite an imagining technique, and because alternative elements are positively recited in the specification, they may be explicitly excluded in the claims, therefore the US 2011/024511 patent possessed the invention disclosed in the instant application's claims which include the negative limitation "the method does not depend on an imaging technique").

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 59, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises a target-binding moiety capable of binding to the probe's corresponding biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]);
allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering at least one adaptor to the multiple sites in the sample, wherein the at least one adaptor specifically binds to the probe (Following ligation, the encoded probes are eluted, pooled, and, optionally, sequencing adapters are added to the encoded probes, Para. [0056]), wherein the probe and/or the adaptor comprises an identity tag that identifies the probe's and/or adaptor's corresponding biological target or target-binding moiety (The first encoded probe 420 comprises coding tag 408, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies, Para. [0062]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; independently actuated nozzles can be used to deliver multiple reagents at the same time, Para. [0082]);
delivering an address tag to each of the multiple sites in the sample (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]), wherein the address tag is to be coupled to the at least one adaptor bound to the probe bound to the biological target and identifies the site to which the address tag is delivered (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); analyzing the adaptor/address tag conjugate, the analysis comprising: (1) determining abundance, expression, and/or activity of each of the one or more biological targets by assessing the amount of the adaptor/address tag conjugate bound to the probe bound to the biological target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (2) determining the identities of the identity tag, and the address tag of the adaptor/address tag conjugate; and determining a spatial pattern of abundance, expression, and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]), wherein the method does not depend on an imaging technique for determining spatial information of the one or more biological targets in the sample (In an optional embodiment, the assay system comprises imaging means to determine features and organization of the biological sample of interest, Para. [0080]; US 2011/024511 Claims: 1-57, do not explicitly cite an imagining technique, and because alternative elements are positively recited in the specification, they may be explicitly excluded in the claims, therefore the US 2011/024511 patent possessed the invention disclosed in the instant application's claims which include the negative limitation "the method does not depend on an imaging technique").

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044196

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 60, Chee discloses a method of determining a spatial pattern of abundance, expression, and/or activity of one or more biological targets across multiple sites in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: delivering a probe for each of one or more biological targets to multiple sites in a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), wherein each probe comprises a target-binding moiety capable of binding to the probe's corresponding biological target Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); allowing each probe to bind to its corresponding biological target in the sample (allowing the encoded probes to interact With the biological targets, Para. [0008]); delivering at least one adaptor to the multiple sites in the sample, wherein the at least one adaptor specifically binds to the probe (Following ligation, the encoded probes are eluted, pooled, and, optionally, sequencing adapters are added to the encoded probes, Para. [0056]), wherein the probe and/or the adaptor comprises an identity tag that identifies the probe's and/or adaptor's corresponding biological target or target-binding moiety (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); delivering an address tag to each of the multiple sites in the sample (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]), wherein the address tag is to be coupled to the at least one adaptor bound to the probe bound to the biological target and identifies the site to which the address tag is delivered (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); analyzing the adaptor/address tag conjugate by sequencing, wherein the amount of a sequencing product indicates abundance, expression, and/or activity of each of the one or more biological targets, the sequencing product comprising all or a portion of the address tag sequence and all or a portion of the identity tag sequence (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and determining a spatial pattern of abundance, expression, and/or activity of the one or more biological targets across the multiple sites in the sample based on the analysis (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample, Para. [0014]).

Regarding claim 61, Chee discloses the method of claim 57, wherein at least two adaptors are delivered to each of the multiple sites in the sample (The final construct comprising first adapter 327, first primer region 319, first coding tag 315, ligation regions 311 and 301, target region 309, ligation regions 313 and 303, second coding tag 317, second primer region 325 and second adapter 329 is now ready for input into a digital high-throughput sequencing process, Para. [0056]; See FIG. 3), wherein the at least two adaptors each specifically binds to one probe that specifically binds to the biological target (shows two target-specific/encoding oligonucleotide constructs 420 and 422 (e.g., formed betWeen steps 302 and 304 of FIG. 3) specifically bound to a target nucleic acid 402 of interest, Para. [0062]).

Regarding claim 62, Chee discloses the method of claim 61, wherein the at least two adaptors are joined (As seen in FIG. 3, each sequencing adapter comprises primer region 319 or 321, compatible With the primer regions 319 and 321 on the encoded probes. The ? nal construct comprising first adapter 327, first primer region 319, first coding tag 315, ligation regions 311 and 301, target region 309, ligation regions 313 and 303, second coding tag 317, second primer region 325 and second adapter 329, Para. [0056]; See FIG. 3).

Regarding claim 63, Chee discloses the method of claim 62, wherein the at least two adaptors are ligated by using a portion of the probe sequence as a splint (As seen in FIG. 3, each sequencing adapter comprises primer region 319 or 321, compatible With the primer regions 319 and 321 on the encoded probes. The ?nal construct comprising first adapter 327, first primer region 319, first coding tag 315, ligation regions 311 and 301, target region 309, ligation regions 313 and 303, second coding tag 317, second primer region 325 and second adapter 329, Para. [0056]; See FIG. 3).

Claims 1-5, 12-17, and 52-63 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 699932000640	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2014/064588	International filing date *(day/month/year)* 07 November 2014	(Earliest) Priority Date *(day/month/year)* 08 November 2013
Applicant PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 4

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/064588

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/064588

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-27, 31-59, 68-71, 82, 83, 88-107, 111, 113, 115
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/064588

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/064588

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C40B 30/04 (2015.01)
CPC - C40B 30/04 (2015.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C40B 20/04, 30/04, 40/06, 50/06 (2015.01)
USPC - 506/9; 506/4; 506/16, 506/26

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C40B 20/04, 30/04, 40/06, 50/06 (2015.01) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google Scholar, Google, PubMed

Search terms used: polynucleotide polypeptide conjugate, antibody, antigen, aptamer, probe, manufacture, hybridization

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0245101 A1 (CHEE et al) 06 October 2011 (06.10.2011) entire document	1-3, 28-30, 60-67, 72-74, 84-87, 108-110, 112, 114, 116
--		----------
Y		76-81
Y	US 2012/0258871 A1 (KOZLOV et al) 11 October 2012 (11.10.2012) entire document	75
Y	US 2013/0096033 A1 (PROGNOSYS BIOSCIENCES, INC) 18 April 2013 (18.04.2013) entire document	75-81

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2015	11 MAR 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: BU YIN
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	11 MAR 2015
Applicant's or agent's file reference 699932000640	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2014/064588	07 November 2014	08 November 2013

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 30/04 (2015.01)
CPC - C40B 30/04 (2015.01)

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	11 February 2015	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2014/064588

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 4-27, 31-59, 68-71, 82, 83, 88-107, 111, 113, 115

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. see below are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 4-27, 31-59, 68-71, 82, 83, 88-107, 111, 113, and 115 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 4-27, 31-59, 68-71, 82, 83, 88-107, 111, 113, 115

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	75-81	YES
	Claims	1-3, 28-30, 60-67, 72-74, 84-87, 108-110, 112, 114, 116	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-3, 28-30, 60-67, 72-81, 84-87, 108-110, 112, 114 and 116	NO
Industrial applicability (IA)	Claims	1-3, 28-30, 60-67, 72-81, 84-87, 108-110, 112, 114 and 116	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-3, 28-30, 60-67, 72-74, 84-87, 108-110, 112, 114, and 116 lack novelty under PCT Article 33(2) as being anticipated by Chee et al. (hereafter Chee).

Regarding claim 1, Chee discloses a pair of polypeptide-polynucleotide conjugates (a binding pair comprising a protein and a different chemical moiety, Para. [0008]), comprising: a first polypeptide-polynucleotide conjugate comprising (providing a first set of binding constructs immobilized on a support surface, Para. [0009]): (a) a first polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a first identifying sequence (and a first nucleic acid tag unique to the first binding agent, Para. [0009]) that identifies the first polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]); and a second polypeptide-polynucleotide conjugate comprising (providing a second set of binding constructs in solution, Para. [0009]), (a) a second polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a second epitope for specific binding of the antibody or antigen binding fragment thereof (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a second identifying sequence (and a second nucleic acid tag unique to the second binding agent, Para. [0009]) that identifies the second polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]), wherein the first and second engaging sequences are capable of engaging the first and second polynucleotides, and thereby engaging the first and second polypeptide-polynucleotide conjugates (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]).

Regarding claim 2, Chee discloses the pair of claim 1, wherein the first engaging sequence (The term "binding agent" as used herein refers to any binding agent that selectively binds to a molecule of interest, Para. [0033]) comprises a sequence hybridizable (The term also includes complementary nucleic acid molecules that selectively hybridize at or above a desired melting temperature, Para. [0034]) to the second engaging sequence, and/or the second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]) comprises a sequence hybridizable (The term also includes complementary nucleic acid molecules that selectively hybridize at or above a desired melting temperature, Para. [0034]) to the first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]).

Regarding claim 3, Chee discloses the pair of claim 1, wherein the first engaging sequence comprises a sequence complementary ("Complementary" or "substantially complementary" refers to the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, Para. [0035]) to all or a portion of the second engaging sequence (The term "binding pair" means any two molecules (binding agents) that are known to bind selectively to one another. In the case of two proteins, the proteins bind selectively to one another with a high affinity as described in more detail herein, Para. [0034]), and/or the second engaging sequence comprises a sequence complementary to all or a portion of the first engaging sequence.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 28, Chee discloses a method for analyzing an antibody or antigen binding fragment thereof in a sample (a method for identifying binding agents that form a binding pair, Para. [0016]), comprising: (i) immobilizing a first polypeptide-polynucleotide conjugate on a substrate (comprising: providing a first set of binding constructs immobilized on a support surface, Para. [0016]), the first polypeptide-polynucleotide conjugate comprising: (a) a first polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof (the first and second binding agents (binding pair) are provided as antibodies or variable domains of antibodies that bind to different epitopes on a common molecule, Para. [0077]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (a first primer region, Para. [0016]), a first identifying sequence (a first nucleic acid tag unique to the first binding agent, Para. [0016]) that identifies the first polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0016]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0016]); (ii) contacting the substrate having the immobilized first polypeptide-polynucleotide conjugate (providing a first set of binding constructs immobilized on a support surface, Para. [0016]) with a sample containing or suspected of containing the antibody or antigen binding fragment thereof (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]), whereby the first epitope specifically binds to the antibody or antigen binding fragment thereof in the sample (the method further comprises the step of adding a third binding agent in the combining step, Para. [0035]), (iii) contacting the substrate having the immobilized first polypeptide-polynucleotide conjugate with a second polypeptide-polynucleotide conjugate (providing a second set of binding constructs in solution, Para. [0016]), the second polypeptide-polynucleotide conjugate comprising: (a) a second polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a second epitope for specific binding of the antibody or antigen binding fragment thereof (the first and second binding agents (binding pair) are provided as antibodies or variable domains of antibodies that bind to different epitopes on a common molecule, Para. [0077]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (a second primer region, Para. [0016]), a second identifying sequence (a second nucleic acid tag unique to the second binding agent, Para. [0016]) that identifies the second polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0016]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0016]), whereby the second epitope specifically binds to the antibody or antigen binding fragment thereof specifically bound to the first epitope (The presence and binding of both the first (A) and second (S) binding agents is necessary for the detection of the third molecule, Para. [0077]), and whereby the first and second polynucleotides are engaged via the first and second engaging sequences (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, Para. [0016]); and (iv) analyzing the first and/or/or second polynucleotides engaged via the first and second engaging sequences (determining a sequence of at least one thousand binding event identifiers, where the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair, Para. [0016]), wherein the analysis in step (iv) indicates the presence, absence, and/or amount (and determining the frequency of each binding event identifier sequenced, Para. [0016]) of the antibody or antigen binding fragment thereof in the sample (In certain other assay schemes of the invention, however, the first and second binding agents may be analyzed for their ability to bind a third agent or analyte in addition to or in place of binding to one another, Para. [0076]).

Regarding claim 29, Chee discloses the method of claim 28, wherein the substrate (a support surface comprising multiple anchors, Para. 0067]) comprises a microarray (the binding event identifiers could be amplified and hybridized to a microarray, Para. [0089]; wherein the support is a microarray, Claim 22) and/or a bead (a flowcell or a bead, Para. [0067]; wherein the support is a bead, Claim 23).

Regarding claim 30, Chee discloses the method of claim 28, wherein the first engaging sequence (The term "binding agent" as used herein refers to any binding agent that selectively binds to a molecule of interest, Para. [0033]) comprises a sequence hybridizable (The term also includes complementary nucleic acid molecules that selectively hybridize at or above a desired melting temperature, Para. [0034]) to the second engaging sequence, and/or the second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0016]) comprises a sequence hybridizable (The term also includes complementary nucleic acid molecules that selectively hybridize at or above a desired melting temperature, Para. [0034]) to the first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0016]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 60, Chee discloses a method for analyzing an antibody or antigen binding fragment thereof in a sample (for detection of an agent or analyte using two sets of binding constructs, Para. [0087]), comprising: (i) immobilizing a first polypeptide-polynucleotide conjugate on a substrate (where the binding construct is ligated using a splint sequence 1333 to a anchor 1305 attached to a solid support, Para. [0087], Fig. 13), the first polypeptide-polynucleotide conjugate comprising (the first binding construct used in the assay is illustrated, Para. [0087]): (a) a first polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof (An antibody 1301 is attached to a nucleic acid portion of the first binding construct via a cleavable linker 1335, Para. [0087]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (Sequence 1311 is a primer sequence, Para. [0087]), a first identifying sequence that identifies the first polypeptide (second unique nucleic acid tag 1323, the complement to second unique nucleic acid tag 1313, Para. [0088]), and a first engaging sequence (and primer/hybridization region 1325, the complement to primer/hybridization region 1315, Para. [0088]); (ii) providing a second polypeptide-polynucleotide conjugate (a second binding construct, Para. [0088]), the second polypeptide-polynucleotide conjugate comprising: (a) a second polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a second epitope for specific binding of the antibody or antigen binding fragment thereof (Binding of the binding agent 1303 of the second set of binding constructs to target agent 1337, Para. [0087]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (a second binding construct, blocked at the 3' end 1329, Para. [0088]) a second identifying sequence that identifies the second polypeptide (The second binding construct comprises a unique nucleic acid tag 1313 that identifies the second binding agent present in the second binding construct, Para. [0088]), and a second engaging sequence (the complement to primer/hybridization region 1315, Para. [0088]), wherein the second polypeptide-polynucleotide conjugate forms a complex with the immobilized first polypeptide-polynucleotide conjugate (a second binding construct, blocked at the 3' end 1329 (black square) so that it cannot be extended, is bound to the first binding construct via target agent 1337, Para. [0088]); (iii) contacting the substrate having the complex between the first and second polypeptide-polynucleotide conjugates with a sample containing or suspected of containing the antibody or antigen binding fragment thereof (In the first part of the assay, a target agent 1337 is captured, Para. [0087]), wherein at least one molecule of the second polypeptide-polynucleotide conjugate specifically binds to the antibody or antigen binding fragment thereof specifically bound to the first polypeptide-polynucleotide conjugate (Binding of the binding agent 1303 of the second set of binding constructs to target agent 1337 may be done in solution prior to exposing the second set of binding constructs to the first set of binding constructs, Para. [0087]), thereby forming a ternary complex, (In the first part of the assay, a target agent 1337 is captured, Para. [0087]); (iv) washing the substrate under conditions that release molecules of the second polypeptide-polynucleotide conjugate that are not specifically bound to the antibody or antigen binding fragment thereof, while maintaining the ternary complex formed by specific binding (the cleavable linker 1335 attaching the first binding agent 1301 to the first binding construct is cleaved, and washing removes the second binding construct, the first antibody and target agent. The primer-extended construct attached to the support surface can now be assayed, Para. [0088]); (v) providing conditions under which the first and second polynucleotides are engaged via the first and second engaging sequences in the ternary complex formed by specific binding (The binding event identifiers comprise a combination of two unique nucleic acid tags, one present on each of the binding constructs, and the association of these unique nucleic acid tags, e.g., through incorporation into a single oligonucleotide. Para. [0089]); and (vi) analyzing the first and/or second polynucleotides engaged via the first and second engaging sequences, wherein the analysis in step (vi) indicates the presence, absence, and/or amount of the antibody or antigen binding fragment thereof in the sample (Numerous methods can be used to identify the binding event identifiers used in the assay systems of the invention, Para. [0089]).

Regarding claim 61, Chee discloses the method of claim 60, wherein the complex between the first and second polypeptide-polynucleotide conjugates in step (ii) (a second binding construct, blocked at the 3' end 1329 (black square) so that it cannot be extended, is bound to the first binding construct via target agent 1337, Para. [0088], Fig. 13) is formed by hybridization between the first and second polynucleotides (and primer/hybridization region 1325, the complement to primer/hybridization region 1315, Para. [0088], Fig. 13).

Regarding claim 62, Chee discloses the method of claim 61, wherein the washing step is performed under conditions that destabilize the hybridization between the first and second polynucleotides thereby releasing molecules of the second polypeptide-polynucleotide conjugate that are not specifically bound to the antibody or antigen binding fragment thereof (the cleavable linker 1335 attaching the first binding agent 1301 to the first binding construct is cleaved, and washing removes the second binding construct, the first antibody 1301 and target agent 1337, Para. [0088], Fig. 13).

Regarding claim 63, Chee discloses the method of claim 61, wherein the sequences involved in the hybridization between the first and second polynucleotides comprise one or more cleavable sites (An antibody 1301 is attached to a nucleic acid portion of the first binding construct via a cleavable linker 1335, Para. [0087], Fig. 13).

Regarding claim 64, Chee discloses the method of claim 63, wherein the sequences involved in the hybridization are cleaved at the one or more cleavable sites (In 13D, the cleavable linker 1335 attaching the first binding agent 1301 to the first binding construct is cleaved, Para. [0088], Fig. 13) before or during the washing step thereby destabilizing the hybridization between the first and second polynucleotides (and washing removes the second binding construct, the first antibody 1301 and target agent 1337, Para. [0088], Fig. 13).

Regarding claim 65, Chee discloses the method of claim 61, wherein the second polypeptide-polynucleotide conjugate is kept in physical proximity to the first polypeptide-polynucleotide conjugate by a cleavable linker (An antibody 1301 is attached to a nucleic acid portion of the first binding construct via a cleavable linker 1335, Para. [0087], Fig. 13) before or after the first and second polypeptide-polynucleotide conjugates form the complex in step (ii) (may be applied separately or simultaneously to the support surface (i.e., without a previous opportunity to interact), Para. [0087], Fig. 13).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 66, Chee discloses the method of claim 65, wherein the cleavable linker is between the first and second polypeptide-polynucleotide conjugates, or between the second polypeptide polynucleotide conjugate and the substrate (An antibody 1301 is attached to a nucleic acid portion of the first binding construct via a cleavable linker 1335, Para. [0087]), Fig. 13).

Regarding claim 67, Chee discloses the method of claim 66, wherein the cleavable linker is cleaved (In 13D, the cleavable linker 1335 attaching the first binding agent 1301 to the first binding construct is cleaved, Para. [0088]) before or during the washing step (and washing removes the second binding construct, the first antibody 1301 and target agent 1337, Para. [0088]), optionally under conditions that destabilize the hybridization between the first and second polynucleotides.

Regarding claim 72, Chee discloses a method for analyzing an antibody or antigen binding fragment thereof in a sample (a method for identifying binding agents that form a binding pair, Para. [0016]), comprising: (i) providing a first polypeptide-polynucleotide conjugate immobilized on a substrate (comprising: providing a first set of binding constructs immobilized on a support surface, Para. [0016]), the first polypeptide-polynucleotide conjugate comprising: (a) a first polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof (the first and second binding agents (binding pair) are provided as antibodies or variable domains of antibodies that bind to different epitopes on a common molecule, Para. [0077]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a primer sequence (a first primer region, Para. [0016]), a first identifying sequence (a first nucleic acid tag unique to the first binding agent, Para. [0016]) that identifies the first polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0016]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0016]); (ii) contacting the substrate having the immobilized first polypeptide-polynucleotide conjugate (providing a first set of binding constructs immobilized on a support surface, Para. [0016]) with a sample containing or suspected of containing the antibody or antigen binding fragment thereof (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]), whereby the first epitope specifically binds to the antibody or antigen binding fragment thereof in the sample (the method further comprises the step of adding a third binding agent in the combining step, Para. [0035]); (iii) contacting the substrate having the immobilized first polypeptide-polynucleotide conjugate with a second polypeptide-polynucleotide conjugate (providing a second set of binding constructs in solution, Para. [0016]), the second polypeptide-polynucleotide conjugate comprising: (a) a second polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a second epitope for specific binding of the antibody or antigen binding fragment thereof (the first and second binding agents (binding pair) are provided as antibodies or variable domains of antibodies that bind to different epitopes on a common molecule, Para. [0077]); and (b) a second polynucleotide comprising a second identifying sequence (a second nucleic acid tag unique to the second binding agent, Para. [0016]) that identifies the second polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0016]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0016]), whereby the second epitope specifically binds to the antibody or antigen binding fragment thereof specifically bound to the first epitope (The presence and binding of both the first (A) and second (S) binding agents is necessary for the detection of the third molecule, Para. [0077]), and whereby the first and second polynucleotides are engaged via the first and second engaging sequences (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, Para. [0016]); and (iv) analyzing the first and/or second polynucleotides engaged via the first and second engaging sequences (determining a sequence of at least one thousand binding event identifiers, where the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair, Para. [0016]), wherein the analysis in step (iv) indicates the presence, absence, and/or amount (and determining the frequency of each binding event identifier sequenced, Para. [0016]) of the antibody or antigen binding fragment thereof in the sample (In certain other assay schemes of the invention, however, the first and second binding agents may be analyzed for their ability to bind a third agent or analyte in addition to or in place of binding to one another, Para. [0076]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 73, Chee discloses a method for analyzing an antibody or antigen binding fragment thereof in a sample (a method for identifying binding agents that form a binding pair, Para. [0016]), comprising: (i) providing a first polypeptide-polynucleotide conjugate immobilized on a substrate (comprising: providing a first set of binding constructs immobilized on a support surface, Para. [0016]), the first polypeptide-polynucleotide conjugate comprising: (a) a first polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof (the first and second binding agents (binding pair) are provided as antibodies or variable domains of antibodies that bind to different epitopes on a common molecule, Para. [0077]); and (b) a first polynucleotide comprising a first identifying sequence (a first nucleic acid tag unique to the first binding agent, Para. [0016]) that identifies the first polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0016]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0016]); (ii) contacting the substrate having the immobilized first polypeptide-polynucleotide conjugate (providing a first set of binding constructs immobilized on a support surface, Para. [0016]) with a sample containing or suspected of containing the antibody or antigen binding fragment thereof (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]), whereby the first epitope specifically binds to the antibody or antigen binding fragment thereof in the sample (the method further comprises the step of adding a third binding agent in the combining step, Para. [0035]); (iii) contacting the substrate having the immobilized first polypeptide-polynucleotide conjugate with a second polypeptide-polynucleotide conjugate (providing a second set of binding constructs in solution, Para. [0016]), the second polypeptide-polynucleotide conjugate comprising: (a) a second polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a second epitope for specific binding of the antibody or antigen binding fragment thereof (the first and second binding agents (binding pair) are provided as antibodies or variable domains of antibodies that bind to different epitopes on a common molecule, Para. [0077]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a primer sequence (a second primer region, Para. [0016]), a second identifying sequence (a second nucleic acid tag unique to the second binding agent, Para. [0016]) that identifies the second polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0016]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0016]), whereby the second epitope specifically binds to the antibody or antigen binding fragment thereof specifically bound to the first epitope (The presence and binding of both the first (A) and second (S) binding agents is necessary for the detection of the third molecule, Para. [0077]), and whereby the first and second polynucleotides are engaged via the first and second engaging sequences (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, Para. [0016]); and (iv) analyzing the first and/or second polynucleotides engaged via the first and second engaging sequences determining a sequence of at least one thousand binding event identifiers, where the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair, Para. [0016]), wherein the analysis in step (iv) indicates the presence, absence, and/or amount (and determining the frequency of each binding event identifier sequenced, Para. [0016]) of the antibody or antigen binding fragment thereof in the sample (In certain other assay schemes of the invention, however, the first and second binding agents may be analyzed for their ability to bind a third agent or analyte in addition to or in place of binding to one another, Para. [0076]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 74, Chee discloses a method for analyzing a plurality (at least ten different binding agents, Para. [0009]) of antibodies or antigen binding fragments thereof in a sample (a method for identifying binding agents that form a binding pair, Para. [0009]), comprising: (i) immobilizing a plurality of first polypeptide-polynucleotide conjugates on a substrate (providing a first set of binding constructs immobilized on a support surface, Para. [0009]), each of the first polypeptide-polynucleotide conjugates comprising: (a) a first polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof in a plurality (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]) of antibodies or antigen binding fragments thereof (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a first identifying sequence (and a first nucleic acid tag unique to the first binding agent, Para. [0009]) that identifies the first polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]); (ii) contacting the substrate having the immobilized plurality of first polypeptide-polynucleotide conjugates (providing a first set of binding constructs immobilized on a support surface, Para. [0016]) with a sample containing or suspected of containing at least one of the plurality (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]) of antibodies or antigen binding fragments (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]), whereby the at least one antibody or antigen binding fragment in the sample specifically binds to a first epitope of the immobilized plurality of first polypeptide-polynucleotide conjugates (the method further comprises the step of adding a third binding agent in the combining step, Para. [0035]); (iii) contacting the substrate having the immobilized plurality of first polypeptide-polynucleotide conjugates with a plurality of second polypeptide-polynucleotide conjugates (providing a second set of binding constructs in solution, Para. [0009]), each of the second polypeptide-polynucleotide conjugates comprising: (a) a second polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a second epitope for specific binding of an antibody or antigen binding fragment thereof in the plurality (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]) of antibodies or antigen binding fragments (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a second identifying sequence (and a second nucleic acid tag unique to the second binding agent, Para. [0009]) that identifies the second polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]), whereby the at least one antibody or antigen binding fragment specifically bound to the first epitope specifically binds to a second epitope of the plurality of second polypeptide-polynucleotide conjugates (The presence and binding of both the first (A) and second (S) binding agents is necessary for the detection of the third molecule, Para. [0077]), and whereby the first and second polypeptide-polynucleotide conjugates specifically bound to the same antibody or antigen binding fragment are engaged via the first and second engaging sequences (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]); and (iv) analyzing the first and/or second polypeptide-polynucleotide conjugates engaged via the first and second engaging sequences (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), wherein the analysis in step (iv) indicates the presence, absence, and/or amount of the plurality of antibodies or antigen binding fragments thereof in the sample (and determining a sequence of each binding event identifier; wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair, Para. [0009]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2014/064588

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 84, Chee discloses a set of polypeptide-polynucleotide conjugates (detection of an agent or analyte using two sets of binding constructs, Para. [0087]), Fig. 13), comprising: a first polypeptide-polynucleotide conjugate (the first binding construct used in the assay, Para. [0087]) comprising: (a) a first polypeptide comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof (An antibody 1301 is attached to a nucleic acid portion of the first binding construct via a cleavable linker 1335, Para. [0087]); and (b) a first polynucleotide comprising a first primer sequence (Sequence 1311 is a primer sequence, Para. [0087]), a first identifying sequence that identifies the first polypeptide, and a first engaging sequence (The binding construct further comprises a unique nucleic acid tag 1319 that identifies the binding agent, Para. [0087]); a second polypeptide-polynucleotide conjugate comprising: (a) a second polypeptide (a second binding construct, blocked at the 3' end 1329 (black square) so that it cannot be extended, Para. [0088]) comprising a second epitope for specific binding of the antibody or antigen binding fragment thereof (is bound to the first binding construct via target agent 1337, Para. [0088]); and (b) a second polynucleotide comprising a second identifying sequence that identifies the second polypeptide (The second binding construct comprises a unique nucleic acid tag 1313 that identifies the second binding agent present in the second binding construct, Para. [0088]), a second engaging sequence (a primer/hybridization region 1315, Para. [0088]), and optionally a second primer sequence; and a third polypeptide-polynucleotide conjugate comprising (in C, primer extension is carried out resulting in the first binding construct extended to comprise, Para. [0088]): (a) an antibody portion (first unique nucleic acid sequence tag 1319, Para. [0088]) that specifically binds to the Fe portion of the antibody or antigen binding fragment thereof (The binding construct further comprises a unique nucleic acid tag 1319 that identifies the binding agent (in this case, an antibody), Para. [0087]); and (2) a third polynucleotide that comprises a third primer sequence (primer 1311, Para. [0088]), a third identifying sequence that identifies the antibody portion (the complement to second unique nucleic acid tag 1313, Para. [0088]), and a third engaging sequence (the complement to primer/hybridization region 1315, Para. [0088]), wherein the first and second engaging sequences are capable of engaging the first and second polynucleotides (Binding of the binding agent 1303 of the second set of binding constructs to target agent 1337, Para. [0087]), wherein the first or second polynucleotide is capable of being extended by a polymerase after the first and second polynucleotides are engaged (The primer-extended construct attached to the support surface can now be assayed. For example, it can be amplified using either surface PCR, Para. [0088]), wherein the extended (primer extension is carried out resulting in the first binding construct extended, Para. [0088]) first or second polynucleotide is capable of engaging the third polynucleotide (the second set of binding constructs comprising binding agents 1303 and target agent 1337 may be applied separately or simultaneously to the support surface, Para. [0087]), and wherein the extended first or second polynucleotide is capable of being further extended by a polymerase (The primer-extended construct attached to the support surface can now be assayed. For example, it can be amplified using either surface PCR or an emulsion PCR, followed by sequencing, Para. [0088]).

Regarding claim 85, Chee discloses a pair of polynucleotide conjugates (a binding pair comprising a protein and a different chemical moiety, Para. [0008]), comprising: a first polynucleotide conjugate comprising (providing a first set of binding constructs immobilized on a support surface, Para. [0009]): (a) a first binder for specific binding of an analyte (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a first identifying sequence (and a first nucleic acid tag unique to the first binding agent, Para. [0009]) that identifies the first binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]); and a second polynucleotide (an oligonucleotide, Para. [0039]) conjugate comprising (providing a second set of binding constructs in solution, Para. [0009]): (a) a second binder for specific binding of the analyte (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a second identifying sequence (and a second nucleic acid tag unique to the second binding agent, Para. [0009]) that identifies the second binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]), wherein the first and second engaging sequences are capable of engaging the first and second polynucleotides, thereby engaging the first and second polynucleotide conjugates (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]).

Regarding claim 86, Chee discloses the pair of claim 85, wherein the first engaging sequence (The term "binding agent" as used herein refers to any binding agent that selectively binds to a molecule of interest, Para. [0033]) comprises a sequence hybridizable (The term also includes complementary nucleic acid molecules that selectively hybridize at or above a desired melting temperature, Para. [0034]) to the second engaging sequence, and/or the second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]) comprises a sequence hybridizable (The term also includes complementary nucleic acid molecules that selectively hybridize at or above a desired melting temperature, Para. [0034]) to the first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]).

Regarding claim 87, Chee discloses the pair of claim 85, wherein the first engaging sequence comprises a sequence complementary ("Complementary" or "substantially complementary" refers to the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, Para. [0035]) to all or a portion of the second engaging sequence (The term "binding pair" means any two molecules (binding agents) that are known to bind selectively to one another. In the case of two proteins, the proteins bind selectively to one another with a high affinity as described in more detail herein, Para. [0034]), and/or the second engaging sequence comprises a sequence complementary to all or a portion of the first engaging sequence.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 108, Chee discloses a method for analyzing an analyte in a sample (a method for identifying binding agents that form a binding pair, Para. [0009]), comprising: (i) immobilizing a first polynucleotide conjugate on a substrate (providing a first set of binding constructs immobilized on a support surface, Para. [0009]), the first polynucleotide conjugate comprising: (a) a first binder for specific binding of an analyte (In yet alternative aspects, either the first or second binding agent is an aptamer, Para. [0014]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first identifying sequence (and a first nucleic acid tag unique to the first binding agent, Para. [0009]) that identifies the first binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]), and optionally a first primer sequence; (ii) contacting the substrate having the immobilized first polynucleotide conjugate with a sample containing or suspected of containing the analyte (the method further comprises the step of adding a third binding agent in the combining step, Para. [0035]), whereby the first binder specifically binds to the analyte in the sample (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); (iii) contacting the substrate having the immobilized first polynucleotide conjugate (providing a first set of binding constructs immobilized on a support surface, Para. [0016]) with a second polynucleotide conjugate (providing a second set of binding constructs in solution, Para. [0009]), the second polynucleotide conjugate comprising: (a) a second binder for specific binding of the analyte (In yet alternative aspects, either the first or second binding agent is an aptamer, Para. [0014]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second identifying sequence (and a second nucleic acid tag unique to the second binding agent, Para. [0009]) that identifies the second binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]), and optionally a second primer sequence, whereby the second binder specifically binds to the analyte specifically bound to the first binder (The presence and binding of both the first (A) and second (S) binding agents is necessary for the detection of the third molecule, Para. [0077]), and whereby the first and second polynucleotides are engaged via the first and second engaging sequences (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]); and (iv) analyzing the first and/or second polynucleotides engaged via the first and second engaging sequences (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), wherein the analysis in step (iv) indicates the presence, absence, and/or amount of the analyte in the sample (and determining a sequence of each binding event identifier; wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair, Para. [0009]).

Regarding claim 109, Chee discloses a method for analyzing a plurality (at least ten different binding agents, Para. [0009]) of analytes in a sample (a method for identifying binding agents that form a binding pair, Para. [0009]), comprising: (i) immobilizing a plurality of first polynucleotide conjugates on a substrate (providing a first set of binding constructs immobilized on a support surface, Para. [0009]), each of the first polynucleotide conjugates comprising: (a) a first binder for specific binding of an analyte (In yet alternative aspects, either the first or second binding agent is an aptamer, Para. [0014]) in a plurality of analytes (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a first identifying sequence (and a first nucleic acid tag unique to the first binding agent, Para. [0009]) that identifies the first binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]); (ii) contacting the substrate having the immobilized plurality of first polynucleotide conjugates with a sample containing or suspected of containing at least one of the plurality of analytes (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]), whereby the at least one analyte in the sample specifically binds to a first binder of the immobilized plurality of first polynucleotide conjugates (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); (iii) contacting the substrate having the immobilized plurality of first polynucleotide conjugates (providing a first set of binding constructs immobilized on a support surface, Para. [0016]) with a plurality (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]) of second polynucleotide conjugates (providing a second set of binding constructs in solution, Para. [0009]), each of the second polynucleotide conjugates comprising: (a) a second binder for specific binding of an analyte (In yet alternative aspects, either the first or second binding agent is an aptamer, Para. [0014]) in the plurality of analytes (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a second identifying sequence (and a second nucleic acid tag unique to the second binding agent, Para. [0009]) that identifies the second binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]), whereby the at least one analyte specifically bound to the first binder specifically binds to a second binder of the plurality of second polynucleotide conjugates (The presence and binding of both the first (A) and second (S) binding agents is necessary for the detection of the third molecule, Para. [0077]), and whereby the first and second polynucleotide conjugates specifically bound to the same anlayte are engaged via the first and second engaging sequences (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]); and (iv) analyzing the first and/or second polynucleotide conjugates engaged via the first and second engaging sequences (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), wherein the analysis in step (iv) indicates the presence, absence, and/or amount of the plurality of analytes in the sample (and determining a sequence of each binding event identifier; wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair, Para. [0009]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 109, Chee discloses a method for analyzing a plurality (at least ten different binding agents, Para. [0009]) of analytes in a sample (a method for identifying binding agents that form a binding pair, Para. [0009]), comprising: (i) immobilizing a plurality of first polynucleotide conjugates on a substrate (providing a first set of binding constructs immobilized on a support surface, Para. [0009]), each of the first polynucleotide conjugates comprising: (a) a first binder for specific binding of an analyte (In yet alternative aspects, either the first or second binding agent is an aptamer, Para. [0014]) in a plurality of analytes (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a first identifying sequence (and a first nucleic acid tag unique to the first binding agent, Para. [0009]) that identifies the first binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]); (ii) contacting the substrate having the immobilized plurality of first polynucleotide conjugates with a sample containing or suspected of containing at least one of the plurality of analytes (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]), whereby the at least one analyte in the sample specifically binds to a first binder of the immobilized plurality of first polynucleotide conjugates (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); (iii) contacting the substrate having the immobilized plurality of first polynucleotide conjugates (providing a first set of binding constructs immobilized on a support surface, Para. [0016]) with a plurality (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]) of second polynucleotide conjugates (providing a second set of binding constructs in solution, Para. [0009]), each of the second polynucleotide conjugates comprising: (a) a second binder for specific binding of an analyte (In yet alternative aspects, either the first or second binding agent is an aptamer, Para. [0014]) in the plurality of analytes (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a second identifying sequence (and a second nucleic acid tag unique to the second binding agent, Para. [0009]) that identifies the second binder (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]), whereby the at least one analyte specifically bound to the first binder specifically binds to a second binder of the plurality of second polynucleotide conjugates (The presence and binding of both the first (A) and second (S) binding agents is necessary for the detection of the third molecule, Para. [0077]), and whereby the first and second polynucleotide conjugates specifically bound to the same anlayte are engaged via the first and second engaging sequences (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]); and (iv) analyzing the first and/or second polynucleotide conjugates engaged via the first and second engaging sequences (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), wherein the analysis in step (iv) indicates the presence, absence, and/or amount of the plurality of analytes in the sample (and determining a sequence of each binding event identifier; wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair, Para. [0009]).

Regarding claim 110, Chee discloses the method of claim 109, wherein the plurality of first polynucleotide conjugates are immobilized (a first set of binding constructs comprising binding agents is associated with a support surface, e.g., immobilized on the support surface or provided in a discrete feature on the support surface, Para. [0049]) or directly synthesized on the substrate at a density that substantially reduces or prevents interaction between adjacent first polynucleotide conjugates on the substrate (Members of first binding construct set are immobilized on a surface 1121 so that each molecule is well spaced from another, Para. [0080]).

Regarding claim 112, Chee discloses the method of claim 108, wherein the density of the first polynucleotide conjugate molecules immobilized (a first set of binding constructs comprising binding agents is associated with a support surface, e.g., immobilized on the support surface or provided in a discrete feature on the support surface, Para. [0049]) or directly synthesized on the substrate is sufficiently low to: (1) substantially reduce or prevent interaction between adjacent first polynucleotide conjugate molecules on the substrate (Members of first binding construct set are immobilized on a surface 1121 so that each molecule is well spaced from another, Para. [0080]); (2) substantially reduce or prevent interaction between an analyte molecule bound by one first polynucleotide conjugate molecule and an adjacent first polynucleotide conjugate molecule on the substrate (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); (3) substantially reduce or prevent interaction between a second polynucleotide conjugate molecule non-specifically bound to the substrate (For example, the first set of binding constructs 1117 may hybridize to anchor 1105 on surface 1121, e.g., the surface of a flowcell or a micro bead, but may not hybridize to anchors 1107, Para. [0080]) and an immobilized first polynucleotide conjugate molecule (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); (4) substantially reduce or prevent interaction between a second polynucleotide conjugate molecule bound by an analyte molecule non-specifically bound to the substrate (For example, the first set of binding constructs 1117 may hybridize to anchor 1105 on surface 1121, e.g., the surface of a flowcell or a micro bead, but may not hybridize to anchors 1107, Para. [0080]), and an immobilized first polynucleotide conjugate molecule (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); and/or (5) substantially reduce or prevent interaction between a second polynucleotide conjugate molecule specifically bound to an analyte molecule in a ternary complex with a first polynucleotide conjugate molecule, and an adjacent immobilized first polynucleotide conjugate molecule (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 114, Chee discloses the method of claim 108, wherein the second polynucleotide conjugate is provided in solution (This assay utilizes an anchored first set of binding constructs, a second set of binding constructs in solution, Para. [0079]), and the concentration of the second polynucleotide conjugate is sufficiently low to: (1) substantially reduce or prevent interaction between a second polynucleotide conjugate molecule non-specifically bound to the substrate and an immobilized first polynucleotide conjugate molecule (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); and/or (2) substantially reduce or prevent interaction between a second polynucleotide conjugate molecule bound by an analyte molecule nonspecifically bound to the substrate, and an immobilized first polynucleotide conjugate molecule (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]).

Regarding claim 116, Chee discloses the method of claim 108, wherein the concentration of the second polynucleotide conjugate provided in solution (This assay utilizes an anchored first set of binding constructs, a second set of binding constructs in solution, Para. [0079]) and/or the ratio between the second polynucleotide conjugate and the first polynucleotide conjugate is sufficiently low to: (1) substantially reduce or prevent interaction between a second polynucleotide conjugate molecule non-specifically bound to the substrate and an immobilized first polynucleotide conjugate molecule (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]); and/or (2) substantially reduce or prevent interaction between a second polynucleotide conjugate molecule bound by an analyte molecule non-specifically bound to the substrate, and an immobilized first polynucleotide conjugate molecule (the binding agent of the first set of binding constructs (A) and the binding agent of the second set of binding constructs provided in solution (S) bind to a third agent or analyte (L) and do not bind directly to one another. Figure 8 and 8B, Para. [0076]).

Claim 75 lacks an inventive step under PCT Article 33(3) as being obvious over Chee et al (hereafter Chee), in view of Kozlov et al (hereafter Kozlov).

Regarding claim 75, Chee discloses the method of claim 74, but fails to explicitly disclose wherein the plurality of the first epitopes are overlapping epitopes, and/or the plurality of the second epitopes are overlapping epitopes. Kozlov is in the field of creating libraries of molecular tools for peptide analysis (methods for constructing peptide construct sets and methods of use of these peptide construct sets in assay systems for peptide analysis, Abstact) and discloses wherein the plurality of the first epitopes (the invention provides a set of at least 5,000 distinct peptide constructs, Para. [0013]) are overlapping epitopes (the peptide sequences of the peptide constructs are grouped into one or more sets of partially overlapping sequences, Para. [0015]), and/or the plurality of the second epitopes are overlapping epitopes. It would have been obvious to one of ordinary skill in the art at the time of invention to modify the multi-antigen analysis method of Chee to include use of overlapping epitopes as taught by Kozlov. The motivation would have been to create molecular tools in particular for use in high throughput peptide analysis (Abstract).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Claims 76-81 lack an inventive step under PCT Article 33(3) as being obvious over Prognosys Biosciences, Inc., (hereafter Prognosys), in view of Chee et al (hereafter Chee).

Regarding claim 76, Prognosys discloses a method of manufacturing a system for analyzing an antibody or antigen binding fragment thereof (a method of manufacturing an array of products on a substrate, Para. [0010]), the method comprising (comprising providing partitioned reaction volumes on the substrate, Para. [0010]): (i) generating an array (an array of products, Para. [0010]) by immobilizing or directly synthesizing (immobilizing the products from the partitioned reaction volumes on capture moieties on the substrate, Para. [0010]) but fails to explicitly disclose a plurality of first polypeptide-polynucleotide conjugates on a substrate, each first polypeptide-polynucleotide conjugate comprising: (a) a first polypeptide comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof; and (b) a first polynucleotide comprising a first primer sequence, a first identifying sequence that identifies the first polypeptide, and a first engaging sequence; (ii) generating a library comprising a plurality of second polypeptide-polynucleotide conjugates, each second polypeptide-polynucleotide conjugate comprising: (a) a second polypeptide comprising a second epitope for specific binding of an antibody or antigen binding fragment thereof; and (b) a second polynucleotide comprising a second primer sequence, a second identifying sequence that identifies the second polypeptide, and a second engaging sequence, wherein at least one pair of first and second polypeptide-polynucleotide conjugates is capable of specific binding to the same antibody molecule or antigen binding fragment thereof, wherein specific binding of the at least one pair to the same antibody molecule or antigen binding fragment thereof engages the first and second engaging sequences of the at least one pair. Chee is in the field of high throughput molecular assay platforms (a new assay format for high throughput molecular binding studies at a single molecule level, Abstract) and discloses a plurality of first polypeptide-polynucleotide conjugates on a substrate (providing a first set of binding constructs immobilized on a support surface, Para. [0009]), each first polypeptide-polynucleotide conjugate comprising: (a) a first polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a first epitope for specific binding of an antibody or antigen binding fragment thereof (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a first polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a first primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a first identifying sequence (and a first nucleic acid tag unique to the first binding agent, Para. [0009]) that identifies the first polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a first engaging sequence (where each binding construct of the first set of binding constructs comprises a first binding agent, Para. [0009]); (ii) generating a library (either or both of the first and second sets of binding constructs comprises at least one hundred different binding agents....at least one million different binding agents or more, Para. [0011]) comprising a plurality of second polypeptide-polynucleotide conjugates (where either or both of the first and second sets of binding constructs comprises at least ten different binding agents, Para. [0009]), each second polypeptide-polynucleotide conjugate comprising (providing a second set of binding constructs in solution, Para. [0009]): (a) a second polypeptide (In some aspects, at least one of the first and second binding agents is a peptide, Para. [0013]) comprising a second epitope for specific binding of an antibody or antigen binding fragment thereof (In yet other aspects, the first binding agent is a peptide and the second binding agent is an antibody, Para. [0014]); and (b) a second polynucleotide ("Primer" means an oligonucleotide, Para. [0039]) comprising a second primer sequence (one or both of the first and second binding constructs further comprise a primer sequence, Para. [0012]), a second identifying sequence (and a second nucleic acid tag unique to the second binding agent, Para. [0009]) that identifies the second polypeptide (creating binding event identifiers from the co-located first and second nucleic acid tags, Para. [0009]), and a second engaging sequence (where each binding construct of the second set of binding constructs comprises a second binding agent, Para. [0009]), wherein at least one pair of first and second polypeptide-polynucleotide conjugates is capable of specific binding to the same antibody molecule or antigen binding fragment thereof (the method further comprises the step of adding a third binding agent in the combining step, Para. [0035]), wherein specific binding of the at least one pair to the same antibody molecule or antigen binding fragment thereof engages the first and second engaging sequences of the at least one pair (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]). It would have been obvious to one of ordinary skill in the art at the time of invention to modify the molecular analysis manufacturing method of Prognosys to include use of polypeptide-polynucleotide conjugates as taught by Chee. The motivation would have been to create a molecular assay platform which enables creation of binding event identifiers in a highly parallel way (Abstract).

Regarding claim 77, Prognosys discloses the method of claim 76, but fails to explicitly disclose wherein the plurality of first polypeptide-polynucleotide conjugates are immobilized or directly synthesized on the substrate at a density that substantially reduces or prevents interaction between adjacent first polypeptide-polynucleotide conjugates on the substrate. Chee is in the field of high throughput molecular assay platforms (a new assay format for high throughput molecular binding studies at a single molecule level, Abstract) and discloses wherein the plurality of first polypeptide-polynucleotide conjugates are immobilized (a first set of binding constructs comprising binding agents is associated with a support surface, e.g., immobilized on the support surface or provided in a discrete feature on the support surface, Para. [0049]) or directly synthesized on the substrate at a density that substantially reduces or prevents interaction between adjacent first polypeptide-polynucleotide conjugates on the substrate (Members of first binding construct set are immobilized on a surface 1121 so that each molecule is well spaced from another, Para. [0080]). It would have been obvious to one of ordinary skill in the art at the time of invention to modify the molecular analysis manufacturing method of Prognosys to include use of spaced polypeptide-polynucleotide conjugates as taught by Chee. The motivation would have been to create a molecular assay platform which enables creation of binding event identifiers in a highly parallel way (Abstract).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/064588

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 78, Prognosys discloses the method of claim 76, further comprising contacting the array with the library (immobilizing the products from the partitioned reaction volumes from the master substrate on capture moieties disposed on the replica array that is in contact with the partitioned reaction volumes, Para. [0016]), but fails to explicitly disclose wherein at least one pair of first and second polypeptide-polynucleotide conjugates forms a conjugate complex. Chee is in the field of high throughput molecular assay platforms (a new assay format for high throughput molecular binding studies at a single molecule level, Abstract) and discloses wherein at least one pair of first and second polypeptide-polynucleotide conjugates forms a conjugate complex (combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs thereby co-locating the first nucleic acid tags and the second nucleic acid tags, Para. [0009]). It would have been obvious to one of ordinary skill in the art at the time of invention to modify the molecular array manufacturing method of Prognosys to include use of interacting polypeptide-polynucleotide conjugates as taught by Chee. The motivation would have been to create a molecular assay platform which enables creation of binding event identifiers in a highly parallel way (Abstract).

Regarding claim 79, Prognosys discloses the method of claim 78, but fails to explicitly disclose wherein the conjugate complex is formed by hybridization between the first and second polynucleotides of the at least one pair of first and second polypeptide-polynucleotide conjugates. Chee is in the field of high throughput molecular assay platforms (a new assay format for high throughput molecular binding studies at a single molecule level, Abstract) and discloses wherein the conjugate complex is formed (a second binding construct, blocked at the 3' end 1329 (black square) so that it cannot be extended, is bound to the first binding construct via target agent 1337, Para. [0088], Fig. 13) by hybridization between the first and second polynucleotides of the at least one pair of first and second polypeptide-polynucleotide conjugates (and primer/hybridization region 1325, the complement to primer/hybridization region 1315, Para. [0088], Fig. 13). It would have been obvious to one of ordinary skill in the art at the time of invention to modify the molecular array manufacturing method of Prognosys to include use of hybridizing polypeptide-polynucleotide conjugates as taught by Chee. The motivation would have been to create a molecular assay platform which enables creation of binding event identifiers in a highly parallel way (Abstract).

Regarding claim 80, Prognosys discloses the method of claim 79, but fails to explicitly disclose wherein the sequences involved in the hybridization between the first and second polynucleotides comprise one or more cleavable sites. Chee is in the field of high throughput molecular assay platforms (a new assay format for high throughput molecular binding studies at a single molecule level, Abstract) and discloses wherein the sequences involved in the hybridization between the first and second polynucleotides comprise one or more cleavable sites (An antibody 1301 is attached to a nucleic acid portion of the first binding construct via a cleavable linker 1335, Para. [0087], Fig. 13). It would have been obvious to one of ordinary skill in the art at the time of invention to modify the molecular array manufacturing method of Prognosys to include use of cleavable polypeptide-polynucleotide conjugates as taught by Chee. The motivation would have been to create a molecular assay platform which enables creation of binding event identifiers in a highly parallel way (Abstract).

Regarding claim 81, Prognosys discloses the method of claim 78, but fails to explicitly disclose wherein the second polypeptide-polynucleotide conjugate of the at least one pair that forms a conjugate complex is immobilized on the substrate via a cleavable linker. Chee is in the field of high throughput molecular assay platforms (a new assay format for high throughput molecular binding studies at a single molecule level, Abstract) and discloses wherein the second polypeptide-polynucleotide conjugate of the at least one pair that forms a conjugate complex is immobilized on the substrate (where the binding construct is ligated using a splint sequence 1333 to a anchor 1305 attached to a solid support. Para. [0087]) via a cleavable linker (the cleavable linker 1335 attaching the first binding agent 1301 to the first binding construct is cleaved, and washing removes the second binding construct, the first antibody 1301 and target agent 1337, Para. [0088], Fig. 13). It would have been obvious to one of ordinary skill in the art at the time of invention to modify the molecular array manufacturing method of Prognosys to include use of cleavable polypeptide-polynucleotide conjugates as taught by Chee. The motivation would have been to create a molecular assay platform which enables creation of binding event identifiers in a highly parallel way (Abstract).

Claims 1-4, 28-30, 60-67, 72-81, 84-87, 108-110, 112, 114 and 116 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
PGNS002PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2010/033064	29/04/2010	30/04/2009
Applicant		
PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/033064

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/073171 A2 (KEYGENE NV [NL]; VAN EIJK MICHAEL JOSEPHUS THER [NL]) 28 June 2007 (2007-06-28) the whole document, in particular p. 25, last paragraph, p.29-30 and claims, especially claims 7 and 13-14	1-29
X	WO 2007/076726 A1 (LOK SI [CN]) 12 July 2007 (2007-07-12) the whole document	1-29
A	XIAO MING ET AL: "Direct determination of haplotypes from single DNA molecules" NATURE METHODS, vol. 6, no. 3, March 2009 (2009-03), pages 199-201, XP002591312 ISSN: 1548-7091 cited in the application	1-29

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 July 2010	30/07/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bassias, Ioannis

Curio EX1010-p. 337
Curio v Prognosys
IPR2024-01289

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/033064

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/032167 A1 (ILLUMINA CAMBRIDGE [GB]; BIGNELL HELEN [GB]; GORMLEY NIALL ANTHONY [GB) 12 March 2009 (2009-03-12)	1-29
A	WO 2007/145612 A1 (454 LIFE SCIENCES CORP [US]; BERKA JAN [US]; CHEN ZHOUTAO [US]; EGHOLM) 21 December 2007 (2007-12-21)	1-29

Curio EX1010-p. 338
Curio v Prognosys
IPR2024-01289

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/033064

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007073171 A2	28-06-2007	CN 101365803 A	11-02-2009
		EP 1966394 A2	10-09-2008
		JP 2009520500 T	28-05-2009
		US 2009247415 A1	01-10-2009
WO 2007076726 A1	12-07-2007	EP 1969146 A1	17-09-2008
WO 2009032167 A1	12-03-2009	EP 2191011 A1	02-06-2010
		US 2009093378 A1	09-04-2009
WO 2007145612 A1	21-12-2007	CA 2615323 A1	21-12-2007
		EP 1910537 A1	16-04-2008

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/033064	29.04.2010	30.04.2009

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68

Applicant
PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0	see form PCT/ISA/210	Bassias, Ioannis Telephone No. +49 89 2399-8196

Curio EX1010 p. 340
Curio v Prognosys
IPR2024-01289

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-29
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V:

1 The present application relates to nucleic acid contructs which comprise a region of interest (ROI), at least of unique identifier and at least one amplification site. Said contructs are used in methods for determining the sequence of nucleic acids, for identifying nucleic acids having a desired sequence and in methods for selecting nucleic acids having a desired sequence.

2 Reference is made to the following documents:

D1 WO 2007/073171 A2 (KEYGENE NV [NL]; VAN EIJK MICHAEL JOSEPHUS THER [NL]) 28 June 2007 (2007-06-28)

D2 WO 2007/076726 A1 (LOK SI [CN]) 12 July 2007 (2007-07-12)

3 The application is drafted in a very broad manner. The terms used, "region of interest", "unique identifier", etc. are very undefined and open for interpretation in various directions. Consequently, the claims need to be interpreted in the broadest possible sense. This approach leads to objections under Articles 33(2) and (3) in view of the teaching of D1 and/or D2.

3.1 D1 describes methods for transcript profiling using high throughput sequencing techniques where adaptors are used that can be used as a template for PCR oligonucleotide primers and for sequencing. The constructs can be immobilized on a solid support such as beads. The adaptors may comprise identifier sequences that can be unique and can vary in the length. Any nucleic acid to be analyzed in D1 represents the region of interest (ROI). The unique identifiers are not further characterized in the application. Hence, the unique identifiers of D1 fully fall into the scope of this expression. These unique identifiers can vary in the length. Consequently, any ROI can be attached to different identifiers and thus the concept of having the same ROI with different unique identifiers is fulfilled. The adaptors represent the amplification site since they are used as a template for PCR oligonucleotide primers and for sequencing. Restrictions sites within the constructs are mentioned. Sequencing of the constructs anticipates the method as described in claims 11-17. The determination of the sequence and further analysis for transcript profiling requires the identification of nucleic acids with desired sequences and also the selection thereof. Consequently, the subject-matter of all claims (1-29) is anticipated by the teaching of D1 (Article 33(2) PCT).

3.2 In D2 methods are described for nucleic acid mapping and identification of fine-structural variations in nucleic acids wherein so called "Genomic Variations Tags" (GVTs) are produced that bind to target nucleic acids. Said GVTs comprise one or more restriction endonucleases sites. They can be used as unique genomic positional identifiers. Different restriction endonucleases sites can be used in the GVTs which are tagged to the ROIs. Hence, the same ROI can have different unique identifiers. Amplification and sequencing via these tags is described. Immobilisation on solid support is not specifically mentioned in this prior art document. However, this is not considered something special which would not be within the ordinary skills of a person in the art.

Consequently, claims 1-6, 9-25, 28, and 29 are not in accordance with Article 33(2) PCT in view of D2. Claims 7, 8, 26, and 27 contravene the requirements of Article 33(3) PCT in view of the teaching of D2 combined with general knowledge.

4 Claim 28 lacks clarity (Article 6 PCT) apparently due to a wrong syntax.

5 Expressions like "incorporated herein/hereby by reference" used in the description (e.g. on pages 12 and 24) render the true scope of the application unclear and are thus not in accordance with Article 6 PCT.

The same applies for the use of expressions like "spirit of the invention" (see e.g. on pages 30, 42, 43).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
PGNS003PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2010/044134	02/08/2010	31/07/2009
Applicant		
PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed
      ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (See Box No. II)

3. ☒ **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   ☒ the text is approved as submitted by the applicant
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   ☐ the text is approved as submitted by the applicant
   ☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. 1
      ☒ as suggested by the applicant
      ☐ as selected by this Authority, because the applicant failed to suggest a figure
      ☐ as selected by this Authority, because this figure better characterizes the invention
   b. ☐ none of the figures is to be published with the abstract

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The present invention provides assay tools for the detection of biological or chemical activity in a sample. The assay tools of the invention provide direct detection using a positive signal generated on a surface of the assay tool. These assay tools provide improved methods for detection and/or identification of multiple agents (e.g., enzymes) in a sample, analysis of substrate specificity of such agents, and binding affinities and specificities of such agents.
Upon activity a component is released from a first immobilised construct and then captured by a capture surface. At least two different immobilised constructs are used.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/044134

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/044134

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/573 C12Q1/34 C12Q1/37 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/003394 A1 (SONG XUEDONG [US]) 5 January 2006 (2006-01-05) the whole document in particular: paragraphs [0004] - [0006], [0015], [0018], [0020], [0030], [0060], [0061] figure 3 claim 24 -----	1-6
X	WO 2007/060599 A1 (KONINKL PHILIPS ELECTRONICS NV [NL]; VOSSENAAR ERIK R [NL]; PRINS MENN) 31 May 2007 (2007-05-31) the whole document in particular: figures 5,6,9,10 paragraphs [0042], [0060] - [0067], [0080], [0089] -----	1-6

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2011	18/03/2011

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer
Tuynman, Antonin

Curio EX1010-p. 347
Curio v Prognosys
IPR2024-01289

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/044134

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006003394 A1	05-01-2006	CN 1977167 A	06-06-2007
		EP 1782069 A1	09-05-2007
		KR 20070026691 A	08-03-2007
		WO 2006006961 A1	19-01-2006
WO 2007060599 A1	31-05-2007	CN 101313218 A	26-11-2008
		EP 1957979 A1	20-08-2008
		JP 2009517651 T	30-04-2009
		US 2008311598 A1	18-12-2008

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6

   An assay tool for detecting biological or chemical activity in a sample.

   ---

2. claims: 7-9, 19

   An assay tool for detecting activity in a sample involving constructs with cleavage agent substrate region, affinity region and detectable marker.

   ---

3. claims: 10-18, 34

   An assay tool for detecting cleavage agent activity in a sample involving constructs with cleavage agent substrate and detectable marker.

   ---

4. claims: 20-33, 41

   An assay tool and method for detecting activity of a cleavage agent in a sample wherein the proximity of first and second surface allow for the detection of the positive signal.

   ---

5. claim: 35

   An assay tool for detecting activity of a cleavage agent in a sample, wherein association of a first released component of a combined detectable marker with a second component thereof on the capture surface creates a positive signal though interaction of first and second components of the combined detectable marker

   ---

6. claims: 36, 37, 42, 43

   An assay tool and methods for detecting competitive binding of a molecule in a sample.

   ---

7. claims: 38, 39

   An assay tool for detecting activity of an enzyme.

   ---

8. claim: 40

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for detecting cleavage agent activity in a sample involving a surface with releasable component constructs and capture agents.

---

9. claims: 44-47

A method for detecting a modulator of enzyme activity

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2010/044134	02.08.2010	31.07.2009

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/573 C12Q1/34 C12Q1/37 G01N33/543

Applicant
PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Tuynman, Antonin Telephone No. +31 70 340-3741

Curio EX1010-p. 30
Curio v Prognosys
IPR2024-01289

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 7-47

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 7-47

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-6

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-6
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6
Industrial applicability (IA)	Yes:	Claims	1-6
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item IV

Lack of unity of invention

The problem to be solved in the present application (PA) relates to the detection of biological and/or chemical activity in a sample. The single general concept which can be identified a priori as linking the various claimed inventions and which forms a solution to the above mentioned problem relates to "the notion that an assay tool comprising a set of two or more immobilised constructs comprising a releasable component and a capture surface, wherein release of the component allows for the detection of a positive signal"

However, US2006/003394 A (hereinafter referred to as D1, relevant passages as cited in the search report) and WO2007/060599 A (hereinafter referred to as D2, relevant passages as cited in the search report) disclose such a notion.

In the light of D1 and D2, each document if taken alone, the above identified single general concept is not novel and inventive and can thus not be the single general inventive concept as required by Rule 13.1 PCT. The present application is therefore considered not to fulfill the requirement of unity as laid down in Rule 13.1 PCT.

Consequently the groups of inventions are split up as follows:

1) An assay tool for detecting biological or chemical activity in a sample (claims 1-6)

2) An assay tool for detecting activity in a sample involving constructs with cleavage agent substrate region, affinity region and detectable marker (claims 7-9,19)

3) An assay tool for detecting cleavage agent activity in a sample involving constructs with cleavage agent substrate and detectable marker (claims 10-18,34)

4) An assay tool and method for detecting activity of a cleavage agent in a sample wherein the proximity of first and second surface allow for the detection of the positive signal (claims 20-33 and 41)

5) An assay tool for detecting activity of a cleavage agent in a sample, wherein association of a first released component of a combined detectable marker with a second component thereof on the capture surface creates a positive signal though interaction of first and second components of the combined detectable marker (claim 35)

6) An assay tool and methods for detecting competitive binding of a molecule in a sample (claims 36,37,42 and 43)

7) An assay tool for detecting activity of an enzyme (claims 38 and 39)

8) A method for detecting cleavage agent activity in a sample involving a surface with releasable component constructs and capture agents (claim 40)

9) A method for detecting a modulator of enzyme activity (claims 44-47)

No other technical features could be identified that form a technical relationship among each of the separate inventions claimed and which could be considered as special technical feature within the meaning of Rule 13.2 PCT. As no additional search fees have been paid upon invitation, the present opinion is limited to group of inventions 1 (claims 1-6).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 US 2006/003394 A1 (SONG XUEDONG [US]) 5 January 2006 (2006-01-05)

D2 WO 2007/060599 A1 (KONINKL PHILIPS ELECTRONICS NV [NL]; VOSSENAAR ERIK R [NL]; PRINS MENN) 31 May 2007 (2007-05-31)

1 The present application does not meet the criteria of Article 33(2) PCT, because the subject-matter of independent claim 1 is not new.

D1 discloses : An assay tool (Figure 3, claim 24, pars. 4-6,15,18,20,30,60 and 61) for detecting biological or chemical activity in a sample, comprising: a set of at least two different immobilized constructs (e.g. Figure 3 items 47 and 49: immobilised on particle 43), wherein the constructs comprises a releasable component (item 45, figure 3); and a capture surface (Figure 3, item 31); wherein the exposure of the immobilized constructs to a biological or chemical activity may result in release of a releasable component from one or more of the immobilized constructs, and wherein the interaction of a releasable component with the capture surface directly or indirectly enables the generation of a positive signal (claim 24).

2 The present application does not meet the criteria of Article 33(3) PCT, because the subject-matter of independent claim 1 does not involve an inventive step.

2.1 As claim 1 is not novel (see point 1) it cannot be inventive either.

2.2 Also in view of D2 alone claim 1 is not inventive: D2 discloses a similar assay tool for detecting biological or chemical activity in a sample as described in detail under point 1 (see D2, figures 5-10) which differs from presently claimed assay tool in that there is only one type of immobilised construct. The effect thereof is that the tool does not allow for multiplexing assays.

The problem to be solved by the present invention may therefore be regarded as how to provide an assay tool so as to allow multiplexing assays.

The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons: D2 already suggests that different labels can be used (par. 89) in the methods, systems and devices disclosed therein. If this solution is applied to the embodiments involving the label as part of the substrate to be cleaved as well as part of the product (pars. 60-67; figures 5-10), a tool is provided which falls within the scope of present claim 1.

3 Dependent claims 2-6 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, see D1 and/or D2 (relevant passages as cited in the search report). The incorporation of the features of claims 2-6 into independent claim 1 is either known or obvious from D1-D2 or falls within the knowledge and ability of a person skilled in the art.

Re Item VII

Certain defects in the international application

4 The phrase " incorporated by reference" on several pages of the description should be deleted. According to the PCT Guidelines, II, 4.26, the patent specification should be self contained i.e. capable of being understood without reference to any other document.

5 The statement which refers to the extent of protection being expanded to cover the spirit of the invention on several pages of the description should be deleted (PCT Guidelines II, 5.30).

Re Item VIII

Certain observations on the international application

6 The application does not meet the requirements of Article 6 PCT, because claim 1 is not clear.

6.1 The phrase "constructs comprises" is unclear as it is not clear whether it was intended that only one of the constructs comprises a releasable component or whether both comprise a releasable component.

6.2 Claim 2 is unclear as only the releasable component can bind to the capture surface and not the complete immobilised constructs comprising a releasable component as the claim presently suggests.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: DIANNA L. DEVORE
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	29 MAR 2011
Applicant's or agent's file reference PGNS005PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 10/59327	07 December 2010 (07.12.2010)	07 December 2009 (07.12.2009)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68; G01N 33/50 (2011.01)
USPC - 435/6, 435/7.1

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	14 March 2011 (14.03.2011)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-21,23,30	YES
	Claims	1,22,24-29,31-35	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-35	NO
Industrial applicability (IA)	Claims	1-35	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by US 2008/0312103 A1 to NEMOTO et al. (hereinafter "Nemoto").

As to claim 1, Nemoto teaches an array comprising a substrate having a surface;
two or more individual constructs associated on the surface of the substrate (para [0020]; "An mRNA chip comprising a plurality of the immobilized mRNA-puromycin conjugates described"), wherein the individual constructs comprise: an oligonucleotide encoding a peptide of interest (para [0020] mRNA); and the peptide of interest (para [0039]; "synthesizing a protein by adding the mRNA-PM conjugate immobilized on a solid phase to a translation system"); wherein the oligonucleotide is associated with the substrate surface (para [0008]; "a solid phase binding site for binding an mRNA-puromycin conjugate"; sheet 1 fig 1a, b), and the peptide is associated with the oligonucleotide (para [0039]; "synthesizing a protein by adding the mRNA-PM conjugate immobilized on a solid phase to a translation system. Namely, when a conjugate in which puromycin is ligated to mRNA through the linker is contacted with a translation system, an in vitro virus virion is known to be formed in which the mRNA is ligated with a translated protein through the puromycin"; sheet 1 fig 1a, b).

Claims 22, 24-29, 31-35 lack novelty under PCT Article 33(2) as being anticipated by US 2005/0260653 A1 (LABAER et al.) (hereinafter "LaBaer").

As to claim 22, LaBaer teaches a method for constructing an array (abstract; "Arrays of polypeptides can be generated by translation of nucleic acid sequences encoding the polypeptides at individual addresses on the array") comprising:
a) synthesizing a set of nucleic acid sequences of known sequence on a solid substrate (para [0005]; " In one aspect, the invention features a method that includes: disposing, on a substrate, one or more nucleic acids that include a coding region and an anchoring agent, maintaining the substrate under conditions which enable the anchoring agent of each disposed nucleic acid to stably attached to the substrate"; para [0015]; " In one embodiment, the coding region encodes a polypeptide that includes a first amino acid sequence, e.g., an amino acid sequence of interest").
b) attaching one or more additional nucleic acid regions to the nucleic acids on the solid substrate to provide extended nucleic acids on the substrate (para [0445-0446]; "[0445] Capture Probes. The substrate can include an attached nucleic acid capture probe at each address. In one aspect, capture probes can be used to create a self-assembling array. A unique capture probe at each address selectively hybridizes to a nucleic acid encoding a test amino acid sequence, thereby organizing each encoding nucleic acid to a unique address. The capture nucleic acid can be covalently attached or bound, e.g., to a polycationic surface on the substrate. [0446] The capture probe can itself be synthesized in situ, e.g. by a light-directed method ..., or by being spotted or disposed at the addresses. The capture probe can hybridize to the nucleic acid encoding the test polypeptide. In a preferred embodiment, the capture probe anneals to the T7 promoter region of a single stranded nucleic acid encoding the test amino acid sequence. In another embodiment, the capture probe is ligated to the encoding nucleic acid sequence")
c) performing in vitro transcription and translation utilizing the extended nucleic acids as a template for production of peptides (para [0458]; " The fusion proteins will be generated with commercially available in vitro transcription/translation kits such as the Promega TNT Coupled Reticulocyte Lysate Systems or TNT Coupled Wheat Germ Extract Systems"; para [0022]); and
d) attaching the produced peptides on known locations on the solid substrate (para [0015]; " In one embodiment, the coding region encodes a polypeptide that includes a first amino acid sequence, e.g., an amino acid sequence of interest, and an affinity tag. The affinity tag binds to a binding agent. The method can also include disposing the binding agent on the substrate. In some cases, it is useful to prepare a solution that includes the nucleic acid and the binding agent, and to dispose the solution onto the substrate"; sheet 1 fig 1).

----------continued in Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
No valid CRF was filed in response to the ISA/225 of 10 December 2010, therefore no sequence information was available for the ISR.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V-2 (Citations and Explanations)

As to claim 24, LaBaer further teaches that the nucleic acids are chemically synthesized directly on the solid substrate (para [0446]; "The capture probe can itself be synthesized in situ, e.g., by a light-directed method").

As to claim 25, LaBaer further teaches that the additional nucleic acids in step b) are attached via enzymatic ligation (para [0446]; "In another embodiment, the capture probe is ligated to the encoding nucleic acid sequence").

As to claim 26, LaBaer further teaches that the set of nucleic acids comprises at least 1,000 nucleic acid sequences on the substrate (para [0367]; For example, a single array containing 10,000 arrayed elements, for example, can be sufficient to yield 10,000 results (e.g., quantitative results)").

As to claim 27, LaBaer further teaches that the set of nucleic acids comprises at least 5,000 nucleic acid sequences on the substrate (para [0367]).

As to claim 28, LaBaer further teaches that the set of nucleic acids comprises at least 10,000 nucleic acid sequences on the substrate (para [0367]).

As to claim 29, LaBaer teaches a method for constructing a peptide array (abstract), comprising:
a) manufacturing a nucleic acid microarray comprising a set of chemicall synthesized oligonucleotides encoding amino acid sequences (para [0005], [0015]);
b) attaching one or more additional nucleic acid regions to the nucleic acids on the solid substrate to provide extended nucleic acids on the substrate (para [0446]);
c) performing in vitro transcription and translation utilizing the extended nucleic acids as a template for production of peptides (para [0458], [0022]) ; and
d) attaching the produced peptides on the solid substrate (para [0015]; sheet 1 fig 1).

As to claim 31, LaBaer further teaches that the nucleic acids are chemically synthesized directly on the solid substrate (para [0446]; "The capture probe can itself be synthesized in situ, e.g., by a light-directed method").

As to claim 32, LaBaer further teaches that the additional nucleic acids in step b) are attached via enzymatic ligation (para [0446]; "In another embodiment, the capture probe is ligated to the encoding nucleic acid sequence").

As to claim 33, LaBaer further teaches that the set of nucleic acids comprises at least 1,000 nucleic acid sequences on the substrate (para [0367]; For example, a single array containing 10,000 arrayed elements, for example, can be sufficient to yield 10,000 results (e.g., quantitative results)").

As to claim 34, LaBaer further teaches that the set of nucleic acids comprises at least 5,000 nucleic acid sequences on the substrate (para [0367]).

As to claim 35, LaBaer further teaches that the set of nucleic acids comprises at least 10,000 nucleic acid sequences on the substrate (para [0367]).

Claims 2-10, 12-15, 23,30 lack an inventive step under PCT Article 33(3) as being obvious over US 2005/0260653 A1 (LABAER et al.) (hereinafter "LaBaer") in view of the publication titled " The developments of semisynthetic DNA.protein conjugates" By NIEMEYER et al. (hereinafter "Niemeyer").

As to claim 2, LaBaer teaches a substrate having a surface; two or more individual constructs associated on the surface of the substrate, wherein the individual constructs comprise: an oligonucleotide comprising a region encoding a peptide of interest, a region encoding an affinity tag and an untranslated region (para [0005]; " In one aspect, the invention features a method that includes: disposing, on a substrate, one or more nucleic acids that include a coding region and an anchoring agent, maintaining the substrate under conditions which enable the anchoring agent of each disposed nucleic acid to stably attached to the substrate"; para [0015]; " In one embodiment, the coding region encodes a polypeptide that includes a first amino acid sequence, e.g., an amino acid sequence of interest, and an affinity tag. The affinity tag binds to a binding agent"; para [0056]; "The nucleic acid can further include one or more of: a transcription promoter; a transcription regulatory sequence; a untranslated leader sequence; a sequence encoding a cleavage site; a recombination site; a 3' untranslated sequence") the peptide of interest and an affinity tag (para [0022]; " In one embodiment, the step of contacting the substrate with a translation effector includes disposing or flowing the translation effector onto the surface, for example, using a single dispensing action or multiple dispensing actions. In one embodiment, the substrate is also contacted with a transcription effector"); and a capture agent that selectively binds to the affinity tag (para [0046]; " In another aspect, the invention features a method that includes: contemporaneously providing (e.g., depositing)----------continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
previous Supplemental Box

(claim 2 continued): (i) a binding agent that can interact with a tag and (ii) a nucleic acid that can be stably attached to a substrate and that includes a sequence encoding a first amino acid sequence and the tag onto a substrate"; sheet 1 fig 1) wherein the oligonucleotide is associated with the substrate surface (para [0005]). LaBaer does not teach that the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Consequently it would have been obvious to one of ordinary skill in the art to combine an oligonucleotide comprising a region encoding a peptide of interest, a region encoding an affinity tag and an untranslated region, as taught by LaBaer, with the peptide of interest and an affinity tag, again as taught by LaBaer, with a capture agent that selectively binds to the affinity tag, again as taught by LaBaer, with the oligonucleotide is associated with the substrate surface, again as taught by LaBaer, with the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent, as taught by Niemeyer, because it would have enabled construction of a oligonucleotide-peptide microarray.

As to claim 3, LaBaer further teaches that the untranslated region comprises a transcriptional start site and a ribosomal binding site (para [0115]; " The nucleic acid can further include one or more of: a transcription promoter; a transcription regulatory sequence; a untranslated leader sequence; a transcriptional terminator; and an internal ribosome entry site").

As to claim 4, Niemeyer further teaches that the capture agent is associated with the oligonucleotide (pg 400 fig 6).

As to claim 5, LaBaer teaches that the oligonucleotide is associated with the substrate surface via a linker (para [0040]; " In another aspect, the invention features a method that includes: providing a substrate that includes a plurality of addresses, each addresses including a nucleic acid that includes a coding region and an anchoring agent that stably attaches the nucleic acid to the substrate").

As to claim 6, LaBaer further teaches that the capture agent is associated with the linker (para [0129]; " "The binding agent can be attached to the substrate. For example, the substrate can be derivatized and the binding agent covalent attached thereto").

As to claim 7, LaBaer further teaches that the substrate is a planar support (para [0008]; " The substrate can be planar, e.g., have a horizontal plane in which the addresses are located at different discrete locations. The surface of the substrate can be flat (e.g., a glass slide) ").

As to claim 8, LaBaer further teaches that the substrate is a bead (para [0088]; " In yet another embodiment, an insoluble substrate (e.g., a bead or particle), is disposed at each address of the plurality, and the binding agent is attached to the insoluble substrate").

As to claim 9, LaBaer further teaches that the oligonucleotide is double-stranded (para [0055]; "The nucleic acid can be a RNA, or a DNA (e.g., a single-stranded DNA, or a double stranded DNA").

As to claim 10, Niemeyer further teaches that the oligonucleotide further comprises a region used for introduction of the capture agent to the oligonucleotide (pg 400 fig 6; A microarray of capture oligonucleotides is used as the immobilization matrix. Note that owing to the specificity of Watson.Crick base pairing, many different compounds can be sites pecifically immobilized simultaneously in a single step").

As to claim 12, LaBaer teaches a method of constructing an array (abstract), comprising the steps of:
(a) providing a surface comprising two or more constructs, the constructs
comprising a single-stranded oligonucleotide region encoding a peptide of interest
and an affinity tag (para [0005], [0015]; [0055]-" (para [0055]; "The nucleic acid can be a RNA, or a DNA (e.g., a single-stranded DNA, or a double stranded DNA");
(d) subjecting the double-stranded oligonucleotide region to a transcription
and translation event to produce a peptide comprising the peptide of interest and
the affinity tag (para [0458]; [0022]) wherein the protein produced in step (d) is captured by the capture agent of the construct following translation, thereby forming individual constructs
comprising the peptide associated with a substrate encoding the peptide (sheet 1 fig 1; para [0015]). LaBaer does not teach that the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). In addition, LaBaer does not teach (b) converting the single-stranded oligonucleotide region into a double stranded oligonucleotide region.----------claim continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
previous Supplemental Box

(continuation of claim 12)-----However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation. Furthermore, LaBaer does not teach (c); introducing an untranslated region comprising a ribosomal start site to the 5' end of the double-stranded oligonucleotide region, wherein the untranslated region is associated with a capture agent with the ability to form a binding pair with the affinity tag. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (b) and (d), as taught by LaBaer, with claim limitation (c), as taught by LaBaer in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a microarray by way of a two oligonucleotide ligation at each addressable position of the microarray, with oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent, as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 13, LaBaer teaches a method of constructing an array (abstract), comprising the steps of:
(a) providing a substrate comprising a surface with two or more constructs associated on the surface , the constructs comprising a single-stranded oligonucleotide encoding a peptide of interest and an affinity tag (para [0005], [0015], [0055]);
(e) subjecting the double-stranded oligonucleotide to a transcription and translation event to produce a peptide from the double-stranded oligonucleotide; wherein the protein produced in step (e) is captured by the capture agent of the construct; and wherein the resulting array comprises discrete units comprising a nucleic acid associated with substrate and the peptide encoded by the nucleotide also associated with the substrate through a capture agent (sheet 1 fig 1; para [0015]). LaBaer does not specifically teach the peptide encoded by the nucleic acid is associated by the nucleic acid. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). In addition, LaBaer does not teach (d) converting the single-stranded oligonucleotide region into a double stranded oligonucleotide region. However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation (i.e. claim limitation (e)). Furthermore, LaBaer does not teach (b) ligating a second, universal oligonucleotide to the oligonucleotides of the constructs, wherein the universal oligonucleotide comprises an untranslated region comprising a ribosomal binding site. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6), thus fulfilling claim limitation (c). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (d) and (e), as taught by LaBaer, with claim limitation (b), as taught by LaBaer, in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a two oligonucleotide ligation of a microarray, with claim limitation (c), as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 14, LaBaer teaches method of constructing an array (abstract), comprising the steps of:
(a) providing a surface comprising two or more constructs, the constructs comprising a single-stranded oligonucleotide region (para [0055]) having a sequence that is the sequence encoding a peptide of interest and an affinity tag (para [0005], [0015]); LaBaer does not teach the single stranded nucleotide is a reverse complement sequence encoding the peptide of interest. However, an artisan of ordinary skill in the art would have readily appreciated that a reverse complement sequence could have readily hybridized with a mRNA molecule and could have utilized it to hybridize with mRNA introduced to the microRNA. Furthermore, LaBaer does not teach (b) converting the single-stranded oligonucleotide region into a double stranded oligonucleotide region. However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation (i.e. claim limitation (d)). LaBaer further teaches (d) subjecting the double-stranded oligonucleotide region to a transcription and translation event to produce a peptide comprising the peptide of interest and the affinity tag wherein the protein produced in step (d) is captured by the capture agent of the construct following translation, thereby forming individual constructs comprising the peptide associated with the substrate and an oligonucleotide encoding the peptide associated with the substrate. LaBaer does not specifically teach the peptide encoded by the nucleic acid is associated with the nucleic acid. ----------claim continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
previous Supplemental Box

(continuation of claim 14)----------However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). LaBaer does not teach (c); introducing an untranslated region comprising a ribosomal start site to the 5' end of the double-stranded oligonucleotide region, wherein the untranslated region is associated with a capture agent with the ability to form a binding pair with the affinity tag. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (b) and (d), as taught by LaBaer, in view of the observations of an artisan of ordinary skill, with claim limitation (c), as taught by LaBaer, in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a microarray by way of a two oligonucleotide ligation step of the microarray, with attachment of a peptide of interest to an oligonucleotide encoding the peptide via a capture agent, as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 15, LaBaer teaches a method of constructing an array (abstract), comprising the steps of: (a) providing a surface comprising two or more constructs, the constructs
comprising a single-stranded oligonucleotide region (para [0055]) having a sequence that is the
sequence encoding a peptide of interest and an affinity tag (para [0005], [0015]); LaBaer does not teach the single stranded nucleotide is a reverse complement sequence encoding the peptide of interest. However, an artisan of ordinary skill in the art would have readily appreciated that a reverse complement sequence could have readily hybridized with a mRNA molecule and could have utilized it to hybridize with mRNA introduced to the microRNA. LaBaer further does not teach (d) converting the single-stranded oligonucleotide regions of the constructs into double-stranded oligonucleotide regions. However, an artisan of ordinary skill in the art would have readily appreciated that sequence specific regulators of eukaryotic gene expression (e.g. transcription binding, recognition and promoter sites), axiomatically, act through double stranded DNA targets only. Consequently, it would have been obvious that single-stranded oligonucleotides would have had to have been converted to a double stranded oligonucleotide before initiating transcription/translation (i.e. claim limitation (e)). LaBaer further teaches (e) subjecting the double-stranded oligonucleotide region to a transcription and translation event to produce a peptide comprising the peptide of interest and the affinity tag wherein the protein produced in step (e) is captured by the capture agent of the construct following translation, thereby forming individual constructs comprising the peptide associated with the substrate and an oligonucleotide encoding the peptide associated with the substrate. LaBaer does not specifically teach the peptide encoded by the nucleic acid is associated by the nucleic acid. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Furthermore, LaBaer does not teach (b) ligating a second, universal oligonucleotide to the oligonucleotides of the constructs, wherein the universal oligonucleotide comprises an untranslated region comprising a ribosomal binding site. However, LaBaer does teach modular construction of the oligonucleotide microarray involving hybridization, possible extension, and ligation of 2 separate oligonucleotides at each addressable position on the microarray (para [0445-0446]. An artisan of ordinary skill in the art would have readily appreciated that such universal elements as ribosomal binding sites and/or an untranslated region for attachment of a capture agent with the ability to form a binding pair with the affinity tag could have been engineered into the modular second oligonucleotide. Indeed, Niemeyer teaches association of an oligonucleotide attached to a capture agent through hybridization to the microarray attached oligonucleotide (pg 400 fig 6), thus fulfilling claim limitation (c). Consequently, it would have been obvious to one of ordinary skill in the art to combine claim limitations (a), (d) and (e), as taught by LaBaer, with claim limitation (b), as taught by LaBaer, in view of the observations of an artisan of ordinary skill in the art concerning introducing modular elements such as a ribosomal binding site into a two oligonucleotide ligation of a microarray, with claim limitation (c), as taught by Niemeyer, because it would have enabled construction of a supramolecular array that would function as a protein biochip.

As to claim 23 and 30, LaBaer teaches that the produced peptides are bound to the substrate (para [0015], sheet 1 fig 1). LaBaer does not teach that the produced peptides are attached to their respective nucleic acid substrates. However, Niemeyer teaches that the produced peptides are attached to their respective nucleic acid substrates (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Consequently it would have been obvious to one of ordinary skill in the art to combine peptides produced by in vitro transcription/translation of an array, as taught by LaBaer, with the produced peptides are attached to their respective nucleic acid substrates, as taught by Niemeyer, because it would have enabled construction of a protein array where the produced peptides are at discrete spatially addressable locations on the array.
----------continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
previous Supplemental Box

Claim 11 lacks an inventive step under PCT Article 33(3) as being obvious over LaBaer, in view of Niemeyer, in further view of the publication titled " High sensitivity multianalyte immunoassay using covalent DNA-labeled antibodies and polymerase chain reaction" by HENDERSON et al. (hereinafter "Henderson").

As to claim 11, Niemeyer teaches association of capture agent with substrate attached oligonucleotide by DNA annealing (pg 400 fig 6). Neither Niemeyer nor LaBaer teaches that the region is associated to the oligonucleotide via a linker molecule. However, Henderson teaches a region is associated to the oligonucleotide via a linker molecule (pg 523 fig 2; "Design of DNA-antibody reporter conjugates--chemical crosslinker). An artisan of ordinary skill in the art would have readily appreciated that a linker region was well known in the art for covalently linking DNA directly to a protein molecule such as an antibody (i.e. capture agent). Consequently, it would have been obvious to one of ordinary skill in the art to combine association of capture agent with substrate attached oligonucleotide, as taught by Niemeyer, with region of association is a linker, as taught by Henderson, because it would have enabled attaching the capture agent directly to the oligonucleotide.

Claims 16-21 lack an inventive step under PCT Article 33(3) as being obvious over LaBaer, in view of Niemeyer, in further view of the publication titled " Next-generation high-density self-assembling functional protein arrays" by RAMACHANDRAN et al. (hereinafter "Ramachandran").

As to claim 16, LaBaer teaches a method of identifying an agent that binds to a peptide of interest, comprising: providing an array (abstract) comprising:
a substrate having a surface (para [0005]);
two or more individual constructs associated on the surface of the substrate, wherein the individual constructs comprise:
(i) an oligonucleotide encoding a peptide of interest (para [0005], [0015]) and
(ii) the peptide of interest (para [0022]; wherein the oligonucleotide is associated with the substrate surface (para [0005]). LaBaer does not teach that the peptide is associated with the oligonucleotide. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Furthermore, LaBaer does not specifically teach exposing the array to an agent; and detecting the presence or absence of binding of the agent to the peptide of interest. However, Ramachandran teaches exposing the array to an agent; and detecting the presence or absence of binding of the agent to the peptide of interest (pg 537 left col para 1; " To confirm protein function on high-density arrays, we printed an array expressing 647 unique genes in duplicate, including 449 genes that we had not previously tested (Fig. 3a and Supplementary Table 3 online). We tested for binary interactions between several well characterized interacting pairs including Jun-Fos and p53-MDM2 (Fig. 3b). We co-expressed the query protein along with the arrayed proteins by adding the appropriate cDNA clone (without a GST tag) to the cell-free expression lysate. After protein expression and washing, we treated the arrays with protein-specific antibodies to detect the query protein, revealing the positions where it bound. Using Jun, Fos and MDM2 as queries, we detected selective binding to their appropriate interacting partners"; pg 538 fig 3B). An artisan of ordinary skill in the art would have readily appreciated that binding of a protein through protein-protein interaction was well known in the art at the time of the invention. Consequently, it would have been obvious to one of ordinary skill in the art to combine an oligonucleotide bound to a substrate encoding a protein of interest, as taught by LaBaer, with a peptide of interest associated with an array, also taught by LaBaer, with peptide is associated with the oligonucleotide, as taught by Niemeyer, with detecting the presence or absence of binding of an agent to the peptide of interest, as taught by Ramachandran, because it would have enabled a functional protein biochip that identifies an agent that binds to a peptide of interest.

As to claim 17, Ramachandran further teaches the agent is provided in a sample, and wherein presence of binding to the peptide of interest indicates the presence of the agent in a sample (pg 537 left col para 1; pg 538 fig 3B).

As to claim 18, Ramachandran further teaches that the agent is provided in a sample, and wherein absence of binding to the peptide of interest indicates an absence of the agent in a sample (pg 537 left col para 1; pg 538 fig 3B).
----------continued in next Supplemental Box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/59327

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
previous Supplemental Box

As to claim 19, LaBaer teaches a method of identifying an agent that binds to a peptide of interest, comprising:
providing an array (abstract) comprising:
a substrate having a surface (para [0005]); two or more individual constructs associated on the surface of the substrate, wherein the individual constructs comprise:
(i) an oligonucleotide comprising a region encoding a peptide of interest, a region encoding an affinity tag, and an untranslated region (para [0005], [0015], [0056])
(ii) a peptide comprising the peptide of interest and an affinity tag (para [0022]); and
(iii) a capture agent that selectively binds to the affinity tag (para [0046]); wherein the oligonucleotide is associated with the substrate surface (para [0005]). LaBaer does not teach that the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent. However, Niemeyer teaches oligonucleotide-antibody array where the peptide is associated with the oligonucleotide through binding of the affinity tag to the capture agent (pg 400 fig 6; antibody microarray where antibody is the capture agent). An artisan of ordinary skill in the art would have readily appreciated that the capture agent could have been attached to the oligonucleotide directly, as taught by Niemeyer, or attached directly to the substrate, as taught by LaBaer (sheet 1 fig 1). Furthermore, LaBaer does not teach exposing the array to an agent; and detecting the presence or absence of binding of the agent to the peptide of interest. However, Ramachandran teaches exposing the array to an agent (pg 537 left col para 1); and detecting the presence or absence of binding of the agent to the peptide of interest (pg 537 left col para 1, pg 538 fig 3B). An artisan of ordinary skill in the art would have readily appreciated that binding of a protein through protein-protein interaction was well known in the art at the time of the invention. Consequently, it would have been obvious to one of ordinary skill in the art to combine an oligonucleotide bound to a substrate encoding a protein of interest, as taught by LaBaer, with a peptide of interest associated with an array, also taught by LaBaer, with capture agent that selectively binds to affinity tag, also as taught by LaBaer, with peptide is associated with the oligonucleotide, as taught by Niemeyer, with detecting the presence or absence of binding of an agent to the peptide of interest, as taught by Ramachandran, because it would have enabled a functional protein biochip that identifies an agent that binds to a peptide of interest.

As to claim 20, Ramachandran further teaches that the agent is provided in a sample (pg 537 left col para 1), and wherein the presence of binding to the peptide of interest indicates the presence of the agent in a sample (pg 538 fig 3B).

As to claim 21, Ramachandran the agent is provided in a sample (pg 537 left col para 1), and wherein absence of binding to the peptide of interest indicates an absence of the agent in a sample (pg 538 fig 3B).

Claims 1-35 have industrial applicability as defined in PCT Article 33(4) because the subject matter can be made or used by industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PGNS008PCT	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 11/31163	International filing date *(day/month/year)* 05 April 2011 (05.04.2011)	(Earliest) Priority Date *(day/month/year)* 05 April 2010 (05.04.2010)
Applicant PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 9 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☒ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/31163

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6, 287.2, 7.1, 91.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 435/6, 287.2, 7.1, 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C12Q 1/68; C12M 3/00 (2011.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,EPAB,JPAB) and Google (non-patent literature): high-throughput, binding event, co-localized, affinity, COLA, array, microarray, bead, sequence, digital, computer, electronic, software, DNA, RNA, nucleotide, nucleic acid, aptamer, identify, ascertain, determine, count, measure

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0134669 A1 (Casasanta) 22 June 2006 (22.06.2006). Para [0006], [0012]-[0014].	1-37
Y	US 2003/0224419 A1 (Corcoran et al.) 4 December 2003 (04.12.2003). Fig 5; Para [0002], [0021], [0022], [0082], [0083], [0096].	1-37
Y	US 7,378,242 B2 (Hurt) 27 May 2008 (27.05.2008). Col 3, ln 64-col 4, ln 16; col 6, ln 55-60.	13-15, 30-37
A, P	US 2010/0184614 A1 (Ye et al.) 22 July 2010 (22.07.2010), entire document.	1-37
A	US 2009/0291854 A1 (Wiesinger-Mayr et al.) 26 November 2009 (26.11.2009). Para [0035].	1-37

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 May 2011 (13.05.2011)	23 MAY 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SARAH BRASHEARS
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	23 MAY 2011
Applicant's or agent's file reference PGNS008PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/31163	05 April 2011 (05.04.2011)	05 April 2010 (05.04.2010)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6, 287.2, 7.1, 91.2

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	16 May 2011 (16.05.2011)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-37	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-37	NO
Industrial applicability (IA)	Claims	1-37	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-12 and 16-29 lack an inventive step under PCT Article 33(3) as being obvious over US 2006/0134669 A1 (Casasanta), in view of US 2003/0224419 A1 to Corcoran et al. (hereinafter "Corcoran").

As to claim 1, Casasanta teaches a method for identifying binding agents that form a binding pair, comprising:
a) providing a first set of binding constructs immobilized on a support surface, wherein each binding construct of the first set of binding constructs comprises a first binding agent and a first nucleic acid tag unique to the first binding agent (para [0014]...first component of the binding pair is immobilized on a spot of the microarray and the second component is bound to a protein...binding pairs can include...complementary strands of DNA or RNA);
b) providing a second set of binding constructs in solution, wherein each binding construct of the second set of binding constructs comprises a second binding agent and a second nucleic acid tag unique to the second binding agent, and wherein either or both of the first and second sets of binding constructs comprises at least ten different binding agents (para [0014]...second component of the binding pair is introduced to the microarray by...solution flooding);
c) combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, thereby co-locating the first nucleic acid tags and the second nucleic acid tags (para [0014]...complementary strands of DNA or RNA are bound together, therefore are co-located);
d) creating binding event identifiers from the co-located first and second nucleic acid tags (para [0012]...spectral shifts can be measured for respective molecular binding events).

Casasanta does not specify what Corcoran does in an alternative ligation-based sequencing scheme (para [0022]), namely:
e) determining a sequence of each binding event identifier, wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair (para [0002]...massively parallel signature sequencing).

Neither Casasanta nor Corcoran specify at least ten different binding agents. Casasanta does teach testing a variety of binding pair types (para [0014]...antigen/antibody, peptide/peptide, complementary DNA/RNA), discriminating between different spots (para [0018]) and testing up to 1000 spots (para [0013]). It would have been obvious to one skilled in the art to use at least ten different binding agents, for greater economy of testing scale. Further, it would have been obvious to one skilled in the art to combine the binding pair sequence of Corcoran with the method of Casasanta to enable more specific detection of binding events between a variety of biological molecules.

As to claim 2, Corcoran further teaches wherein the sequence of the binding event identifier is determined by digital readout 36, 38 (Fig 3...display controller, display device).

As to claim 3, Corcoran further teaches wherein the sequence of the binding event identifier is determined by high throughput digital sequencing (para [0022]...massively parallel signature sequencing...identify simultaneously signatures of very large numbers of DNAs attached to microbeads disposed in closely packed arrays).

As to claims 4-7, Casasanta and Corcoran make obvious the method of claim 1. Neither specifies wherein either or both of the first and second sets of binding constructs comprises at least twenty-five, one hundred, one thousand or at least five thousand different binding agents, respectively. Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches each binding pair has a unique signature (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound.

As to claims 8, Corcoran further teaches wherein the sequences of the binding event identifiers are determined in parallel (para [0002]...massively parallel signature sequencing)

As to claims 9-10, Casasanta and Corcoran make obvious the method of claim 8. Casasanta teaches microarrays having 1008 spots (para [0013]). Corcoran teaches identifying in parallel (para [0002]) sequences of binding pairs on a microarray (para [0002]; [0083]...microbead array display). Neither specifies the sequence of 100,000 binding events determined in parallel. However, it would have been obvious to one skilled in the art that the technique of Corcoran could be applied to microarrays of any dimension.

Please continue to the supplemental page

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Citations and Explanations, Box V(2):

As to claim 11, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches wherein the binding event identifier is created by coupling the first and second nucleic acid tags (Fig 5...note the sticky ends and the two ligation steps).

As to claim 12, Casasanta and Corcoran make obvious the method of claim 10. Corcoran further teaches wherein the coupling of the first and second tags is accomplished by ligation (Fig 5...note the sticky ends and the two ligation steps).

As to claim 16, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches wherein at least one of the first and second binding agents is a peptide (para [0006]...peptide-peptide pair).

As to claim 17, Casasanta and Corcoran make obvious the method of claim 16. Casasanta further teaches wherein the first and second binding agents are peptides (para [0006]...peptide-peptide pair).

As to claim 18, Casasanta and Corcoran make obvious the method of claim 16. Casasanta further teaches wherein the second binding agent is an antibody (para [0006]...hapten-antibody pair, antigen-antibody pair).

As to claim 19, Casasanta and Corcoran make obvious the method of claim 16, wherein the second binding agent is a small molecule (para [0006]...peptides can be small molecules; avidin and biotin are small molecules).

As to claim 20, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches wherein the first or second binding agent is an aptamer (para [0014]...biotin, antigen, antibody, oligonucleotides are all aptamers).

As to claim 21, Casasanta and Corcoran make obvious the method of claim 20. Casasanta further teaches wherein the first binding agent is a peptide and the second binding agent is an aptamer (para [0014]...first component immobilized on a spot in the microarray...second component introduced by solution flooding...combinations can include antigen/antibody, peptide/peptide, or complementary strands of DNA or RNA...all of these potential components of the binding pair can function as aptamers).

As to claim 22, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches wherein the support is a microarray 8 (para [0012]).

As to claim 23, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches wherein the support is a bead (Fig 5...see 'image microbeads').

As to claim 24, Casasanta and Corcoran make obvious the method of claim 1. Casasanta further teaches the step of adding a third binding agent in the combining step (para [0014]...third chemical component can bind the complex of the first component and the second component).

As to claim 25, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches the step of identifying binding agents that bind promiscuously (para [0096]...optical measurements were adjusted for system background noise, which can result from nonspecific binding of probes...or incomplete ligation).

As to claim 26, Casasanta and Corcoran make obvious the method of claim 25. Corcoran further teaches wherein data from promiscuous binding agents is subtracted from binder identifier results of the determining step (para [0096]...optical measurements were adjusted for system background noise, which can result from nonspecific binding of probes...or incomplete ligation).

As to claim 27, Casasanta and Corcoran make obvious the method of claim 25. Corcoran further teaches wherein a quantitative metric can be derived for the extent of promiscuity of promiscuous binding agents (para [0096]...computing the background noise for each signal set...and subtracting that computed value from each of the four fluorescence values...).

As to claim 28. Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches wherein false positives are identified within the binding event identifiers and data from the false positives subtracted from binder identifier results of the determining step (para [0096]...computing the background noise for each signal set...and subtracting that computed value from each of the four fluorescence values...).

As to claim 29, Casasanta and Corcoran make obvious the method of claim 1. Corcoran further teaches the step of determining the number of each binding event identifier sequenced (para [0021]...counting of tags or signatures of DNA fragments).

Claims 13-15 and 30-37 lack an inventive step under PCT Article 33(3) as being obvious over Casasanta, in view of Corcoran and further in view of US 7,378,242 B2 (Hurt).

As to claim 13, Casasanta and Corcoran make obvious the method of claim 10. Neither specifies wherein the coupling of the first and second tags is accomplished by primer extension. Hurt teaches coupling strands of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control the nucleotide sequence.

Please continue

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Citations and Explanations, Box V(2):

As to claim 14, Casasanta and Corcoran make obvious the method of claim 1. Neither teaches wherein one or both of the first and second binding constructs comprise a primer sequence. Hurt teaches binding two pieces of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control the nucleotide sequence.

As to claim 15, Casasanta, Corcoran and Hurt make obvious the method of claim 13. Hurt further teaches the step of amplifying the binding event identifier after the creating step and before the determining step (col 6, ln 55-60...multiple temperature cycles can be used to amplify the extension product...different temperatures can be used for the three steps: association of the primer to the template, extension, and separation of the LPE product from the template).

As to claim 30, Casasanta teaches a method for identifying binding agents that form a binding pair, comprising:

a) providing a first set of binding constructs immobilized on a support surface, wherein each binding construct of the first set of binding constructs comprises a first binding agent, and a first nucleic acid tag unique to the first binding agent (para [0014]...first component of the binding pair is immobilized on a spot of the microarray and the second component is bound to a protein...binding pairs can include...complementary strands of DNA or RNA);
b) providing a second set of binding constructs in solution, wherein each binding construct of the second set of binding constructs comprises a second binding agent, and a second nucleic acid tag unique to the second binding agent, and wherein either or both of the first and second sets of binding constructs comprises at least ten different binding agents (para [0014]...second component of the binding pair is introduced to the microarray by...solution flooding);
c) combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, thereby co-locating the first nucleic acid tags and the second nucleic acid tags (para [0014]...complementary strands of DNA or RNA are bound together, therefore are co-located);
d) creating binding event identifiers from the co-located first and second nucleic acid tags (para [0012]...spectral shifts can be measured for respective molecular binding events).

Casasanta does not specify:
wherein the first set of binding constructs also comprises a first primer region,
wherein the second set of binding constructs comprises a second primer region, or
e) determining a sequence of at least one thousand binding event identifiers, wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair; and
f) determining the frequency of each binding event identifier sequenced.

Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches each binding pair has a unique signature (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound. Corcoran further teaches the step of determining the number of each binding event identifier sequenced (para [0021]...counting of tags or signatures of DNA fragments). It would have been obvious to one skilled in the art to determine the frequency of each binding event identifier sequences, to enable analysis of broader trends in binding events.

Hurt teaches coupling strands of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control the nucleotide sequence.

As to claim 31, Casasanta, Corcoran and Hurt make obvious the method of claim 30. Corcoran further teaches wherein the sequences of the binding event identifiers are determined in parallel (para [0002]...massively parallel signature sequencing).

As to claims 32-33, Casasanta, Corcoran, and Hurt make obvious the method of claim 31. None specifies wherein the sequence of at least 1,000 or 100,000 binding event identifiers, respectively, are determined in parallel. Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches determining the sequences of the binding event identifiers in parallel (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound.

Please continue

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31163

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Citations and Explanations, Box V(2):

As to claim 34, Casasanta teaches a method for characterizing the specificity of binding between binding agents that form a binding pair, comprising:
a) providing a first set of binding constructs immobilized on a support surface, wherein each binding construct of the first set of binding constructs comprises a first binding agent, and a first nucleic acid tag unique to the first binding agent (para [0014]...first component of the binding pair is immobilized on a spot of the microarray and the second component is bound to a protein...binding pairs can include...complementary strands of DNA or RNA);
b) providing a second set of binding constructs in solution, wherein each binding construct of the second set of binding constructs comprises a second binding agent, and a second nucleic acid tag unique to the second binding agent, and wherein either or both of the first and second sets of binding constructs comprises at least ten different binding agents (para [0014]...second component of the binding pair is introduced to the microarray by...solution flooding);
c) combining the first and second sets of binding constructs under conditions to allow the first binding agents and the second binding agents to form binding pairs, thereby co-locating the first nucleic acid tags and the second nucleic acid tags (para [0014]...complementary strands of DNA or RNA are bound together, therefore are co-located);
d) creating binding event identifiers from the co-located first and second nucleic acid tags (para [0012]...spectral shifts can be measured for respective molecular binding events).

Casasanta does not specify what Corcoran does in an alternative ligation-based sequencing scheme (para [0022]), namely:
e) determining a sequence of each binding event identifier, wherein the sequence of each binding event identifier identifies the binding pair and the binding agents that form the binding pair (para [0002]...massively parallel signature sequencing). It would have been obvious to one skilled in the art to determine the frequency of each binding event identifier sequences, to enable analysis of broader trends in binding events.

Neither Casasanta nor Corcoran specifies:
wherein the first set of binding constructs also comprises a first primer region,
wherein the second set of binding constructs comprises a second primer region. Hurt teaches coupling strands of DNA by primer extension (col 3, ln 64-col 4, ln 16). It would have been obvious to one skilled in the art to couple the first and second tags by primer extension, as taught by Hurt, with the method of Casasanta and Corcoran, to more precisely control and track the nucleotide sequence.

As to claims 35-36, Casasanta, Corcoran and Hurt make obvious the method of claim 34, but do not specify wherein the sequence of at least 1,000 or 100,000 binding event identifiers, respectively, are determined in parallel. Casasanta teaches a 36x28 microarray having 1008 spots (para [0013]) and that the binding pairs may comprise complementary strands of DNA or RNA (para [0014]). Corcoran teaches determining the sequences of the binding event identifiers in parallel (para [0002]...massively parallel signature sequencing; [0021]...element-to-element differences within microarrays; [0022]...ligation-based sequencing scheme). It would have been obvious to one skilled in the art to use the Corcoran digital sequencing method of an array of any number of spots, to uniquely identify the sequences that are bound.

As to claim 37, Casasanta, Corcoran and Hurt make obvious the method of claim 34. Hurt further teaches the step of amplifying the binding event identifier after the creating step and before the determining step (col 6, ln 55-60...multiple temperature cycles can be used to amplify the extension product...different temperatures can be used for the three steps: association of the primer to the template, extension, and separation of the LPE product from the template).

Claims 1-37 have industrial applicability as defined by PCT Article 33(4) because the subject can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PGNS007PCT	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 11/31308	International filing date *(day/month/year)* 05 April 2011 (05.04.2011)	(Earliest) Priority Date *(day/month/year)* 05 April 2010 (05.04.2010)
Applicant PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/31308

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
435/7.9,287.2,7.1,91.2,4

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST - DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=YES; OP=ADJ; Google Scholar
search terms: probe, analyt$, gene or marker, biomarker, oligonucle$, polymer, oligomer, polynucleo$, nucleotid$, nucleic, dna, rna, deoxyribonuc$, protein, affix$, attach$, immobil$, bound, tether$, teather$, support, surface, substrate, solid, multip$, many, differen$,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2006/0046313 A1 (ROTH et al.) 02 March 2006 (02.03.2006) para [0027]; [0031]-[0033]; [0036]; [0038]; [0042]; [0043]; [0047]; [0053]; [0055]; [0069]; [0073]; [0083]-[0085]; [0090]-[0092]; [0097]; [0098]; [0102]-[0105]; [0122]-[0124]; [0138]; [0140].	1-3, 6-37, 39-55, 57 ------ 4, 5, 38, 56
Y	US 6,344,316 B1 (LOCKHART et al.) 05 February 2002 (05.02.2002) abstract; col. 24, ln 8-61.	4, 5, 38, 56

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2011 (25.05.2011)	0 7 JUN 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SARAH BRASHEARS
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	07 JUN 2011
Applicant's or agent's file reference: PGNS007PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/31308	05 April 2011 (05.04.2011)	05 April 2010 (05.04.2010)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2011.01)
USPC - 435/6

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	25 May 2011 (25.05.2011)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Curio EX1010-p. 380
Curio v Prognosys
IPR2024-01289

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	4, 5, 38, 56	YES
	Claims	1-3, 6-37, 39-55, 57	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-57	NO
Industrial applicability (IA)	Claims	1-57	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-3, 6-37, 39-55 and 57 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0046313 A1, Roth et al., (hereinafter Roth).

Regarding claim 1, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033] - 'The invention reverse phase protein assay includes forming one or more arrays of cell lysates obtained from one or more tissue samples from a patient. For example, individual cells of a cell population obtained from a patient tissue sample may be lysed and extracted 310 to form an array. If cell lysates are obtained from more than one tissue sample, two or more arrays may be formed, with an array containing the cell lysates from a particular tissue sample of the patient In an array of defined locations 320 on a substrate, a mixture of extracted cell proteins 330, 331, 332 is immobilized at (for example, spotted onto) individual defined locations,');
delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered (para [0033] - 'Then the cell lysate spots at the defined locations are contacted with a mixture of COIN-labeled probes 330, 332 under conditions suitable to form analyte-COIN complexes 341, 342, 343 as the probes bind individual protein in the spots through both specific and degenerate binding.', and para [0097]);
allowing the encoded probes to interact with the biological targets (para [0033]);
separating encoded probes that interact with the biological targets from encoded probes that do not interact with the biological targets (para [0033] - 'After removal of unbound COIN-labeled probes from the array');
determining all or a portion of a sequence of the encoded probes (para [0104] and [0122]), and
associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample (para [0033] - 'the array 350 may be scanned 340 to detect SERS signals at the defined locations 320 so as to identify and compile data regarding the presence of the known analytes in the tissue sample.').

Regarding claim 27, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple nucleic acid targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033]);
delivering oligonucleotide probes for multiple nucleic acid targets to the multiple sites in the sample in a known spatial pattern (para [0033] and [0097]);
allowing the oligonucleotide probes to hybridize with the nucleic acid targets (para [0033] and [0102]-[0103]);
washing unhybridized encoded oligonucleotide probes from the sample (para [0033] and [0102]-[0103]);
delivering one or more encoding agents to locations of the multiple sites in the sample according to a known spatial pattern, wherein the combination of encoding agents delivered to each site is different (para [0086] and [0097] - encoding agents are 'Raman-active organic compounds');
coupling the encoding agents and the oligonucleotide probes to form encoded probes (para [0086]);
determining all or a portion of a sequence of the encoded probes using high throughput sequencing (para [0104] and [0122]), and
associating the abundance or activity or both of multiple biological targets to the locations of multiple sites in the sample (para [0033], [0104] and [0122]).

----------continued in next supplemental box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
citations and explanations from box V.2

Regarding claim 40, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple protein targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033]);
delivering encoded probes for the multiple protein targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a protein probe region that may interact with the protein targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and the protein probe region of the encoding probe of which the coding tag is part (para [0033], [0092] and [0097]);
allowing the encoded probes to interact with the protein targets (para [0033]);
separating encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets (para [0033]);
determining all or a portion of a sequence of the encoded probes by high throughput sequencing (para [0104] and [0122]), and
associating the abundance or activity or both of the multiple protein targets to the locations of the multiple sites in the sample (para [0033]).

Regarding claim 2, Roth teaches the biological targets are nucleic acids and the encoded probes are oligonucleotides (para [0102]-[0103]).

Regarding claim 3, Roth teaches there are two encoded probes for each of the multiple nucleic acid targets (para [0047] and [0091]).

Regarding claim 6, Roth teaches probe regions of the encoded probes comprise antibodies (para [0069]).

Regarding claim 7, Roth teaches the probe regions of the encoded probes are aptamers (para [0069]).

Regarding claim 8, Roth teaches the probe regions of the encoded probes are small molecules (para [0069]).

Regarding claims 9, 39 and 50, Roth teaches an amplification step between the separating step and the determining step (para [0105]).

Regarding claim 10, Roth teaches the determining step is performed by nucleic acid sequencing (para [0104] and [0122]).

Regarding claim 11, Roth teaches the sequencing is high-throughput digital nucleic acid sequencing (para [0039], [0122]-[0124]).

Regarding claims 12-19, 28-33, 41-46, Roth teaches the product of the multiple biological targets being assayed and the multiple sites in the sample is greater than 1,000,000 (para [0097]-[0098] and [0138]).

Regarding claims 20-22, 34, 35, 47-49, Roth teaches the sequence of at least one million encoding probes are determined in parallel (para [0097]-[0098], [0104] and [0138]).

Regarding claim 23, Roth teaches the known spatial pattern is determined by histological features of the sample (para [0036] and [0038]).

Regarding claim 24, Roth teaches software programmed hardware performs at least two steps of the delivering step, the separation step, the determining step and the associating step (para [0123]-[0124]).

Regarding claim 25, Roth teaches the probe regions of the encoded probes are proteins (para [0092]), and the separating step is accomplished by encoded probes that interact with the biological targets being captured by an affinity capture agent (para [0027] - 'contacting a biological sample 110 with a substrate 100 having a set of capture antibodies 120 that bind specifically to different proteins attached at defined locations on the substrate 100 to allow formation of antibody-protein complexes 130. The antibody-protein complexes are then contacted with a set of COIN-labeled probes 140 comprising a probe moiety 150 that binds specifically to a known protein analyte').

Regarding claim 26, Roth teaches the probe regions of the encoding probes are nucleic acids and the separating step is accomplished by a washing of the sample (para [0084]-[0085]).

Regarding claim 36, Roth teaches two oligonucleotide probes are delivered for each of the multiple nucleic acid targets (para [0083] - 'the term "oligonucleotide" is used herein to refer to a polynucleotide that is used as a primer or a probe. Generally, an oligonucleotide useful as a probe or primer that selectively hybridizes to a selected nucleotide sequence is at least 6 nucleotides to about 9 nucleotides in length.' --oligonucleotide probes are used as primers; later amplification would require the primers are in pairs).

----------continued in next supplemental box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 11/31308

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
previous supplemental box

Regarding claim 37, Roth teaches the coupling step is performed by ligation (para [0083] - 'Polynucleotide probes used in the invention methods are useful for detecting and hybridizing under suitable conditions to complementary polynucleotides in a biological sample by pairing a known polynucleotide probe with a known Raman-active COIN comprising one or more Raman-active organic compounds, as described herein. A covalent phosphodiester bond generally ligates the nucleotides of a polynucleotide sequence. However, the covalent bond also may be any of numerous other bonds, including a thiodiester bond, a phosphorothioate bond, a peptide-like amide bond or any other bond known to those in the art as useful for linking nucleotides to produce synthetic polynucleotides').

Regarding claim 51, Roth teaches the protein targets are enzymes (para [0090]), and the probe regions of the encoded probes are substrates, putative substrates of both for the enzymes (para [0069] and [0090]).

Regarding claim 52, Roth teaches the probe regions of the encoded probes are affinity capture agents (para [0043]).

Regarding claim 53, Roth teaches the probe regions of the encoded probes are antibodies (para [0069]).

Regarding claim 54, Roth teaches the probe regions of the encoded probes are aptamers (para [0069]).

Regarding claim 55, Roth teaches separating encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets is accomplished by an affinity capture agent that distinguishes encoded probes that interact with the protein targets from encoded probes that do not interact with the protein targets (para [0027] and [0042]-[0043]).

Regarding claim 57, Roth teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (para [0032] and [0140]), wherein the assay system performs the following steps:
providing a sample affixed to a support (para [0033]);
delivering encoded probes for the multiple biological targets to the multiple sites in the sample in a known spatial pattern, wherein each encoded probe comprises a probe region that may interact with the biological targets and a coding tag that identifies a location of the site to which the encoded probe was delivered and identifies the biological target (para [0033], [0092] and [0097]);
allowing the encoded probes to interact with the biological targets (para [0033]);
determining all or a portion of a sequence of the encoded probes (para [0104] and [0122]), and
associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample (para [0033]).

Claims 4, 5, 38 and 56 lack an inventive step under PCT Article 33(3) as being obvious over Roth in view of US 6,344,316 B1, Lockhart et al., (hereinafter Lockhart).

Regarding claim 4, Roth teaches the multiple biological targets are proteins (para [0069]), the probe regions of the encoding probes are proteins (para [0073]). Roth does not specifically teach the coding tags comprise oligonucleotides. However, Roth does teach the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]). Lockhart teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (abstract), comprising encoded probes for the multiple biological targets (abstract), wherein the coding tags comprise oligonucleotides (abstract and col. 24, ln 8-30). It would have been obvious to one of ordinary skill in the art to have prepared an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the coding tags comprise oligonucleotides, in view of Roth teaching the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]), and in light of Lockhart teaching unique and florescent coding tags comprising oligonucleotides (col. 24, ln 37-61), since a skilled artisan would have readily appreciated use of the labeling methods taught by Lockhart applied to the probes taught by Roth, since they can be detected in a microarray.

Regarding claim 5, Roth teaches the multiple biological targets comprise enzymes (para [0090]).

Regarding claim 38, Roth does not specifically teach the coupling step is performed by extension followed by ligation. Lockhart teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (abstract), comprising coupling the one or more encoding agents and one or more oligonucleotide probes to form encoded probes (abstract and col. 24, ln 8-30), wherein the coupling step is performed by extension followed by ligation (col. 24, ln 8-30). It would have been obvious to one of ordinary skill in the art to have prepared an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the coupling step is performed by extension followed by ligation, in view of Roth teaching the probe is coupled to the encoding agents by various methods including ligation (para [0083]), and in light of Lockhart teaching coupling of one or more encoding agents and one or more oligonucleotide probes to form encoded probes (col. 24, ln 8-30), wherein the coupling step is performed by extension followed by ligation (col. 24, ln 8-30), since a skilled artisan would have readily appreciated use of the coupling methods taught by Lockhart applied to the probes taught by Roth, since they are both polynucleotide based probes, and could therefore be coupled via extension.

----------continued in next supplemental box----------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/31308

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
previous supplemental box

Regarding claim 56, Roth does not specifically teach the coding tags are oligonucleotides. However, Roth does teach the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]). Lockhart teaches an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample (abstract), comprising encoded probes for the multiple biological targets (abstract), wherein the coding tags comprise oligonucleotides (abstract and col. 24, ln 8-30). It would have been obvious to one of ordinary skill in the art to have prepared an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, wherein the coding tags comprise oligonucleotides, in view of Roth teaching the coding tags are unique and florescent for scanning based detection (para [0031] and [0055]), and in light of Lockhart teaching unique and florescent coding tags comprising oligonucleotides (col. 24, ln 37-61), since a skilled artisan would have readily appreciated use of the labeling methods taught by Lockhart applied to the probes taught by Roth, since they can be detected in a microarray.

Claims 1-57 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in the industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PGNS009PCT	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/32759	International filing date *(day/month/year)* 09 April 2012 (09.04.2012)	(Earliest) Priority Date *(day/month/year)* 08 April 2011 (08.04.2011)
Applicant PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/32759

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-8, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. The first named invention (claims 1-3, 6-8), is limited to wherein the species are eukaryotic species. Applicant is invited to elect an additional limitations including: wherein the species are bacterial species or human pathogens (claims 4-5), to be searched by paying additional fee for each election.

**********Continued in the extra sheet**********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 6-8

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/32759

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The disclosure provides methods for constructing peptide construct sets and methods of use of these peptide construct sets in assay systems for peptide analysis, and in particular for use in high throughput peptide analysis. The methods allow for analysis of large sets of peptide constructs in a cost-effective manner, employing molecular biological techniques that are both robust and easily parallelized. Thus, the methods allow for the construction of peptide construct sets encompassing, e.g., the human proteome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/32759

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C40B 40/08; C40B 40/10; C40B 50/06 (2012.01)
USPC - 506/17; 506/18; 506/26
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C40B 40/08; C40B 40/10; C40B 50/06; A61K 38/00; A61K 38/04; G01N 33/566 (2012.01)
USPC - 506/17; 506/18; 506/26; 530/300; 530/324, 530/325, 530/326; 530/327; 436/501

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C40B 40/08; C40B 40/10; C40B 50/06; A61K 38/00; A61K 38/04; G01N 33/566 (2012.01) - see keyword below
USPC - 506/17; 506/18; 506/26; 530/300; 530/324, 530/325, 530/326; 530/327; 436/501 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: peptide, nucleotide, encoding, fusion, conjugate, library, translation, ribosome, collection, array, micro-array, distinct, diversity, different, construct, identical, species, organism, eukaryotic, mammalian, human, overlapping, amino acid, length, 5000,10000, in-frame, stop codon, elongation, relea

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2003/0235852 A1 (ROBERTS et al.) 25 December 2003 (25.12.2003), para [0004], [0005], [0008], [0009], [0057], [0059], [0065], [0066], [0083], [0090], [0092], [0094], and [0127]	1-3, 6-8
Y	US 2009/0280487 A1 (HUNG et al.) 12 November 2009 (12.11.2009), para [0019],[0039], [0056], [0058], [0062], [0066], [0073], [0091], [0096], [0103], and [0129]	1-3, 6-8
Y	US 2007/0014810 A1 (BAKER et al.) 18 January 2007 (18.01.2007), para [0359], and [0361]	6
A	US 2007/0020625 A1 (DUCHAUD et al.) 25 January 2007 (25.01.2007), para [0093], and [0253]	1-3, 6-8
A	US 2005/0026188 A1 (VAN KESSEL et al.) 03 February 2005 (03.02.2005), para [0029], [0060], and [0062]	1-3, 6-8
A	Atkinson, Insert Overview of Translation here 2 pages, Between sections 5 and 6, University of Texas, Lecture manuscript, 2000, [online]. [Retrieved on 2012.09.11]. Retrieved from the Internet: <URL: http://www.sbs.utexas.edu/herrin/bio344/lectures/lecturespdf/Background/gSection%206.pdf> Entire documentation, especially pg 6.5, Elongation; and pg 6.6, Translocation and Termination	1-3, 6-8
A	US 2010/0113302 A1 (Williams) 06 May 2010 (06.05.2010), para [0008], [0014], [0018], [0024], [0046], [0054], and [0056]	1-3, 6-8
A	US 2008/0220981 A1 (McGregor) 11 September 2008 (11.09.2008), Abstract, para [0009], [0011], and [0022]	1-3, 6-8

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
12 September 2012 (12.09.2012)

Date of mailing of the international search report
28 SEP 2012

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/32759

Continuation of:
Box No III (unity of invention is lacking)

Group II, claims 9-14, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed such that the peptide sequence diversity is no less than 1 % of the encoding oligonucleotide sequence diversity.

Group III, claims 15-20 drawn to a method for analyzing a sample using a set of peptide constructs, each peptide construct comprising a custom-designed peptide portion and an identifying nucleic acid portion, comprising the steps of: a. performing an assay on the set of peptide constructs, where some of the peptide constructs are acted upon by assay agents, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs of either the peptide constructs that were acted upon by the assay agents or the peptide constructs that were not acted upon by the assay agents by analyzing the nucleic acid portion of the peptide constructs.

The inventions listed as Groups I+-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+- II do not include the inventive concept of analyzing a sample using a set of peptide constructs, comprising the steps of: a. performing an assay on the set of peptide constructs, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs, as required by Group III.

Groups II-III do not include the inventive concept of a set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, as required by Groups I+.

Furthermore, among Group I+, identifying oligonucleotide sequence from eukaryotic species (claims 2-3) are structurally different from identifying oligonucleotide sequence from bacterial species or human pathogens (claims 4-5) and are collected in different databases.

The inventions of Groups I+ and III share the technical feature of a set of peptide constructs, each peptide construct comprising a custom-designed peptide portion and an identifying nucleic acid portion; and Group I+ and II further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2003/0235852 A1 to ROBERTS et al. (hereinafter 'Roberts') as follows:

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules', wherein '10.sup.9 different molecules' comprising at least 5,000 distinct peptide constructs; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,... prepare peptide libraries, wherein the encoding nucleic acid molecule remains physically associated with the peptide. Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide')
---that are custom-designed (para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed'). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups I+ further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set
of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. However, this shared technical feature does not represent a contribution over prior art as being obvious over Roberts, in view of US 2009/0280487 A1 to HUNG et al. (hereinafter 'Hung') as follow:

**********Continued in the next extra sheet**********

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/32759

Continuation of:
The previous extra sheet - Box No III (unity of invention is lacking)

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090]; para [0008]; para [0127], as discussed above)
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009]; para [0005], as discussed above)
---that are custom-designed (para [0004],as discussed above)
--- so that the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids (para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues').

Although Roberts does not specifically teach wherein the peptide sequences of at least 12 amino acids are at least 10% of the set of peptide that have more than 80% amino acid identity to the protein sequences of interest, one of ordinary skill in the art at the time the invention was made would have known to do so, because Roberts teaches detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'), which requires the peptide having enough amino acids and requires enough percentage of peptides to have enough amino acids, including to have at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids to include a functional group that have more than 80% amino acid identity to the protein sequences of interest. And furthermore, it is a common practice to include peptides with known sequences in consensus regions that have at least 80% amino acid identity to the same proteins from different species when preparing a library (please see US 2007/0020625 A1 to DUCHAUD et al.: para [0253] - 'a DNA library prepared from the genome of a bacterium belonging to... species different from the Photorhabdus strain used to construct the DNA library'; para [0093] - 'The expression "polypeptide exhibiting a certain percentage identity with another", ... "homologous polypeptide" will also be used... the amino acid sequence exhibits at least 80%, preferably 85%, 90%, 95%, 98% or 99%, identity'; please also see US 2005/0026188 A1 to van KESSEL et al.: para [0029] - 'developing a library of nucleotide sequences ... corresponding to variable regions of the cpn60 gene'; para [0060] - 'a "cnp60-like" sequence ... encoding a protein ... has a biological function similar to that of cpn60, ... homology of at least 70%, 80% or 90% may be used'; para [0062]- 'performs substantially the same role ... the homology will be at least about 80%, 85%, 90% or 95%').

Roberts does not specifically teach wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism. Hung discloses a peptide library comprising G-protein coupled receptor polypeptides from up to 100 different species (para [0062] - 'a library contains a plurality of members, ...up to about 1000 or more olfactory GPCR polypeptides.... derived from a plurality of species'; para para [0019] - '"G-protein coupled receptors", or "GPCRs" are polypeptides'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal', wherein 'up to about 100...species of animal' is 'up to 100 different species of organism'; para [0129] - 'producing a library ... up to about 1000 or more...different olfactory GPCRs'; para [0056]; para [0058]) for activity determination and modulator identification (para [0096] - 'screening for olfactory GPCR modulators'; para [0103] - 'GPCR activity may be measured by assessing a reporter signal').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences of interest, based on the teaching of Roberts and the common practice, wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism, based on the combination of Hung and Roberts, in order to include protein of interests in the same family from different species for facilitating identifying the peptide sequence associated with activity for characterization of protein of interest. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SARAH J. BRASHEARS
CONVERGENT LAW GROUP LLP
475 N. WHISMAN ROAD, SUITE 400
MOUNTAIN VIEW, CA 94043

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 SEP 2012
Applicant's or agent's file reference PGNS009PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/32759	09 April 2012 (09.04.2012)	08 April 2011 (08.04.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C40B 40/08; C40B 40/10; C40B 50/06 (2012.01)
USPC - 506/17; 506/18; 506/26

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	12 September 2012 (12.09.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-8, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. The first named invention (claims 1-3, 6-8), is limited to wherein the species are eukaryotic species. Applicant is invited to elect an additional limitations including: wherein the species are bacterial species or human pathogens (claims 4-5), to be searched by paying additional fee for each election.

Group II, claims 9-14, drawn to a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed such that the peptide sequence diversity is no less than 1 % of the encoding oligonucleotide sequence diversity.

Group III, claims 15-20 drawn to a method for analyzing a sample using a set of peptide constructs, each peptide construct comprising a custom-designed peptide portion and an identifying nucleic acid portion, comprising the steps of: a. performing an assay on the set of peptide constructs, where some of the peptide constructs are acted upon by assay agents, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs of either the peptide constructs that were acted upon by the assay agents or the peptide constructs that were not acted upon by the assay agents by analyzing the nucleic acid portion of the peptide constructs.

The inventions listed as Groups I+-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+- II do not include the inventive concept of analyzing a sample using a set of peptide constructs, comprising the steps of: a. performing an assay on the set of peptide constructs, b. separating the peptide constructs that are acted upon by the assay agents from the peptide constructs that are not acted upon by the assay agents, and c. determining the identities of substantially all of the peptide constructs, as required by Group III.

Groups II-III do not include the inventive concept of a set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, as required by Groups I+.

Furthermore, among Group I+, identifying oligonucleotide sequence from eukaryotic species (claims 2-3) are structurally different from identifying oligonucleotide sequence from bacterial species or human pathogens (claims 4-5) and are collected in different databases.

The inventions of Groups I+ and III share the technical feature of a set of peptide constructs, each peptide construct comprising a custom -designed peptide portion and an identifying nucleic acid portion; and Group I+ and II further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2003/0235852 A1 to ROBERTS et al. (hereinafter 'Roberts') as follows:

**********Continued in the supplmental box**********

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-3, 6-8

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-3, 6-8	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-3, 6-8	NO
Industrial applicability (IA)	Claims	1-3, 6-8	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-3 and 7-8 lack an inventive step under PCT Article 33(3) as being obvious over US 2003/0235852 A1 to ROBERTS et al. (hereinafter 'Roberts'), in view of US 2009/0280487 A1 to HUNG et al. (hereinafter 'Hung').

Regarding claim 1, Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules', wherein '10.sup.9 different molecules' comprising at least 5,000 distinct peptide constructs; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
— each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,... prepare peptide libraries, wherein the encoding nucleic acid molecule remains physically associated with the peptide. Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide')
—that are custom-designed (para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed')
— so that the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids (para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues').

Although Roberts does not specifically teach wherein the peptide sequences of at least 12 amino acids are at least 10% of the set of peptide that have more than 80% amino acid identity to the protein sequences of interest, one of ordinary skill in the art at the time the invention was made would have known to do so, because Roberts teaches detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'), which requires the peptide having enough amino acids and requires enough percentage of peptides to have enough amino acids, including to have at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids to include a functional group that have more than 80% amino acid identity to the protein sequences of interest. And furthermore, it is a common practice to include peptides with known sequences in consensus regions that have at least 80% amino acid identity to the same proteins from different species when preparing a library (please see US 2007/0020625 A1 to DUCHAUD et al.: para [0253] - 'a DNA library prepared from the genome of a bacterium belonging to... species different from the Photorhabdus strain used to construct the DNA library'; para [0093] - "The expression "polypeptide exhibiting a certain percentage identity with another", ... "homologous polypeptide" will also be used... the amino acid sequence exhibits at least 80%, preferably 85%, 90%, 95%, 98% or 99%, identity'; please also see US 2005/0026188 A1 to van KESSEL et al.: para [0029] - 'developing a library of nucleotide sequences ... corresponding to variable regions of the cpn60 gene'; para [0060] - 'a "cnp60-like" sequence ... encoding a protein ... has a biological function similar to that of cpn60, ... homology of at least 70%, 80% or 90% may be used'; para [0062]- 'performs substantially the same role ... the homology will be at least about 80%, 85%, 90% or 95%').

Roberts does not specifically teach wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism. Hung discloses a peptide library comprising G-protein coupled receptor polypeptides from up to 100 different species (para [0062] - 'a library contains a plurality of members, ...up to about 1000 or more olfactory GPCR polypeptides.... derived from a plurality of species'; para [0019] - '"G-protein coupled receptors", or "GPCRs" are polypeptides'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal', wherein 'up to about 100... species of animal' is 'up to 100 different species of organism'; para [0129] - 'producing a library ... up to about 1000 or more...different olfactory GPCRs'; para [0056]; para [0058]) for activity determination and modulator identification (para [0096] - 'screening for olfactory GPCR modulators'; para [0103] - 'GPCR activity may be measured by assessing a reporter signal').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences of interest, based on the teaching of Roberts and common practice, wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism, based on the combination of Hung and Roberts, in order to include protein of interests in the same family from different species for facilitating identifying the peptide sequence associated with activity for characterization of protein of interest.

**********Continued in the supplemental box**********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fu lly supported by the description, are made:

For dependent claims 2-6, 10-14, 16, 19-20, the word 'where' in each claim is assumed to read as 'wherein'.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No IV (Lack of unity of invention)

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules', wherein '10.sup.9 different molecules' comprising at least 5,000 distinct peptide constructs; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,... prepare peptide libraries, wherein the encoding nucleic acid molecule remains physically associated with the peptide. Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide')
---that are custom-designed (para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed'). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups I+ further share the technical feature of a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set
of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism. However, this shared technical feature does not represent a contribution over prior art as being obvious over Roberts, in view of US 2009/0280487 A1 to HUNG et al. (hereinafter 'Hung') as follow:

Roberts discloses a set of at least 5,000 distinct peptide constructs (para [0090]; para [0008]; para [0127], as discussed above)
--- each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences (para [0009]; para [0005], as discussed above)
---that are custom-designed (para [0004],as discussed above)
--- so that the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids (para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues').

Although Roberts does not specifically teach wherein the peptide sequences of at least 12 amino acids are at least 10% of the set of peptide that have more than 80% amino acid identity to the protein sequences of interest, one of ordinary skill in the art at the time the invention was made would have known to do so, because Roberts teaches detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'), which requires the peptide having enough amino acids and requires enough percentage of peptides to have enough amino acids, including to have at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids to include a functional group that have more than 80% amino acid identity to the protein sequences of interest. And furthermore, it is a common practice to include peptides with known sequences in consensus regions that have at least 80% amino acid identity to the same proteins from different species when preparing a library (please see US 2007/0020625 A1 to DUCHAUD et al.: para [0253] - 'a DNA library prepared from the genome of a bacterium belonging to... species different from the Photorhabdus strain used to construct the DNA library'; para [0093] - 'The expression "polypeptide exhibiting a certain percentage identity with another", ... "homologous polypeptide" will also be used... the amino acid sequence exhibits at least 80%, preferably 85%, 90%, 95%, 98% or 99%, identity'; please also see US 2005/0026188 A1 to van KESSEL et al.: para [0029] - 'developing a library of nucleotide sequences ... corresponding to variable regions of the cpn60 gene'; para [0060] - 'a "cnp60-like" sequence ... encoding a protein ... has a biological function similar to that of cpn60, ... homology of at least 70%, 80% or 90% may be used'; para [0062]- 'performs substantially the same role ... the homology will be at least about 80%, 85%, 90% or 95%').

Roberts does not specifically teach wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism. Hung discloses a peptide library comprising G-protein coupled receptor polypeptides from up to 100 different species (para [0062] - 'a library contains a plurality of members, ...up to about 1000 or more olfactory GPCR polypeptides.... derived from a plurality of species'; para para [0019] - '"G-protein coupled receptors", or "GPCRs" are polypeptides'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal', wherein 'up to about 100...species of animal' is 'up to 100 different species of organism'; para [0129] - 'producing a library ... up to about 1000 or more...different olfactory GPCRs'; para [0056]; para [0058]) for activity determination and modulator identification (para [0096] - 'screening for olfactory GPCR modulators'; para [0103] - 'GPCR activity may be measured by assessing a reporter signal').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences of interest, based on the teaching of Roberts and the common practice, wherein the peptides of interest are protein sequences encoded by up to 100 different species of organism, based on the combination of Hung and Roberts, in order to include protein of interests in the same family from different species for facilitating identifying the peptide sequence associated with activity for characterization of protein of interest. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V No 2 (citations and explanations)

Regarding claim 2, the combination of Roberts and Hung discloses the set of peptide constructs from claim 1, and the said combination further discloses wherein the species are eukaryotic species (Roberts: para [0066] - 'nucleic acid-peptide fusion molecules, ...the initiator AUG codon in eukaryotic systems', wherein 'the initiator AUG codon in eukaryotic systems' indicating the peptide constructs are for eukaryotic species; para [0083] - 'RNA-peptide fusions then are generated using an in vitro or in situ translation system. ... eukaryotic systems are preferred'; Hung: para [0056] - 'the olfactory GPCR may be of human origin or of non-human animal origin... may be a mouse, a rat, a dog, or any other non-human animal'; para [0066] - 'a particular species, particularly a mammalian, e.g., human or mouse species'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal').

Regarding claim 3, the combination of Roberts and Hung discloses the set of peptide constructs from claim 2, and Hung further discloses wherein the species are mammalian species (para [0056] - 'the olfactory GPCR may be of human origin or of non-human animal origin... may be a mouse, a rat, a dog, or any other non-human animal'; para [0066] - 'a particular species, particularly a mammalian, e.g., human or mouse species'; para [0091] - 'The olfactory GPCRs may be of known identity...may be derived from ... up to about 100... species of animal').

Regarding claim 7, the combination of Roberts and Hung discloses the set of peptides from claim 1, and Roberts further discloses wherein the peptide portion is dissociated from the ribosome before being linked to the identifying nucleic acid (para [0009] - 'The encoding nucleic acid is translationally linked at or near its 3' terminus to the C-terminus of its encoded peptide, ... via a peptide acceptor such as puromycin'; para [0057]; para [0059] - 'puromycin acts as a universal inhibitor of protein synthesis by binding the translation complex A site, accepting the growing peptide chain, and falling off the ribosome ...methods for translationally linking an encoding nucleic acid and encoded peptide ... effecting the joining using a peptide acceptor such as puromycin, which can be linked at or near the 3' terminus of the encoding nucleic acid such that it can enter the ribosome complex during translation and be incorporated at the C-terminus of the growing (nascent) peptide, thereby terminating translation and linking the encoding nucleic acid and encoded peptide', wherein ' puromycin ... falling off the ribosome... terminating translation and linking the encoding nucleic acid and encoded peptide' indicates 'wherein the peptide portion is dissociated from the ribosome before being linked to the identifying nucleic acid'; Please see Alkinson for the translation steps: Entire documentation, especially pg 6.5, Elongation; and pg 6.6, Translocation and Termination).

Regarding claim 8, the combination of Roberts and Hung discloses the set of peptide constructs from claim 1, and the said combination further obviates wherein the set of peptide constructs comprises at least 100,000 distinct peptide constructs (Roberts: para [0090] - 'libraries of molecules having a diversity of at least about 10.sup.9 different molecules'; para [0008] - 'a library of nucleic acid-peptide fusion molecules'; para [0127] - the starting library contained approximately 3.times.10.sup.12 different peptides')
---each comprising a peptide portion of at least 10 amino acids and an identifying oligonucleotide portion (Roberts: para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide', wherein 'an encoding nucleic acid' is 'an identifying oligonucleotide portion'; para [0005] - 'it is easier to determine a nucleotide sequence of a nucleic acid molecule encoding a peptide than it is to determine the amino acid sequence of a peptide,...Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide'; para [0065] - 'the nucleic acid-peptide fusion molecules can include any number of amino acid resides...at least... 15, 20, 25, 30, or more amino acid residues')
---wherein the peptide portions are encoded within oligonucleotide sequences that are custom designed (Roberts: para [0004] - 'Libraries of peptides ... be prepared based on a known peptide', wherein 'be prepared based on a known peptide' is 'custom-designed'; para [0009] - 'a library of diverse nucleic acid-peptide fusion molecules, wherein each molecule of the library comprises an encoding nucleic acid ... linked to an encoded peptide').
----such that at least 90% of the sequences contain no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs (Roberts: para [0092] - 'the orthogonal aminoacylated tRNA, which is charged with an unnatural amino acid residue, recognizes a stop codon... a start codon operably linked to a protein coding sequence, and each of which is linked to a peptide acceptor at the 3'-end of the peptide coding sequence, which includes or can include one or a series of stop codons, ... to produce a population of RNA-peptide fusion molecules comprising peptides', wherein additional stop codons are used for translating unatural amino acids, one of ordinary skill in the art at the time the invention was made would have known to include no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs because is the basic principle for successful protein translation; para [0094] - 'an amber suppressor tRNA, which recognizes a UAG stop codon. ...other unnatural residues such as N-methyl glycine and N--(S)-phenylethyl glycine similarly can be used'; Hung: para [0073] - 'a fusion protein may contain a heterologous N-terminal domain (e.g. an epitope tag) fused in-frame with a GPCR that has had its N-terminal methionine residue either deleted or substituted with an alternative amino acid', wherein 'fused in-frame' is the basic practice including 'in-frame codon that can function as a stop codon' for protein translation; para [0039] - 'The boundaries of the coding sequence are typically determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus', wherein 'a translation stop codon at the 3' (carboxy) terminus' indicates each conding sequence having 'one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs', one of ordinary skill in the art at the time the invention was made would have known to have ' at least 90% of the sequences contain no more than one in-frame codon that can function as a stop codon in the translation system used to make the peptide constructs' for producing sufficient amount of distinct peptides, but not high percentage of premature peptides due to the presence of undesired stop codon).

**********Continued in the next supplemental box**********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/32759

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
The previous supplemental box - Box V No 2 (citations and explanations)

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Roberts, in view of Hung, as above, and further in view of US 2007/0014810 A1 to BAKER et al. (hereinafter 'Baker').

Regarding claim 6, the combination of Roberts and Hung discloses a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, as discussed in the discussion of claim 1. Roberts further discloses using the set of peptide constructs for detecting a desired activity of a peptide for identifying the peptide sequence associated with the activity (para [0005] - 'Upon identifying a peptide having a desired activity, the sequence of the encoding nucleic acid associated with the peptide can be determined, thus providing information as to the amino acid sequence of the encoded peptide').

Said combination does not specifically teach wherein the peptide sequences are grouped into one or more sets of partially overlapping sequences. Baker discloses a method for identifying ligand binding domains of a sequence of interest, the method comprising producing a set of peptide sequences that are partially overlapping sequences that cover the entire sequence of the sequence of interest for identifying ligand binding domains (para [0359] - 'The identification of motifs and/or super motifs that correlate with high and intermediate affinity binding ... identification of immunogenic peptide epitopes ... all possible peptides of 9 amino acids in length and overlapping by eight amino acids ... which cover the entire sequence of the ... proteins ...were evaluated for binding to five allele-specific HLA molecules'; para [0361] - 'a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets'). Although Baker does not specifically teach the peptide sequences are grouped into one or more sets of partially overlapping sequences, one of ordinary skill in the art at the time the invention was made would have known to do so by grouping partially overlapping sequences generated from the same protein in the same group for facilitating characterization of the protein function associated with the peptide fragments in the protein.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Roberts and Hung with that of Baker, to obtain a set of at least 5,000 distinct peptide constructs, each comprising a peptide portion and an identifying oligonucleotide portion, wherein the peptide portions are encoded by oligonucleotide sequences that are custom-designed so that at least 10% of the set of peptide constructs produced contain contiguous peptide sequences of at least 12 amino acids that have more than 80% amino acid identity to protein sequences encoded by up to 100 different species of organism, based on the combination of Roberts and Hung, and further wherein the peptide sequences are grouped into one or more sets of partially overlapping sequences, based on the combination of Baker, Roberts and Hung, in order to obtain a set of distinct peptide constructs that include all factional fragments of the targeted protein sequences for facilitating characterization of the functional domain of the targeted protein sequences in different species of organism.

Claims 1-3 and 6-8 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 699932000340	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2014/044191	International filing date *(day/month/year)* 25 June 2014	(Earliest) Priority Date *(day/month/year)* 25 June 2013
Applicant PROGNOSYS BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. FIG.8

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/044191

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-41, 45-53
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/044191

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2014.01)
CPC - C12Q 1/6809 (2014.09)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12N 15/11, 15/115; C12Q 1/68; C40B 30/04, 60/04; G01N 33/50, 33/53, 33/543, 37/00 (2014.01)
USPC - 506/8, 9, 15, 24, 32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12Q 1/6809, 1/6834, 1/6837, 1/6841, 1/6869; C40B 60/04, 30/04; G01N 33/6845, 2458/10 (2014.09)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0245111 A1 (CHEE) 06 October 2011 (06.10.2011) entire document	1-4, 42-43
Y	ROUTENBERG et al. "Microfluidic probe: a new tool for integrating microfluidic environments and electronic wafer-probing," Lab Chip, 29 October 2009 (29.10.2009), Vol. 10, Pgs. 123-127. entire document	1-4, 42-43
A	US 2012/0270748 A1 (CHEE) 25 October 2012 (25.10.2012) entire document	1-4, 42-43
A	US 2012/0195810 A1 (COHEN et al) 02 August 2012 902.08.2012) entire document	1-4, 42-43
A	US 2003/0162216 A1 (GOLD et al) 28 August 2003 (28.08.2003) entire document	1-4, 42-43

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2014	07 NOV 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: BU YIN
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	07 NOV 2014
Applicant's or agent's file reference 699932000340	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2014/044191	25 June 2014	25 June 2013

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/86 (2014.01)
CPC - C12Q 1/8609 (2014.09)

Applicant PROGNOSYS BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	10 October 2014	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044191

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2014/044191

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 5-41, 45-53

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 5-41, 45-53 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 5-41 and 45-53 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 5-41, 45-53

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and mannacceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044191

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-4, 42-44	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-4, 42-44	NO
Industrial applicability (IA)	Claims	1-4, 42-44	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4, and 42-44 lack an inventive step under PCT Article 33(3) as being obvious over Chee in view of Routenberg et al. (hereafter Routenberg).

Regarding claim 1, Chee discloses a method of determining a spatial pattern of abundance, expression and/or activity of a biological target in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: (a) delivering a probe for a biological target to a sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]), the probe comprising a binding moiety capable of binding to the biological target, wherein the probe comprises an identity tag that identifies the biological target or target-binding moiety (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); (b) affixing the sample from step (a) to a first microfluidic device having multiple first addressing channels, wherein each first addressing channel identifies a first area in the sample (Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; biological sample in ten discrete areas, Para. [0063]); (c) delivering a first address tag through each of the first addressing channels to each first area in the sample, wherein each first address tag is to be coupled to the probe and/or the adaptor (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; Biological targets can include…proteins, Para. [0042]); (d) affixing the sample from step (c) to a second microfluidic device having multiple second addressing channels, wherein each second addressing channel identifies in the sample a second area that intersects with a first area identified by the first (Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; biological sample in ten discrete areas, Para. [0063]); (e) delivering a second address tag through each of the second addressing channels to each second area in the sample (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]; Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]), wherein each second address tag is to be coupled to the probe and/or the adaptor, whereby the first address tag and the second address tag at an intersection between a first area and a second area determine the intersection's address (delivering probes and encoding agents Where the encoding agents (in some embodiments pre-coupled to the probes) are delivered to the sample according to a known spatial pattern, Para. [0051]; a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a); (f) analyzing the probe and/or the adaptor bound to the biological target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), the analysis comprising: (1) determining the abundance, expression and/or activity of the biological target by assessing the amount of the probe and/or the adaptor bound to the biological target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and (2) determining the identities of the identity tag and the first and second address tags at each address (the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid, Para. [0033]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and (g) determining a spatial pattern of the biological target abundance, expression and/or activity in the sample based on the analysis of step (f) (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), but fails to explicitly disclose addressing channels at an angle greater than 0 degree.
However, Routenberg is in the field of integrated microfluidic addressing systems (Abstract) and teaches addressing channels at an angle greater than 0 degree (Schematic cross-section of patterning probe; (b) patterned channels and through holes; (c) fan-out connects to patterning channels and (d) resulting pattern on substrate after selective area functionalization, Pg. 126, right-hand column, top of page; See FIG. 5, esp. 5c).
It would have been obvious to one of ordinary skill in the art at the time of invention to modify the method of Chee to include the addressing channels at an angle greater than 0 degree as taught by Routenberg. The motivation would have been to allow measurements to be taken in spatial patterns (Routenberg, left-hand column, first paragraph).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044191

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 2 Chee discloses a method of determining a spatial pattern of abundance, expression and/or activity of a biological target in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: (a) affixing a sample to a first microfluidic device having multiple first addressing Channels (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; provide a biological sample affixed to a support, See FIG. 1), wherein each first addressing channel identifies a first area in the Sample (coding tags, which identify the positions in the sample of the biological targets being assayed, Para. [0042]; delivered to the sample according to a known spatial pattern, allowing the probes to interact or react with biological targets, Para. [0051]);
(b) delivering a first probe for a biological target through each of the first addressing channels to each first area in the sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; Biological tar gets can include... proteins, Para. [0042]; invention can include instru mentation such as one or more pumps, valves, fluid reservoirs, channels, and/or reagent storage cells. Reagent delivery systems are configured to move fluid to contact a discrete section of the biological sample, Para. [0074]), the probe comprising: (1) a first binding moiety capable of binding to the biological target (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); (2) a first address tag that identifies an area in the sample to which the first probe is delivered (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); and (3) a first identity tag that identifies the biological target or the first binding moiety (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); (c) affixing the sample from step (b) to a second microfluidic device having multiple second addressing channels, wherein each second addressing channel identifies in the sample a second area that intersects with a first area identified by the first (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; provide a biological sample affixed to a support, See FIG. 1; Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; biological sample in ten discrete areas, Para. [0063]); (d) delivering a second probe for the biological target through each of the second addressing channels to each second area in the sample (delivering probes and encoding agents Where the encoding agents (in some embodiments pre-coupled to the probes) are delivered to the sample according to a known spatial pattern, Para. [0051]; a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a, the probe comprising: (1) a second binding moiety capable of binding to the biological target (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; Biological targets can include...proteins, Para. [0042]); (2) a second address tag that identifies an area in the sample to which the first probe is delivered; and (3) a second identity tag that identifies the biological target or the second binding moiety, whereby the first address tag and the second address tag at an intersection between a first area and a second area determine the intersection's address (a coding tag that identifies a location of the site to which the encoded probe was delivered, Para. [0008]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); (e) analyzing the probes bound to the biological target target (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), the analysis comprising: (1) determining the abundance, expression and/or activity of the biological target by assessing the amount of the probes bound to the biological target; and (2) determining the identities of the first and second identity tags and the first and second address tags at each address (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and
(f) determining a spatial pattern of the biological target abundance, expression and/or activity in the sample based on the analysis of step (e) (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), but fails to explicitly disclose addressing channels at an angle greater than 0 degree.
However, Routenberg is in the field of integrated microfluidic addressing systems (Abstract) and teaches addressing channels at an angle greater than 0 degree (Schematic cross-section of patterning probe; (b) patterned channels and through holes; (c) fan-out connects to patterning channels and (d) resulting pattern on substrate after selective area functionalization, Pg. 126, right-hand column, top of page; See FIG. 5, esp. 5c).
It would have been obvious to one of ordinary skill in the art at the time of invention to modify the method of Chee to include the addressing channels at an angle greater than 0 degree as taught by Routenberg. The motivation would have been to allow measurements to be taken in spatial patterns (Routenberg, left-hand column, first paragraph).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044191

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 3, Chee discloses a method of determining a spatial pattern of abundance, expression and/or activity of a biological target in a sample (the invention provides an assay system to determine spatial patterns of abundance or activity or both of multiple biological targets at multiple sites in a sample, Para. [0008]), comprising: (a) affixing a sample to a first microfluidic device having multiple first addressing Channels (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; provide a biological sample affixed to a support, See FIG. 1), wherein each first addressing channel identifies a first area in the sample (coding tags, which identify the positions in the sample of the biological targets being assayed, Para. [0042]; delivered to the sample according to a known spatial pattern, allowing the probes to interact or react with biological targets, Para. [0051]); (b) delivering a probe for a biological target through each of the first addressing channels to each first area in the sample, the probe comprising a binding moiety capable of binding to the biological target (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; Biological tar gets can include...proteins, Para. [0042]; invention can include instru mentation such as one or more pumps, valves, fluid reservoirs, channels, and/or reagent storage cells. Reagent delivery systems are configured to move fluid to contact a discrete section of the biological sample, Para. [0074]), wherein the probe and/or the adaptor comprises: (1) a first address tag that identifies an area in the sample to which the probe is delivered (Where each encoded probe comprises a probe region that may interact with the biological targets, Para. [0008]); and (2) an identity tag that identifies the biological target or the binding moiety (coding tags, Which identify the positions in the sample of the biological targets being assayed, and thus can be used to link assay results back to locations in the sample, Para. [0042]); (c) affixing the sample from step (b) to a second microfluidic device having multiple second addressing channels, wherein each second addressing channel identifies in the sample a second area that intersects with a first area identified by the first (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; provide a biological sample affixed to a support, See FIG. 1; Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; biological sample in ten discrete areas, Para. [0063]); (d) delivering a second address tag through each of the second addressing channels to each second area in the sample, wherein each second address tag is to be coupled to the probe and/or the adaptor, whereby the first address tag and the second address tag at an intersection between a first area and a second area determine the intersection's address (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); (e) analyzing the probe and/or the adaptor bound to the biological target, the analysis comprising: (1) determining the abundance, expression and/or activity of the biological target by assessing the amount of the probe and/or the adaptor bound to the biological target; and (2) determining the identities of the identity tag and the first and second address tags at each address (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]); and (f) determining a spatial pattern of the biological target abundance, expression and/or activity in the sample based on the analysis of step (e) (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), but fails to explicitly disclose addressing channels at an angle greater than 0 degree.
However, Routenberg is in the field of integrated microfluidic addressing systems (Abstract) and teaches addressing channels at an angle greater than 0 degree (Schematic cross-section of patterning probe; (b) patterned channels and through holes; (c) fan-out connects to patterning channels and (d) resulting pattern on substrate after selective area functionalization, Pg. 126, right-hand column, top of page; See FIG. 5, esp. 5c).
It would have been obvious to one of ordinary skill in the art at the time of invention to modify the method of Chee to include the addressing channels at an angle greater than 0 degree as taught by Routenberg. The motivation would have been to allow measurements to be taken in spatial patterns (Routenberg, left-hand column, first paragraph).

Regarding claim 4, modified Chee discloses the method of claim 3. Chee fails to explicitly disclose wherein the angle is about 90 degrees, about 80 degrees, about 70 degrees, about 60 degrees, about 50 degrees, about 40 degrees, about 30 degrees, about 20 degrees, or about 10 degrees.
However, Routenberg teaches the angle is about 90 degrees (Schematic cross-section of patterning probe; (b) patterned channels and through holes; (c) fan-out connects to patterning channels and (d) resulting pattern on substrate after selective area functionalization, Pg. 126, right-hand column, top of page; See FIG. 5, esp. 5b, Patterning channels are perpendicular to the through-hold channels).
It would have been obvious to one of ordinary skill in the art at the time of invention to modify the method of Chee to include the angle is about 90 degrees as taught by Routenberg. The motivation would have been to allow measurements to be taken in spatial patterns (Routenberg, left-hand column, first paragraph).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/044191

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 42, Chee discloses a method of address coding multiple sites in a sample (delivering encoded probes for the multiple protein targets to the multiple sites in the sample in a known spatial pattern, Para. [0015]; encoded probe comprises a probe region that may interact With the biological targets and a coding tag that identifies a location of the site to Which the encoded probe Was delivered, Para. [0008]), comprising: (a) providing a sample affixed to a first microfluidic device having multiple first addressing channels (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; provide a biological sample affixed to a support, See FIG. 1), wherein each first addressing channel identifies a first area in the sample (coding tags, which identify the positions in the sample of the biological targets being assayed, Para. [0042]; delivered to the sample according to a known spatial pattern, allowing the probes to interact or react with biological targets, Para. [0051]); (b) delivering a first probe capable of binding to a target in the sample through each of the first addressing channels to each first area in the sample (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; (coding tags, which identify the positions in the sample of the biological targets being assayed, Para. [0042]; delivered to the sample according to a known spatial pattern, allowing the probes to interact or react with biological targets, Para. [0051]); (c) affixing the sample from step (b) to a second microfluidic device having multiple second addressing channels (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; provide a biological sample affixed to a support, See FIG. 1, wherein each second addressing channel identifies in the sample a second area that intersects with a first area identified by the first (delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; provide a biological sample affixed to a support, See FIG. 1; Microfluidic passages for holding and carrying fluid may be formed on and/ or above the planar substrate by a fluidics layer abutted to the substrate. Fluid reagents can be selected and delivered according to selective opening and closing of valves disposed between reagent reservoirs, Para. [0076]; delivering encoded probes for the multiple biological targets to the multiple sites in the sample, Para. [0008]; biological sample in ten discrete areas, Para. [0063]); (d) delivering a second probe capable of binding to the target in the sample through each of the second addressing channels to each second area in the sample (determining all or a portion of a sequence of the encoded probes, and associating the abundance or activity or both of the multiple biological targets to the locations of the sites in the sample, Para. [0008]; Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]; The second encoded probe 422 comprises coding tag 406, associated With, e.g., a universal priming site for amplification of the assay products or an adapter to enable identification of the coding identifiers using sequencing technologies 410, Para. [0062]; See FIG. 4a; Tag 410 can be a universal priming site for amplification of the assay products or an adaptor to enable identification of the address tag 406; as defined in Para. [0146] and FIG. 4a of the instant application); and (e) determining an address of an intersection between a first area and a second area in the sample based on the first probe and the second probe bound to the target at the intersection (Following hybridization, the relative amounts of a particular target mapping to different spatial locations in the sample can be determined by the relative abundances of the resolvable labels, Para. [0086]), but fails to explicitly disclose addressing channels at an angle greater than 0 degree. However, Routenberg is in the field of integrated microfluidic addressing systems (Abstract) and teaches addressing channels at an angle greater than 0 degree (Schematic cross-section of patterning probe; (b) patterned channels and through holes; (c) fan-out connects to patterning channels and (d) resulting pattern on substrate after selective area functionalization, Pg. 126, right-hand column, top of page; See FIG. 5, esp. 5c).
It would have been obvious to one of ordinary skill in the art at the time of invention to modify the method of Chee to include the addressing channels at an angle greater than 0 degree as taught by Routenberg. The motivation would have been to allow measurements to be taken in spatial patterns (Routenberg, left-hand column, first paragraph).

Regarding claim 43, modified Chee discloses the method of claim 42. Chee fails to explicitly disclose wherein the angle is about 90 degrees, about 80 degrees, about 70 degrees, about 60 degrees, about 50 degrees, about 40 degrees, about 30 degrees, about 20 degrees, or about 10 degrees.
However, Routenberg teaches the angle is about 90 degrees (Schematic cross-section of patterning probe; (b) patterned channels and through holes; (c) fan-out connects to patterning channels and (d) resulting pattern on substrate after selective area functionalization, Pg. 126, right-hand column, top of page; See FIG. 5, esp. 5b, Patterning channels are perpendicular to the through-hold channels).
It would have been obvious to one of ordinary skill in the art at the time of invention to modify the method of Chee to include the angle is about 90 degrees as taught by Routenberg. The motivation would have been to allow measurements to be taken in spatial patterns (Routenberg, left-hand column, first paragraph).

Regarding claim 44, modified Chee discloses the method of claim 42. Chee further discloses wherein the first addressing channels substantially parallel each other and the second addressing channels substantially parallel each other (The flow-cell can include inlet and outlet tubes connected thereto and optionally an external pump is used to
deliver reagents to the flow-cell and across the biological sample. The flow cells are configured to deliver reagents only to certain portions of the biological sample, restricting the amount and type of reagent delivered to any specific section
of the biological sample, Para. [0075]; Coding tag a2 is deposited on the biological sample in ten spots on the second horizontal line in 412. Coding tag a3 is
deposited on the biological sample in ten spots on the third horizontal line in 412, and so on. Whereas the "a" tags are deposited in ten horizontal rows, the "b" tags are deposited in ten vertical rows as shown in 414, Para. [0063]; See FIG. 4).

Claims 1-4 and 42-44 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Electronic Acknowledgement Receipt

EFS ID:	35428822
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. CHEE
Customer Number:	159367
Filer:	Cameron Mark Luitjens/Kirsten Neuman
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	47706-0004003
Receipt Date:	15-MAR-2019
Filing Date:	14-FEB-2019
Time Stamp:	14:42:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Tian_AntigenPeptideBased_2009.pdf	4922623 d67be31417fc8129d6df5e4477b12cdaabe57db2	no	8

Warnings:

Information:					
2	Non Patent Literature	Valencia_mRNADisplayBased_2008.pdf	5760101 80c2890bb9572bef3245716414c6111e0969f32f	no	9
Warnings:					
Information:					
3	Non Patent Literature	VanGelder_AmplifiedRNA_1990.pdf	1648446 9258fc1f5374c45aa0198775d67e4fd087bc2b04	no	5
Warnings:					
Information:					
4	Non Patent Literature	VanNess_AVersatileSolid_1991.pdf	7260883 790a1486bb1fddac8a9f3475679d9a4e27332f7b	no	6
Warnings:					
Information:					
5	Non Patent Literature	Velculescu_SerialAnalysisOf_1995.pdf	1320828 33466270bf0fc53524920c5982ea8dd0f6c954ca	no	4
Warnings:					
Information:					
6	Non Patent Literature	VICKOVIC_MassiveandParallelExpression.pdf	7178227 6cd052c2f82b23b7b5d49eafe82cf8b39e81a363	no	9
Warnings:					
Information:					
7	Non Patent Literature	Vogelstein_DigitalPCR_1999.pdf	4119190 fa95f2023238baa0825165921a8683b7f6761609	no	6
Warnings:					
Information:					
8	Non Patent Literature	WADE_GenomeSequence.pdf	1341738 8d18ed1631042385a5d4e6d71d1b698e65e1098c	no	6
Warnings:					
Information:					

9	Non Patent Literature	Waichman_FunctionalImmobilizationAnd_2010.pdf	4520203 a23febc87474de27d0180cc83ffbeddbd37473ae	no	8
Warnings:					
Information:					
10	Non Patent Literature	Walker_StrandDisplacementAmplification_1992.pdf	1689059 d8c0c0c3cd1d157c3e3565c6fd2f5ea1a823da8e	no	6
Warnings:					
Information:					
11	Non Patent Literature	Wang_SingleCellAnalysis_2010.pdf	11844370 58b0ba49cd6e943b1f4d7dfced1a7cade086f0a6	no	19
Warnings:					
Information:					
12	Non Patent Literature	Wang_ThreeDimensional_2018.pdf	9641364 9ec9f6f6ee84cc0207333737d8cc261c4edd33fd	no	22
Warnings:					
Information:					
13	Non Patent Literature	Wang_HighFidelitymRNA_2000.pdf	2298682 deb18753f25658a4d2cdd8aee51a2b337d772ed8	no	3
Warnings:					
Information:					
14	Non Patent Literature	Weichhart_FunctionalSelectionOf_2003.pdf	5134507 fd2b1f18ed0ae773aef56e5861ba9b476a342bb7	no	9
Warnings:					
Information:					
15	Non Patent Literature	Weinstein_DNAmicroscopy_2018.pdf	10613851 1668015659ddf799ba8017ec1ff8bca7a404cc8b	no	41
Warnings:					
Information:					

16	Non Patent Literature	WILLI-MONNERAT_ComprehensiveSpatiotemporalTranscriptomic.pdf	1774143 07ab1dc14e74c9501cf6ecc05e8c5686bab7f147	no	12
Warnings:					
Information:					
17	Non Patent Literature	Wong_DirectSiteSelective_2008.pdf	5659730 9a3b5553e359ff55885e70c1773d92b8557314f2	no	9
Warnings:					
Information:					
18	Non Patent Literature	WOO_AComparisonofcDNA.pdf	5000360 0ba417db3847195e48bd7e47d9c2a3dc5b1b578a	no	9
Warnings:					
Information:					
19	Non Patent Literature	WORTHINGTON_CloningOfRandom.pdf	7087054 154071275b3bf4fe512cac576ef3f392e1bcb21c	no	7
Warnings:					
Information:					
20	Non Patent Literature	XIAO_DirectDeterminationOf.pdf	3947956 fc3b35a3bb6808735867a36df373a39b835e51d5	no	3
Warnings:					
Information:					
21	Non Patent Literature	Yin_GeneticallyEncodedShort2005.pdf	4271805 ccbd8c52bc4aa86c0ec3d2f36e0233fa0aa931a7	no	6
Warnings:					
Information:					
22	Non Patent Literature	YONEZAWA_DNADisplayFor.pdf	5787401 d79cf387745230a74aec32717fe5d2a6e37227b4	no	5
Warnings:					
Information:					

23	Non Patent Literature	Zhang_AssemblingDNAThrough_2013.pdf	4340907 1b559663de8280d3d4f331ca9c09864302b288e6	no	9
Warnings:					
Information:					
24	Non Patent Literature	ZHANG_BindingInducedDNA.pdf	7962744 533abf1b6f934aef3a27b660f4feced11a32f755	no	8
Warnings:					
Information:					
25	Non Patent Literature	Zheng_OriginsOfHuman_2006.pdf	4685345 791fd86536b7975f1d7cbc94869bdfb45be03375	no	10
Warnings:					
Information:					
26	Non Patent Literature	Zhou_GeneticallyEncodedShort_2007.pdf	4467639 4a16ded15f3f7bab89829c4ccf8a706754ae1dbd	no	10
Warnings:					
Information:					
27	Non Patent Literature	ZILBERMAN_GenomeWideAnalysis.pdf	7841945 a7588b8513fc5451a5ba3548976bee8ca6cb61a9	no	7
Warnings:					
Information:					
28	Non Patent Literature	ZLOBEC_NextGenerationTissue.pdf	7391121 be3810c4a471f3c18e258766c7023113663cd61c	no	7
Warnings:					
Information:					
		Total Files Size (in bytes):	149512222		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	35429427
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. CHEE
Customer Number:	159367
Filer:	Cameron Mark Luitjens/Kirsten Neuman
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	47706-0004003
Receipt Date:	15-MAR-2019
Filing Date:	14-FEB-2019
Time Stamp:	14:47:45
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	DEAN_ComrpehensiveHuman Genome.pdf	8573191 436028db50309f6e1f73b6ab65a37503bd36688a	no	6

Warnings:

Information:					
2	Non Patent Literature	Dhindsa_VirtualElectrowettingChannels_2010.pdf	3273974 2658ba07c0bc40984e745689d145c733df834508	no	6
Warnings:					
Information:					
3	Non Patent Literature	Dressman_TransformingSIngleDNA_2003.pdf	4148180 e6372517708a70717e722a8a697af4c27008dcaf	no	6
Warnings:					
Information:					
4	Non Patent Literature	Drmanac_AccurateSequencing_1998.pdf	909741 d972a955960 4e6186f6f0b10ca444b385635170e	no	5
Warnings:					
Information:					
5	Non Patent Literature	Druley_QuantificationOfRare_2009.pdf	1263587 f11029ae51b66fe9724df2d9874b8248cf792aba	no	7
Warnings:					
Information:					
6	Non Patent Literature	Eagen_PrinciplesOfChromosome_2018.pdf	407686 b561674047ff74260b85d4b579a04f65a2c7af63	no	10
Warnings:					
Information:					
7	Non Patent Literature	Eberwine_AnalysisOfGene_1992.pdf	1607230 d19fe6315a71d73194a33bd455a11c17058f736a	no	5
Warnings:					
Information:					
8	Non Patent Literature	Eberwine_AmplificationOfmRNA_1996.pdf	7309279 b7ce66c3a2488602fb6d863fd33c065998478106	no	5
Warnings:					
Information:					

9	Non Patent Literature	Eguiluz_2002.pdf	1008869 5103df9dd45cac8536fae3b02c9f58bd718a29f9	no	8
Warnings:					
Information:					
10	Non Patent Literature	Eldridge_AnInVitroSelection_2009.pdf	7360438 5b49aa716798555df26639cca7fa1c034258073e	no	8
Warnings:					
Information:					
11	Non Patent Literature	Ellington_AntibodybasedProtein_2010.pdf	4958334 174c0bd91d9de15f13bf506107489cf1cd5a2389	no	8
Warnings:					
Information:					
12	Non Patent Literature	Eng_ProfilingThe_2017.pdf	1329978 cb154d15109787bdd7e7aeba5e9f048651b5f766	no	13
Warnings:					
Information:					
13	Non Patent Literature	AUOA_ESR_2010278710_2-11-2014.pdf	258338 0d3430d45a95c63f39442aa3003e795cf95af2ef	no	4
Warnings:					
Information:					
14	Non Patent Literature	AUOA_ESR_2010328226_5-9-2013.pdf	253627 d26232f77d6e96875614452de1767b5d90e44b57	no	4
Warnings:					
Information:					
15	Non Patent Literature	AUOA_ESR_2011237729_7-9-2013.pdf	274015 a327f1083d8c4235dddcdf7c1c30b3f7b285d514	no	3
Warnings:					
Information:					

16	Non Patent Literature	AUOA_ESR_2014203638_10-1-2015.pdf	199456 5801d7c6e830562a437dbae51d1f62ab574bde68	no	2
Warnings:					
Information:					
17	Non Patent Literature	EPOCOMM_ESR_10836568_2-13-2013.pdf	369397 16092230aaf642fe07329c77f73dbed4d31aaf8a	no	6
Warnings:					
Information:					
18	Non Patent Literature	EPOEESR_11766613_1-15-2014.pdf	198468 33bcc562b6b44928ac44d376c0a6030a99bc0bab	no	4
Warnings:					
Information:					
19	Non Patent Literature	EPOEESR_12767937_11-18-2014.pdf	342335 686feaabbc54173085954 5b91b38d8f0ba4e7d81	no	5
Warnings:					
Information:					
20	Non Patent Literature	EPOEESR_14765026_9-26-2016.pdf	532924 128533259be67e4f356ec95c91b46b1248fd02ca	no	9
Warnings:					
Information:					
21	Non Patent Literature	EPOEESR_14816674_2-3-2017.pdf	484557 99424cfbe6eb5172b71e13d1124ff73715057899	no	8
Warnings:					
Information:					
22	Non Patent Literature	EPOEESR_14818012_2-3-2017.pdf	481158 fb491287c699014a909ff583aac7ab9bfe0c9204	no	8
Warnings:					
Information:					

23	Non Patent Literature	EPOEESR_16183356_4-24-2017.pdf	941608 5d1daf89a69a2beb1c5a743bec749a5561075600	no	12
Warnings:					
Information:					
24	Non Patent Literature	Fan_HighlyParallel_2003.pdf	7005844 3786260aedc5558600abab82138881236fd80d60	no	11
Warnings:					
Information:					
25	Non Patent Literature	Fire_Xu_Rolling_replication_1995.pdf	1474069 309be2730d21dfc78108125e30a6d5a47525b767	no	5
Warnings:					
Information:					
26	Non Patent Literature	Flanigon_MultiplexProtein_2013.pdf	1398756 f04d6043da2c8ceeb3d9420afa8447ee2bd3aa5c	no	10
Warnings:					
Information:					
27	Non Patent Literature	Fodor_LightDirectedSpatially_1995.pdf	2341684 63ad04192aca1e78285cc4b00359af1206f5c9c2	no	7
Warnings:					
Information:					
28	Non Patent Literature	Forcucci_AllPlasticMiniature_2015.pdf	3457359 807b725e8dc4c504fc86ba72dc7d86f22d6341a6	no	15
Warnings:					
Information:					
29	Non Patent Literature	Fredriksson_MultiplexedProtein_2007.pdf	2078426 eff0bf544e9a480c22d0c577a130e3aea71fdd77	no	3
Warnings:					
Information:					

30	Non Patent Literature	Fredriksson_MultiplexedProximityLigation_2008.pdf	4434733 58cf0869434dea5e7603126ce007a94191d4a3e7	no	8
Warnings:					
Information:					
31	Non Patent Literature	Fredriksson_ProteinDetection_2002.pdf	3562652 94685812e972e8fc96d2849c5b08f28f2c407219	no	5
Warnings:					
Information:					
32	Non Patent Literature	Frese_FormylglycineAldehyd_2009.pdf	1297472 d787494767712ab67df6c3ef8bdbb0ce3821a5a2	no	3
Warnings:					
Information:					
33	Non Patent Literature	Fu_CountingIndividualDNA_2011.pdf	7858560 5e4b120bb6185f426c01f391f377890ed0e37a2b	no	6
Warnings:					
Information:					
34	Non Patent Literature	Fullwood_NextGenerationDNA_2009.pdf	4224227 2e6abc451b601e84bbe25c43c464259dd58dd901	no	12
Warnings:					
Information:					
35	Non Patent Literature	Gao_2004.pdf	1373695 8d47f4fafe7259b741b38de314968dbe42b48874	no	11
Warnings:					
Information:					
36	Non Patent Literature	Gao_QandA_2017.pdf	2173527 54600d81293eba089f9c4aacaae0a4020f62d0da	no	9
Warnings:					
Information:					

37	Non Patent Literature	Giacomello_SpatiallyResolved_2017.pdf	4371585 f917a972ffb777aed8657e73eaa168fe49d0f3fc	no	11
Warnings:					
Information:					
38	Non Patent Literature	Gnanapragasam_2009.pdf	3406525 e1bb2435d7bc3b779201dbcea4ca955b3ea3946d	no	5
Warnings:					
Information:					
39	Non Patent Literature	Goldkom_Prockop_ASimple_1986.pdf	10819594 ea115a998ce5e9b7f621595375ca4f4e3b7b6333	no	21
Warnings:					
Information:					
40	Non Patent Literature	Gu_ProteinTagMediated_2013.pdf	2055839 a2819db6be09fcd4dee993e319a9c8b22d8bae7c	no	9
Warnings:					
Information:					
41	Non Patent Literature	Gunderson_DecodingRandomly_2004.pdf	8471406 4a982aab5e6b1dd295c24bd13255a81efac9a7a6	no	9
Warnings:					
Information:					
42	Non Patent Literature	Hammond_ProfilingCellularProtein_2012.pdf	6804095 0f556ceed1f0b6372ee29952687d8e3002c59fdc	no	9
Warnings:					
Information:					
43	Non Patent Literature	Han_3Cand_2018.pdf	977856 07b8385a6ec40ced73ad544441de6aa93f6a7ecc	no	10
Warnings:					
Information:					

44	Non Patent Literature	He_InSituSynthesis_2008.pdf	5643525 5a2fbbcfbde347846bddd926052d0ed1f8354293	no	6
Warnings:					
Information:					
45	Non Patent Literature	He_PrintingProteinArrays_2008.pdf	3694405 4c1ea719e7126023aff5201344aaa1a42f3a456b	no	3
Warnings:					
Information:					
46	Non Patent Literature	He_CellFreeProteinSynthesis_2008.pdf	7758277 ecdd260080fbe744ca49768d8f20596f980c3a46	no	7
Warnings:					
Information:					
47	Non Patent Literature	Hedskog_DynamicsOfHIV1_2010.pdf	8034427 fc0137a8018f035e47e92026c6ad3ac48b8260ec	no	14
Warnings:					
Information:					
48	Non Patent Literature	CAOA_ESR_2794522_4-28-2015.pdf	156740 d1150caa92c3dd657577c38a76923e6e75a7280f	no	3
Warnings:					
Information:					
		Total Files Size (in bytes):	151361648		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	35433460
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. CHEE
Customer Number:	159367
Filer:	Cameron Mark Luitjens/Kirsten Neuman
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	47706-0004003
Receipt Date:	15-MAR-2019
Filing Date:	14-FEB-2019
Time Stamp:	14:52:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	WO2010126614.pdf	1123389 b519b92c761407485256aef37e657c55766a3b52	no	121

Warnings:

Information:					
2	Foreign Reference	WO2010127186.pdf	572334 b7dfda83f439964ec5bb857da605744d69a69492	no	65
Warnings:					
Information:					
3	Foreign Reference	WO2011014879.pdf	562214 c7115b1e4d4006c3adb4a04a844839163cad8682	no	102
Warnings:					
Information:					
4	Foreign Reference	WO2011071943.pdf	571332 b906e3e841ee8c938c95cc54c75ad91d8a6c86bb	no	93
Warnings:					
Information:					
5	Foreign Reference	WO2011127006.pdf	271885 fce3965e603ceec43930748e4383ea4c8afdab8a	no	44
Warnings:					
Information:					
6	Foreign Reference	WO2011127099.pdf	314846 6afe04e575cddc6f5fc571892a3a472005ed2cfb	no	45
Warnings:					
Information:					
7	Foreign Reference	WO2011155833.pdf	267716 7c1b584dcb0ad5503ebe0639ce0ff33d2ad89403	no	51
Warnings:					
Information:					
8	Foreign Reference	WO2012022975.pdf	282544 ed3798306fc9534af4dbce81b89406d42df7938f	no	44
Warnings:					
Information:					

9	Foreign Reference	WO2012058096.pdf	921845 f29d3e3f642177fd83bba0c65070abafd20f2062	no	99
Warnings:					
Information:					
10	Foreign Reference	WO2012071428.pdf	3807808 7836e4047390d11c901b35aefea4195499207b55	no	333
Warnings:					
Information:					
11	Foreign Reference	WO2012139110.pdf	580794 36b5175192ad38a7e809758028f2b90d7046662e	no	79
Warnings:					
Information:					
12	Foreign Reference	WO2012140224.pdf	1421554 7d57a69b533d333ec30223683f36138f12918a15	no	153
Warnings:					
Information:					
13	Foreign Reference	WO2012142213.pdf	1535983 6516c1c14eb22e38d9259f633c5a76f021f1ef85	no	68
Warnings:					
Information:					
14	Foreign Reference	WO2012148477.pdf	1536712 b85702d16c6cdc7f0ba4b05980f74bf09dbd92e3	no	135
Warnings:					
Information:					
15	Foreign Reference	WO2013123442.pdf	312498 b00008b3d0e379e6e0927adec44657a008fb01fb	no	48
Warnings:					
Information:					

16	Foreign Reference	WO2013138510.pdf	3744709 11bb5856c1af2f3901811f9ae0c4d243342cb8a1	no	305
Warnings:					
Information:					
17	Foreign Reference	WO2013142389.pdf	547328 6dd10089b744d0a19e512dda623723f705f9d8b0	no	70
Warnings:					
Information:					
18	Foreign Reference	WO2013150083.pdf	599117 3193772f0128dc66b76871a641c1b275de97975f	no	82
Warnings:					
Information:					
19	Foreign Reference	WO2014060483.pdf	1165115 838bc5ce0b3ded68b783fb24dc945a3c98ca9903	no	144
Warnings:					
Information:					
20	Foreign Reference	WO2014142841.pdf	276234 81fd0ea20a75cdc224c1526f83c46b8d0ccceec96	no	41
Warnings:					
Information:					
21	Foreign Reference	WO2014210223.pdf	1034007 20ca46171c952cf410f54af84fe8ea4c67939d7f	no	133
Warnings:					
Information:					
22	Foreign Reference	WO2014210225.pdf	900064 01252c061bd090a29ee7abf0701a9e9acf8457ff	no	113
Warnings:					
Information:					

23	Foreign Reference	WO2016007839.pdf	5151215 069e5ac5d8ea5809341672e224504349fe3de8c3	no	36
Warnings:					
Information:					
24	Foreign Reference	WO2016162309.pdf	700542 47c5b583c68b5fd24864d7cc0cca97feff39cb54	no	70
Warnings:					
Information:					
25	Foreign Reference	WO2014163886.pdf	6643318 6ae2ab16f59c1957e31f14058534e1b61facd082	no	40
Warnings:					
Information:					
26	Foreign Reference	WO2018045186.pdf	12908291 cbed940ca679376fdf2c011e21ff381470b703c7	no	70
Warnings:					
Information:					
27	Foreign Reference	WO2018045181.pdf	13491122 3c0181b00210bb4622385d3c2d4121f6bbc5da71	no	75
Warnings:					
Information:					
		Total Files Size (in bytes):	61244516		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	35429388
Application Number:	16276235
International Application Number:	
Confirmation Number:	7944
Title of Invention:	Spatially Encoded Biological Assays
First Named Inventor/Applicant Name:	Mark S. CHEE
Customer Number:	159367
Filer:	Cameron Mark Luitjens/Kirsten Neuman
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	47706-0004003
Receipt Date:	15-MAR-2019
Filing Date:	14-FEB-2019
Time Stamp:	14:43:46
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	DE102008025656A1.pdf	14642830 053877a3480c7e54b994cbeda0200625bfb7b77c	no	85

Warnings:

Information:					
2	Foreign Reference	EP1712623.pdf	697468 bbcda2fd01b166302fd989f536a3c2c4ec60d8e5	no	49
Warnings:					
Information:					
3	Foreign Reference	EP1910562.pdf	8420 21359efbff2306af26502b4ecbf0f751afb75446	no	1
Warnings:					
Information:					
4	Foreign Reference	EP1929039.pdf	19002 8736496b2b533faa9a4877363565f68035c5bda8	no	1
Warnings:					
Information:					
5	Foreign Reference	EP1966393.pdf	19160 c51233a531a56d4cba408ec2b99e8bc049317b7a	no	1
Warnings:					
Information:					
6	Foreign Reference	EP2002017.pdf	8870 e18ef35de1badff27263f6f278a539746f6ba929	no	1
Warnings:					
Information:					
7	Foreign Reference	EP2292788.pdf	615996 f10467f20e4d79b04342576bd6cd08cf35e8d23c	no	52
Warnings:					
Information:					
8	Foreign Reference	EP2302070.pdf	580756 a91b7d2183035b3340b58d16ac09a45ef84f1665	no	51
Warnings:					
Information:					

9	Foreign Reference	EP2363504.pdf	655435 db5250a0508e945b19baf6e373964b7bdd d67454	no	48
Warnings:					
Information:					
10	Foreign Reference	EP2580351.pdf	8748 1962d845272935cb2da016bb4d77a5cb94 b44cdc	no	1
Warnings:					
Information:					
11	Foreign Reference	EP2789696.pdf	555466 8cd8018965c0b79aadcf80a9996a99d60e5 e4725	no	43
Warnings:					
Information:					
12	Foreign Reference	EP2963127.pdf	229413 4107fa6647d99fea93b4f1e416476df9932d 8f74	no	23
Warnings:					
Information:					
13	Foreign Reference	EP3045544.pdf	650854 19ca374c5dafcc5977883887e337820c76b b20ff	no	43
Warnings:					
Information:					
14	Foreign Reference	EP3239304.pdf	269450 ae0baf5fefe9e4b923634ef18925097936ff9 af5	no	24
Warnings:					
Information:					
15	Foreign Reference	JP2011182702.pdf	674700 0dfec21c94f29342953605a7e6e25874af1f0 da2	no	29
Warnings:					
Information:					

16	Foreign Reference	JP2014217381A.pdf	7133864 a2b406706727bdff7b041763a038b2be27472772	no	161
Warnings:					
Information:					
17	Foreign Reference	RU2145635.pdf	74941 cace1ec631af88ff16dfc48ad186f9152a07eac9	no	1
Warnings:					
Information:					
18	Foreign Reference	RU2270254.pdf	81332 abf5b0b0261ec7580d760acd24d1725791870916	no	2
Warnings:					
Information:					
19	Foreign Reference	WO1989010977.pdf	440132 5af20b2504a474bf78f4be2fe05511f4163f3cad	no	31
Warnings:					
Information:					
20	Foreign Reference	WO1991006678.pdf	727790 1209f673cac36dab43d01aa06d80bd978d7df265	no	62
Warnings:					
Information:					
21	Foreign Reference	WO1995025116.pdf	342996 adce702a50d1d6881cee10c7281d0b02cc6771c8	no	40
Warnings:					
Information:					
22	Foreign Reference	WO1995035505.pdf	645805 35bcf2ef2f511957d12143753a0ee56900019e1e	no	53
Warnings:					
Information:					

23	Foreign Reference	WO1998044151.pdf	1096908 36469d21f31b451d2fd4172d9d57d28e049fbeda	no	109
Warnings:					
Information:					
24	Foreign Reference	WO1999032654.pdf	471083 c57c1764ec02d7272959e2346205e32864bb383b	no	34
Warnings:					
Information:					
25	Foreign Reference	WO2000018957.pdf	913115 30e8bb6e81f43cfbb6ef014ac672b35fed7a5fdf	no	104
Warnings:					
Information:					
26	Foreign Reference	WO2000024940.pdf	775480 915dc16cad56dcc431c5c0eddb722e1179cb93f1	no	87
Warnings:					
Information:					
27	Foreign Reference	WO2000063437.pdf	3327342 012a5a598c3257417feebb7efdbffb7820f355f6	no	327
Warnings:					
Information:					
28	Foreign Reference	WO2001007915.pdf	637032 89e51d883a92c6b65d060d2b579f96b94fb5d2ae	no	61
Warnings:					
Information:					
29	Foreign Reference	WO2001042796.pdf	1518115 0764ab9f2f0071c449225306315057c55aff35a4	no	136
Warnings:					
Information:					

30	Foreign Reference	WO2002024952.pdf	928482 e6743e941613ccb062b50e55478a89c570da910e	no	70
Warnings:					
Information:					
31	Foreign Reference	WO2002059355.pdf	1024258 2d357f44a403a3340ed0d45256295df426f95484	no	127
Warnings:					
Information:					
32	Foreign Reference	WO2002077283.pdf	559881 111a8a0873917ae657919ddb6ff5840bb513cf93	no	42
Warnings:					
Information:					
33	Foreign Reference	WO2002088396.pdf	1369649 befd099636a5265260254dd414813d60340b6ac4	no	113
Warnings:					
Information:					
34	Foreign Reference	WO2003010176.pdf	313377 006afe20ee598fff64d4e8eb6c387796b70a5091	no	31
Warnings:					
Information:					
35	Foreign Reference	WO2004018497.pdf	1545202 cd895686cca8ee41e69232e00db1c21ea862e70e	no	142
Warnings:					
Information:					
36	Foreign Reference	WO2004028955.pdf	1269796 aa1b14b4487bcf7f6e9dd8f3c71c258d53cc255b	no	107
Warnings:					
Information:					

37	Foreign Reference	WO2005010145.pdf	634479 787a769e11763fa4a72937c98e54dbf0b1b63777	no	50
Warnings:					
Information:					
38	Foreign Reference	WO2005026387.pdf	1530244 c60bab245428c6d5f7cdbc7cfa82fa76067f09be	no	150
Warnings:					
Information:					
39	Foreign Reference	WO2005042759.pdf	1132653 11c33c2f4ae262cce4a2e8d335a29f648e587572	no	101
Warnings:					
Information:					
40	Foreign Reference	WO2006074351.pdf	956221 4bc33905d6cfa801e1f4a2e5ac94c0ffe257d74c	no	84
Warnings:					
Information:					
41	Foreign Reference	WO2006084130.pdf	4884715 dead06ed719b0fcb5de5760c5060d8364e2387c5	no	432
Warnings:					
Information:					
42	Foreign Reference	WO2006117541.pdf	1967950 72df34f3b487331660e4f86848a8cc4d5c4876a9	no	103
Warnings:					
Information:					
43	Foreign Reference	WO2006137733.pdf	774825 a314abe2646ebb948c4c1a1bfce3d88b4f98b3e1	no	75
Warnings:					
Information:					

44	Foreign Reference	WO2007030373.pdf	343268 32f45085222a2a1aed9a928c71a6cd2d1fba1443	no	37
Warnings:					
Information:					
45	Foreign Reference	WO2007037678.pdf	886456 337537c11cc1f993d728f6d53683ee546cd4721b	no	51
Warnings:					
Information:					
46	Foreign Reference	WO2007041689.pdf	1898878 fc3a028b31705814befa199006ac49308b847915	no	129
Warnings:					
Information:					
47	Foreign Reference	WO2007060599.pdf	174215 562a027165115ae1e85d1c6e6d64be6a349cd446	no	21
Warnings:					
Information:					
48	Foreign Reference	WO2007073165.pdf	793694 5d31ba5fc5ceca7f351a200b446ef3b7f644d6a4	no	57
Warnings:					
Information:					
49	Foreign Reference	WO2007073171.pdf	791065 430bb67759455a7540ed902b3886a94ad953b347	no	67
Warnings:					
Information:					
50	Foreign Reference	WO2007076726.pdf	744902 f0f067bc60125baadd820363097389a7db0bc2ff	no	58
Warnings:					
Information:					

51	Foreign Reference	WO2007114693.pdf	403654 bdd7e2981ad1a70d43fbaa3cd4832c0067f07d9b	no	36
Warnings:					
Information:					
52	Foreign Reference	WO2007120241.pdf	3905253 95eb25dcd166ea7e508a13e3b026f938e540545e	no	233
Warnings:					
Information:					
53	Foreign Reference	WO2007123744.pdf	2728597 c480ae0d9b52eb4e47beecd3abf67f1948887841	no	139
Warnings:					
Information:					
54	Foreign Reference	WO2007145612.pdf	1394738 4defbdde3e377246fbd1f31ce157a39d0d2472b8	no	110
Warnings:					
Information:					
55	Foreign Reference	WO2008093098.pdf	1255467 4d380d570e43fd2071ddaf0d52928df40421ef8a	no	119
Warnings:					
Information:					
56	Foreign Reference	WO2009032167.pdf	1032055 c42f28088bac5bf145024c48c3dd441a56228e45	no	119
Warnings:					
Information:					
57	Foreign Reference	WO2009036525.pdf	468087 07cdad0786450708c0c9712a17245b971f8e0da2	no	40
Warnings:					
Information:					

58	Foreign Reference	WO2009152928.pdf	881183 2b13d13f9a31d5fa5a544dd63480d90fd7da7bd5	no	80
Warnings:					
Information:					
59	Foreign Reference	WO2010019826.pdf	753135 31360835e562bd11da259329ba74921805a9c6f5	no	92
Warnings:					
Information:					
60	Foreign Reference	WO2010027870.pdf	884895 a3eacedd3bc35de57edb699bbab0a319aa3a6a00	no	77
Warnings:					
Information:					
			Total Files Size (in bytes):	76053777	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/276,235	02/14/2019	Mark S. CHEE	47706-0004003	7944

159367 7590 04/18/2019

FISH & RICHARDSON P.C.
10x Genomics
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
MARTINELL, JAMES	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
04/18/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com
ipadmin@10xgenomics.com

Office Action Summary	Application No. 16/276,235	Applicant(s) CHEE, Mark S.	
	Examiner JAMES MARTINELL	Art Unit 1634	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 15 March 2019.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 2-31 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 2-31 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 14 February 2019 is/are: a)☐ accepted or b)☑ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date 3/15/19 (2 sets).

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Regarding the Information Disclosure Statement filed March 15, 2019 listing U.S Patent applications. These applications are not considered nor cited by the examiner because they are either applications of published U.S. Patent Applications that are already listed on the longer Information Disclosure Statement filed March 15, 2019 which cites 698 references or they are not shown by applicants to be by the same inventive entity or have the same assignee or owner as the instant application. See MPEP 901.03.

The following comments are in regard to the Information Disclosure Statement filed March 15, 2019 and listing 698 references.

(a) Reference Numbers 165, 345, 346, and 347 have been crossed out because they are not U.S. Patents or U.S. Patent Application Publications.

(b) Reference Number 438 is a duplicate listing.

(c) Number 535 is inaccurate. This is actually Eberwine, already cited.

(d) Reference Number 644 is a foreign language document. No English language translation or explanation is in the record. See MPEP 609.

The drawings are objected to as failing to comply with 37 CFR 1.84(p)(5) because they include the following reference character(s) not mentioned in the description: **150**, **160**, **200**, **306**, and **550**. Corrected drawing sheets in compliance with 37 CFR 1.121(d), or amendment to the specification to add the reference character(s) in the description in compliance with 37 CFR 1.121(b) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If

the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal

Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 2-31 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-12, 20, 24-28, 34, 36-39, and 58-77 of copending Application No. 15/187,661 in view of any one of Fu et al (U.S. Patent Application Publication 2009/0117573 A1, particularly paragraph 0116), Rothberg et al (U.S. Patent Application Publication 2009/0026082 A1, particularly paragraph 0339), Chee et al (U.S. Patent Application Publication 2009/0117573 A1, particularly paragraph 0116), or Leamon et al (U.S. Patent Application Publication 2005/0130173 A1, particularly paragraph 0083). Claims 1-12, 20, 24-28, 34, 36-39, and 58-77 of copending Application No. 15/187,661 are directed to detection of biological targets by binding to encoded binding agents attached to a substrate and determining the spatial pattern of the binding. The instant clams use the same methodology while employing beads as binding agents. Claims 1-12, 20, 24-28, 34, 36-39, and 58-77 of copending Application No. 15/187,661 do not employ beads. Each of the secondary references uses beads as solid supports to detect binging of biological molecules. It would have been obvious for one of ordinary skill in the art to use the beads of any one of the secondary references in the detection methods of claims 1-12, 20, 24-28, 34, 36-39, and 58-77 of copending Application No. 15/187,661 for the known and expected results.

This is a provisional nonstatutory double patenting rejection.

Claims 2-31 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2-23of copending Application No. 16/276,260 in view of any one of Fu et al (U.S. Patent Application Publication 2009/0117573 A1, particularly paragraph 0116), Rothberg et al (U.S. Patent Application Publication 2009/0026082 A1, particularly paragraph 0339), Chee et al (U.S. Patent Application Publication 2009/0117573 A1, particularly paragraph 0116), or Leamon et al (U.S. Patent

Application Publication 2005/0130173 A1, particularly paragraph 0083). Claims 2-23 of copending Application No. 16/276,260 are directed to detection of biological targets by binding to encoded binding agents attached to a substrate and determining the spatial pattern of the binding. The instant clams use the same methodology while employing beads as binding agents. Claims 2-23 of copending Application No. 16/276,260 do not employ beads. Each of the secondary references uses beads as solid supports to detect binging of biological molecules. It would have been obvious for one of ordinary skill in the art to use the beads of any one of the secondary references in the detection methods of claims 2-23 of copending Application No. 16/276,260 for the known and expected results.

This is a provisional nonstatutory double patenting rejection.

Claims 2-31 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-46 of U.S. Patent No. 9,371,598 in view of any one of Fu et al (U.S. Patent Application Publication 2009/0117573 A1, particularly paragraph 0116), Rothberg et al (U.S. Patent Application Publication 2009/0026082 A1, particularly paragraph 0339), Chee et al (U.S. Patent Application Publication 2009/0117573 A1, particularly paragraph 0116), or Leamon et al (U.S. Patent Application Publication 2005/0130173 A1, particularly paragraph 0083).

Claims 1-46 of U.S. Patent No. 9,371,598 are directed to detection of biological targets by binding to encoded binding agents attached to a substrate and determining the spatial pattern of the binding. The instant clams use the same methodology while employing beads as binding agents. Claims 1-46 of U.S. Patent No. 9,371,598 do not employ beads.

Each of the secondary references uses beads as solid supports to detect binging of biological molecules. It would have been obvious for one of ordinary skill in the art to use the beads of any one of the secondary references in the detection methods of claims 1-46 of U.S. Patent No. 9,371,598 are for the known and expected results.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JAMES MARTINELL whose telephone number is (571) 272-0719.

The examiner works a flexible schedule and can be reached by phone and voice mail. Alternatively, a request for a return telephone call may be e-mailed to james.martinell@uspto.gov. Since e-mail communications may not be secure, it is suggested that information in such requests be limited to name, phone number, and the best time to return the call.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave T. Nguyen, can be reached on (571) 272-0731.

OFFICIAL FAX NUMBER

The fax phone number for the organization where this application or proceeding is assigned is (571) 273-8300. Any Official Communication to the USPTO should be faxed to this number.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

/JAMES MARTINELL/
Primary Examiner, Art Unit 1634

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/276,235	CHEE, Mark S.
	Examiner	Art Unit
	JAMES MARTINELL	1634

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Parent SN 13/080,616 (PN 9,371,598)	04/10/2019	JM
Parent SN 15/187,661 (pending)	04/10/2019	JM
STN search (2 sets of printouts attached)	04/12/2019	JM
EAST text search (printout attached)	04/14/2019	JM
Inventor search (PALM)	04/14/2019	JM

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/JAMES MARTINELL/ Primary Examiner, Art Unit 1634	

Curio EX1010 p. 448
Curio v Prognosys
IPR2024-01289

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/276,235	CHEE, Mark S.
	Examiner	Art Unit
	JAMES MARTINELL	1634

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	04/14/2019								
	1	-								
	2	✓								
	3	✓								
	4	✓								
	5	✓								
	6	✓								
	7	✓								
	8	✓								
	9	✓								
	10	✓								
	11	✓								
	12	✓								
	13	✓								
	14	✓								
	15	✓								
	16	✓								
	17	✓								
	18	✓								
	19	✓								
	20	✓								
	21	✓								
	22	✓								
	23	✓								
	24	✓								
	25	✓								
	26	✓								
	27	✓								
	28	✓								
	29	✓								
	30	✓								
	31	✓								

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Mark S. Chee	Art Unit	:	Unknown
Application No.	:	16/276,235	Examiner	:	Unknown
Filed	:	February 14, 2019	Conf. No.	:	7944
Title	:	SPATIALLY ENCODED BIOLOGICAL ASSAYS			

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the documents listed on the disclosure form submitted with this statement. Copies of the documents are being submitted with this statement to the extent required under 37 C.F.R. § 1.98(a)(2).

The applicant wishes to make the examiner aware of the following related applications. The prosecution histories for these applications are available on PAIR, and are not provided with this statement.

Application No.	Filing Date	First Named Inventor	Attorney Docket No.
13/080,616	4/5/2011	Mark S. Chee	47706-0004001
15/187,661	6/20/2016	Mark S. Chee	47706-0004002
16/276,260	2/14/2019	Mark S. Chee	47706-0004004
14/900,602	12/21/2015	Mark S. Chee	47706-0005US1
14/900,604	12/21/2015	Mark S. Chee	47706-0006US1
15/831,158	12/4/2017	Mark S. Chee	47706-0006002
15/565,637	10/10/2017	Jonas Frisen	47706-0009US1
14/111,482	10/11/2013	Jonas Frisen	47706-0011US1
16/013,654	6/20/2018	Jonas Frisen	47706-0011002
16/042,950	7/23/2018	Jonas Frisen	47706-0011003
16/043,038	7/23/2018	Jonas Frisen	47706-0011004
16/254,443	1/22/2019	Jonas Frisen	47706-0011005
14/434,274	4/8/2015	Jonas Frisen	47706-0012US1

This statement is being filed within three months of the filing date of the application.

/JAMES MARTINELL/ 04/14/2019

Apply any necessary charges or credits applicable under 37 C.F.R. § 1.17(p) to deposit account 06-1050, referencing the above attorney docket number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: March 15, 2019

/Cameron Luitjens/

Cameron M. Luitjens, Ph.D., J.D.
Reg. No. 58,674

Customer Number 159367
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

61870675.doc

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	1.	4,683,195	7/28/1987	Mullis			
	2.	4,683,202	7/28/1987	Mullis			
	3.	4,800,159	1/24/1989	Mullis			
	4.	4,883,867	11/28/1989	Lee			
	5.	4,965,188	10/23/1990	Mullis			
	6.	5,002,882	3/26/1991	Lunnen			
	7.	5,130,238	7/14/1992	Malek			
	8.	5,308,751	5/3/1994	Ohkawa			
	9.	5,321,130	6/14/1994	Yue			
	10.	5,410,030	4/25/1995	Yue			
	11.	5,436,134	7/25/1995	Haugland			
	12.	5,455,166	10/3/1995	Walker			
	13.	5,512,439	4/30/1996	Hornes			
	14.	5,512,462	4/30/1996	Cheng			
	15.	5,582,977	12/10/1996	Yue			
	16.	5,599,675	2/4/1997	Brenner			
	17.	5,641,658	6/24/1997	Adams			
	18.	5,658,751	8/19/1997	Yue			
	19.	5,750,341	5/12/1998	Macevicz			
	20.	5,763,175	6/1/1998	Brenner			
	21.	5,863,753	1/26/1999	Haugland			
	22.	5,912,148	6/15/1999	Eggerding			
	23.	6,013,440	1/11/2000	Lipshutz			
	24.	6,130,073	10/10/2000	Eggerding			
	25.	6,143,496	11/7/2000	Brown			
	26.	6,153,389	11/28/2000	Haarer			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Curio EX1010-p. 452
Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	27.	6,210,891	4/3/2001	Nyren			
	28.	6,210,894	4/3/2001	Brennan			
	29.	6,214,587	4/10/2001	Dattagupta			
	30.	6,258,558	7/10/2001	Szostak			
	31.	6,258,568	7/10/2001	Nyren			
	32.	6,261,804	7/17/2001	Szostak			
	33.	6,266,459	7/24/2001	Walt			
	34.	6,274,320	8/14/2001	Rothberg			
	35.	6,281,804	8/28/2001	Haller			
	36.	6,309,824	10/30/2001	Drmanac			
	37.	6,344,316	2/5/2002	Lockhart			
	38.	6,355,431	3/12/2002	Chee			
	39.	6,401,267	6/11/2002	Drmanac			
	40.	6,404,907	6/11/2002	Gilchrist			
	41.	6,416,950	7/9/2002	Lohse			
	42.	6,485,982	11/26/2002	Charlton			
	43.	6,518,018	2/11/2003	Szostak			
	44.	6,565,727	5/20/2003	Shenderov			
	45.	6,579,695	6/17/2003	Lambalot			
	46.	6,632,641	10/14/2003	Brennan			
	47.	6,770,441	8/3/2004	Dickinson			
	48.	6,773,566	8/10/2004	Shenderov			
	49.	6,773,886	8/10/2004	Kaufman			
	50.	6,787,308	9/7/2004	Balasubramanian			
	51.	6,800,453	10/5/2004	Labaer			
	52.	6,828,100	12/7/2004	Ronaghi			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents							
Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	53.	6,833,246	12/21/2004	Balasubramanian			
	54.	6,859,570	2/22/2005	Walt			
	55.	6,864,052	3/8/2005	Drmanac			
	56.	6,867,028	3/15/2005	Janulaitis			
	57.	6,878,515	4/12/2005	Landegren			
	58.	6,897,023	5/24/2005	Fu			
	59.	6,911,132	6/28/2005	Pamula			
	60.	6,911,345	6/28/2005	Quake			
	61.	6,969,488	11/29/2005	Bridgham			
	62.	6,969,589	11/29/2005	Patil			
	63.	6,977,033	12/20/2005	Becker			
	64.	7,052,244	5/30/2006	Fouillet			
	65.	7,057,026	6/6/2006	Barnes			
	66.	7,115,400	10/3/2006	Adessi			
	67.	7,118,883	10/10/2006	Inoue			
	68.	7,163,612	1/16/2007	Sterling			
	69.	7,192,735	3/20/2007	Lambalot			
	70.	7,211,414	5/1/2007	Hardin			
	71.	7,229,769	6/12/2007	Kozlov			
	72.	7,244,559	7/17/2007	Rothberg			
	73.	7,264,929	9/4/2007	Rothberg			
	74.	7,270,950	9/18/2007	Szostak			
	75.	7,297,518	11/20/2007	Quake			
	76.	7,315,019	1/1/2008	Turner			
	77.	7,328,979	2/12/2008	Decre			
	78.	7,329,492	2/12/2008	Hardin			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	79.	7,378,242	5/27/2008	Hurt			
	80.	7,393,665	7/1/2008	Brenner			
	81.	7,405,281	7/29/2008	Xu			
	82.	7,407,757	8/5/2008	Brenner			
	83.	7,462,449	12/9/2008	Quake			
	84.	7,501,245	3/10/2009	Quake			
	85.	7,537,897	5/26/2009	Brenner			
	86.	7,544,473	6/9/2009	Brennan			
	87.	7,547,380	6/16/2009	Velev			
	88.	7,579,153	8/25/2009	Brenner			
	89.	7,595,883	9/29/2009	Gamal			
	90.	7,601,498	10/13/2009	Mao			
	91.	7,635,566	12/22/2009	Brenner			
	92.	7,641,779	1/5/2010	Becker			
	93.	7,666,612	2/23/2010	Johnsson			
	94.	7,674,752	3/9/2010	He			
	95.	7,754,429	7/13/2010	Rigatti			
	96.	7,776,547	8/17/2010	Roth			
	97.	7,776,567	8/17/2010	Mao			
	98.	7,803,943	9/28/2010	Mao			
	99.	7,858,321	12/28/2010	Glezer			
	100.	7,960,120	6/14/2011	Rigatti			
	101.	8,092,784	1/10/2012	Mao			
	102.	8,148,068	4/3/2012	Brenner			
	103.	8,148,518	4/3/2012	Buchanan			
	104.	8,207,093	6/26/2012	Szostak			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Curio EX1010-p. 455
Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	105.	8,288,103	10/16/2012	Oliphant			
	106.	8,337,851	12/25/2012	Aukerman			
	107.	8,343,500	1/1/2013	Wraith			
	108.	8,383,338	2/26/2013	Kitzman			
	109.	8,460,865	6/11/2013	Chee			
	110.	8,481,257	7/9/2013	Van Eijk			
	111.	8,481,292	7/9/2013	Casbon			
	112.	8,486,625	7/16/2013	Gunderson			
	113.	8,586,310	11/19/2013	Mitra			
	114.	8,614,073	12/24/2013	Van Eijk			
	115.	8,637,242	1/28/2014	Shen			
	116.	8,685,889	4/1/2014	Van Eijk			
	117.	8,741,606	6/3/2014	Casbon			
	118.	8,778,849	7/15/2014	Bowen			
	119.	8,785,353	7/22/2014	Van Eijk			
	120.	8,815,512	8/26/2014	Van Eijk			
	121.	8,835,358	9/16/2014	Fodor			
	122.	8,865,410	10/21/2014	Shendure			
	123.	8,895,249	11/25/2014	Shen			
	124.	8,911,945	12/16/2014	Van Eijk			
	125.	8,936,912	1/20/2015	Mitra			
	126.	8,951,781	2/10/2015	Reed			
	127.	9,023,768	5/5/2015	Van Eijk			
	128.	9,062,348	6/23/2015	Van Eijk			
	129.	9,080,210	7/14/2015	Van Eijk			
	130.	9,085,798	7/21/2015	Chee			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 456
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	131.	9,121,069	9/1/2015	Lo			
	132.	9,194,001	11/24/2015	Brenner			
	133.	9,290,808	3/22/2016	Fodor			
	134.	9,290,809	3/22/2016	Fodor			
	135.	9,328,383	5/3/2016	Van Eijk			
	136.	9,334,536	5/10/2016	Van Eijk			
	137.	9,340,830	5/17/2016	Lipson			
	138.	9,371,598	6/21/2016	Chee			
	139.	9,376,716	6/28/2016	Van Eijk			
	140.	9,376,719	6/28/2016	Van Eijk			
	141.	9,404,156	8/2/2016	Hicks			
	142.	9,447,459	9/20/2016	Van Eijk			
	143.	9,453,256	9/27/2016	Van Eijk			
	144.	9,493,820	11/15/2016	Van Eijk			
	145.	9,506,061	11/29/2016	Brown			
	146.	9,574,230	2/21/2017	Van Eijk			
	147.	9,593,365	3/14/2017	Frisen et al.			
	148.	9,657,335	5/23/2017	Van Eijk			
	149.	9,670,542	6/6/2017	Van Eijk			
	150.	9,702,004	7/11/2017	Van Eijk			
	151.	9,727,810	8/8/2017	Fodor et al.			
	152.	9,745,627	8/29/2017	Van Eijk			
	153.	9,777,324	10/3/2017	Van Eijk			
	154.	9,868,979	1/16/2018	Chee et al.			
	155.	9,879,313	1/30/2018	Chee et al.			
	156.	9,896,721	2/20/2018	Van Eijk			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 457
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	157.	9,898,576	2/20/2018	Van Eijk			
	158.	9,898,577	2/20/2018	Van Eijk			
	159.	10,002,316	6/19/2018	Fodor et al.			
	160.	10,023,907	7/17/2018	Van Eijk			
	161.	10,030,261	7/24/2018	Frisen et al.			
	162.	10,095,832	10/9/2018	Van Eijk			
	163.	10,138,509	11/27/2018	Church et al.			
	164.	10,179,932	1/15/2019	Church et al.			
	165.	~~13/080,616~~	~~10/6/2011~~	~~Chee~~			
	166.	2002/0055100	5/9/2002	Kawashima			
	167.	2002/0064779	5/30/2002	Landegren			
	168.	2002/0150909	10/17/2002	Stuelpnagel			
	169.	2002/0168645	11/14/2002	Taylor			
	170.	2003/0022207	1/30/2003	Balasubramanian			
	171.	2003/0040035	2/27/2003	Slamon			
	172.	2003/0064398	4/3/2003	Barnes			
	173.	2003/0087232	5/8/2003	Christians			
	174.	2003/0096323	5/22/2003	James			
	175.	2003/0113713	6/19/2003	Glezer			
	176.	2003/0138879	7/24/2003	Lambalot			
	177.	2003/0148335	8/7/2003	Shen et al.			
	178.	2003/0162216	8/28/2003	Gold			
	179.	2003/0170637	9/11/2003	Pirrung et al.			
	180.	2003/0205632	11/6/2003	Kim et al.			
	181.	2003/0224419	12/4/2003	Corcoran			
	182.	2003/0232382	12/18/2003	Brennan			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 458
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	183.	2003/0235852	12/25/2003	Roberts			
	184.	2004/0002090	1/1/2004	Mayer et al.			
	185.	2004/0067492	4/8/2004	Peng et al.			
	186.	2004/0096853	5/20/2004	Mayer			
	187.	2004/0106110	6/3/2004	Balasubramanian			
	188.	2004/0112442	6/17/2004	Maerkl			
	189.	2005/0003431	1/6/2005	Wucherpfennig			
	190.	2005/0019776	1/27/2005	Callow et al.			
	191.	2005/0026188	2/3/2005	Van Kessel			
	192.	2005/0037362	2/17/2005	Remacle et al.			
	193.	2005/0037393	2/17/2005	Gunderson et al.			
	194.	2005/0048580	3/3/2005	Labaer			
	195.	2005/0064460	3/24/2005	Holliger et al.			
	196.	2005/0130173	6/16/2005	Leamon et al.			
	197.	2005/0164292	7/28/2005	Farooqui			
	198.	2005/0179746	8/18/2005	Roux et al.			
	199.	2005/0191656	9/1/2005	Drmanac et al.			
	200.	2005/0191698	9/1/2005	Chee et al.			
	201.	2005/0239192	10/27/2005	Nasarabadi et al.			
	202.	2005/0255548	11/17/2005	Lipovsek et al.			
	203.	2005/0260653	11/24/2005	LaBaer			
	204.	2006/0003394	1/5/2006	Song			
	205.	2006/0039823	2/23/2006	Yamakawa et al.			
	206.	2006/0046313	3/2/2006	Roth			
	207.	2006/0079453	4/13/2006	Sidney et al.			
	208.	2006/0134669	6/22/2006	Casasanta			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 459
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	209.	2006/0164490	7/27/2006	Kim et al.			
	210.	2006/0188875	8/24/2006	Cox et al.			
	211.	2006/0194331	8/31/2006	Pamula et al.			
	212.	2006/0199207	9/7/2006	Matysiak			
	213.	2006/0211001	9/21/2006	Yu et al.			
	214.	2006/0216721	9/28/2006	Kozlov et al.			
	215.	2006/0216775	9/28/2006	Burkart et al.			
	216.	2006/0228758	10/12/2006	Muchhal et al.			
	217.	2006/0263789	11/23/2006	Kincaid			
	218.	2006/0275799	12/7/2006	Banerjee et al.			
	219.	2007/0014810	1/18/2007	Baker et al.			
	220.	2007/0020625	1/25/2007	Duchaud et al.			
	221.	2007/0020640	1/25/2007	McCloskey et al.			
	222.	2007/0020669	1/25/2007	Ericsson			
	223.	2007/0023292	2/1/2007	Kim et al.			
	224.	2007/0026430	2/1/2007	Andersen et al.			
	225.	2007/0036511	2/15/2007	Lundquist et al.			
	226.	2007/0054288	3/8/2007	Su et al.			
	227.	2007/0099208	5/3/2007	Drmanac et al.			
	228.	2007/0128624	6/7/2007	Gormley et al.			
	229.	2007/0166725	7/19/2007	McBride et al.			
	230.	2007/0172873	7/26/2007	Brenner et al.			
	231.	2007/0178503	8/2/2007	Jiang			
	232.	2007/0207482	9/6/2007	Church et al.			
	233.	2007/0254305	11/1/2007	Paik, et al.			
	234.	2007/0269805	11/22/2007	Hogers			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 460
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents							
Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	235.	2008/0009420	1/10/2008	Schroth et al.			
	236.	2008/0032301	2/7/2008	Rank et al.			
	237.	2008/0071071	3/20/2008	LaBaer et al.			
	238.	2008/0108082	5/8/2005	Rank et al.			
	239.	2008/0108804	5/8/2008	Hayashizaki et al.			
	240.	2008/0124252	5/29/2008	Marchand et al.			
	241.	2008/0128627	6/5/2008	Lundquist et al.			
	242.	2008/0220434	9/11/2008	Thomas			
	243.	2008/0220981	9/11/2008	McGregor			
	244.	2008/0261204	10/23/2008	Lexow			
	245.	2008/0293591	11/27/2008	Taussig et al.			
	246.	2008/0312103	12/18/2008	Nemoto et al.			
	247.	2009/0005252	1/1/2009	Drmanac et al.			
	248.	2009/0006002	1/1/2009	Honisch et al.			
	249.	2009/0018024	1/15/2009	Church et al.			
	250.	2009/0026082	1/29/2009	Rothberg et al.			
	251.	2009/0036323	2/5/2009	van Eijk et al.			
	252.	2009/0082212	3/26/2009	Williams			
	253.	2009/0099041	4/16/2009	Church et al.			
	254.	2009/0105959	4/23/2009	Braverman et al.			
	255.	2009/0117573	5/7/2009	Fu et al.			
	256.	2009/0127589	5/21/2009	Rothberg et al.			
	257.	2009/0155781	6/18/2008	Drmanac et al.			
	258.	2009/0170713	7/2/2009	van Eijk et al.			
	259.	2009/0192044	7/30/2009	Fouillet			
	260.	2009/0197326	8/6/2009	El Gamal et al.			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 461
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	261.	2009/0215633	8/27/2009	van Eijk et al.			
	262.	2009/0233802	9/17/2009	Bignell et al.			
	263.	2009/0253581	10/8/2009	van Eijk et al.			
	264.	2009/0264299	10/22/2009	Drmanac et al.			
	265.	2009/0280487	11/12/2009	Hung et al.			
	266.	2009/0283407	11/19/2009	Shah et al.			
	267.	2009/0291854	11/26/2009	Weisinger-Mayr et al.			
	268.	2009/0305237	12/10/2009	Cantor et al.			
	269.	2009/0321262	12/31/2009	Adachi et al.			
	270.	2010/0035249	2/11/2010	Hayashizaki et al.			
	271.	2010/0069263	3/18/2010	Shendure et al.			
	272.	2010/0096266	4/22/2010	Kim et al.			
	273.	2010/0099103	4/22/2010	Hsieh et al.			
	274.	2010/0105052	4/29/2010	Drmanac et al.			
	275.	2010/0111768	5/6/2010	Banerjee et al.			
	276.	2010/0113302	5/6/2010	Williams			
	277.	2010/0120098	5/13/2010	Grunenwald et al.			
	278.	2010/0129874	5/27/2010	Mitra et al.			
	279.	2010/0137143	6/3/2010	Rothberg et al.			
	280.	2010/0151464	6/17/2010	Stuelpnagel et al.			
	281.	2010/0159446	6/24/2010	Haff et al.			
	282.	2010/0173384	7/8/2010	Johnsson et al.			
	283.	2010/0184614	7/22/2010	Ye et al.			
	284.	2010/0227329	9/9/2010	Cuppens			
	285.	2010/0282617	11/11/2010	Rothberg et al.			
	286.	2011/0033854	2/10/2011	Drmanac et al.			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 462

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	287.	2011/0048951	3/3/2011	Wu			
	288.	2011/0059436	3/10/2011	Hardin et al.			
	289.	2011/0059865	3/10/2011	Smith et al.			
	290.	2011/0244448	10/6/2011	Shirai et al.			
	291.	2011/0245101	10/6/2011	Chee et al.			
	292.	2011/0245111	10/6/2011	Chee			
	293.	2012/0046175	2/23/2012	Rodesch et al.			
	294.	2012/0065081	3/15/2012	Chee			
	295.	2012/0129248	5/24/2012	Chee et al.			
	296.	2012/0135871	5/31/2012	van Eijk et al.			
	297.	2012/0195810	8/2/2012	Cohen et al.			
	298.	2012/0202698	8/9/2012	van Eijk et al.			
	299.	2012/0245053	9/27/2012	Shirai et al.			
	300.	2012/0258871	10/11/2012	Kozlov et al.			
	301.	2012/0270748	10/25/2012	Chee et al.			
	302.	2012/0289414	11/15/2012	Mitra et al.			
	303.	2013/0096033	4/18/2013	Routenberg			
	304.	2013/0109595	5/2/2013	Routenberg			
	305.	2013/0261019	10/3/2013	Lin et al.			
	306.	2013/0296174	11/7/2013	Peumans			
	307.	2014/0065609	3/6/2014	Hicks et al.			
	308.	2014/0066318	3/6/2014	Frisen et al.			
	309.	2014/0079923	3/20/2014	George et al.			
	310.	2014/0121118	5/1/2014	Warner			
	311.	2014/0155274	6/5/2014	Xie et al.			
	312.	2014/0227705	8/14/2014	Vogelstein et al.			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 463
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	313.	2014/0243224	8/28/2014	Barnard et al.			
	314.	2014/0274731	9/18/2014	Raymond et al.			
	315.	2014/0296081	10/2/2014	Diehn et al.			
	316.	2014/0342921	11/20/2014	Weiner			
	317.	2014/0378345	12/25/2014	Hindson et al.			
	318.	2015/0005447	1/1/2015	Berti et al.			
	319.	2015/0051085	2/19/2015	Vogelstein et al.			
	320.	2015/0087027	3/26/2015	Makarov et al.			
	321.	2015/0344942	12/3/2015	Frisen et al.			
	322.	2015/0376609	12/31/2015	Hindson et al.			
	323.	2016/0003812	1/7/2016	Porreca et al.			
	324.	2016/0024576	1/28/2016	Chee			
	325.	2016/0138091	5/19/2016	Chee et al.			
	326.	2016/0145677	5/26/2016	Chee et al.			
	327.	2016/0298180	10/13/2016	Chee			
	328.	2016/0333403	11/17/2016	Chee			
	329.	2017/0058339	3/2/2017	Chee			
	330.	2017/0058340	3/2/2017	Chee			
	331.	2017/0058345	3/2/2017	Chee			
	332.	2017/0088881	3/30/2017	Chee			
	333.	2017/0166962	6/15/2017	van Eijk et al.			
	334.	2017/0242020	8/24/2017	Yamauchi et al.			
	335.	2017/0343545	11/30/2017	HADRUP et al.			
	336.	2018/0179591	6/28/2018	Belgrader et al.			
	337.	2018/0180601	6/28/2018	Pedersen et al.			
	338.	2018/0201980	7/19/2018	Chee et al.			

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	339.	2018/0217094	8/2/2018	Herr et al.			
	340.	2018/0247017	8/30/2018 ~~8/30/2017~~	van Eijk et al.			
	341.	2018/0291439	10/11/2018	van Eijk et al.			
	342.	2019/0017106	1/17/2019	Frisen et al.			
	343.	2019/0024153	1/24/2019	Frisen et al.			
	344.	2019/0024154	1/24/2019	Frisen et al.			
	345.	~~61/839,313~~	~~6/25/2013~~	~~Chee et al.~~			
	346.	~~61/839,320~~	~~6/25/2013~~	~~Chee et al.~~			
	347.	~~61/902,105~~	~~11/8/2013~~	~~Kozlov et al.~~			
	348.	RE44596	11/12/2013	Stroun et al.			

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	349.	DE 102008025656	12/3/2009	DE			Machine Translation	
	350.	EP 1712623	10/18/2006	EP				
	351.	EP 1910562	4/16/2008	EP			Corresponds to WO2006/137733	
	352.	EP 1929039	6/11/2008	EP			Corresponds to WO2007/037678	
	353.	EP 1966393	9/10/2008	EP			Corresponds to WO2007/073165	
	354.	EP 2002017	12/17/2008	EP			Corresponds to WO2007/114693	
	355.	EP 2292788	3/9/2011	EP				
	356.	EP 2302070	3/30/2011	EP				
	357.	EP 2363504	9/7/2011	EP				
	358.	EP 2580351	4/17/2013	EP			Corresponds to WO2007/155833	
	359.	EP 2789696	10/15/2014	EP				
	360.	EP 2963127	1/6/2016	EP				

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 465
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation	
							Yes	No
	361.	EP 3045544	7/20/2016	EP				
	362.	EP 3239304	11/1/2017	EP				
	363.	JP 2011-182702	9/22/2011	JP			English Abstract	
	364.	JP 2014-217381	11/20/2014	JP			English Abstract	
	365.	RU 2145635	2/20/2000	RU			English Translation	
	366.	RU 2270254	2/20/2006	RU			English Abstract	
	367.	WO 1989/010977	11/16/1989	WO				
	368.	WO 1991/006678	5/16/1991	WO				
	369.	WO 1995/025116	9/21/1995	WO				
	370.	WO 1995/035505	12/28/1995	WO				
	371.	WO 1998/044151	10/8/1998	WO				
	372.	WO 1999/032654	7/1/1999	WO				
	373.	WO 2000/018957	4/6/2000	WO				
	374.	WO 2000/024940	5/4/2000	WO				
	375.	WO 2000/063437	10/26/2000	WO				
	376.	WO 2001/007915	2/1/2001	WO				
	377.	WO 2001/042796	6/14/2001	WO				
	378.	WO 2002/024952	3/28/2002	WO				
	379.	WO 2002/059355	8/1/2002	WO				
	380.	WO 2002/077283	10/3/2002	WO				
	381.	WO 2002/088396	11/7/2002	WO				
	382.	WO 2003/010176	2/6/2003	WO				
	383.	WO 2004/018497	3/4/2004	WO				
	384.	WO 2004/028955	4/8/2004	WO				
	385.	WO 2005/010145	2/3/2005	WO				
	386.	WO 2005/026387	3/24/2005	WO				

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 466
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	387.	WO 2005/042759	5/12/2005	WO				
	388.	WO 2006/074351	7/13/2006	WO				
	389.	WO 2006/084130	8/10/2006	WO				
	390.	WO 2006/117541	11/9/2006	WO				
	391.	WO 2006/137733	12/28/2006	WO				
	392.	WO 2007/030373	3/15/2007	WO				
	393.	WO 2007/037678	4/5/2007	WO				
	394.	WO 2007/041689	4/12/2007	WO				
	395.	WO 2007/060599	5/31/2007	WO				
	396.	WO 2007/073165	6/28/2007	WO				
	397.	WO 2007/073171	6/28/2007	WO				
	398.	WO 2007/076726	7/12/2007	WO				
	399.	WO 2007/114693	10/11/2007	WO				
	400.	WO 2007/120241	10/25/2007	WO				
	401.	WO 2007/123744	11/1/2007	WO				
	402.	WO 2007/145612	12/21/2007	WO				
	403.	WO 2008/093098	8/7/2008	WO				
	404.	WO 2009/032167	3/12/2009	WO				
	405.	WO 2009/036525	3/26/2009	WO				
	406.	WO 2009/152928	12/23/2009	WO			English Abstract	
	407.	WO 2010/019826	2/18/2010	WO				
	408.	WO 2010/027870	3/11/2010	WO				
	409.	WO 2010/126614	11/4/2010	WO				
	410.	WO 2010/127186	11/4/2010	WO				
	411.	WO 2011/014879	2/3/2011	WO				
	412.	WO 2011/071943	6/16/2011	WO				

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 467
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	413.	WO 2011/127006	10/13/2011	WO				
	414.	WO 2011/127099	10/13/2011	WO				
	415.	WO 2011/155833	12/15/2011	WO				
	416.	WO 2012/022975	2/13/2012 ~~2/13/2012~~	WO				
	417.	WO 2012/058096	5/3/2012	WO				
	418.	WO 2012/071428	5/31/2012	WO				
	419.	WO 2012/139110	10/11/2012	WO				
	420.	WO 2012/140224	10/18/2012	WO				
	421.	WO 2012/142213	10/18/2012	WO				
	422.	WO 2012/148477	11/1/2012	WO				
	423.	WO 2013/123442	8/22/2013	WO				
	424.	WO 2013/138510	9/19/2013	WO				
	425.	WO 2013/142389	9/26/2013	WO				
	426.	WO 2013/150083	10/10/2013	WO				
	427.	WO 2014/060483	4/24/2014	WO				
	428.	WO 2014/142841	9/18/2014	WO				
	429.	WO 2014/163886	10/9/2014	WO				
	430.	WO 2014/210223	12/31/2014	WO				
	431.	WO 2014/210225	12/31/2014	WO				
	432.	WO 2016/162309	10/13/2016	WO				
	433.	WO 2016/007839	1/14/2016	WO				
	434.	WO 2018/045186	3/8/2018 ~~3/5/2018~~	WO				
	435.	WO 2018/045181	3/8/2018 ~~3/5/2018~~	WO				

Other Documents (include Author, Title, Date, and Place of Publication)

Examiner Initial	Desig. ID	Document

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 468
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	436.	Affymetrix, "GeneChip Human Genome U133 Set," retrieved from the Internet: on the World Wide Web at affymetrix.com/support/technical/datasheets/hgu133_datasheet.pdf, retrieved on February 26, 2003.
	437.	Affymetrix, "Human Genome U95Av2," INTERNET CITATION, retrieved from the internet: on the World Wide Web affymetrix.com, retrieved on October 2, 2002
	438.	Albretsen et al., "Applications of magnetic beads with covalently attached oligonucleotides in hybridization: Isolation and detection of specific measles virus mRNA from a crude cell lysate", Anal. Biochem. 189: 40-50, 1990
	439.	~~ALEXANDER VALENCIA et al., "mRNA-display-based selections for proteins with desired functions: A protease-substrate case study," Biotechnology Progress, 2008, 24(3): 561-569~~
	440.	Allawi and SantaLucia, "Thermodynamics and NMR of Internal GâT Mismatches in DNA," Biochemistry, 36:10581-94
	441.	ANDERSON et al., "Microarrayed Compound Screening to Identify Activators and Inhibitors of AMP-Activated Protein Kinase," J. of Biomolecular Screening, 2004, 9:112
	442.	Andersson et al., "Analysis of protein expression in cell microarrays: a tool for antibody-based proteomics.," J Histochem Cytochem., 4(12): 1413-1423, 2006
	443.	Andresen et al., "Deciphering the Antibodyome - Peptide Arrays for Serum Antibody Biomarker Diagnostics," Current Proteomics, 6(1), 1-12, 2009
	444.	ANGENENDT et al., "Cell-free expression and functional assay in a nanowell chip format," Analytical Chemistry, 2004, 76(7):1844-49
	445.	ANGENENDT et al.,"Generation of High Density Protein Microarrays by Cell-free in Situ Expression of Unpurified PCR Products," Molecular and Cellular Proteomics, (2006) Ch. 5.9, pp. 1658-66
	446.	Armani et al, "2D-PCR: a method of mapping DNA in tissue sections," Lab on a Chip, 2009, 9(24):3526-34
	447.	Asp et al., "Spatial detection of fetal marker genes expressed at low level in adult human heart tissue", Scientific Reports, 7: 12941, 10 pages, 2017
	448.	Atkinson and Wells, "An Updated Protocol for High Throughput Plant Tissue Sectioning.", Front Plant Sci, 8: 1721, 2017
	449.	ATKINSON, Overview of Translation: Lecture Manuscript, U of Texas (2000) DD. 6.1-6.8
	450.	Bains et al, "A Novel Method for Nucleic Acid Sequence Determination ," Journal of Theoretical Biology, 1988, 135(3), 303-7
	451.	BAIRD et al., "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD markers," PLOS One, 2008, 3(1 0):e3376
	452.	Barnes, "PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates.," Proc. Natl. Acad. Sci USA, 91: 2216-2220, 1994
	453.	Baugh et al, "Quantitative analysis of mRNA amplification by in vitro transcription," Nucleic Acids Res., 2001, 29:5:e29
	454.	BELL, "A Simple Way to Treat PCR Products Prior to Sequencing Using ExoSAP-IT," Biotechniques, 2008, Vol. 44, No. 6

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 469
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	455.	Bentley et al, "Accurate whole human genome sequencing using reversible terminator chemistry", Nature, 2008, 456:53-59
	456.	BIELAS et al., "Human cancers express a mutator phenotype," Proc. Natl. Acad. Sci. USA, 2006, 103(48): 18238-18242
	457.	BIELAS et al., "Quantification of random genomic mutations," Nat. Methods, 2005, 2(4):285-290
	458.	Birney, et al, "Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project," Nature, 2007, 447:799-816
	459.	BLOKZIJL et al., "Profilinq protein expression and interactions: proximity liqation as a tool for personalized medicine," J Intern. Med., 2010, 268:232-245
	460.	Blow, "Tissue Issues," Nature, 448(23), 959-962, 2007
	461.	BONFIELD et al., "The application of numerical estimates of base calling accuracy to DNA sequencing projects," Nucleic Acids Research, 1995, 23(8):1406-1410
	462.	Boulgakov et al., "From Space to Sequence and Back Again: Iterative DNA Proximity Ligation and its Applications to DNA-Based Imaging", bioRxiv, 24 pages, 2018
	463.	BOWTELL, "The genesis and evolution of high-grade serous ovarian cancer," Nat. Rev. Cancer, 2010, (11):803-808 ABSTRACT
	464.	BRANDON et al., "Mitochondrial mutations in cancer," Oncogene, 2006, 25(34):4647-4662
	465.	BRENNER et al, "In vitro cloning of complex mixtures of DNA on microbeads: physical separation of differentially expressed cDNAs," Proc. Natl. Acad. Sci. USA, 2000, 97, 1665-1670
	466.	Brenner et al., "Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays", Nat. Biotech. 18: 630-634, 2000
	467.	BROCKMAN et al., "Quality scores and SNP detection in sequencing-by-synthesis systems," Methods, 2008, 18:763-770
	468.	Burgess, "A space for transcriptomics", Nature Reviews Genetics | Published online 15 Jul 2016; doi:10.1038/nrg.2016.94
	469.	Burgess, "Finding structure in gene expression", Nature Reviews Genetics | Published online 13 Apr 2018; doi:10.1038/nrg.2018.19
	470.	BURNS et al., "Well-less, gel-permeation formats for ultra-HTS," DDT, 2001, 6(12):S40-S47
	471.	Burton et al., "Coverslip Mounted-Immersion Cycled in Situ RT-PCR for the Localization of mRNA in Tissue Sections," Biotechniques, 1998, 24; pages 92-100
	472.	CARLSON et al., "Formylglycine-generating Enzyme," J. of Biological Chemistry, 2008, 283(29):20117-125
	473.	Carter et al., "Stabilization of an optical microscope to 0.1 nm in three dimensions," Applied Optics, 2007, 46:421-427
	474.	CERUTTI et al., "Generation of sequence-specific, high affinity anti-DNA antibodies," Journal of Biological Chemistry, 2001, 276(16):12769-12773
	475.	CHA et al., "Specificity, Efficiency and Fidelity of PCR," Genome Res., 1993, 3:518-29
	476.	CHANDRA et al., "Cell-free synthesis-based protein microarrays and their applications," Proteomics, 2009, 5(6):717-30.

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 470

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	477.	CHATTERJEE et al., "Protein Microarray On-Demand: A Novel Protein Microarray System," PLos One, 2008, 3(9):e3265
	478.	CHATTERJEE, et al., "Mitochondrial DNA mutations in human cancer. Oncogene," 2006, 25(34):4663-4674
	479.	CHEN et al., "DNA hybridization detection in a microfluidic Channel using two fluorescently labelled nucleic acid probes," Biosensors and Bioelectronics, 2008, 23:1878-1882
	480.	Chen et al., "Expansion Microscopy," 2015, Science. 347(6221):543-548
	481.	Chen et al., "Spatially resolved, highly multiplexed RNA profiling in single cells", Science 348(6233), 36 pages, 2015
	482.	CHENG et al., "Sensitive Detection of Small Molecules by Competitive Immunomagnetic-Proximity Ligation Assay," Anal Chem, 2012, 84:2129-2132
	483.	CONDINA et al., "A sensitive magnetic bead method for the detection and identification of tyrosine phosphorylation in proteins by MALDI-TOF/TOF MS," Proteomics, 2009, 9:3047-3057
	484.	Constantine et al.,"Use of genechip high-density oligonucleotide arrays for gene expression monitoring," LIFE SCEIENCE NEWS, AMERSHAM LIFE SCIENCE; 11-14, 1998
	485.	COPELAND et al., "Mitochondrial DNA Alterations in Cancer," Cancer Invest., 2002, 557-569
	486.	Corces et al., "An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues", Nat. Methods. vol. 14(10): pp.959–962, 2017
	487.	Crosetto et al, "Spatially resolved transcriptomics and beyond," Nature Review Genetics, 2015, 57-66
	488.	CUJEC et al. "Selection of v-abl tyrosine kinase substate sequences from randomnized peptide and cellular proteomic libraries using mRNA display," Chemistry and Biology, 2002, 9:253-264
	489.	Dahl et al., "Circle-to-circle amplification for precise and sensitive DNA analysis," Proc. Natl. Acad. Sci., 2004, 101:4548-4553
	490.	DARMANIS, et al.,"ProteinSeq: High-Performance Proteomic Analyses by Proximity, Ligation and Next Generation Sequencing," PLos One, 2011, 6(9):e25583
	491.	Daubendiek et al., "Rolling-Circle RNA Synthesis: Circular Oligonucleotides as Efficient Substrates for T7 RNA Polymerase," J. Am. Chem. Soc., 1995, 117:77818-7819
	492.	Davies et al., "How best to identify chromosomal interactions: a comparison of approaches.", Nat. Methods 14, 125–134, 2017
	493.	Dean et al., "Comprehensive human genome amplification using multiple displacement amplification," Proc Natl. Acad. Sci. USA 99:5261-66 (2002
	494.	Dhindsa et al., "Virtual Electrowetting Channels: Electronic Liquid Transport with Continuous Channel Functionality," Lab Chip, 2010, 10:832-836
	495.	Dressman et al., "Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations," Proc. Natl. Acad. Sci. USA, 2003, 100:8817-8822
	496.	Drmanac et al., "Accurate sequencing by hybridization for DNA diagnostics and individual genomics," Nature Biotechnology, 16:54-58, 1998
	497.	Druley et al., "Quantification of rare allelic variants from pooled genomic DNA," Nat. Methods 6: 263-65, 2009

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 471
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	498.	Duncan et al., "Affinity chromatography of a sequence-specific DNA binding protein using teflon-linked oligonucleotides", Anal. Biochem. 169: 104-108, 1988
	499.	Eagen, "Principles of Chromosome Architecture Revealed by Hi-C", Trends in Biochemical Sciences, Vol. 43, No. 6, 10 pages, 2018
	500.	Eberwine et al., "Analysis of gene expression in single live neurons," Proc. Natl. Acad. Sci., USA 89, 3010-3014, 1992
	501.	Eberwine, "Amplification of mRNA Populations Using aRNA Generated from Immobi- lized Oligo(dT)-T7 Primed cDNA," BioTechniques 20 (4), 584, 1996
	502.	Eguiluz et al., "Multitissue array review: A chronological description of tissue array techniques, applications and procedures," Pathology Research and Practice, : 8: 561-568, 2002
	503.	Eldridge et al. "An in vitro selection strategy for conferring protease resistance to ligand binding peptides," Protein Eng Des Sel., 22(11): 691-698, 2009
	504.	Ellington et al., "Antibody-based protein multiplex platforms: technical and operational challenges," Clin Chem 56(2): 186-193, 2010
	505.	Eng et al., "Profiling the transcriptome by RNA SPOTs", Nat Methods., 14(12): 1153–1155, 2017
	506.	Examination Search Report for Canadian Appln. No. 2,794,522, dated 4/28/2015, 3 pages
	507.	Examination Search Report issued in Australian Appln. No. 2010278710, dated 2/11/2014, 4 pages
	508.	Examination Search Report issued in Australian Appln. No. 2010328226, dated 5/9/2013, 4 pages
	509.	Examination Search Report issued in Australian Appln. No. 2011237729, dated 7/9/2013, 3 pages
	510.	Examination Search Report issued in Australian Appln. No. 2014203638, dated 10/1/2015, 2 pages
	511.	Examination Search Report issued in Australian Appln. No. 2014203638, dated 10/1/2015, 2 pages
	512.	Extended European Search Report issued in European Appln. No. 10836568.5, dated 2/13/2013, 6 pages
	513.	Extended European Search Report issued in European Appln. No. 11766613.1, dated 1/15/2014, 4 pages
	514.	Extended European Search Report issued in European Appln. No. 12767937, dated 11/18/2014, 5 pages
	515.	Extended European Search Report issued in European Appln. No. 14765026.1, dated 9/26/2016, 9 pages
	516.	Extended European Search Report issued in European Appln. No. 14816674.7, dated 2/3/2017, 8 pages
	517.	Extended European Search Report issued in European Appln. No. 16183356.1, dated 4/24/2017, 12 pages
	518.	Fan et al., " Highly parallel SNP genotyping," Cold Spring Symp. Quant. Biol., 68: 69-78, 2003
	519.	Fire and Xu, "Rolling replication of short DNA circles," Proc. Natl. Acad. Sci., 92: 4641-4645, 1995

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	520.	Flanigon et al., "Multiplex protein detection with DNA readout via mass spectrometry," N. Biotechnol., 30(2): 153-158, 2013
	521.	Fodor et al., "Light-Directed, Spatially Addressable Parallel Chemical Synthesis," Science, 251(4995), 767-773, 1995
	522.	Forcucci et al., "All-plastic miniature fluorescence microscope for point-of-care readout of bead-based bioassays.," J. Biomed Opt. 20 (10): 105010, 15 pages, 2015
	523.	Fredriksson et al., "Multiplexed protein detection by proximity ligation for cancer detection," Nature Methods, 4(4): 327-29, 2007
	524.	Fredriksson et al., "Multiplexed proximity ligation assays to profile putative plasma biomarkers relevant to pancreatic and ovarian cancer," Clin. Chem., 5(3): 582-89, 2008
	525.	Fredriksson et al., "Protein detection using proximity-dependent DNA ligation assays," Nature Biotech., 20: 473-77, 2002
	526.	Frese et al., "Formylglycine Aldehyde Tag-Protein Engineering through a Novel Posttranslational Modification," ChemBioChem., 10: 425-27, 2009
	527.	Fu et al., "Counting individual DNA molecules by the stochastic attachment of diverse labels," PNAS, 108: 9026-9031, 2011
	528.	Fullwood et al., "Next-generation DNA sequencing of paired-end tags (PET) for transcriptome and genome analyses," Genome Res., 19: 521-532, 2009
	529.	Gao et al., "High density peptide microarrays. In situ synthesis and applications," Molecular Diversity, 8, 177-187, 2004
	530.	Gao et al., "Q&A: Expansion microscopy", BMC Biology, 15: 50, 9 pages, 2017
	531.	Gerdtsson et al., "Evaluation of Solid Supports for Slide- and Well-Based Recombinant Antibody Microarrays", Microarrays (2016) 5:16, 2016
	532.	Giacomello et al., "Spatially resolved transcriptome profiling in model plant species", NATURE PLANTS 3, 17061, 11 pages, 2017
	533.	Gnanapragasam, "Unlocking the molecular archive: the emerging use of formalin-fixed paraffin-embedded tissue for biomarker research in urological cancer," BJU International,105, 274-278, 2009
	534.	Goldkom and Prockop, "A simple and efficient enzymatic method for covalent attachment of DNA to cellulose. Application for hybridization-restriction analysis and for in vitro synthesis of DNA probes.", Nucleic Acids Res. 14: 9171-9191, 1986
	535.	~~Gow et al., "Amplifications of mRNA populations using aRNA generated from immobilized oligo(dT)-T7 primed cDNA," BioTech., vol. 20, no. 4, 584-591, 1996~~
	536.	Gu et al., "Protein tag-mediated conjugation of oligonucleotides to recombinant affinity binders for proximity ligation," N Biotechnol., 30(2): 144-152, 2013
	537.	Gunderson et al., "Decoding Randomly Ordered DNA Arrays," Genome Research 14: 870-877, 2004
	538.	Hammond et al., "Profiling cellular protein complexes by proximity ligation with dual tag microarray readout," 7(7): e40405, 2012
	539.	Han et al., "3C and 3C-based techniques: the powerful tools for spatial genome organization deciphering. Mol. Cytogenet. 11, 21, 10 pages, 2018

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	540.	He et al., "In situ synthesis of protein arrays," Current Opinion in Biotechnology, 19: 4-9, 2008
	541.	He et al., "Printing protein arrays from DNA arrays," Nature Methods, 5: 175-77, 2008
	542.	He, "Cell-free protein synthesis: applications in proteomics and biotechnology," New Biotechnology 25: 126-132, 2008
	543.	Hedskog et al., "Dynamics of HIV-1 Quasispecies during Antiviral Treatment Dissected usinq Ultra-Deep Pyrosequencinq," PLoS One, 5(7): e11345, 2010
	544.	Hein et al., "Click Chemistry, A Powerful Tool for Pharmaceutical Sciences", Pharm Res., 25(10): 2216–2230, 2008
	545.	Hiatt et al., "Parallel, tag-directed assembly of locally-derived short sequence reads," Nature Methods, 7(2): 119-25, 2010
	546.	Inoue and Wittbrodt, "One for All—A Highly Efficient and Versatile Method for Fluorescent Immunostaining in Fish Embryos," PLoS One 6, e19713, 2011
	547.	Jabara et al., "Accurate sampling and deep sequencing of the HIV-1 protease gene using a Primer ID. PNAS 108(50); 20166-20171, 2011
	548.	Jamur and Oliver, "Permeabilization of cell membranes.," Method Mal. Biol., 588: 63-66, 2010
	549.	Jemt et al., "An automated approach to prepare tissue-derived spatially barcoded RNA-sequencing libraries", Scientific Reports, 6: 37137, 10 pages, 2016
	550.	Jones et al., Comparative lesion sequencing provides insights into tumor evolution. Proc. Natl. Acad. Sci. USA 105(11): 4283-4288, 2008
	551.	Kanehisa, "Use of sttistical cteria for sreening potential homologies in nudeic add sequences," Nucleic Acids Res. 12: 203-213, 1984
	552.	Kap et al., "Histological Assessment of PAXgene Tissue Fixation and Stabilization Reagents," PLoS One 6, e27704, 10 pages, 2011
	553.	Kolb et al., "Click Chemistry: Diverse Chemical Function from a Few Good Reactions.", Angew. Chem. Int. Ed., 40(11): 2004-2021, 2001
	554.	Kolovos et al., "Investigation of the spatial structure and interactions of the genome at sub-kilobasepair resolution using T2C.", Nat. Protoc. 13, 459–477, 2018
	555.	Korbel et al., "Paired-End Mapping Reveals Extensive Structural Variation in the Human Genome," Science, 318(5849): 420-426, 2007
	556.	Korlach et al., "Selective aluminum passivation for targeted immobilization of single DNA polymerase molecules in zero-mode waveguide nanostructures," Proc. Natl. Acad. Sci. USA 105, 1176-1181, 2008
	557.	Kozlov et al., "A High-Complexity Multiplexed Solution-Phase Assay for Profiling Protease Activity on Microarrays," Comb. Chem. And Hiah Throuahout, 11: 24-35, 2008
	558.	Kozlov et al., "A Highly Scalable Peptide-Based Assay System for Proteomics," PLoS ONE, 7(6): e37441, 2012
	559.	Kozlov et al., "A Method for Rapid Protease Substrate Evaluation and Optimization," Comb. Chem. and Hiah Throuahout, 9: 481-87, 2006
	560.	Kurz et al., "eDNA-Protein Fusions: Covalent Protein-Gene Conjugates for the In Vitro Selection of Peptides and Proteins," ChemBioChem., 2: 666-72, 2001

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 474
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	561.	Lage et al., "Whole Genome Analysis of Genetic Alterations in Small DNA Samples Using Hyperbranched Strand Displacement Amplification and Array–CGH," Genome Research 13: 294-307, 2003
	562.	Langdale et al., "A rapid method of gene detection using DNA bound to Sephacryl", Gene 36: 201-210, 1985
	563.	Larman et al., "Autoantigen discovery with a synthetic human peptidome," Nature Biotechnology, doi:1 0.1038/nbt.1856, 2011 vol. 29, No. 6, pp. 535-541
	564.	Lassmann et al., A Novel Approach For Reliable Microarray Analysis of Microdissected Tumor Cells From Formalin-Fixed and Paraffin-Embedded Colorectal Cancer Resection Specimens, J Mol Med, 87, 211-224, 2009
	565.	Lee et al., "Fluorescent in situ sequencing (FISSEQ) of RNA for gene expression profiling in intact cells and tissues", Nature Protec, 10(3): 442-458, 2015
	566.	Lee et al., "Highly multiplexed subcellular RNA sequencing in situ", Science, 343(6177): 1360–1363, 2014
	567.	Lein et al., "The promise of spatial transcriptomics for neuroscience in the era of molecular cell typing", Science 358, 64–69, 2017
	568.	Leriche et al., "Cleavable linkers in chemical biology.", Bioorganic & Medicinal Chemistry, 20: 571–582, 2012
	569.	Levene et al., "Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations," Science 299, 682-686, 2003
	570.	Li et al., "A photocleavable fluorescent nucleotide for DNA sequencing and analysis," Proc. Natl. Acad. Sci., 100: 414-419, 2003
	571.	Linnarsson, "Recent advances in DNA sequencing methods - general principles of sample preparation," Experimental Cell Research, 316: 1339-1343, 2010
	572.	Lizardi et al., "Mutation detection and single-molecule counting using isothermal rolling-circle amplification," Nat. Genet. 19: 225-232, 1998
	573.	Lovatt et al., "Transcriptome in vivo analysis (TIVA) of spatially defined single cells in live tissue.", Nature Methods 11, 190–196, 2014
	574.	Lund et al., "Assessment of methods for covalent binding of nucleic acids to magnetic beads, Dynabeads, and the characteristics of the bound nucleic acids in hybridization reactions.", Nucleic Acids Res., 16: 10861-80, 1988
	575.	Lundberg et al., "High-fidelity amplification using a thermostable DNA polymerase isolated from Pyrococcus furiosus.," Gene., 108(1): 1-6, 1991
	576.	Lundberg et al., "Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood," Nucleic Acids Res., 39(15): e102, 2011
	577.	Lundberg et al., "Multiplexed homogeneous proximity ligation assays for high-throughput protein biomarker research in serological material," Mol Cell Proteomics, 10(4): M110.004978, 2011
	578.	Lundquist et al., "Parallel confocal detection of single molecules in real time," Opt. Lett. 33, 1026-1028, 2008
	579.	Lyck, et al., "Immunohistochemical Markers for Quantitative Studies of Neurons and Glia in Human Neocortex," J Histochem Cytochem 56, 201-21, 2008

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 475
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	580.	MacIntyre, "Unmasking antigens for immunohistochemistry.," Br J Biomed Sci. 58, 190-6, 2001
	581.	Macosko et al., "Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets," Cell 161, 1202-1214, 2015
	582.	Maniatis et al., "Spatiotemporal Dynamics of Molecular Pathology in Amyotrophic Lateral Sclerosis", 54 pages, 2018
	583.	McCloskey et al., "Encoding PCR Products with Batch-stamps and Barcodes.," Biochem. Genet. 45: 761-767, 2007
	584.	Mcgee, "Structure and Analysis of Affymetrix Arrays," UTSW Microarray Analysis Course, October 28, 2005
	585.	McKernan et al., "Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding," Genome Res., 19: 1527-41, 2009
	586.	Metzker "Sequencing technologies - the next generation," Nature Reviews Genetics, 11: 31-46, 2010
	587.	Miller et al., "Basic Concepts of Microarrays and Potential Applications in Clinical Microbiology," Clinical Microbiology Reviews, Vol. 22, No. 4, pp. 611-633, 2009
	588.	Mir et al., "Sequencing by cyclic ligation and cleavage (CycliC) directly on a microarray captured template," Nucleic Acids Research, 37(1): e5-1, 2009
	589.	Mishra and Hawkins, "Three-dimensional genome architecture and emerging technologies: looping in disease. Genome Med. 9, 87, 14 pages, 2017
	590.	Mishra, "Three-dimensional genome architecture and emerging technologies: looping in disease", Genome Medicine, 9: 87, 14 pages, 2017
	591.	Mitsuhashi et al., "Gene manipulation on plastic plates," Nature 357: 519-520, 1992
	592.	Mizusawa et al., "A bacteriophage lambda vector for cloning with BamHI and Sau3A," Gene, 20: 317-322, 1982
	593.	Moncada et al., "Building a tumor atlas: integrating single-cell RNA-Seq data with spatial transcriptomics in pancreatic ductal adenocarcinoma", Institute for Computational Medicine, bioRxiv, 28 pages, 2018
	594.	Moor et al., "Spatial transcriptomics: paving the way for tissue-level systems biology", Science Direct, Current Opinion in Biotechnology, 46: 126–133, 2017
	595.	Mortazavi et al., "Mapping and quantifying mammalian transcriptomes by RNA-Seq," Nature Methods, 5(7): 621-8, 2008
	596.	Nawy, "Spatial transcriptomics", Nature Methods, Vol. 15, No. 1, 2018
	597.	Ng et al., "Gene identification signature (GIS) analysis for transcriptome characterization and genome annotation," Nature Methods, 2(2): 105-111, 2005
	598.	Ng et al., "Massively parallel sequencing and rare disease," Human Malec. Genetics, 19(2): R119-R124, 2010
	599.	NG et al., "Multiplex sequencing of paired-end ditags (MS-PET): a strategy for the ultra-high-throughput analysis of transcriptomes and genomes," Nucleic Acids Research, July 2006, 34(12): e84, 10 pages
	600.	Niemeyer, "The developments of semisynthetic DNA-protein conjugates," TrendsBiotechnol, September 2002, 20(9): 395-401

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 476
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	601.	Nuovo, "In situ PCR: protocols and applications.," Genome Res, February 1995, 4 (4):151-167
	602.	Oleinikov et al., "Self-assembling protein arrays using electronic semiconductor microchips and in vitro translation," J Proteome Res, May-June 2003, 2(3): 313-319
	603.	Olink Proteomics AB, Proseek® Multiplex 96x96 User Manual, 2016, 12 pages
	604.	Osada et al., "Epitope mapping using ribosome display in a resconstituted cell-free protein synthesis system," J Biochem, May 2009, 145(5): 693-700
	605.	O-Shannessy et al., "Detection and quantitation of hexa-histidine-tagged recombinant proteins on western blots and by a surface plasmon resonance biosensor technique," Anal Biochem, 229(1): 119-124, 1995
	606.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2010/033064, dated 11/1/2011, 6 pages
	607.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2010/044134, dated 1/31/2012, 20 pages
	608.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2010/059327, dated 6/12/2012, 12 pages
	609.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2011/031163, dated 10/9/2012, 7 pages
	610.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2011/031308, dated 10/9/2012, 7 pages
	611.	PCT International Preliminary Report on Patentability issued in International Appln. No. PCT/US2012/032759, dated 10/8/2013, 17 pages
	612.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US14/29691, dated 8/19/2014, 10 pages
	613.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US14/44196, dated 11/7/2014, 15 pages
	614.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US14/64588, dated 3/11/2015, 20 pages
	615.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2010/033064, dated 7/30/2010, 8 pages
	616.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2010/044134, dated 3/18/2011, 16 pages
	617.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2010/059327, dated 3/29/2011, 12 pages
	618.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2011/031163, dated 5/23/2011, 8 pages
	619.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2011/031308, dated 6/7/2011, 8 pages
	620.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2012/032759, dated 9/28/2012, 14 pages
	621.	PCT International Search Report and Written Opinion issued in International Appln. No. PCT/US2014/044191, dated 11/7/2014, 10 pages

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	622.	Perler et al., "Intervening sequences in an Archaea DNA polymerase gene," PNAS USA, June 1992, 89(12): 5577-5581
	623.	Petterson et al., "Generations of sequencing technologies," Genomics, 2009, 105-111
	624.	Polsky-Cynkin et al., "Use of DNA Immobilizedon Plastic and Agarose Supports to DetectDNA by SandwichHybridization", Clin. Chem. 31: 1438-1443, 1985
	625.	Rajeswari et al., "Multiple pathogen biomarker detection using an encoded bead array in droplet PCR," J. Microbial Methods, August 2017, 139:22-28
	626.	Ramachandran et al., "Next-generation high-density self-assembling functional protein arrays," Nature Methods, June 2008, 5(6):535-538
	627.	Ranki et al., "Sandwich hybridization as a convenient method for the detection of nucleic acids in crude samples", Gene 21: 77-85, cellulose, 1983
	628.	ROBERTS et al., "RNA-peptide fusions for the in vitro selection of peptides and proteins," PNAS USA, November 1997, 94: 12297-122302
	629.	Ronaghi et al., "A sequencing method based on real-time pyrophosphate," Science, July 1998, 281(5375): 363-365
	630.	Ronaghi et al., "Real-time DNA sequencing using detection of pyrophosphate release," Analytical Biochemistry, November 1996, 242(1): 84-89
	631.	Ronaghi, "Pyrosequencing sheds light on DNA sequencing," Genome Res, January 2001, 11(1):3-11
	632.	ROUILLARD et al., "OligoArray 2.0: design of oligonucleotide probes for DNA microarrays usina a thermodynamic approach," Nuc. Acid Research, June 2003, 31(12): 3057-3062
	633.	ROUNTENBERG et al., "Microfluidic probe: a new tool for integrating microfluidic environments and electronic wafer-orobina," Lab Chip, October 2009, 10: 123-127
	634.	Rubin et al., "Whole-genome resequencing reveals loci under selection during chicken domestication.," Nature, March 2010, 464: 587-591
	635.	RUSH et al., "New Aldehyde Tag Sequences Identified by Screening Formylglycine Generating Enzymes in Vitro and in Vivo," J. of American Chemical Society, August 2008, 130(37): 12240-12241
	636.	Rusk, "Spatial transcriptomics", Nature Methods, vol. 13, no. 9, 2016
	637.	Salem et al., "Multidimensional transcriptomics provides detailed information about immune cell distribution and identity in HER2+ breast tumors", bioRxiv, 41 pages, 2018
	638.	Schaus et al., "A DNA nanoscope via auto-cycling proximity recording.", Nat. Commun. 8, 696, 10 pages, 2017
	639.	Schaus et al., "A DNA nanoscope via auto-cycling proximity recording", NATURE COMMUNICATIONS, 8: 696, 9 pages, 2017
	640.	Schena et al., "Quantitative monitoring of gene expression patterns with a complementary DNA microarray," Science, October 1995, 270(5235): 467-470
	641.	Schena et al., "Entering the Postgenome Era," Science, 1995, 270:368-9, 371
	642.	Schlapak et al., "Glass surfaces grafted with high-density poly (ethylene glycol) as substrates for DNA oligonucleotide microarrays," Langinuir, January 2006, 22: 277-285

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 478
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	643.	Schmitt et al., "Detection of ultra-rare mutations by next-generation sequencing," PNAS (2012) 109:14508-14523
	644.	~~Science Tools: results, reports and refinement for life science researchers, Amersham Pharmacia Biotech, 1998, Vol. 3(4): 16 pages~~
	645.	SERGEEVA et al., "Display technologies: Application for the discovery of drug and gene delivery aaents," Advanced Drua Delivery Reviews (2006) 58(15):1622-1654
	646.	Seurynck-Servoss et al., "Evaluation of Surface Chemistries for Antibody Microarrays", Anal Biochem., 371(1): 105–115, 2007
	647.	Shelbourne et al., "Fast copper-free click DNA ligation by the ring-strain promoted alkyne-azide cycloaddition reaction.", Chem. Commun., 47: 6257-6259, 2011
	648.	Shendure et al., "Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome," Science, 2005, 309:1728-1732
	649.	Shi et al., "The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements," Nature Biotechnology, 2006, 24(9):1151-61
	650.	Shirai et al., "Novel Tools for Analyzing Gene Expressions in Single Cells," The 5th International Workshop on Approaches to Single-Cell Analysis, The University of Tokyo, March 3-4th 2011, 1 page
	651.	SHOEMAKER et al., "Quantitative phenotypic analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy," Nature genetics (1996) 14:450-456
	652.	SHULTS et al., "A multiplexed protein kinase assay," Chem Bio Chem (2007) 8:933-942
	653.	Sievertzon et al., "Transcriptome analysis in primary neural stem cells using a tag cDNA amplification method," BMC Neuroscience, December 2005, 6: 28
	654.	Slonim and Yanai, "Getting started in gene expression microarray analysis," Plos Computational Biology, 2009, 5(10):e1000543
	655.	Soni and Meller, "Progress toward ultrafast DNA sequencing using solid-state nanopores," Clin Chem., 2007, 53: 1996-2001
	656.	Spitale et al., "Structural imprints in vivo decode RNA regulatory mechanisms", Nature, 519(7544): 486-90, 2015
	657.	Spurgeon et al., "High Throughput Gene Expression Measurement with Real Time PCR in a Microfluidic Dynamic Array," PlosONE, 2008, 3(2):e1662
	658.	Stahl et al., "Visualization and analysis of gene expression in tissue sections by spatial transcriptomics.", Science 353, 78-82, 2016
	659.	Strell et al., "Placing RNA in context and space – methods for spatially resolved transcriptomics", The FEBS Journal, 14 pages, 2017
	660.	Summersgill et al., "Fluorescence In Situ Hybridization Analysis of Formalin Fixed Paraffin Embedded Tissues, Including Tissue Microarrays," Chapter 4, Bridger, J. Ed., Methods in Molecular Biology 659, 2010, 51-70, 2010
	661.	Sun et al., "Direct immobilization of DNA probes on non-modified plastics by UV irradiation and integration in microfluidic devices for rapid bioassay," Anal. Bio. Chem., 402: 741-748, 2012
	662.	TAKAHASHI et al., "In Vitro Selection of Protein and Peptide Libraries Using mRNA Display," Nucleic Acid and Peptide Aptamers: Methods and Protocols (2009) 535:293-314 (Ch.17).

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Curio EX1010-p. 479
ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/ Curio v Prognosys IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	663.	Tang et al., "RNA-Seq analysis to capture the transcriptome landscape of a single cell.," Nat Protoc., 5: 516-35, 2010
	664.	Taniguchi et al., ""Quantitative analysis of gene expression in a single cell by qPCR,"" Nature Methods, 6, pp 503-506, 2009"
	665.	Taylor et al., "Mitochondrial DNA mutations in human disease." Nature Reviews Genetics. May 2005, 6(5):389-402
	666.	Tentori et al., "Detection of Isoforms Differing by a Single Charge Unit in Individual Cells.", Chem. Int. Ed., 55, 12431, 2016
	667.	Thornton, "High rate thick film growth." Annual review of materials science, August 1977, 7(1):239-60
	668.	Tian et al., "Antigen peptide-based immunosensors for rapid detection of antibodies and antigens," Anal Chem May 26, 2009, 81 (13):5218-5225
	669.	Tijssen, "Overview of principles of hybridization and the strategy of nucleic acid assays" in Techniques in 5 Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes 1993
	670.	Tolbert et al., "New Methods for Proteomic Research: Preparation of Proteins with N-Terminal Cysteines for Labeling and Conjugation" Angewandte Chemie International Edition, June 17, 2002, 41(12):2171-4
	671.	Valencia et al., "mRNA-Display-Based Selections for Proteins with Desired Functions: A Protease-Substrate Case Study." Biotechnology progress, May 2008, 24(3):561-9
	672.	Van Gelder et al., "Amplified RNA synthesized from limited quantities of heterogeneous cDNA," Proc. Natl. Acad. Sci. USA 87, 1663-1667, 1990
	673.	Van Ness et al., "A versatile solid support system for oligodeoxynucleotide probe-based hybridization assays", Nucleic Acids Res. 19: 3345-3350, 1991
	674.	Velculescu et al., "Serial analysis of gene expression." Science, October 20, 1995, 270(5235):484-7
	675.	Vickovic et al., "Massive and parallel expression profiling using microarrayed single-cell sequencing," Nature Communications, 2016 Oct 14; 7:13182. doi: 10.1038/ncomms13182
	676.	Vogelstein et al., "Digital PCR," Proceedings of the National Academy of Sciences, August 3, 1999, 96:9236-9241
	677.	Wade et al., "Genome sequence, comparative analysis, and population genetics of the domestic horse.," Science., 326: 865-7, 2009
	678.	Waichman et al., "Functional immobilization and patterning of proteins by an enzymatic transfer reaction." Analytical chemistry, January 21, 2010, 82(4):1478-85
	679.	Walker et al., "Strand displacement amplification—an isothermal, in vitro DNA amplification technique." Nucleic acids research. 1992 April 11, 1992, 20(7):1691-1696
	680.	Wang et al., "Single cell analysis: the new frontier in 'omics'.,"Trends Biotechnol., 28: 281-90, 2010
	681.	Wang et al., "Three-dimensional intact-tissue sequencing of single-cell transcriptional states", Science, 361(6400), 22 pages, 2018
	682.	Wang et al., "High-fidelity mRNA amplification for gene profiling." Nature biotechnology. April 2000, 18(4):457-459

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.M/

Curio EX1010-p. 480
Curio v Prognosys
IPR2024-01289

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 47706-0004003	Application No. 16/276,235
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Prognosys Biosciences, Inc.	
(37 CFR §1.98(b))		Filing Date February 14, 2019	Group Art Unit

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
	683.	WEICHHART et al., "Functional selection of vaccine candidate peptides from Staphylococcus aureus whole-genome expression libraries in vitro," Infection and Immunity, 2003, 71 (8):4333-4641
	684.	Weinstein et al., "DNA microscopy: Optics-free spatio-genetic imaging by a stand-alone chemical reaction", bioRxiv, 41 pages, 2018
	685.	Willi-Monnerat et al., "Comprehensive spatiotemporal transcriptomic analyses of the ganglionic eminences demonstrate the uniqueness of its caudal subdivision," MOLECULAR AND CELLULAR NUEORSCIENCES 37: 845-856, 2008
	686.	Wolf et al., "Rapid hybridization kinetics of DNA attached to submicron latex particles", Nucleic Acids Res. 15: 2911-2926, 1987
	687.	WONG et al., "Direct Site-Selective Covalent Protein Immobilization Catalyzed by a Phosphopantetheinyl Transferase," J. Am. Chem Soc., 2008, 130:12456-64
	688.	Woo et al., "A Comparison of cDNA, Oligonucleotide, and Affymetrix GeneChip Gene Expression Microarray Platforms," Journal of Biomolecular Techniques, 2004, 15(4), 276-284
	689.	WORTHINGTON et al., "Cloning of random oligonucleotides to create single-insert plasmid libraries," Analyt. Biochem, 2001, 294:169-175
	690.	XIAO et al., "Direct determination of haplotypes from single DNA molecules," Nature Methods, 2009, 6(3):199-01
	691.	YIN et al., "Genetically encoded short peptide tag for versatile protein labeling by Sfp phosphopantetheinyl transferase," PNAS, 2005, 102(44):15815-20
	692.	YONEZAWA et al., "DNA display for in vitro selection of diverse peptide libraries," Nucleic Acids Research , 2003, 31 (19):e118
	693.	ZHANG et al., "Assembling DNA through Affinity Binding to Achieve Ultrasensitive Protein Detection," Anaew Chem Int Ed (2013) 52:2-10
	694.	ZHANG et al., "Binding-induced DNA assembly and its application to yoctomole detection of proteins," Anal Chem (2012) 84(2):877-884
	695.	ZHENG et al., Origins of human mitochondrial point mutations as DNA polymerase mediated errors. Mutat. Res. 599(1-2): 11-20 (2006
	696.	ZHOU et al., "Genetically Encoded Short Peptide Tags for Orthogonal Protein Labeling by Sfp and Ac S Phos ho entethein I Transferases," ACS Chemical Biolo 2007 2 5 : 337-346
	697.	ZILBERMAN et al., "Genome-wide analysis of DNA methylation patterns," Development (2007) 134: 3959-3965
	698.	ZLOBEC et al., "Next-generation tissue microarray (ngTMA) increases the quality of biomarker studies: an example using CD3, CD8, and CD45RO in the tumor microenvironment of six different solid tumor t es," Journal of Translational Medicine 2013 11:104

Examiner Signature	Date Considered
/JAMES MARTINELL/	04/14/2019

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J. M./

```
* * * * * * * * * * *  Welcome to STN International  * * * * * * * * * * *

 NEWS  1  DEC 20  2019 MeSH Version Now Available in MEDLINE on STN
 NEWS  2  JAN  4  DWPI Manual Code revision in 2019
 NEWS  3  JAN 17  First 2019 Emtree(R) update now available in Embase(TM)
 NEWS  4  JAN 29  The 2019 Version of MEDLINE Now Available on STN(TM)
 NEWS  5  FEB  4  INPADOC/INPAFAM: Major enhancements for PCT
 NEWS  6  FEB 26  FRFULL and GBFULL reloaded
 NEWS  7  MAR  7  Search Limits Significantly Expanded Exclusively in STNext(R)
 NEWS  8  MAR  7  New Key Terms field supports efficient full-text searching
                  and results evaluation in Patent Full-text Databases
 NEWS  9  MAR  8  CPC Combination Sets Now Fully Available in Derwent
                  World Patents Index
 NEWS 10  MAR 25  Derwent World Patent Index Coverage Expanded to Include
                  Eight Former Soviet Republics and the Eurasian Patent
                  Organization (EAPO)
 NEWS 11  APR  3  Derwent World Patents Index and Derwent Markush
                  Resource (DWPIM) Now Updated Twice Weekly
 NEWS 12  APR 11  Updates in database World Surface Coatings Abstracts
                  (file WSCA) have resumed

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS LOGIN    Welcome Banner and News Items
 NEWS TRAINING Find instructor-led and self-directed training opportunities

Enter NEWS followed by the item number or name to see news on that
specific topic.

For pricing information enter HELP PRICE or HELP COST at any arrow prompt.

  All use of STN is subject to the provisions of the STN customer
  agreement.  This agreement limits use to scientific research.  Use
  for software development or design, implementation of commercial
  gateways, or use of CAS and STN data in the building of commercial
  products is prohibited and may result in loss of user privileges
  and other penalties.

Access the latest version of STN.
Log into STNext with your STN login ID at http://next.stn.org

* * * * * * * * * * * * * * * * STN  Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:36:30 ON 12 APR 2019

=> file biosis caplus medline
COST IN U.S. DOLLARS                        SINCE FILE      TOTAL
                                          ENTRY    SESSION
FULL ESTIMATED COST                          0.40       0.40

FILE 'BIOSIS' ENTERED AT 13:36:48 ON 12 APR 2019
Copyright (c) 2019 Clarivate Analytics

FILE 'CAPLUS' ENTERED AT 13:36:48 ON 12 APR 2019
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
COPYRIGHT (C) 2019 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'MEDLINE' ENTERED AT 13:36:48 ON 12 APR 2019

=> s ((locat? or address? or spac?)(20a)(bead? or micorbead? or sphere? or microsphere?) and (cod? or barcod? or tag?))/bi,ab
L1          392 ((LOCAT? OR ADDRESS? OR SPAC?)(20A)(BEAD? OR MICORBEAD? OR SPHER
                E? OR MICROSPHERE?) AND (COD? OR BARCOD? OR TAG?))/BI,AB
```

```
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          326 DUP REM L1 (66 DUPLICATES REMOVED)

=> s ((locat? or address or spac?)(20a)(bead? or microbead? or sphere? or microsphere?) and target? and (cod? or barcod? or
tag?))/bi,ab
L3          85 ((LOCAT? OR ADDRESS OR SPAC?)(20A)(BEAD? OR MICROBEAD? OR SPHERE
              ? OR MICROSPHERE?) AND TARGET? AND (COD? OR BARCOD? OR TAG?))/BI
              ,AB

=> s l2 not l3
L4          252 L2 NOT L3

=> s l4 not 2019/py
L5          245 L4 NOT 2019/PY

=> s l5 not 2018/py
L6          232 L5 NOT 2018/PY

=> s l6 not 2017/py
L7          224 L6 NOT 2017/PY

=> s l7 not 2016/py
L8          210 L7 NOT 2016/PY

=> s l8 not 2015/py
L9          205 L8 NOT 2015/PY

=> s l9 not 2014/py
L10         193 L9 NOT 2014/PY

=> s l10 not 2013/py
L11         174 L10 NOT 2013/PY

=> s l11 not 2012/py
L12         163 L11 NOT 2012/PY

=> d his

    (FILE 'HOME' ENTERED AT 13:36:30 ON 12 APR 2019)

    FILE 'BIOSIS, CAPLUS, MEDLINE' ENTERED AT 13:36:48 ON 12 APR 2019
L1          392 S ((LOCAT? OR ADDRESS? OR SPAC?)(20A)(BEAD? OR MICORBEAD? OR SP
L2          326 DUP REM L1 (66 DUPLICATES REMOVED)
L3           85 S ((LOCAT? OR ADDRESS OR SPAC?)(20A)(BEAD? OR MICROBEAD? OR SPH
L4          252 S L2 NOT L3
L5          245 S L4 NOT 2019/PY
L6          232 S L5 NOT 2018/PY
L7          224 S L6 NOT 2017/PY
L8          210 S L7 NOT 2016/PY
L9          205 S L8 NOT 2015/PY
L10         193 S L9 NOT 2014/PY
L11         174 S L10 NOT 2013/PY
L12         163 S L11 NOT 2012/PY

=> s l12 and (aptamer# or oligonucleotide# or nucleotide# or probe# or protein# or peptide# or antibod?)/bi,ab
L13          43 L12 AND (APTAMER# OR OLIGONUCLEOTIDE# OR NUCLEOTIDE# OR PROBE#
              OR PROTEIN# OR PEPTIDE# OR ANTIBOD?)/BI,AB

=> d his

    (FILE 'HOME' ENTERED AT 13:36:30 ON 12 APR 2019)
```

```
    FILE 'BIOSIS, CAPLUS, MEDLINE' ENTERED AT 13:36:48 ON 12 APR 2019
L1          392 S ((LOCAT? OR ADDRESS? OR SPAC?)(20A)(BEAD? OR MICORBEAD? OR SP
L2          326 DUP REM L1 (66 DUPLICATES REMOVED)
L3           85 S ((LOCAT? OR ADDRESS OR SPAC?)(20A)(BEAD? OR MICROBEAD? OR SPH
L4          252 S L2 NOT L3
L5          245 S L4 NOT 2019/PY
L6          232 S L5 NOT 2018/PY
L7          224 S L6 NOT 2017/PY
L8          210 S L7 NOT 2016/PY
L9          205 S L8 NOT 2015/PY
L10         193 S L9 NOT 2014/PY
L11         174 S L10 NOT 2013/PY
L12         163 S L11 NOT 2012/PY
L13          43 S L12 AND (APTAMER#  OR OLIGONUCLEOTIDE#  OR NUCLEOTIDE#  OR PROBE

=> d l13 bib ab
```

L13 ANSWER 1 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2012:107054 BIOSIS Full-text
DN PREV201200107054
TI An exonic insertion within Tex14 gene causes spermatogenic arrest in pigs.
AU Sironen, Anu [Reprint Author]; Uimari, Pekka; Venhoranta, Heli; Andersson, Magnus; Vilkki, Johanna
CS Agrifood Res Finland, MTT, Biotechnol and Food Res, Genom, FI-36100 Jokioinen, Finland
anu.sironen@mtt.fi
SO BMC Genomics, (DEC 2 2011) Vol. 12, pp. Article No.: 591.
http://www.biomedcentral.com/bmcgenomics.
ISSN: 1471-2164. E-ISSN: 1471-2164.
DOI 10.1186/1471-2164-12-591
DT Article
LA English
ED Entered STN: 25 Jan 2012
Last Updated on STN: 4 Jul 2018
AB Background: Male infertility is an increasing problem in all domestic species including man. Localization and identification of genes involved in defects causing male infertility provide valuable information of specific events in sperm development. Sperm development is a complex process, where diploid spermatogonia develop into haploid, highly specialized spermatozoa. Correct expression and function of various genes and their protein products are required for production of fertile sperm. We have identified an infertility defect in Finnish Yorkshire boars caused by spermatogenic arrest. The aim of this study was to locate the disease associated region using genome wide screen with the PorcineSNP60 Beadchip and identify the causal mutation by candidate gene approach.Results: In the Finnish Yorkshire pig population the spermatogenic arrest (SA) defect appears to be of genetic origin and causes severe degeneration of germ cells and total absence of spermatozoa. Genome wide scan with the PorcineSNP60 Beadchip localized the SA defect to porcine chromosome 12 in a 2 Mbp region. Sequencing of a candidate gene Tex14 revealed a 51 bp insertion within exon 27, which caused differential splicing of the exon and created a premature translation stop codon. The expression of Tex14 was markedly down regulated in the testis of a SA affected boar compared to control boars and no protein product was identified by Western blotting. The SA insertion sequence was also found within intron 27 in all analyzed animals, thus the insertion appears to be a possible duplication event.Conclusion: In this study we report the identification of a causal mutation for infertility caused by spermatogenic arrest at an early meiotic phase. Our results highlight the role of TEX14 specifically in spermatogenesis and the importance of specific genomic remodeling events as causes for inherited defects.

=> d l13 2-43 bib ab

L13 ANSWER 2 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2011:692038 BIOSIS Full-text
DN PREV201100692038
TI A Study of the Spatial Protein Organization of the Postsynaptic Density Isolated from Porcine Cerebral Cortex and Cerebellum.
AU Yun-Hong, Yen; Chih-Fan, Chuang; Chia-Wei, Chang; Yen-Chung, Chang [Reprint Author]
CS Natl Tsing Hua Univ, Inst Mol Med, 101 Sec 2,Kung Fu Rd, Hsinchu 30013, Taiwan
ycchang@life.nthu.edu.tw
SO Molecular & Cellular Proteomics, (OCT 2011) Vol. 10, No. 10, pp. Article No.: M110.007138. http://www.mcponline.org/.
ISSN: 1535-9476. E-ISSN: 1535-9484.
DOI 10.1074/mcp.M110.007138
DT Article
LA English
ED Entered STN: 2 Nov 2011
Last Updated on STN: 31 Aug 2016
AB Postsynaptic density (PSD) is a protein supramolecule lying underneath the postsynaptic membrane of excitatory synapses and has been implicated to play important roles in synaptic structure and function in mammalian central nervous system. Here, PSDs were isolated from two distinct regions of porcine brain, cerebral cortex and cerebellum. SDS-PAGE and Western blotting analyses indicated that cerebral and cerebellar PSDs consisted of a similar set of proteins with noticeable differences in the abundance of various proteins between these samples. Subsequently, protein localization in these PSDs was analyzed by using the Nano-Depth-Tagging method. This method involved the use of three synthetic reagents, as agarose beads whose surface was covalently linked with a fluorescent, photoactivable, and cleavable chemical crosslinker by spacers of varied lengths. After its application was verified by using a synthetic complex consisting of four layers of different proteins, the Nano-Depth-Tagging method was used here to yield information concerning the depth distribution of various proteins in the PSD. The results indicated that in both cerebral and cerebellar PSDs, glutamate receptors, actin, and actin binding proteins resided in the peripheral regions within similar to 10 nm deep from the surface and that scaffold proteins, tubulin subunits, microtubule-binding proteins, and membrane cytoskeleton proteins found in mammalian erythrocytes resided in the interiors deeper than 10 nm from the surface in the PSD. Finally, by using the immunoabsorption method, binding partner proteins of two proteins residing in the interiors, PSD-95 and alpha-tubulin, and those of two proteins residing in the peripheral regions, elongation factor-1 alpha and calcium, calmodulin-dependent protein kinase II alpha subunit, of cerebral and cerebellar PSDs were identified. Overall, the results indicate a striking similarity in protein organization between the PSDs isolated from porcine cerebral cortex and cerebellum. A model of the molecular structure of the PSD has also been proposed here. Molecular & Cellular Proteomics 10: 10.1074/mcp.M110.007138, 1-20, 2011.

L13 ANSWER 3 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2011:622500 BIOSIS Full-text
DN PREV201100622500
TI Modulation of the distribution of small proteins within porous matrixes by smart-control of the immobilization rate.
AU Bolivar, Juan M.; Hidalgo, Aurelio; Sanchez-Ruiloba, Lucia; Berenguer, Jose; Guisan, Jose M. [Reprint Author]; Lopez-Gallego, Fernando
CS Inst Catalisis and Petroleoquim CSIC, Dept Biocatalisis, Campus UAM, Madrid 28049, Spain
jmguisan@icp.csic.es; flopez@icp.csic.es
SO Journal of Biotechnology, (OCT 10 2011) Vol. 155, No. 4, pp. 412-420.
CODEN: JBITD4. ISSN: 0168-1656. E-ISSN: 1873-4863.
DOI 10.1016/j.jbiotec.2011.07.039
DT Article
LA English
ED Entered STN: 28 Sep 2011
Last Updated on STN: 23 Nov 2011
AB The distribution of enzymes attached to porous solid supports is a major concern in multienzymatic bioreactors. Herein, as proof of the concept that protein localization on porous surfaces can be controlled by tuning the protein immobilization rate. We study the distribution of two poly-histidine-tagged fluorescent proteins (His-GFP and His-mCherryFP) immobilized on different 4% crosslinked agarose-type carriers by confocal laser scanning microscopy. In this context, immobilization rate is easily modulated by controlling the (i) nature of physico-chemical interaction between protein and surface (reactive groups on surface), (ii) by controlling the reactive group density and (iii) by adding competitors to the immobilization process. His-GFP is 350-fold more rapid immobilized on agarose surfaces activated with either glyoxyl groups or chelates than the same matrix activated with primary amine groups instead. A similar effect is seen with agarose matrixes activated with lower glyoxyl densities that immobilize His-GFP roughly 350-fold slower than the corresponding highly activated matrix. When His-GFP is immobilized on agarose activated with chelates groups in presence of imidazol which competes with the protein for the reactive groups on the support, the immobilization rate is again 400-fold slower than when the same protein was immobilized on the same support but with no imidazol during the immobilization process. In all cases, it was observed that rapid immobilizations (quantitative immobilization in less than 10 min) located 100% of the loaded protein at the crown of the carrier beads, meaning that only the 10% of the bead radius was colonized by the protein. On the contrary, when immobilization is much slower, a homogeneous distribution is obtained, resulting in beads whose whole radius is occupied by the protein. Therefore, we set that the more rapid immobilization, the more heterogeneous distribution. All the knowledge gained in protein distribution by immobilization rate alteration of a single protein is applied to the co-immobilization of the two fluorescent proteins in order to develop four different co-immobilization patterns with an enormous applied potential to other multi-protein systems. (C) 2011 Elsevier B.V. All rights reserved.

L13 ANSWER 4 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2011:292049 BIOSIS Full-text
DN PREV201100292049
TI Fluorescent semiconductor nanocrystals (quantum dots) in protein biochips.
AU Oleinikov, V. A. [Reprint Author]
CS Russian Acad Sci, Shemyakin Ovchinnikov Inst Bioorgan Chem, Ul Miklukho Maklaya 16-10, Moscow 117997, Russia
voleinik@mail.ru
SO Russian Journal of Bioorganic Chemistry, (MAR 2011) Vol. 37, No. 2, pp. 151-167. http://www.springerlink.com/content/1068-1620/.
ISSN: 1068-1620. E-ISSN: 1068-330X.
DOI 10.1134/S1068162011020117
DT Article
General Review; (Literature Review)
LA English
ED Entered STN: 4 May 2011
Last Updated on STN: 13 Jun 2012
AB Understanding the biological processes in cells, tissues, and organisms requires the identification and analysis of multiple biological objects and the mechanisms of their functioning and regulation. The biological chip (biochip) technique is one of the most efficient tools for these tasks. Biochips are highly efficient and can quantitatively register multiple molecules simultaneously in samples of microscopic volume. Biochips allow the parallel genomic or proteomic analysis of normal or pathologically modified cells and tissues and a comparative analysis to elucidate disease-related changes. Fluorescent dyes used for signal readout from biochips have the following disadvantages: low photostability, low brightness, and the presence of a fluorescent background. It was recently shown that these limitations can be removed if fluorescent semiconductor nanocrystals (quantum dots) are used. Individual quantum dots in the form of colloid nanocrystals (QDs) are easily registered by conventional microscopic equipment due to their high brightness; they are extremely resistant to photobleaching and provide unique opportunities for multiplexing. QDs are ideal fluorophores for information readout from biochips and allow for the detection of single molecules.The present work is aimed at developing approaches for the use of QDs in biochip-based detection systems. The possibilities of using QDs in both planar (or matrix) biochips and suspension (or liquid) biochips, which are undergoing intensive development, are demonstrated. The use of the latter in analytical systems for the simultaneous identification of multiple objects in proteomics, genomics, drug testing, and clinical diagnostics is currently increasing. These systems are based on spectrally coded elements (usually polymer microspheres). An advantage of liquid biochips over matrix planar solid biochips is the possibility of the free movement of microspheres in three-dimensional space. Organic fluorophores allow the realization of a limited number of codes, i.e., objects analyzed simultaneously (multiplexing), while semiconductor QDs make possible a significant increase in both biochip multiplexing and the photostability and sensitivity of the biochips. In addition, the use of FRET (Foerster resonance energy transfer) in liquid biochips makes possible an increase in the detection specificity. The absence of a background signal from the fluorescent labels not bound to the microparticles increases the sensitivity of the analysis and provides additional opportunities for multiplex analysis and diagnostics.Thus, a combination of the biochip technique and semiconductor QDs makes it possible to increase the method's sensitivity and the number of objects detected (the degree of multiplexing). This combination is likely to enable a significant breakthrough in proteomics, particularly in the development of new drugs, clinical diagnostics, identification of molecular markers, and elucidation of the intracellular processes.

L13 ANSWER 5 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2011:187957 BIOSIS Full-text
DN PREV201100187957
TI Light transmitted assay beads.
AU Ho, Winston Z. [Inventor]; Anonymous
CS Hacienda Heights, CA USA
ASSIGNEE: Maxwell Sensors Inc
PI US 07871770 20110118
SO Official Gazette of the United States Patent and Trademark Office Patents, (JAN 18 2011)
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 30 Mar 2011
Last Updated on STN: 30 Mar 2011
AB A micro bead having a digitally coded structure that is partially transmissive and opaque to light. The pattern of transmitted light is determined by to decode the bead. The coded bead may be structured a series of alternating light transmissive and opaque sections, with relative positions, widths and spacing resembling a 1D or 2D bar code image. To decode the image, the alternating transmissive and opaque sections of the body are scanned in analogous fashion to bar code scanning. The coded bead may be coated or immobilized with a capture or probe to effect a desired bioassay. The coded bead may include a paramagnetic material. A bioanalysis system conducts high throughput bioanalysis using the coded bead, including a reaction detection zone and a decoding zone.

L13 ANSWER 6 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2011:64576 BIOSIS Full-text
DN PREV201100064576
TI Light transmitted assay beads.
AU Ho, Winston Z. [Inventor]; Anonymous
CS Hacienda Heights, CA USA
ASSIGNEE: Maxwell Sensors Inc
PI US 07858307 20101228
SO Official Gazette of the United States Patent and Trademark Office Patents, (DEC 28 2010)
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 19 Jan 2011
Last Updated on STN: 19 Jan 2011
AB A micro bead having a digitally coded structure that is partially transmissive and opaque to light. The pattern of transmitted light is determined by to decode the bead. The coded bead may be structured a series of alternating light transmissive and opaque sections, with relative positions, widths and spacing resembling a 1D or 2D bar code image. To decode the image, the alternating transmissive and opaque sections of the body are scanned in analogous fashion to bar code scanning. The coded bead may be coated or immobilized with a capture or probe to effect a desired bioassay. The coded bead may include a paramagnetic material. A bioanalysis system conducts high throughput bioanalysis using the coded bead, including a reaction detection zone and a decoding zone.

L13 ANSWER 7 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2008:247801 BIOSIS Full-text
DN PREV200800240201
TI On-chip oligonucleotide ligation assay using one-dimensional microfluidic beads array for the detection of low-abundant DNA point mutations.
AU Zhang, He; Yang, Xiaohai; Wang, Kemin [Reprint Author]; Tan, Weihong; Li, Huimin; Zuo, Xinbing; Wen, Hanhui
CS Hunan Univ, Coll Chem and Chem Engn,Biomed Engn Ctr, State Key Lab Chemo Biosensing and Chemomet, Key Lab Bio Nanotechnol and Mol Engn Hunan Prov, Changsha 410082, Peoples R China
kmwang@hnu.cn
SO Biosensors & Bioelectronics, (FEB 28 2008) Vol. 23, No. 7, pp. 945-951.
CODEN: BBIOE4. ISSN: 0956-5663.
DOI 10.1016/j.bios.2007.09.012
DT Article
LA English
ED Entered STN: 2 Apr 2008
Last Updated on STN: 12 May 2008
AB The detection of low-abundant DNA point mutations is very important for the early prediction of cancer, diagnostics of disease and clinical prognosis. In this paper, an on-chip oligonucleotide ligation approach that arrayed a series of functionalized beads in a single microfluidic channel was described for detection of low-abundant point mutations in p53 gene. This gene carried the point mutation with high diagnostic value for assessment of tumor progression and resectional borders. This work extended our prior efforts using one-dimensional (1-D) microfluidic beads array for protein and nucleic acid molecular profiling, and displayed high discrimination sensitivity to mutations detection due to the enhanced mass transport capability caused by microfluidic addressing format of beads array. As a demonstration, it was found that the on-chip beads ligation held high mutation discrimination sensitivity in 1 pM quantities at a SNR (signal-to-noise ratio) >2 using synthesized DNA oligonucleotides in accordance with target fragment. The RT-PCR products of tumor cell line A549, CNE2 and SKBr-3 were further examined to distinguish the point mutation at codon 175 of p53 gene. This approach was capable of detecting a point mutation in a p53 oncogene at a level of I mutant in 1000 wild-type sequences using PCR products without the need of LDR amplification. Additionally, this on-chip beads ligation approach also displayed other microfluidic-based advantages of simple handling (one sample injection per test), little reagent quantities, and low potential of contaminations. (C) 2007 Elsevier B.V. All rights reserved.

L13 ANSWER 8 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2008:155703 BIOSIS Full-text
DN PREV200800144871
TI Spatially addressable bead-based biosensor for simple and rapid DNA detection.
AU Ng, Johnson K.; Selamat, Ezrein S.; Lin, Wen-Tso [Reprint Author]
CS Natl Univ Singapore, Div Environm Sci and Engn, 21 Lower Kent Ridge Rd, Singapore 119077, Singapore
eseliuwt@nus.edu.sg
SO Biosensors & Bioelectronics, (JAN 18 2008) Vol. 23, No. 6, pp. 803-810.
CODEN: BBIOE4. ISSN: 0956-5663.
DT Article
LA English
ED Entered STN: 27 Feb 2008
Last Updated on STN: 27 Feb 2008
AB A simple glass-polymer-based biosensor that allows arrays of beads to be immobilized, separated and identified without any prior encoding is developed. To do so, distinct bead types that are conjugated with different oligonucleotide probes are sequentially spotted onto a polymeric matrix (or gel pad) on the surface of the device. The spotted beads are firmly immobilized to the gel pad, acquiring spatial codes that allow them to be identified. Throughput is enhanced by spotting different bead types onto hundreds of different gel pads. The bead-based biosensor was applied for the DNA-based detection of 10 model bacterial species and two single-nucleotide polymorphisms, and based on passive hybridization the final signals are obtained with single-mismatch specificity within 10 min. (C) 2007 Elsevier B.V. All rights reserved.

L13 ANSWER 9 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2007:542069 BIOSIS Full-text
DN PREV200700532507
TI Complex microparticles for delivery of low-molecular weight, water-soluble nutrients and pharmaceuticals to marine fish larvae.
AU Langdon, Chris; Clack, Brendan; Onal, Urnur [Reprint Author]
CS Canakkale Onsekiz Univ, Fac Fisheries, Dept Aquaculture, Terzioglu Campus, TR-17100 Canakkale, Turkey
chris.langdon@oregonstate.edu; umuronal@yahoo.com
SO Aquaculture, (AUG 22 2007) Vol. 268, No. 1-4, Sp. Iss. SI, pp. 143-148.
CODEN: AQCLAL. ISSN: 0044-8486.
DT Article
LA English
ED Entered STN: 17 Oct 2007
Last Updated on STN: 17 Oct 2007
AB Most currently available microparticulate artificial diets for rearing marine suspension feeders, including larval fish, are not effective in retaining low-molecular weight, water-soluble (LMWS) nutrients and other materials [Lopez-Alvarado, J., Langdon, C.J., Teshima, S., Kanazawa, A., 1994. Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. Aquaculture 122, 335-346; Baskerville-Bridges, B., Kling, L.J., 2000. Development and evaluation of microparticulate diets for early weaning of Atlantic cod, Gadus morhua, larvae. Aquae. Nutr. 6, 171-182]. Rapid leaching losses occur as a result of high surface-to-volume ratios and the porosity of the matrix or walls of the particles [Langdon, C.J., 2003. Microparticle types for delivery nutrients to marine fish larvae. Aquaculture 227, 259-275]. In order to address this problem of high leakage losses, a novel complex particle type was developed consisting of LMWS incorporated in lipid beads that are then bound, with other nutrients, in a matrix of the water-insoluble protein zein.A spray method for preparing lipid beads was developed to increase the concentration of incorporated core material as well as to reduce losses due to leakage from beads suspended in water. It was possible to prepare lipid spray beads (LSB) that delivered 8.5% of their dry weight as glycine after I-h suspension in water, resulting in free amino acid concentrations similar to those of commonly used live feeds for fish larvae, such as rotifers and Artemia; however, this delivery efficiency was reduced to 1.2% glycine for complex particles made up of 30% w/w glycine-containing LSB and other dietary nutrients. Further experiments are required to determine if complex particles can replace living feeds for the early larval stages of marine fish species. (C) 2007 Published by Elsevier B.V.

L13 ANSWER 10 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2007:331117 BIOSIS Full-text
DN PREV200700335080
TI Defining the role of "second sphere" residues in the activity of glyoxasomal malate dehydrogenase.
AU Jean, Sophonie [Reprint Author]; Bell, Ellis
CS Univ Richmond, Lab Struct Biol Biophys and Bioinformat, Gottwald Sci Ctr, Richmond, VA 23173 USA
SO FASEB Journal, (APR 2007) Vol. 21, No. 5, pp. A643.
Meeting Info.: Experimental Biology 2007 Annual Meeting. Washington, DC, USA. April 28 -May 02, 2007. Amer Assoc Anatomists; Amer Physiol Soc; Amer Soc Biochem & Mol biol; Amer Soc Investigat Pathol; Amer Soc Nutr; Amer Soc Pharmacol & Expt Therapeut.
CODEN: FAJOEC. ISSN: 0892-6638.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 30 May 2007
Last Updated on STN: 30 May 2007
AB Malate Dehydrogenase catalyses the interconversion of Malate and Oxaloacetate using NAD(H). Although the 3-D structure has long been known and a catalytic mechanism proposed involving a histidine-aspartate pair in the active site, little is known about the roles that residues further from the active site may play in either hydride transfer or proton abstraction. Analysis of the active site cavity, both visually and by QM-MM approaches, led to the construction of "second sphere" mutants involving residues located 5- [OA from the site of catalysis using quickchange mutagenesis, and the resultant proteins purified using Ni-NTA affinity chromatography making use of the histidine tag introduced in the pQE 60 vector. While H220 and D193 play well characterized roles in proton abstraction, other residues, N124, T152, T186, A183, G180,V164,N165, S166 and H90 have been mutated and appear to play various roles in the overall reaction. Several mutants appear to be affecting the pH dependence and substrate inhibition. For example, while the N 165D mutant showed similar Vmax values at PH 7 & 8 there is a marked increase in the effects of substrate inhibition at PH 7 and a reversal of the pH effects on K-m for oxaloacetate relative to the native protein or the S166A mutant. The N165D and the S166A mutants have been selected for crystal screening. It is clear that a number of residues within this second sphere contribute to protein stability and aid in the fine tuning of the regulatory effects of substrate inhibition and PH dependence.

L13 ANSWER 11 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2006:499024 BIOSIS Full-text
DN PREV200600505344
TI Novel interaction between XIAP-Associated Factor 1 (XAF1) and nuclear coactivator clip in colon cancer.
AU Sun, Yunwei; Xia, Harry. H. X.; Zhu, Senlin; Zou, Bing; Lin, Marie Cm; Kung, Hsiang Fu; Wong, Benjamin C.
SO Gastroenterology, (APR 2006) Vol. 130, No. 4, Suppl. 2, pp. A680.
Meeting Info.: Digestive Disease Week Meeting/107th Annual Meeting of the American-Gastroenterological-Association. Los Angeles, CA, USA. May 19 -24, 2006. Amer Gastroenterol Assoc Inst.
CODEN: GASTAB. ISSN: 0016-5085.
DT Conference; (Meeting)
Conference; (Meeting Poster)
LA English
ED Entered STN: 4 Oct 2006
Last Updated on STN: 4 Oct 2006
AB Background: XAF1 (XIAP-Associated Factor 1) is a nuclear protein that directly interacts with endogenous XIAP and results in XIAP sequestration in nuclear inclusions. cAMP response element binding protein (CREB)-binding protein (CBP) play an important role as multifunctional transcriptional coactivators. In this study, we examined the association between XAF1 and CBP in colon cancer cell. Methods: Expressions of XAF1 and CBP were detected by Western blot in colon cancer cells. The association of XAF1 with CBP was confirmed in vitro by glutathione S-transferase (GST) pull-down assay and in vivo by coimmumoprecipitation. Co-localization of XAF1 and CBP was demonstrated by immunofluorescence microscopy. An AP1 reporter assay was used to determine the functional interaction between XAF1 and CBP in the presence or absence of PMA. Results: Low level of XAF1 and CBP was detected in SW1116 cells. Using GST-CBP (1-1099,1098-1758,1098-1858 and 1620-1877) bound to sepharose beads and in vitro expressed 35S-labeled XAF1, we found the XAF1 binding sites on CBP are located in the cysteine-histidine-rich C/H1 domains (residues 1-1098). Plasmids encoding XAF1 and HA-tagged CBP were transfected into SW1116 cells alone or in combination. Immunoprecipitation of over-expressed CBP protein with anti-CBP polyclonal antibody co-precipitated XAF I was mainly located in the nucleus. CBP was located exclusively in the nucleus. XAF1-RFP and CBP-HA co-localization were seen in the nucleus after immunofluorescence staining. Reporter assays demonstrated that co-transfection with pCDNA3-XAF1 and pRc/RSV-CBP led to one fold reduction of AP1 luciferase activity stimulated by PMA, which was further enhanced by co-transfection of CBP's homolog pCMV-P300. Conclusions: Our results identified CBP as a novel binding partner for XAF1 and indicated that CBP, as a coactivator, cooperated with XAF1-mediated inhibition of AP 1 transcription.

Curio EX1010-p. 495
Curio v Prognosys
IPR2024-01289

L13 ANSWER 12 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2006:418747 BIOSIS Full-text
DN PREV200600411198
TI Synthetic modification of silica beads that allows for sequential attachment of two different oligonucleotides.
AU Steinberg-Tatman, Gali; Huynh, Michael; Barker, David; Zhao, Chanfeng [Reprint Author]
CS Illunina Inc, 9885 Towne Ctr Dr, San Diego, CA 92121 USA chanfeng@illumina.com
SO Bioconjugate Chemistry, (MAY 17 2006) Vol. 17, No. 3, pp. 841-848. CODEN: BCCHES. ISSN: 1043-1802.
DT Article
LA English
ED Entered STN: 23 Aug 2006
Last Updated on STN: 23 Aug 2006
AB We developed a simple and elegant synthesis strategy that enables us to attach controlled (equimolar) amounts of two different oligonucleotides onto one silica bead. The method involves addition of orthogonally protected lysine followed by activation and derivatization of each amino group with a different moiety. This sequential oligonucleotide attachment enables the use of a combinatorial scheme to generate millions of bead types, each characterized by its two oligo tags. (In our randomly assembled arrays each bead type can then be identified by a series of hybridizations of fluorescently labeled decoder oligos to the address tags.) To demonstrate feasibility of such a scheme we created over 1000 bead types, which were characterized by their two oligo tags. The method enables genotyping or gene expression assays at multiplex levels of hundreds of thousands to millions.

L13 ANSWER 13 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2006:343605 BIOSIS Full-text
DN PREV200600342737
TI Defining the role of "second sphere" residues in the activity of glyoxasomal malate dehydrogenase.
AU Jean, Sophonie [Reprint Author]; Bell, Ellis
CS Univ Richmond, Gottwald Sci Ctr, Richmond, VA 23173 USA
SO FASEB Journal, (MAR 6 2006) Vol. 20, No. 4, Part 1, pp. A45.
Meeting Info.: Experimental Biology 2006 Meeting. San Francisco, CA, USA. April 01 -05, 2006. Amer Assoc Anatomists; Amer Physiol Soc; Amer Soc Biochem & Mol Biol; Amer Soc Investigat Pathol; Amer Soc Nutr; Amer Soc Pharmacol & Expt Therapeut.
CODEN: FAJOEC. ISSN: 0892-6638.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 12 Jul 2006
Last Updated on STN: 12 Jul 2006
AB Malate Dehydrogenase catalyses the interconversion of Malate and Oxaloacetate with the concommitant oxidation and reduction of the cofactor NAD(H). Although the 3 dimensional structure has been known for many years and a catalytic mechanism proposed involving a histidine-aspartate pair in the active site little is known about the roles that residues furthjer from the active site may play in either the hydride transfer or proton abstraction steps of catalysis. Analysis of the active site cavity of the enzyme led to the design of a number of "second sphere" mutants involving various hydrophilic residues located 5-10A from the site of catalysis. Mutants were constructed using quikchange mutagenesis and the resultant proteins purified using NiNTA affinity chromatography making use of the histidine tag introduced in the pQE 60 vector used for expression.The activity of various mutants has been characterized using initial rate kinetic studies and Kin and Vmax values determined. Activation energies have been calculated from the temperature dependence of Vmax and the stabilities of the protein determined using both heat inactivation and guanidine hydrochloride denaturation. It is clear that a number of residues within this second sphere contribute to both protein stability and activity.

L13 ANSWER 14 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2006:206354 BIOSIS Full-text
DN PREV200600197577
TI Fractionation of chromosome 15 with an affinity-based approach using magnetic beads.
AU Vitharana, Samadhi N.; Wilson, George S. [Reprint Author]
CS Univ Kansas, Dept Chem, 1251 Wescoe Hall Dr, Lawrence, KS 66045 USA
gwilson@ku.edu
SO Genomics, (JAN 2006) Vol. 87, No. 1, pp. 158-164.
CODEN: GNMCEP. ISSN: 0888-7543.
DT Article
LA English
ED Entered STN: 22 Mar 2006
Last Updated on STN: 22 Mar 2006
AB The two main shortcomings of the state-of-the-art method of sorting chromosomes, specificity and the efficiency of fractionating a significant amount of chromosomes, are addressed by this work in the design of a massively parallel approach using magnetic beads binding to a chromosome-specific DNA probe. In an attempt to isolate human chromosome 15 from a lymphoblastoid cell line, a chromosome 15 centromere-specific DNA probe with a fluorescent tag attached was reacted with the chromosomes. Magnetic beads bound to anti-FITC antibody were reacted with the labeled pool of chromosomes and separated by exposure to a magnetic field. The specificity of the fractionated pool was verified by performing fluorescence in situ hybridization on the isolated pool. The chromosome of interest could be enriched to about 75% within a maximum of 3-4 days, regardless of the amount of material. (c) 2005 Elsevier Inc. All rights reserved.

L13 ANSWER 15 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 2005:196329 BIOSIS Full-text
DN PREV200500197698
TI Latex bead immobilisation in PDMS matrix for the detection of p53 gene point mutation and anti-HIV-1 capsid protein antibodies.
AU Marquette, Christophe A. [Reprint Author]; Degiuli, Agnes; Imbert-Laurenceau, Emmanuelle; Mallet, Francois; Chaix, Carole; Mandrand, Bernard; Blum, Loic J.
CS CNRSLab Genie Enzymat and BiomolUMR 5013,EMB2, Univ Lyon 1, Batiment CPE-43,Bd 11 Novembre 1918, F-69622, Villeurbanne, France Christophe.marquette@univ-lyon1.fr
SO Analytical and Bioanalytical Chemistry, (March 2005) Vol. 381, No. 5, pp. 1019-1024. print.
ISSN: 1618-2642 (ISSN print).
DT Article
LA English
ED Entered STN: 25 May 2005
Last Updated on STN: 25 May 2005
AB Two diagnostic chemiluminescent biochips were developed for either the detection of p53 gene point mutation or the serological detection of anti-HIV-1 p24 capsid protein. Both biochips were composed of 24 microarrays of latex beads spots (4x4) (150 mum in diameter, 800 mum spacing) entrapped in a poly(dimethylsiloxane) elastomer (PDMS). The latex beads, bearing oligonucleotide sequences or capsid protein, were spotted with a conventional piezoelectric spotter and subsequently transferred at the PDMS interface. The electron microscopy observation of the biochips showed how homogeneous and well distributed the spots could be. Point mutation detection on the codon 273 of the p53 gene was performed on the basis of the melting temperature difference between the perfect match sequence and the one base pair mismatch sequence. The hybridisation of a 20-mer oligonucleotide form the codon 273 including a one base pair mutation in its sequence on a biochip arrayed with non-muted and the muted complementary sequences, enabled a clear discrimination at 56degreeC between muted and wild sequences. Moreover, the quantitative measurement of the amount of muted sequence in a sample was possible in the range 0.4-4 pmol. Serological measurement of anti-HIV-1 p24 capsid protein on the biochip, prepared with 1-mum-diameter latex beads, enabled the detection of monoclonal antibodies in the range 1.55-775 ng mL-1. Such a range could be lowered to 0.775 ng mL-1 when using 50-nm-diameter beads, which generated a higher specific surface. The validation of the biochip for the detection of anti-HIV-1 capsid protein antibodies was performed in human sera from seropositive and seronegative patients. The positivity of the sera was easily discriminated at serum dilutions below 1:1,000.

L13 ANSWER 16 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 1996:152033 BIOSIS Full-text
DN PREV199698724168
TI Filensin and phakinin form a novel type of beaded intermediate filaments and coassemble de novo in cultured cells.
AU Goulielmos, George; Gounari, Fotini; Remington, Susann; Mueller, Shirley; Haener, Markus; Aebi, Ueli; Georgatos, Spyros D. [Reprint author]
CS Dep. Basis Sci., Univ. Crete Sch. Med., 711 10 Heraklion, Crete, Greece
SO Journal of Cell Biology, (1996) Vol. 132, No. 4, pp. 643-655.
CODEN: JCLBA3. ISSN: 0021-9525.
DT Article
LA English
ED Entered STN: 11 Apr 1996
Last Updated on STN: 11 Apr 1996
AB The fiber cells of the eye lens possess a unique cytoskeletal system known as the "beaded-chain filaments" (BFs). BFs consist of filensin and phakinin, two recently characterized intermediate filament (IF) proteins. To examine the organization and the assembly of these heteropolymeric IFs, we have performed a series of in vitro polymerization studies and transfection experiments. Filaments assembled from purified filensin and phakinin exhibit the characteristic 19-21-nm periodicity seen in many types of IFs upon low angle rotary shadowing. However, quantitative mass-per-length (MPL) measurements indicate that filensin/phakinin filaments comprise two distinct and dissociable components: a core filament and a peripheral filament moiety. Consistent with a nonuniform organization, visualization of unfixed and unstained specimens by scanning transmission electron microscopy (STEM) reveals the existence of a central filament which is decorated by regularly spaced 12-15-nm-diam beads. Our data suggest that the filamentous core is composed of phakinin, which exhibits a tendency to self-assemble into filament bundles, whereas the beads contain filensin/phakinin hetero-oligomers. Filensin and phakinin copolymerize and form filamentous structures when expressed transiently in cultured cells. Experiments in IF-free SW13 cells reveal that coassembly of the lens-specific proteins in vivo does not require a preexisting IF system. In epithelial MCF-7 cells de novo forming filaments appear to grow from distinct foci and organize as thick, fibrous laminae which line the plasma membrane and the nuclear envelope. However, filament assembly in CHO and SV40-transformed lens-epithelial cells (both of which are fibroblast-like) yields radial networks which codistribute with the endogenous vimentin IFs. These observations document that the filaments formed by lens-specific IF proteins are structurally distinct from ordinary cytoplasmic IFs. Furthermore, the results suggest that the spatial arrangement of filensin/phakinin filaments in vivo is subject to regulation by host-specific factors. These factors may involve cytoskeletal networks (e.g., vimentin IFs) and/or specific sites associated with the cellular membranes.

L13 ANSWER 17 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 1995:386857 BIOSIS Full-text
DN PREV199598401157
TI RNA transcription from immobilized DNA templates.
AU Marble, H. Anthony; Davis, Robert H. [Reprint author]
CS Dep. Chem. Eng. Chem. Biochem., Univ. Colorado, Boulder, CO 80309-0424, USA
SO Biotechnology Progress, (1995) Vol. 11, No. 4, pp. 393-396.
CODEN: BIPRET. ISSN: 8756-7938.
DT Article
LA English
ED Entered STN: 13 Sep 1995
Last Updated on STN: 13 Sep 1995
AB We describe an RNA transcription protocol based on the multiple reuse of solid-phase synthetic DNA templates. The templates are assembled onto streptavidin-coated agarose beads via a single 5'-terminal biotin located on the noncoding template strand. Transcription occurs in an aqueous buffered suspension containing solid-phase DNA, dissolved enzyme (T7 RNA polymerase), and nucleoside triphosphate substrates (NTPs). A direct comparison of solution and solid-phase templates under standard transcription conditions reveals similar initial reaction rates and overall yields. Immobilized templates store stably for periods of several months and are easily recovered by mild centrifugation. We demonstrate the successive reuse of these templates throughout 15 rounds of transcription. The templates remained active, although an incremental decay in transcription was observed beyond five rounds. Template activity was partially restored by supplementing the support-bound oligonucleotide with fresh coding-strand DNA. These findings indicate that multiple reuse of template is a viable strategy for reducing the amount of DNA template required in RNA transcription.

L13 ANSWER 18 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 1994:131613 BIOSIS Full-text
DN PREV199497144613
TI The origin of serotoninergic afferents to the cat's cerebellar nuclei.
AU Kitzman, Patrick H.; Bishop, Georgia A. [Reprint author]
CS Ohio State Univ., Dep. Cell Biol. Neurobiol. Anatomy, 333 W. 10th Ave., Columbus, OH 43210, USA
SO Journal of Comparative Neurology, (1994) Vol. 340, No. 4, pp. 541-550.
CODEN: JCNEAM. ISSN: 0021-9967.
DT Article
LA English
ED Entered STN: 24 Mar 1994
Last Updated on STN: 25 Mar 1994
AB In the cat, serotoninergic (5HT) axons and terminals form a dense plexus that is present throughout the granule cell and Purkinje cell layers of the cerebellar cortex and all of the cerebellar nuclei. The intent of the present study was to identify the source of 5HT fibers in the cerebellar nuclei. The medial, interposed, and lateral cerebellar nuclei were selectively injected with either rhodamine or fluorescein-labeled latex microspheres that were retrogradely transported to brainstem neurons. Transverse sections of the brainstem were processed with a primary antibody to 5HT and secondary antibody tagged with either rhodamine or fluorescein. The location of neurons containing both serotonin-like immunoreactivity and retrogradely transported microspheres was plotted. All three of the cerebellar nuclei receive 5HT afferents from the nucleus locus coeruleus, the dorsal raphe nucleus, and the dorsal tegmental nucleus. In addition, the medial nucleus receives projections from the superior central nucleus, the nucleus raphe obscurus, the nucleus raphe magnus, and the periolivary reticular formation. The interposed nuclei receive additional projections from the nucleus raphe magnus, whereas the lateral nucleus receives additional projections from the superior central nucleus. In conclusion, the 5HT projections to the cerebellar nuclei do not appear to be collaterals of those projecting to the cortex (Kerr and Bishop, J Comp Neurol 304:502-515, 1991). These findings suggest that, although the cortex and nuclei are anatomically and physiologically related, they do not process all information in parallel.

Curio EX1010-p. 502
Curio v Prognosys
IPR2024-01289

L13 ANSWER 19 OF 43 BIOSIS COPYRIGHT (c) 2019 Clarivate Analytics on STN
AN 1978:237422 BIOSIS Full-text
DN PREV197866049919; BA66:49919
TI HYDRODYNAMIC PROPERTIES OF MACRO MOLECULAR COMPLEXES PART 4 INTRINSIC VISCOSITY THEORY WITH APPLICATIONS TO ONCE BROKEN RODS AND MULTI SUBUNIT PROTEINS.
AU GARCIA DE LA TORRE J [Reprint author]; BLOOMFIELD V A
CS DEP QUIM FIS, UNIV EXTREMADURA, BADAJOZ, SPAIN
SO Biopolymers, (1978) Vol. 17, No. 7, pp. 1605-1628.
CODEN: BIPMAA. ISSN: 0006-3525.
DT Article
FS BA
LA ENGLISH
AB Previous theories of the translational and rotational frictional properties of multisubunit complexes were extended to calculate the intrinsic viscosity of such structures. This theory is similar to those recently constructed by McCammon and Deutch and by Nakajima and Wada, in that it uses a modified hydrodynamic interaction tensor and solves the system of simultaneous interaction equations by digital computation rather than by successive approximations. There are some differences in the formulation and averaging of these equations. Extensive numerical comparison is made between this theory and others that are available associated with the names of Hearst and Tagami, Abdel-Khalik and Bird, and Tsuda, using as a basis exact results for prolate ellipsoids of revolution. For large axial ratios, only this theory asymptotically approaches the correct limit; but for small axial ratios, only the Tsuda shell-model theory is adequate, because the other theories neglect the preponderant influence of the sphere located at the center of rotation. Intrinsic viscosities, translational frictional coefficients and Scheraga- Mandelkern β parameters, are tabulated for a large number of polygonal and polyhedral subunit structures, with up to 8 elements, using both this theory and Tsuda's. Particular application is made to hemerythrin and aspartate transcarbamylase. Finally, the viscosities and friction coefficients of once-broken rods are calculated and compared with an approximate theory by Wilenski.

L13 ANSWER 20 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2012:98207 CAPLUS Full-text
DN 159:255937
TI An exonic insertion within Tex14 gene causes spermatogenic arrest in pigs
AU Sironen, Anu; Uimari, Pekka; Venhoranta, Heli; Andersson, Magnus; Vilkki, Johanna
CS Biotechnology and Food Research, Genomics, Agrifood Research Finland, MTT, Jokioinen, FI-36100, Finland
SO BMC Genomics (2011), 12, 591
CODEN: BGMEET; ISSN: 1471-2164
DOI 10.1186/1471-2164-12-591
PB BioMed Central Ltd.
DT Journal; (online computer file)
LA English
AB Background: Male infertility is an increasing problem in all domestic species including man. Localization and identification of genes involved in defects causing male infertility provide valuable information of specific events in sperm development. Sperm development is a complex process, where diploid spermatogonia develop into haploid, highly specialized spermatozoa. Correct expression and function of various genes and their protein products are required for production of fertile sperm. We have identified an infertility defect in Finnish Yorkshire boars caused by spermatogenic arrest. The aim of this study was to locate the disease associated region using genome wide screen with the PorcineSNP60 Beadchip and identify the causal mutation by candidate gene approach. Results: In the Finnish Yorkshire pig population the spermatogenic arrest (SA) defect appears to be of genetic origin and causes severe degeneration of germ cells and total absence of spermatozoa. Genome wide scan with the PorcineSNP60 Beadchip localized the SA defect to porcine chromosome 12 in a 2 Mbp region. Sequencing of a candidate gene Tex14 revealed a 51 bp insertion within exon 27, which caused differential splicing of the exon and created a premature translation stop codon. The expression of Tex14 was markedly down regulated in the testis of a SA affected boar compared to control boars and no protein product was identified by Western blotting. The SA insertion sequence was also found within intron 27 in all analyzed animals, thus the insertion appears to be a possible duplication event. Conclusion: In this study we report the identification of a causal mutation for infertility caused by spermatogenic arrest at an early meiotic phase. Our results highlight the role of TEX14 specifically in spermatogenesis and the importance of specific genomic remodeling events as causes for inherited defects.
OSC.G 7 THERE ARE 7 CAPLUS RECORDS THAT CITE THIS RECORD (7 CITINGS)
RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 21 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2011:1355454 CAPLUS Full-text
DN 157:543534
TI A study of the spatial protein organization of the postsynaptic density isolated from porcine cerebral cortex and cerebellum
AU Yen, Yun-Hong; Chuang, Chih-Fan; Chang, Chia-Wei; Chang, Yen-Chung
CS Institute of Molecular Medicine, National Tsing Hua University, Hsinchu, Taiwan
SO Molecular and Cellular Proteomics (2011), 10(10), M110.007138, 20 pp.
CODEN: MCPOBS; ISSN: 1535-9484
URL: http://www.mcponline.org/content/10/10/M110.007138.full.pdf+html
DOI 10.1074/mcp.M110.007138
PB American Society for Biochemistry and Molecular Biology
DT Journal; (online computer file)
LA English
AB Postsynaptic d. (PSD) is a protein supramol. lying underneath the postsynaptic membrane of excitatory synapses and has been implicated to play important roles in synaptic structure and function in mammalian central nervous system. Here, PSDs were isolated from two distinct regions of porcine brain, cerebral cortex and cerebellum. SDS-PAGE and Western blotting analyses indicated that cerebral and cerebellar PSDs consisted of a similar set of proteins with noticeable differences in the abundance of various proteins between these samples. Subsequently, protein localization in these PSDs was analyzed by using the Nano-Depth-Tagging method. This method involved the use of three synthetic reagents, as agarose beads whose surface was covalently linked with a fluorescent, photoactivable, and cleavable chemical crosslinker by spacers of varied lengths. After its application was verified by using a synthetic complex consisting of four layers of different proteins, the Nano-Depth-Tagging method was used here to yield information concerning the depth distribution of various proteins in the PSD. The results indicated that in both cerebral and cerebellar PSDs, glutamate receptors, actin, and actin binding proteins resided in the peripheral regions within .apprx.10 nm deep from the surface and that scaffold proteins, tubulin subunits, microtubule-binding proteins, and membrane cytoskeleton proteins found in mammalian erythrocytes resided in the interiors deeper than 10 nm from the surface in the PSD. Finally, by using the immunoabsorption method, binding partner proteins of two proteins residing in the interiors, PSD-95 and α-tubulin, and those of two proteins residing in the peripheral regions, elongation factor-1α and calcium, calmodulin-dependent protein kinase II α subunit, of cerebral and cerebellar PSDs were identified. Overall, the results indicate a striking similarity in protein organization between the PSDs isolated from porcine cerebral cortex and cerebellum. A model of the mol. structure of the PSD has also been proposed here.
OSC.G 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)
RE.CNT 96 THERE ARE 96 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 22 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2010:1226916 CAPLUS Full-text
DN 153:422367
TI Microsphere having hot spots and method for identifying chemicals through surface enhanced Raman scattering using the same
IN Park, Se Jin; Chung, Taek Dong; Piao, Lilin; Boo, Han Kil
PA Cubiq, Inc., S. Korea
SO U.S. Pat. Appl. Publ., 11pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 20100245817	A1	20100930	US 2010-750648	20100330
KR 2010109264	A	20101008	KR 2009-27800	20090331
WO 2010114297	A2	20101007	WO 2010-KR1958	20100331
WO 2010114297	A3	20110120		

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA

PRAI KR 2009-27800 A 20090331
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB The present invention relates to the microsphere whose surface is covered with hot spots and the method for identifying chems. through Surface Enhanced Raman Scattering (SERS) using the same. The microsphere having hot spots, according to the present invention, includes a microsphere and metal networks as a shell which covers the surface of the microsphere, and nano-sized pores are distributed randomly on the surface or in the interstitial spaces of the metal networks. The microsphere having hot spots, according to the present invention, can be individually manipulated under a conventional optical microscope. SERS spectra of the monolayer of mols. on Pt or Au can be measured using single microsphere having hot spots mentioned as a sensitive probe. The microsphere having hot spots can be applied for decoding the microspheres with Raman tags flowing in a microfluidic system.
OSC.G 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)

L13 ANSWER 23 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2009:178294 CAPLUS Full-text
DN 151:269252
TI Air velocity characteristics within vented pallets loaded in a refrigerated vehicle with and without air ducts
AU Moureh, J.; Tapsoba, S.; Derens, E.; Flick, D.
CS UMR Genie Industriel Alimentaire, Genie des Procedes AN/Refrigerating Process Engineering Research Unit, Cemagref, AgroParisTech, Cemagref, INRA, Antony, 92163, Fr.
SO International Journal of Refrigeration (2008), Volume Date 2009, 32(2), 220-234
CODEN: IJRFDI; ISSN: 0140-7007
DOI 10.1016/j.ijrefrig.2008.06.006
PB Elsevier Ltd.
DT Journal
LA English
AB During transport using refrigerated vehicles, this being a vital link in the cold chain, the maintaining of even temperature throughout the cargo is essential in order to preserve the quality, safety and shelf life of perishable food. Within the refrigerated container, the temperature level and its homogeneity are directly governed by airflow patterns. The design of the air-distribution system should allow these airflows to compensate heat fluxes exchanged through the insulated walls or generated by the products. In this paper, a reduced-scale model and CFD predictions were used to investigate exptl. and numerically the airflow patterns within a typical refrigerated truck configuration loaded with vented pallets filled with spherical objects. The expts. were carried out using a laser Doppler velocimetry and thermal sphere-shaped probes located inside the pallets. The aim was to investigate air velocity characteristics above and within pallets. The performance of ventilation was characterized with and without supply air duct systems. Both configurations are extensively used in refrigerated transport. Full-scale measurements were also performed within a load of fruit during transport in a refrigerated truck in order to evaluate the temperature distribution under given operating conditions. The numerical modeling of airflow was performed using the computational fluid dynamics (CFD) fluent code and the Reynolds Stress Model (RSM) turbulence model. Numerical and exptl. data make it possible to evaluate the air ventilation levels and their heterogeneity between the pallets. The numerical predictions show reasonable agreement with exptl. data. The studied supply air duct system improves significantly the homogeneity of ventilation in the vehicle.
OSC.G 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)
RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 24 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2009:139767 CAPLUS Full-text
DN 150:186302
TI Measurement of multiple analytes using flow cytometry utilizing antibodies attached to microspheres
IN Thornthwaite, Jerry T.
PA NED, LLC, USA
SO PCT Int. Appl., 14pp.; Chemical Indexing Equivalent to 150:186301 (US)
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2009018210	A1	20090205	WO 2008-US71343	20080728
US 20090029347	A1	20090129	US 2007-829877	20070727

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRAI US 2007-829877 A 20070727
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB The present invention relates generally to laboratory diagnostics as related to the use of microspheres in flow cytometry. The present invention is directed to a method of using antibodies attached to different types of microspheres against different antigens located within a biol. sample. Optical and electronic particle detection may be used to sep. the microspheres via flow cytometry, allowing the subsequent measurement of multiple analytes in a single sample of body fluid by separating and gating such analytes based on the type of microsphere to which the analyte is coupled. According to the present invention, various biol. components may be attached to microspheres of different vols., shapes, conductivity, densities, and/or colors to detect biol. components by gating on the type of microsphere and analyzing the biol. component attached thereto.
RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 25 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2009:116595 CAPLUS Full-text
DN 150:186301
TI Measurement of multiple analytes using flow cytometry utilizing antibodies attached to microspheres
IN Thornthwaite, Jerry T.
PA Ned, LLC, USA
SO U.S. Pat. Appl. Publ., 6 pp., Chemical Indexing Equivalent to 150:186302 (WO)
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 2
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 20090029347	A1	20090129	US 2007-829877	20070727
WO 2009018210	A1	20090205	WO 2008-US71343	20080728

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRAI US 2007-829877 A 20070727
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB The present invention relates generally to laboratory diagnostics as related to the use of microspheres in flow cytometry. The present invention is directed to a method of using antibodies attached to different types of microspheres against different antigens located within a biol. sample. Optical and electronic particle detection may be used to sep. the microspheres via flow cytometry, allowing the subsequent measurement of multiple analytes in a single sample of body fluid by separating and gating such analytes based on the type of microsphere to which the analyte is coupled. According to the present invention, various biol. components may be attached to microspheres of different vols., shapes, conductivity, densities, and/or colors to detect biol. components by gating on the type of microsphere and analyzing the biol. component attached thereto.

L13 ANSWER 26 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2007:1381649 CAPLUS Full-text
DN 148:460869
TI Ion-collecting sphere in a stationary, weakly magnetized plasma with finite shielding length
AU Patacchini, Leonardo; Hutchinson, Ian H.
CS Plasma Science and Fusion Center, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA
SO Plasma Physics and Controlled Fusion (2007), 49(10), 1719-1733
CODEN: PPCFET; ISSN: 0741-3335
DOI 10.1088/0741-3335/49/10/009
PB Institute of Physics Publishing
DT Journal
LA English
AB Collisionless ion collection by a neg. biased stationary spherical probe in a finite shielding length plasma is investigated using the Particle in Cell code SCEPTIC, in the presence of a weak magnetic field B. The overall effect of the magnetic field is to reduce the ion current, linearly in |B| for weak enough fields, with a slope steepness increasing with the electron Debye length. The angular current distribution and space-charge buildup strongly depend on the focusing properties of the probe, hence on its potential and the plasma shielding length. In particular, it is found that the concavity of the ion collection flux distribution can reverse sign when the electron Debye length is comparable to or larger than the probe radius (λDe .gtorsim. rp), provided the ion temperature is much lower than the probe bias (Ti « -ZeVp).
OSC.G 10 THERE ARE 10 CAPLUS RECORDS THAT CITE THIS RECORD (10 CITINGS)
RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 27 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2007:574237 CAPLUS Full-text
DN 147:136723
TI Proteome analysis technologies based on mass spectrometry for quantitation, phosphoproteomics, and drug discovery
AU Oda, Yoshiya
CS Laboratory of Seeds Finding Technology, Eisai Co., Ltd., Tsukuba, Ibaraki, Japan
SO Journal of the Mass Spectrometry Society of Japan (2007), 55(2), 63-75
CODEN: JMSJEY; ISSN: 1340-8097
DOI 10.5702/massspec.55.63
PB Nippon Shitsuryo Bunseki Gakkai
DT Journal; General Review
LA Japanese
AB A review. Protein concentration is one of the most basic and important parameters in functional anal. because the kinetics/dynamics of cellular mols. is described by changes in the concns. of proteins in particular compartments. Mass spectrometry (MS) plays a central role in proteome studies and allows protein identification and quantitation at the same time. An MS-based method allows simultaneous identification and quantitation of individual proteins and determination of changes in the levels of modifications at specific sites on individual proteins. Accurate quantitation is achieved through the use of whole cell stable isotope labeling, and the seminal work on differential metabolic labeling of proteins by Oda et al. has led to many strategies for quant. proteomics including quant. tissue proteome anal. based on culture-derived isotope tag (CDIT). This is a generalized approach and affords a quant. description of cellular differences at the level of protein expression and modification, thus providing information that is critical to the understanding of complex biol. phenomena. In addition to this relative quant. proteomics, MS-based absolute quantitation is also possible by adding a known amount of standard peptides (e.g. isotope dilution), or reverse isotope dilution (CDIT). Although it has become feasible to rapidly identify proteins from crude cell exts. using mass spectrometry, it can be difficult to elucidate protein kinase substrates in the presence of a large excess of relatively abundant non-phosphoproteins. Therefore, for effective proteome anal. it becomes critical to enrich the sample of phosphorylated proteins. Although enrichment of phosphotyrosine-containing proteins has been achieved through the use of high-affinity anti-phosphotyrosine antibodies, we developed a method for enriching phosphoserine/threonine-containing proteins from crude cell exts. after chemical replacement of the phosphate moieties by affinity tags. Although this is an interesting method, the procedure is complicated and of low efficiency. Therefore, we developed a simple, highly specific enrichment procedure for phosphopeptides, by increasing the specificity of an immobilized metal affinity column (IMAC) without using any chemical reaction. The increase in selectivity was achieved by (a) using a strong acid to discriminate phosphates from carboxyl groups of peptides, (b) using a high concentration of acetonitrile to remove hydrophobic non-phosphopeptides, and (c) desalting the phosphate buffer used for competitive elution from the IMAC. By applying this efficient approach, we were able to identify more than one thousand phosphopeptides. Chemical proteomics is an effective approach to focused proteomics, having the potential to find specific interactors in moderate-scale comprehensive anal. Unlike chemical genetics, chemical proteomics directly and comprehensively identifies proteins that bind specifically to candidate compds. by means of affinity chromatog. purification using the immobilized candidate, combined with mass spectrometric identification of interacting proteins. This is an effective approach for discovering unknown protein functions, identifying the mol. mechanisms of drug action, and obtaining information for optimization of lead compds. However, immobilized-small mol. affinity chromatog. always suffers from the problem of nonspecific binders. Although several approaches have been reported to reduce nonspecific binding proteins, these are mainly focused on the use of low-binding-affinity beads or insertion of a spacer between the bead and the compound Stable isotope labeling strategies have proven particularly advantageous for the discrimination of true interactors from many nonspecific binders, including carrier proteins such as serum albumin, and are expected to be valuable for drug discovery.

L13 ANSWER 28 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2006:408793 CAPLUS Full-text
DN 144:428416
TI Device and method for analytical cell imaging
IN Trainer, Michael Norman
PA Immunivest Corporation, USA
SO U.S. Pat. Appl. Publ., 19 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 20060094109	A1	20060504	US 2004-979388	20041102

PRAI US 2004-979388 20041102

AB In patients with carcinomas tumor cells are shed into the blood, enumeration and characterization of these cells offers the opportunity to obtain a "real time" biopsy of the tumor and may improve the management of the disease. The frequency of circulating tumor cells is rare (<1 cell/mL) and technol. is needed that has sufficient sensitivity and specificity to enumerate and characterize these cells. The present system was developed to provide an immunophenotype, fluorescence wave forms as well as images of immunomagnetically enriched cells. Blood vols. ranging from 7.5-30 mL are immunomagnetically enriched for epithelial cells. The sample volume is reduced to 320 μl and inserted into an anal. chamber. Upon introduction of the chamber in a magnetic field, the immunomagnetically tagged cells rise out of the sample and align between nickel lines (period 30 μm, space 15 μm) that are present on the viewing surface of the chamber. A multi laser system is used to detect the fluorescence emitted by DAPI, Phycoerythrin and Allophycocyan labeled and magnetically aligned cells. Compact disk optics are used to maintain alignment and focus of the laser beams onto nickel lines while moving the chamber. The chamber is scanned with a speed of 10 mm/s and the entire chamber is analyzed in approx. 5 min. The fluorescent signals obtained from the events provide an immunophenotype similar to that of a flow cytometer. The fluorescence waveforms improve the characterization of the events and add to the classification as background, cellular debris and cells. Since the cell locations are preserved, objects that immunophenotypically classify as epithelial cells can be revisited for further anal. Bright field and fluorescent images of the selected objects are captured to confirm that the identified objects are tumor cells.

OSC.G 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)

L13 ANSWER 29 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2006:180045 CAPLUS Full-text
DN 144:428318
TI Cleavable Hydrophilic Linker for One-Bead-One-Compound Sequencing of Oligomer Libraries by Tandem Mass Spectrometry
AU Paulick, Margot G.; Hart, Kathryn M.; Brinner, Kristin M.; Tjandra, Meiliana; Charych, Deborah H.; Zuckermann, Ronald N.
CS Chiron Corporation, Emeryville, CA, 94608, USA
SO Journal of Combinatorial Chemistry (2006), 8(3), 417-426
CODEN: JCCHFF; ISSN: 1520-4766
DOI 10.1021/cc0501460
PB American Chemical Society
DT Journal
LA English
OS CASREACT 144:428318
AB The authors have developed a method for the rapid and unambiguous identification of sequences of hit compds. from one-bead-one-compound combinatorial libraries of peptide and peptoid ligands. The approach uses a cleavable linker that is hydrophilic to help reduce nonspecific binding to biol. samples and allows for the attachment of a halogen tag, which greatly facilitates post-screening sequencing by tandem mass spectrometry (MS/MS). The linker is based on a tartaric acid unit, which, upon cleavage from resin, generates a C-terminal aldehyde. This aldehyde can then be derivatized with a bromine-containing amino-oxy compound that serves as an isotope tag for subsequent MS/MS anal. of y-ion fragments. The authors have applied this linker and method to the syntheses of a number of peptoids that vary in sequence and length and have also demonstrated single-bead sequencing of a peptoid pentamer. The linker is also shown to have very low levels of nonspecific binding to proteins.
OSC.G 52 THERE ARE 52 CAPLUS RECORDS THAT CITE THIS RECORD (52 CITINGS)
RE.CNT 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 30 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2006:81815 CAPLUS Full-text
DN 144:288658
TI Microsphere sedimentation arrays for multiplexed bioanalytics
AU Moser, Christoph; Mayr, Torsten; Klimant, Ingo
CS Institute of Analytical Chemistry and Radiochemistry, Graz University of Technology, Graz, 8010, Austria
SO Analytica Chimica Acta (2006), 558(1-2), 102-109
CODEN: ACACAM; ISSN: 0003-2670
DOI 10.1016/j.aca.2005.11.066
PB Elsevier B.V.
DT Journal
LA English
AB A new measurement platform to analyze multiple assays in parallel in a single well of a standard microplate is presented. The approach is based on optically addressable encoded microbeads as carriers for a series of bioassays, a fluorescence microscope equipped with a LED as excitation source, a fast-gateable CCD-camera and automated image anal. Bead arrays are suspended in the analyte solution and targets are bound to receptors immobilized at the bead surface. Gravitational or magnetical sedimentation of the beads to the transparent bottom of the microplate leads to in situ formation of randomly ordered arrays. Beads are encoded by three features: size, luminescence intensity and luminescence decay time. Luminescence lifetime imaging in the microsecond range is introduced as a new technique for bead identification. Twenty-seven distinguishable bead classes were produced and characterized. The coeffs. of variation were 5.4% for size, 9% intensity and 1.3% for decay time. Based on these results, the number of potential codes is calculated to be about 140. Multiplexed DNA assays at detection limits of 2 nM were carried out to demonstrate the feasibility of the concept presented here.
OSC.G 19 THERE ARE 19 CAPLUS RECORDS THAT CITE THIS RECORD (19 CITINGS)
RE.CNT 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 31 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2005:696494 CAPLUS Full-text
DN 143:169179
TI Method for identifying bead location information
IN Sato, Keiichi
PA Hitachi Software Engineering Co., Ltd., Japan
SO U.S. Pat. Appl. Publ., 8 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 20050170400	A1	20050804	US 2005-41263	20050125
JP 2005214940	A	20050811	JP 2004-25890	20040202
JP 4344624	B2	20091014		

PRAI JP 2004-25890 A 20040202
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB Location information of carriers such as beads arranged inside a capillary represented by a probe array is detected accurately, and an identification method for various materials is developed substituting conventional bar codes for the beads. The arrangement order of a plurality of beads is identified on the basis of stain color information and location information concerning the plurality of stained beads.

L13 ANSWER 32 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2004:634179 CAPLUS Full-text
DN 141:159053
TI Hybrid random bead/chip based microarray
IN Moon, John; Kersey, Alan D.; Putnam, Martin A.; Li, Tuo
PA Cyvera Corporation, USA
SO PCT Int. Appl., 88 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 26
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004066210	A1	20040805	WO 2004-US1685	20040122
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ				
US 20040233485	A1	20041125	US 2003-661234	20030912
US 7106513	B2	20060912		
US 7349158	B2	20080325	US 2003-661031	20030912
US 7399643	B2	20080715	US 2003-661836	20030912
US 20040132205	A1	20040708		
WO 2005079545	A2	20050901	WO 2005-US5745	20050222
WO 2005079545	A3	20061116		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
US 20090034078	A1	20090205	US 2008-144209	20080623
US 7791802	B2	20100907		

PRAI US 2003-60441678 P 20030122
US 2003-661031 A 20030912
US 2003-661234 A 20030912
US 2003-661836 A 20030912
US 2003-60519932 P 20031114
US 2002-60410541 P 20020912
US 2003-645686 A2 20030820
US 2003-645689 A2 20030820
US 2004-60546445 P 20040219
US 2004-60547013 P 20040219
US 2005-63660 A1 20050222
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT

AB A hybrid random bead/chip based microarray includes multiple loose optical identification elements (or beads) comprising a substrate having ≥1 identifiable code. The elements are randomly placed on a substrate, e.g., tray, plate, slide, or chip, in an identifiable position on the slide (but not necessarily immobilized). E.g., the beads are randomly distributed (but oriented) in one dimension along grooves in the slide. Then, each bead code is read and its location determined, i.e., the bead code and location are mapped to its location on the chip. The beads on the chip may then be read/examined by a conventional chip reader. The beads may be functionalized with a desired probe and/or reacted with a desired analyte in the loose state and/or on the chip. After reading the chip, the beads may be removed from the chip for further or alternative processing.

OSC.G 7 THERE ARE 7 CAPLUS RECORDS THAT CITE THIS RECORD (7 CITINGS)
RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

Curio EX1010-p. 517
Curio v Prognosys
IPR2024-01289

L13 ANSWER 33 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2004:451556 CAPLUS Full-text
DN 141:3772
TI Simplified detection process for colored bead random microarrays
IN Kocher, Thomas E.
PA Eastman Kodak Company, USA
SO U.S. Pat. Appl. Publ., 6 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 20040106114	A1	20040603	US 2002-307664	20021202

PRAI US 2002-307664 20021202
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB This article discloses a method of manufacturing a colored microsphere mol. biol./chemical random microarray system, comprising: randomly dispersing on a support a composition including a first set of microspheres modified with a biol./chemical probe and containing an optical bar code generated from at least one colorant associated with the microspheres to produce a biol./chemical active region on the support; scanning the biol./chemical active region with a high-resolution color scanner to produce a color map of the locations of the randomly dispersed first set of microspheres; creating a digital file of the color map; and linking the digital file of the color map with the support.
OSC.G 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)

L13 ANSWER 34 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2002:444423 CAPLUS Full-text
DN 137:16488
TI Analysis of sequence tags with hairpin primers to amplify DNA and add an address sequence to the amplification product
IN Lizardi, Paul M.; Latimer, Darin R.
PA Yale University, USA
SO U.S., 42 pp., Cont.-in-part of U.S. 6,261,782.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 5
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6403319	B1	20020611	US 2000-637384	20000811
US 6261782	B1	20010717	US 2000-544713	20000406
AT 318932	T	20060315	AT 2000-954029	20000811
US 20020106649	A1	20020808	US 2001-855793	20010515
US 6677121	B2	20040113		

PRAI US 1999-60148870 P 19990813
US 2000-544713 A2 20000406
US 1999-60127932 P 19990406
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB Disclosed is a method for the comprehensive anal. of nucleic acid samples and a detector composition for use in the method. The method involves amplifying nucleic acid fragments of interest using a primer that can form a hairpin structure; sequence-based coupling of the amplified fragments to detector probes; and detection of the coupled fragments. The amplified fragments are coupled by hybridization and coupling, preferably by ligation, to detector probes. A hairpin structure formed at the end of the amplified fragments facilitates coupling of the fragments to the probes. The hairpin sequence can be unique to drive the extension product to a specific probe on a microarray. The method allows detection of the fragments where detection provides some sequence information for the fragments. The method allows a complex sample of nucleic acid to be quickly and easily cataloged in a reproducible and sequence-specific manner. The method can also be used to detect amplified fragments having a known sequence.
OSC.G 14 THERE ARE 14 CAPLUS RECORDS THAT CITE THIS RECORD (14 CITINGS)
RE.CNT 73 THERE ARE 73 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 35 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2002:368747 CAPLUS Full-text
DN 136:352271
TI Fluorescence and FRET based assays for biomolecules on beads
IN Buranda, Tione; Huang, Jinman; Perez-Luna, Victor H.; Lopez, Gabriel P.; Simons, Peter; Sklar, Larry A.
PA Science & Technology Corporation UNM, USA
SO PCT Int. Appl., 71 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002039083	A2	20020516	WO 2001-US42983	20011106
WO 2002039083	A3	20020711		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002030419	A	20020521	AU 2002-30419	20011106
US 20020081617	A1	20020627	US 2001-985873	20011106
PRAI US 2000-60246564	P	20001108		
WO 2001-US42983	W	20011106		

ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB The invention concerns a sensing device comprising: a vessel; a plurality of sensor beads located within the vessel to form interstitial spaces therethrough; and a plurality of biomols. bound to at least at portion of the plurality of beads, each of the biomols. having a fluorescent tag. The invention also provides a method for detecting the binding of two biomols. comprising the following steps: providing a plurality of first biomols., each of the first biomols. having a first fluorescent tag, each of the first biomols. being bound to a resp. substrate of a plurality of substrate; providing a plurality of second biomols., each of the second biomols. having a second fluorescent tag, binding at least portion of the second biomols. to at least a portion of the first biomols. to form complexes, wherein the plurality of first biomols. and the plurality of second biomols. prior to the binding step have a pre-complexing total fluorescence and wherein the complexes and free second biomols. after the binding step have a post-complexing total fluorescence; and detecting any difference between the pre-complexing total fluorescence and the post-complexing total fluorescence. A sensing device comprising a suspension of a plurality of sensor beads; and a plurality of biomols. bound to at least a portion of the plurality of beads, each of the biomols. having a fluorescent tag is also provided. Diagrams describing the apparatus assembly and operation are given.
OSC.G 14 THERE ARE 14 CAPLUS RECORDS THAT CITE THIS RECORD (14 CITINGS)
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 36 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2002:291694 CAPLUS Full-text
DN 136:291315
TI Method and device for the integrated synthesis of probes and their usage for analysis on a carrier chip
IN Staehler, Cord F.; Gueimil, Ramon; Scheffler, Matthias; Staehler, Peer F.; Heidbrede, Anke
PA Febit Ferrarius Biotechnology G.m.b.H., Germany
SO Ger. Offen., 20 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 2
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 10051396	A1	20020418	DE 2000-10051396	20001017
WO 2002032567	A1	20020425	WO 2001-EP12027	20011017
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002010552	A	20020429	AU 2002-10552	20011017
EP 1330307	A1	20030730	EP 2001-978431	20011017
EP 1330307	B1	20050907		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
AT 303863	T	20050915	AT 2001-978431	20011017
US 20040043509	A1	20040304	US 2003-399450	20030801
US 7470540	B2	20081230		
US 20090156423	A1	20090618	US 2008-333417	20081212

PRAI DE 2000-10051396 A 20001017
US 2000-60240793 P 20001017
WO 2001-EP12027 W 20011017
US 2003-399450 A1 20030801
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB The invention concerns a method and a device for the synthesis of probes, especially nucleic acids on microspheres that are immobilized onto a carrier chip; the probes on the microspheres are used for anal. in combination with the detection capabilities of the carrier chip. After anal. the beads are discarded and the more valuable carrier chip is reused. The chip can be thermostated, it is usually transparent for spectroscopic detection, and can be involved in the non-permanent immobilization of the microspheres using elec. or electromagnetic field effects. As a first step the microspheres are immobilized onto the carrier chip either permanently or non-permanently. The second step is the synthesis of the probes on the beads; different probes can be formed on predetd. locations of the chip; the procedure involves the microflow of monomers and other reagents to the beads. The final step is the usage of the prepared array for anal., e.g. nucleic acid hybridization and fluorometric detection.
OSC.G 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)
RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 37 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2001:904602 CAPLUS Full-text
DN 136:32666
TI Address/capture oligonucleotide tags on microspheres for flow-cytometry based minisequencing
IN White, P. Scott; Torney, David C.
PA The Regents of the University of California, USA
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001094639	A1	20011213	WO 2001-US18590	20010607
WO 2001094639	A9	20021010		
AU 2001068269	A	20011217	AU 2001-68269	20010607
US 20030190609	A1	20031009	US 2001-877819	20010607
US 20050147998	A1	20050707	US 2004-992971	20041118

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 2000-60210759 P 20000608
US 2001-877819 B1 20010607
WO 2001-US18590 W 20010607
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB A method for generating address/capture tags for use in a sensitive and rapid flow-cytometry based assay for the multiplexed anal. of SNPs based on polymerase-mediated primer extension using microspheres as solid supports is described. Single-nucleotide polymorphisms (SNPs) are the most abundant type of human genetic variation. These variable sites are present at high d. in the genome, making them powerful tools for mapping and diagnosing disease-related alleles. Subnanomolar concns. of sample in small vols. (10 mL) can be analyzed at rates greater than one sample per min, without a wash step. Genomic anal. using multiplexing microsphere arrays, enables the simultaneous anal. of dozens, and potentially hundreds of SNPs per sample. The method has been tested by genotyping the Glu69 variant from the HLA DPB1 locus, an SNP associated with chronic beryllium disease, as well as HLA DPA1 alleles.
RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

Curio EX1010-p. 522
Curio v Prognosys
IPR2024-01289

L13 ANSWER 38 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2000:402289 CAPLUS Full-text
DN 133:174117
TI Capillary Electrochromatography of Proteins on an Anion-Exchanger Column
AU Zhang, Jian; Huang, Xian; Zhang, Shuhong; Horvath, Csaba
CS Department of Chemical Engineering, Yale University, New Haven, CT, 06520, USA
SO Analytical Chemistry (2000), 72(14), 3022-3029
CODEN: ANCHAM; ISSN: 0003-2700
DOI 10.1021/ac000114t
PB American Chemical Society
DT Journal
LA English
AB Capillary electrochromatog. (CEC) of proteins was carried out using 50-μm-i.d. fused-silica capillaries packed with 5-μm silica beads having strong anion-exchanger functions attached to hydrophilic spacers at the chromatog. surface. The siliceous microspheres and the capillary inner wall were treated first with a heterobifunctional silanizing agent and reacted subsequently with a vinyl monomer containing quaternary ammonium groups to form a "tentacular" anion exchanger. A mixture of bovine carbonic anhydrase, α-lactalbumin, soybean trypsin inhibitor, and ovalbumin was separated using CEC by isocratic elution in the codirectional mode with aqueous phosphate buffer, pH 7.0, containing sodium chloride. The retention mechanism of isocratic CEC for proteins on the anion-exchanger column was illustrated by the results of a study on the effect of salt concentration on the separation The potential of CEC for protein separation with high resolution was also demonstrated by electrochromatograms of conalbumin and Hb variants. The results shed light on the mechanism of protein separation by isocratic CEC, which is believed to be a combination of chromatog. retention by electrostatic interactions and electrophoretic migration. Assuming that the contributions of the two mechanisms to the overall migration velocity are additive, an electrochromatog. resolution equation was derived and compared to the resolution equation in HPLC to reveal the constituents responsible for the enhancement of resolution by CEC with respect to that in HPLC. The advantage of CEC was also examined by comparing peak capacities in CEC on an isocratic platform with peak capacities obtained with isocratic and gradient elution HPLC.
OSC.G 59 THERE ARE 59 CAPLUS RECORDS THAT CITE THIS RECORD (59 CITINGS)
RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 39 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 2000:351749 CAPLUS Full-text
DN 132:345123
TI Device and method for analyzing a biologic sample
IN Lea, Peter
PA Biophys Inc., Can.
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000029847	A2	20000525	WO 1999-CA1079	19991112
WO 2000029847	A3	20000908		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6403384	B1	20020611	US 1999-335732	19990618
CA 2350789	A1	20000525	CA 1999-2350789	19991112
AU 2000011440	A	20000605	AU 2000-11440	19991112
BR 9915406	A	20010724	BR 1999-15406	19991112
EP 1131632	A2	20010912	EP 1999-972308	19991112
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002530648	T	20020917	JP 2000-582799	19991112
JP 4544498	B2	20100915		
CA 2289416	C	20050215	CA 1999-2289416	19991112
TW 468046	B	20011211	TW 1999-120038	19991117
WO 2000078917	A1	20001228	WO 2000-US13056	20000512
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000047126	A	20010109	AU 2000-47126	20000512

PRAI CA 1998-2254223 A 19981116
US 1999-335732 A 19990618
WO 1999-CA1079 W 19991112
WO 2000-US13056 W 20000512
ASSIGNMENT HISTORY FOR US PATENT AVAILABLE IN LSUS DISPLAY FORMAT
AB A device for separating fluid from a biol. sample when the sample has a fluid and non-fluid component. The device is a point-of-care device through which data may be electronically collected and electronically transmitted for further evaluation. A method for separating fluid from a biol. sample is provided wherein the method comprises the step of bringing the fluid sample in fluid contact with the microspheres such that the fluid component moves by capillary action between the microspheres along capillary channels formed by the spaces between the spheres and leaving, for example, a cellular fraction behind. In the device of the present invention, the step of separating the fluid may be combined with other assay techniques for detecting and/or measuring one or more analytes which may be present in the fluid sample such as immunoassays and chromatog. assays. These may be further combined with groups of microspheres for use in the analyte detection step as well as the separation step whereby the microspheres act as labels for the analyte or as a source of label for the analyte.
OSC.G 10 THERE ARE 10 CAPLUS RECORDS THAT CITE THIS RECORD (10 CITINGS)
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

Curio EX1010-p. 525
Curio v Prognosys
IPR2024-01289

L13 ANSWER 40 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 1998:119468 CAPLUS Full-text
DN 128:146375
OREF 128:28697a,28700a
TI Reconstruction of spherical torus (low-aspect ratio tokamak) equilibria in absence of magnetic measurements in the central cavity
AU Alladio, F.; Micozzi, P.
CS Centro Ricerche Frascati, Associazione Euratom-ENEA Fusione, Frascati, Italy
SO Nuclear Fusion (1997), 37(12), 1759-1774
CODEN: NUFUAU; ISSN: 0029-5515
DOI 10.1088/0029-5515/37/12/I08
PB International Atomic Energy Agency
DT Journal
LA English
AB Magnetic measurements alone on spherical tori should allow a very good separation of the poloidal beta βP from the internal self-inductance li/2 and should even permit an accurate estimate of the c.d. jΦ profile. However, the reduced space allowed for magnetic sensors near the central conductor in a spherical tokamak, and the possibility of producing flux core spheromak configurations without a central conductor, could imply that magnetic probes are not present in the cavity of the spherical torus. The fluxes and fields of a variety of calculated spherical torus configurations, all endowed with a single or double null separatrix, are analyzed in terms of spherical multipolar moments obtained from simulated magnetic measurements located only upon a sphere surrounding the spherical plasma. The solution to the problem of the absence of magnetic measurements in the cavity of the spherical torus is to fix from non-magnetic measurements (e.g., spectroscopy) the plasma inboard boundary rin on the equatorial plane. This constraint is added to the constraints of matching the spherical multipolar expansion in an iterative solution of the Grad-Shafranov equation, on the basis of a spherical geometry. The convergence of the spherical reconstructive equilibrium code is extremely fast and gives an error on the total plasma current IP of less than 1% at an aspect ratio A = 1.2, an error on the position of the plasma boundary of less than 2% of the radius of the plasma sphere, an error on βP of at most 15% and, finally, the jΦ profile is extremely well reconstructed in peaked, flat and even hollow cases. The effect of an uncertainty ±δrin upon the spectroscopic identification of the plasma inboard boundary on the equatorial plane rin is assessed.
OSC.G 3 THERE ARE 3 CAPLUS RECORDS THAT CITE THIS RECORD (3 CITINGS)

L13 ANSWER 41 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 1994:503633 CAPLUS Full-text
DN 121:103633
OREF 121:18554h,18555a
TI Complex combinatorial chemical libraries encoded with tags
IN Still, W. Clark; OHL-Meyer, Michael H. J.; Wigler, Michael; Dillard, Lawrence; Reader, John
PA Columbia University, USA; Cold Spring Harbor Lab.
SO PCT Int. Appl., 147 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 4
PI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9408051	A1	19940414	WO 1993-US9345	19931001
W: AT, AU, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KR, LU, NL, NO, NZ, PL, RO, RU, SE, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, PT, SE				
AU 9455369	A	19940426	AU 1994-55369	19931001
AU 686579	B2	19980212		
EP 665897	A1	19950809	EP 1994-900350	19931001
EP 665897	B1	20030709		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
HU 72495	A2	19960528	HU 1995-952	19931001
JP 08506175	T	19960702	JP 1994-509330	19931001
IL 107166	A	20001031	IL 1993-107166	19931001
AT 244769	T	20030715	AT 1994-900350	19931001
PT 665897	E	20031128	PT 1994-900350	19931001
ES 2204910	T3	20040501	ES 1994-900350	19931001
CA 2143848	C	20070911	CA 1993-2143848	19931001
NO 9501230	A	19950330	NO 1995-1230	19950330
US 6001579	A	19991214	US 1995-485018	19950607
AU 9745258	A	19980212	AU 1997-45258	19971117
AU 716621	B2	20000302		

PRAI US 1992-955371 A 19921001
US 1993-13948 A 19930204
US 1993-130271 B2 19931001
WO 1993-US9345 W 19931001
US 1993-159861 B2 19931130
US 1994-227007 A3 19940413
OS MARPAT 121:103633
AB Methods and compns. are provided for encoded combinatorial chemical, whereby at each stage of the synthesis, a support such as a particle upon which a compound is being synthesized is uniquely tagged to define a particular event, usually chemical, associated with the synthesis of the compound on the support. The tagging is accomplished using identifier mols. which record the sequential events to which the supporting particle is exposed during synthesis, thus providing a reaction history for the compound produced on the support. Various products can be produced in the multi-stage synthesis, such as oligomers and synthetic nonrepetitive organic mols. Conveniently, nested families of compds. can be employed as identifiers, where number and/or position of a substituent define the choice. Alternatively, detectable functionalities may be employed, such as radioisotopes, fluorescers, halogens, and the like, where presence and ratios of two different groups can be used to define stage or choice. Particularly, pluralities of identifiers may be used to provide a binary or higher code, so as to define a plurality of choices with only a few detachable tags. The particles may be screened for a characteristic of interest, particularly binding affinity, where the products may be detached from the particle or retained on the particle. The reaction history of the particles which are pos. for the characteristic can be determined by the release of the tags and anal. to define the reaction history of the particle. An encoded combinatorial library of 2401 peptides was prepared (by solid phase synthesis) having the sequence (X4)EEDLGGGG (X = Asp, Glu, Ile, Lys, Leu, Gln, or Ser). The 4 Gly served as a spacer between the encoded amino acid sequence and the bead. The library included the sequence KLISEEDL, part of the epitope bound by monoclonal antibody 9E10 to the human C-myc gene product. The identifiers used were 2-nitro-4-carboxybenzyl O-aryl-substituted ω-hydroxyalkyl carbonates (aryl = pentachlorophenyl, 2,4,6-trichlorophenyl, or 2,6-dichloro-4-fluorophenyl) and were attached via their carboxylic acids to tag free amino groups

on each bead. The tags were released from each selected bead by photolysis, silylated, and analyzed by electron capture gas chromatog. The binary synthesis code of the bead was directly determined from the chromatogram of the tags.

OSC.G 75 THERE ARE 75 CAPLUS RECORDS THAT CITE THIS RECORD (82 CITINGS)

L13 ANSWER 42 OF 43 CAPLUS COPYRIGHT 2019 ACS on STN
AN 1986:1614 CAPLUS Full-text
DN 104:1614
OREF 104:299a,302a
TI Exon-intron structure of eukaryote genes can be due to the chromatin nucleosome organization and to the features of the gene expression regulation connected with it
AU Solov'ev, V. V.; Kolchanov, N. A.
CS Inst. Tsitol. Genet., Novosibirsk, USSR
SO Doklady Akademii Nauk SSSR (1985), 284(1), 232-7 [Biochem.]
CODEN: DANKAS; ISSN: 0002-3264
DT Journal
LA Russian
AB A model for chromatin nucleosome formation is proposed wherein coding DNA (exons), which is located in the nucleosome, is split, and noncoding DNA (introns) is inserted to ensure proper bead-on-string organization. In support of the model, contextual anal. of the sequences of several eukaryotic genes showed histone-binding AA, TT, AT, and TA pairs, which are near sites of nucleosome formation initiation, to occur at a 1.5-3-fold greater concentration in introns than in exons. Polytracts (A or T), which packed poorly into nucleosome structures, are found at the 5' end of genes and may insure proper nucleosome distribution along the DNA. Enhancer-like sequences, which are capable of Z-DNA formation, are also detected in intron segments. This specific chromatin structure, dictated by intron-exon organization, ensures the accessibility of regulatory regions to proteins which participate in transcription.
OSC.G 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)

L13 ANSWER 43 OF 43 MEDLINE ® on STN
AN 2011894924 MEDLINE Full-text
DN PubMed ID: 21715321
TI A study of the spatial protein organization of the postsynaptic density isolated from porcine cerebral cortex and cerebellum.
AU Yun-Hong Yen
CS Institute of Molecular Medicine, National Tsing Hua University, Hsinchu, Taiwan.
AU Chih-Fan Chuang; Chia-Wei Chang; Yen-Chung Chang
SO Molecular & cellular proteomics : MCP, (2011 Oct) Vol. 10, No. 10, pp. M110.007138. Electronic Publication Date: 28 Jun 2011
Journal code: 101125647. E-ISSN: 1535-9484. L-ISSN: 1535-9476.
Report No.: PMC-PMC3205859.
DOI http://dx.doi.org/10.1074/mcp.M110.007138
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS MEDLINE; Priority Journals
FS Print; Electronic
EM 201202
ED Entered STN: 11 Oct 2011
Last Updated on STN: 4 Feb 2012
Entered Medline: 3 Feb 2012
OSC.G 5 There are 5 MEDLINE records that cite this record
REM.CNT 92 There are 92 cited references available in MEDLINE for this document.
AB Postsynaptic density (PSD) is a protein supramolecule lying underneath the postsynaptic membrane of excitatory synapses and has been implicated to play important roles in synaptic structure and function in mammalian central nervous system. Here, PSDs were isolated from two distinct regions of porcine brain, cerebral cortex and cerebellum. SDS-PAGE and Western blotting analyses indicated that cerebral and cerebellar PSDs consisted of a similar set of proteins with noticeable differences in the abundance of various proteins between these samples. Subsequently, protein localization in these PSDs was analyzed by using the Nano-Depth-Tagging method. This method involved the use of three synthetic reagents, as agarose beads whose surface was covalently linked with a fluorescent, photoactivable, and cleavable chemical crosslinker by spacers of varied lengths. After its application was verified by using a synthetic complex consisting of four layers of different proteins, the Nano-Depth-Tagging method was used here to yield information concerning the depth distribution of various proteins in the PSD. The results indicated that in both cerebral and cerebellar PSDs, glutamate receptors, actin, and actin binding proteins resided in the peripheral regions within .apprx. 10 nm deep from the surface and that scaffold proteins, tubulin subunits, microtubule-binding proteins, and membrane cytoskeleton proteins found in mammalian erythrocytes resided in the interiors deeper than 10 nm from the surface in the PSD. Finally, by using the immunoabsorption method, binding partner proteins of two proteins residing in the interiors, PSD-95 and α-tubulin, and those of two proteins residing in the peripheral regions, elongation factor-1α and calcium, calmodulin-dependent protein kinase II α subunit, of cerebral and cerebellar PSDs were identified. Overall, the results indicate a striking similarity in protein organization between the PSDs isolated from porcine cerebral cortex and cerebellum. A model of the molecular structure of the PSD has also been proposed here.

=> d his

(FILE 'HOME' ENTERED AT 13:36:30 ON 12 APR 2019)

FILE 'BIOSIS, CAPLUS, MEDLINE' ENTERED AT 13:36:48 ON 12 APR 2019
L1 392 S ((LOCAT? OR ADDRESS? OR SPAC?)(20A)(BEAD? OR MICORBEAD? OR SP
L2 326 DUP REM L1 (66 DUPLICATES REMOVED)
L3 85 S ((LOCAT? OR ADDRESS OR SPAC?)(20A)(BEAD? OR MICROBEAD? OR SPH
L4 252 S L2 NOT L3
L5 245 S L4 NOT 2019/PY
L6 232 S L5 NOT 2018/PY
L7 224 S L6 NOT 2017/PY
L8 210 S L7 NOT 2016/PY
L9 205 S L8 NOT 2015/PY

Curio EX1010-p. 530
Curio v Prognosys
IPR2024-01289

```
L10         193 S L9 NOT 2014/PY
L11         174 S L10 NOT 2013/PY
L12         163 S L11 NOT 2012/PY
L13          43 S L12 AND (APTAMER# OR OLIGONUCLEOTIDE# OR NUCLEOTIDE# OR PROBE

=> log y
COST IN U.S. DOLLARS                      SINCE FILE     TOTAL
                                          ENTRY   SESSION
FULL ESTIMATED COST                         543.50    543.90

STN INTERNATIONAL LOGOFF AT 13:43:42 ON 12 APR 2019
```