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(54) **Title**: FUNCTIONALLY INTEGRATED DEVICE FOR MULTIPLEX GENETIC IDENTIFICATION AND METHOD FOR SEPARATION AND DETECTION OF DNA FRAGMENTS

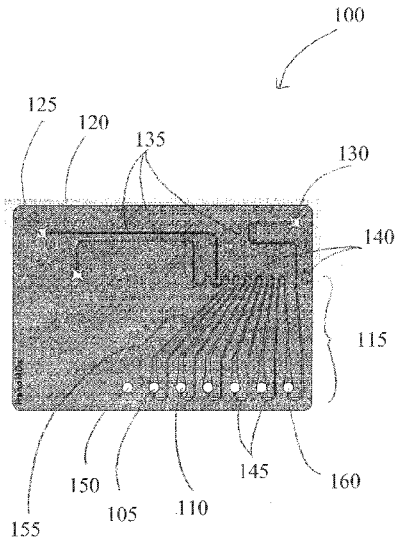



FIG. 1

(57) **Abstract**: A bsochip for multiplex genetic identification is disclosed. An bsochip for separating and detecting a plurality of DNA fragments includes a set of inputs and chambers for receiving a sample matrix of genetic material and reagents needed to conduct a polymerase chain reaction amplification of the genetic material. The bioehip also includes a plurality of separation and detection chambers that physically separate different DNA fragments that have been marked with labels that emit similar colors. A method of detecting nucleic acid fragments is also provided. Each chamber has at least one set of probes disposed therein, each set of probes being capable of binding to a different target nucleic acid sequence relative to the other sets of probes. The method includes providing a sample comprising a plurality of sets of nucleic acid fragments, placing at least a portion of the sample into each of the plurality of chambers.

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TITLE OF THE INVENTION

**Functionally Integrated Device for Multiplex Genetic Identification and Method for Separation and Detection of DNA Fragments**

CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of the following patent applications, the contents of which are incorporated by reference herein:

U.S. Provisional Application No. 61/678,363, entitled "Method for Separation and Detection of DNA Fragment," filed August 1, 2012;

U.S. Provisional Application No. 61/678,364, entitled "Functionally Integrated Device for Multiplex Genetic Identification," filed August 1, 2012;

U.S. Application No. 13/955,690, entitled "Functionally Integrated Device for Multiplex Genetic Identification," filed July 31, 2013;

U.S. Application No. 13/955,641 entitled "Method for Separation and Detection of DNA Fragments", filed July 31, 2013; and

U.S. Application No. 13/955,659 entitled "Enhanced Method for Probe Based Detection of Nucleic Acids", filed July 31, 2013.

BACKGROUND OF THE INVENTION

Field of Invention

**[0002]** The invention generally relates to methods and systems for multiplex DNA analysis in an integrated biochip, and, more specifically, to methods and systems that enable multiple target DNA fragments in a sample to be detected while reducing interference between the detected targets. The invention also relates to nucleic acid amplification, detection, and analysis of nucleotide molecules and sequences.

Description of Related Art

**[0003]** Integrated biochips, also referred to as microfluidics or lab-on-a-chip, have gained increasing attention in recent years especially in clinical diagnostics since they are amiable for self-contained, portable, point of care devices. These devices enable performing several

biotechnology tasks in a single device thereby minimizing the need for a large laboratory setup and skilled laboratory personal to perform diagnostic and research tests. Some of the popular applications of biochips include cancer detection, environmental testing, forensics, pathogen identification, gene-expression, SNP detection, to name a few.

**[0004]** To date there have been several demonstration of integrated biochips, some of which illustrate processing sample for cell lysis, DNA extraction, PCR (polymerase chain reaction) or isothermal amplification and detection. We use the genetic term "amplification" to mean PCR and isothermal amplifications. These terms are interchangeable unless specified otherwise. For example, the integration of sample preparation and amplification to detection strategies such as capillary electrophoresis, mass spectroscopy, qPCR, and microarray have been widely demonstrated. However, it is known that the final DNA fragment detection method determines the limitations on the number and size of the DNA fragments that can be amplified by PCR or isothermal amplification.

**[0005]** Typically, one amplified DNA fragment can correspond to one organism/disease identification. Hence, the ability to amplify several DNA fragments and uniquely detect each of these fragments provides a better platform for identification from a single experiment, both for research and clinical diagnostics. In this regard, in recent years multiplex PCR and isothermal amplification reactions have gained attention since it is capable of generating several DNA fragments to identify many organisms/diseases from a single reaction. Nevertheless, it is the DNA detection strategy employed post multiplex amplification that determines the number (or size) of the DNA fragments that can be uniquely detected.

**[0006]** Capillary electrophoresis (CE) and mass spectroscopy are popular approaches for detecting several DNA fragments generated by multiplex PCR/isothermal amplification. However, both these methods impose the limitation that the amplified DNA fragments must be of unique sizes and the sizes of the DNA fragments should be larger than the separation resolution of the instrument/system. Furthermore, both capillary electrophoresis and mass spectroscopy suffer from an inability to discriminate non-specific amplification if those non-specific DNA fragments are similar in size to any of the other DNA fragments generated by multiplex PCR/isothermal amplification. Also, since capillary or microfluidic electrophoresis in biochips utilize micro fluidic path for DNA migration, they require expensive excitation and emission optics (*e.g.*, lasers, CCD, photomultipliers) to sensitively detect low concentration fluorescence in biochips. Nevertheless, the proven approach of CE for DNA fragment analysis has rendered this method for the widespread use in integrated biochips for over a decade.

[0007] In contrast, traditional qPCR using the TaqMan probe method has better specificity but is limited to the detection of DNA fragments that can be labeled by uniquely colored fluorophore, determined by the instrument color detection capability (e.g., known systems are believed to be limited to nine colors). Hence, integrated biochips that utilize the qPCR method are limited to scanning and identifying fewer than nine DNA fragments, if the capability of a nine color qPCR system is employed. However, qPCR systems typically only demand low cost excitation and emission optics (e.g. LED, flash lamps, and photodiodes) and this enables low-cost instrumentation for this method.

[0008] Another approach that increases detection capability for multiplex PCR/isothermal amplification is the use of microarray technology coupled with PCR/isothermal amplification. In such a method, a biochip that performs PCR or isothermal amplification is then coupled with a microarray method downstream. The feasibility to spot several hundred detection regions in the microarray technology makes it appealing for multiplex PCR/isothermal amplification. However, similar to most high sensitivity electrophoresis systems, microarray detection also requires the use of a high power spot-excitation source for the fluorophores hence demanding the use of expensive fluorescence excitation and optical detection components (e.g. lasers, CCD, photomultipliers).

[0009] From an instrument cost perspective, of the numerous commercially available instruments, qPCR systems (e.g. Applied Biosystems, BioRad, Roche, Eppendorf) are typically several-fold less expensive than electrophoresis (e.g. ABI, Qiagen, Agilent) and microarray systems (e.g. Affymetrix, Molecular Devices, Roche, Tecan) for comparable applications.

[0010] Meanwhile, the DNA fragment detection strategy employed in the integrated biochip, methods, and systems described herein enable a much higher degree of multiplexing as compared to traditional qPCR, while only requiring similar inexpensive optics conventionally used in qPCR. The increased fragment detection capability enables the disclosed techniques to identify more organisms/diseases from a single test as compared to the popular and conventional qPCR technology. Thus, embodiments of the invention enable the use of the relatively low-cost instrumentation of qPCR optics while increasing the fragment detection capability by several folds.

[0011] Furthermore, certain embodiments of the invention include universal fit Luer taper-type connectors integrated into the surface of the biochip for quick connection while maintaining an air/water-tight seal of reagent pre-filled syringes/cartridges to the biochip.

[0012] When a detection method is applied to multiplex-PCR-amplified DNA fragments, similar size molecules will be indistinguishable (i.e., similar size molecules will migrate at identical speeds). Therefore, this size-dependence limitation requires that each unique multiplex-PCR-amplified DNA fragment should be of unique molecular size, and sufficiently unique to distinguish the fragments based on the separation resolution of the instrument. Furthermore, this size-dependence limitation also prevents capillary electrophoresis and mass spectroscopy methods from distinguishing between PCR-amplified DNA fragments featuring specifically-targeted DNA molecules or sequences and non-targeted PCR-amplified DNA fragments that may be of similar size.

[0013] Since the advent of nucleic acid-related technology decades ago, a number of methods have been developed for the detection and analysis of nucleic acid molecules (e.g., DNA). Some examples are capillary electrophoresis (including microfluidic electrophoresis), mass spectroscopy, southern blotting, and quantitative polymerase chain reaction (PCR), which may include real-time PCR methods and the use of TaqMan® probes (Roche Molecular Systems, Inc., Pleasanton, CA).

[0014] These detection methods have applications in, for example, *in vitro* DNA sequencing, gene expression quantification, genetic modification, genetic fingerprinting to identify a person or organism (e.g., for paternity testing, forensic science, and evolutionary studies), and diagnosis of disease (e.g., malignant cancers, hereditary diseases, and infectious agents). Often, the detection methods are paired with a PCR or similar nucleic acid amplification processes, which amplifies (i.e., replicates) the target DNA molecule or sequence in order to generate a sufficient amount of target DNA fragments to be detected. More recently, multiplex PCR was developed to amplify more than one unique target DNA molecule or sequence with a single PCR reaction.

[0015] In both capillary electrophoresis and mass spectroscopy, DNA molecules or sequences are separated and detected based on their molecular size/weight.

[0016] Quantitative or real-time PCR methods, often using TaqMan® probes, are widely used for the detection and analysis of PCR-amplified DNA fragments. A TaqMan® probe produces fluorescence when successfully bonded to a PCR-amplified DNA fragment. However, in a multiplex PCR reaction where more than one unique target DNA molecule or sequence is replicated, unique (i.e., differently colored) TaqMan® probes are necessary to distinguish the fluorescence from each of the DNA fragments. The signal sensitivity of and the capacity of the fluorescence detection instrument to distinguish the different fluorescent colors remain limiting factors, especially when the fluorescence emission spectra overlap.

## BRIEF SUMMARY OF THE INVENTION

**[0017]** Embodiments of the invention include a functionally integrated biochip for multiplex genetic identification. The integrated biochip includes a plurality of separation and detection chambers for receiving a sample material that contains a plurality of sets of DNA fragments of different types. Each of the plurality of separation and detection chambers is spaced apart from each of the other chambers, and each of the separation and detection chambers has a set of DNA probe disposed therein. The set of DNA probes disposed in each of the separation and detection chambers differ in type from those disposed in the other chambers.

**[0018]** In some embodiments, the biochip also includes inputs and chambers for receiving a sample matrix of genetic material and reagents needed to conduct a polymerase chain reaction or isothermal amplification of the genetic material to supply the plurality of collections of DNA fragments.

**[0019]** In other embodiments, to minimize operator intervention and to realize a completely closed biochip system, an integrated reagent cartridge coupled to the biochip which stores all required buffers and waste liquid is provided.

**[0020]** In some embodiments, a biochip for multiplex genetic identification is provided. The biochip includes a sample input port to receive a sample containing a plurality of DNA fragments and a plurality of separation and detection chambers in fluid communication with the sample input port. Each of the plurality of separation and detection chambers is spaced apart from the other chambers, and each chamber contains at least one set of DNA probes immobilized therein. The set of DNA probes in each chamber differs from the sets of DNA probes in the other chambers.

**[0021]** In other embodiments, the biochip includes a wash buffer input port to receive a wash buffer. The wash buffer input port is in fluid communication with the plurality of separation and detection chambers.

**[0022]** In some embodiments, the biochip also includes an amplification chamber to amplify the plurality of DNA fragments. The amplification chamber is in fluid communication with the sample input port and the plurality of separation and detection chambers. In some embodiments, the biochip includes an elution buffer input port to receive an elution buffer and a sample preparation chamber in fluid communication with the elution buffer input port, the sample input port, and the amplification chamber. In other

embodiments, the biochip includes a sample preparation wash buffer input port to receive a sample preparation wash buffer and a sample preparation chamber in fluid communication with the sample preparation wash buffer input port, the sample input port, and the amplification chamber. In other embodiments, the biochip includes a post-amplification buffer input port to receive a post-amplification buffer and a post-amplification vent chamber in fluid communication with the post-amplification buffer input port and the amplification chamber. In some embodiments, the biochip includes a post-amplification vent chamber in fluid communication with the amplification chamber and the plurality of separation and detection chambers. In other embodiments, the biochip also includes a waste output port in fluid communication with the plurality of separation and detection chambers.

**[0023]** In some embodiments, the biochip includes a plurality of vent chambers. Each vent chamber is in fluid communication with its corresponding chamber of the plurality of separation and detection chambers.

**[0024]** In other embodiments, the biochip includes at least one background reference chamber not in fluid communication with the sample input port and the plurality of separation and detection chambers. The at least one background reference chamber and at least one of the plurality separation and detection chambers are composed of a same material. In some embodiments, each of the at least one background reference chamber contains at least one DNA probe identical to the at least one DNA probe of its corresponding separation and detection chamber.

**[0025]** In some embodiments, the plurality of separation and detection chambers are connected in parallel. In alternative embodiments, the plurality of separation and detection chambers are connected in series.

**[0026]** In other embodiments, the biochip includes at least one flow gate to control fluid flow between the sample input port and at least one of the plurality of separation and detection chambers.

**[0027]** In some embodiments, the at least one flow gate has an inlet channel and an outlet channel. The at least one flow gate inhibits fluid flow from the inlet channel to the outlet channel with an absence of fluid pressure and permits fluid flow from the inlet channel to the outlet channel with fluid pressure.

**[0028]** In alternative embodiments, the at least one flow gate has an inlet channel and an outlet channel. The at least one flow gate inhibits fluid flow from the inlet channel to the outlet channel with pneumatic pressure and permits fluid flow from the inlet channel to the outlet channel with an absence of pneumatic pressure.

[0029] In some embodiments, the biochip includes a plurality of transition channels in fluid communication. Each of the plurality of transition channel has an increasing cross-sectional area in a direction to its corresponding separation and detection chamber.

[0030] In other embodiments, the biochip includes a waste output port in fluid communication with the plurality of separation and detection chambers

[0031] In some embodiments, the biochip also includes a cartridge opening on the integrated cartridge to permit access to the at least one reservoir and a membrane affixed to an area in proximity to the cartridge opening, the membrane covering the cartridge opening. The membrane has a sealable opening aligned with the cartridge opening, and the sealable opening is configured to open by external pressure and configured to seal without external pressure.

[0032] Some embodiments of the biochip is provided. The external pressure can be mechanical. The membrane can be a rubber gasket. The rubber gasket can be one of silicone, PTFE, neoprene, polyethelene, polymide, polycarbonate, acrylic, cyclo-olyfin polymers, latex, and cyclo olefin polymers.

[0033] In other embodiments, the biochip includes at least one waste output port in fluid communication with the plurality of separation and detection chambers and an integrated cartridge including at least one corresponding waste reservoir. The at least one waste reservoir has an opening aligned with the corresponding waste reservoir to receive waste from the at least one waste output port.

[0034] In some embodiments, a device including a chamber, an opening to the chamber to receive fluid in the chamber, and a membrane affixed to an area in proximity to the chamber opening, the membrane covering the chamber opening is provided. The membrane has a sealable opening aligned with the chamber opening, and the sealable opening is configured to open by external pressure and configured to seal without external pressure.

[0035] In some embodiments, a flow gate is provided. The flow gate includes a first port and a second port separated by a junction, each port being a small chamber at an end of a channel. The flow gate also includes a thin flexible material adhered across the first and second ports. The flow gate has an open configuration and a closed configuration, wherein pressure applied to the thin flexible material changes between the open configuration and the closed configuration. The open configuration has space between the thin flexible membrane and the junction and permits fluid flow between the two channels through the ports, and the

closed configuration has the thin flexible membrane in contact with the junction and inhibits fluid flow between the two channels.

**[0036]** Some embodiments of the flow gate is provided. The thin flexible material can be at least one of plastic and rubber. The thin flexible material can be at least one of PTFE, Teflon, PVC, polyethylene, polypropylene, polyimide, latex, silicone, and nitrile. The pressure applied to the thin flexible material can be at least one of pneumatic pressure, thrust, and fluid pressure.

**[0037]** In some embodiments, the flow gate includes at least one layer of material disposed above the ports.

**[0038]** In some embodiments, the flow gate is in the open configuration without the pressure, and the pressure changes the flow gate from the open configuration to the closed configuration.

**[0039]** In other embodiments, the flow gate is in the closed configuration without the pressure, and the pressure changes the flow gate from the closed configuration to the open configuration.

**[0040]** In some embodiments, the thin flexible material is adhered to at least a portion of the junction separating the first and second ports to inhibit fluid flow between the two channels. The adhesion is configured to be delaminated by fluid pressure in at least one of the channels above a threshold pressure.

**[0041]** In other embodiments, an adhesive adhering the thin flexible material to the portion of the junction is configured to remain delaminated after delamination by the fluid pressure.

**[0042]** In some embodiments, a method of detecting nucleic acid fragments is provided. The method includes providing a plurality of chambers, each chamber separated from the other chambers of the plurality. Each chamber has at least one set of probes disposed therein, and each set of probes is capable of binding to a different target nucleic acid sequence relative to the other sets of probes. The method includes providing a sample including a plurality of sets of nucleic acid fragments and placing at least a portion of the sample into each of the plurality of chambers and causing the at least one set of probes in each chamber to bind with complementary nucleic acid fragments of the sample. The method also includes detecting the binding of the nucleic acid fragments to the sets of probes in each chamber.

**[0043]** In some embodiments, the placing the at least a portion of the sample into each of the plurality of chambers includes flowing the portions of the sample through a first chamber of the plurality of chambers. The chambers are fluidically coupled in series.

[0044] In other embodiments, the placing the at least a portion of the sample into each of the plurality of chambers comprises flowing the portions of the sample into the plurality of chambers. The chambers are fluidically coupled in parallel.

[0045] In some embodiments, the nucleic acid fragments are DNA fragments, and in other embodiments, the nucleic acid fragments are RNA fragments.

[0046] In some embodiments, the plurality of chambers are disposed in a biochip. The biochip includes an input port in fluid communication with the plurality of chambers.

[0047] In other embodiments, providing the sample includes performing a nucleic acid amplification. In some embodiments, the nucleic acid amplification includes at least one of PCR amplification and isothermal amplification.

[0048] In some embodiments, the sets of probes are fluorescently labeled, and the binding of the complementary nucleic acid fragments of the sample perseveres the fluorescence of the label. The detecting the binding of the nucleic acid fragments to the sets of probes includes detecting the fluorescence of the labels.

[0049] In some embodiments, the method includes quenching the fluorescent labels of unbound probes.

[0050] In some embodiments, the sets of probes are immobilized within the chambers.

[0051] In some embodiments, providing the sample comprises performing a nucleic acid amplification in which copies of amplified nucleic acid fragments are fluorescently labeled. The binding of the complementary nucleic acid fragments of the sample perseveres the fluorescence of the nucleic acid fragments. The detecting the binding of the nucleic acid fragments to the sets of probes includes detecting the fluorescence of the nucleic acid fragments.

[0052] In some embodiments, the method includes quenching the fluorescent labels of unbound nucleic acid fragments. In other embodiments, the method includes washing unbound nucleic acid fragments out of the plurality of plurality of chambers.

[0053] In some embodiments, providing the sample comprises performing a nucleic acid amplification in which copies of amplified nucleic acid fragments are amplified using fluorescently labeled bases of a specified type. At least one of the sets of probes has fluorescently labeled bases that are complementary to the specified type, the fluorescently labeled bases of the probes being quenched by binding to the fluorescently labeled bases of the nucleic acid fragments. The detecting the binding of the nucleic acid fragments to the sets of probes includes detecting when a fluorescently labeled base of the probes is not quenched.

**[0054]** In some embodiments, a method of detecting nucleic acid fragments is provided. The method includes providing a plurality of sets of probes, each set of probes having a nucleic acid sequence. A first portion of the sequence is complementary to a target nucleic acid sequence, and the target nucleic acid sequence differs for each set of probes relative to the other sets of probes. A second portion of the sequence is a specified sequence, and the second portion of the sequence is the same for each set of probes. Each probe has a fluorescent label joined to the second portion of the sequence. The method also includes providing a sample comprising a plurality of sets of nucleic acid fragments and causing the first portion of the sequence of at least one set of probes to bind with nucleic acid fragments of the sample having a sequence complementary to said first portion. The method also includes providing a quenching compound that has a quenching moiety and a nucleic acid sequence that is complementary to the specified sequence of the second portion of the sets of probes, causing the quenching compound to bind to the second portions of the sequences of the sets of probes which are not bound to nucleic acid fragments of the sample, thereby causing the quenching moiety to quench the fluorescent labels of probes to which the quenching compounds are bound, and detecting the binding of the nucleic acid fragments to the sets of probes based on unquenched fluorescent labels of probes.

**[0055]** In some embodiments, the nucleic acid fragments are DNA fragments, and in other embodiments, the nucleic acid fragments are RNA fragments.

**[0056]** In some embodiments, providing the sample includes performing a nucleic acid amplification. The nucleic acid amplification can include at least one of PCR amplification and isothermal amplification.

**[0057]** In other embodiments, the method includes providing a plurality of chambers. Each chamber is separated from the other chambers of the plurality and has at least one set of probes of the plurality disposed therein. The sets of probes in each chamber differs from the sets of probes in the other chambers. The method also includes placing at least a portion of the sample into each of the plurality of chambers prior to causing the first portion of the sequence of at least one set of probes to bind with the nucleic acid fragments.

**[0058]** In some embodiments, the placing the at least a portion of the sample into each of the plurality of chambers includes flowing the portions of the sample through a first chamber of the plurality of chambers. The chambers are fluidically coupled in series.

**[0059]** In other embodiments, the placing the at least a portion of the sample into each of the plurality of chambers includes flowing the portions of the sample into the plurality of chambers. The chambers are fluidically coupled in parallel.

[0060] In some embodiments, the plurality of chambers are disposed in a biochip. The biochip includes an input port in fluid communication with the plurality of chambers.

[0061] In other embodiments, the sets of probes are immobilized within the chambers.

[0062] In some embodiments, the method includes washing unbound nucleic acid fragments out of the plurality of plurality of chambers.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0063] FIG. 1 illustrates a biochip that includes separation and detection chambers and a serial flow path according to an embodiment of the invention.

[0064] FIG. 2 illustrates a single type of probe immobilized within a separation and detection chamber according to an embodiment of the invention.

[0065] FIG. 3 illustrates more than one type of probe immobilized within a separation and detection chamber according to an embodiment of the invention.

[0066] FIG. 4 is a flowchart illustrating a series of steps for using the biochip shown in FIG. 1 according to an embodiment of the invention.

[0067] FIG. 5 illustrates a biochip that includes separation and detection chambers and a parallel flow path according to an embodiment of the invention.

[0068] FIG. 6 is a flowchart illustrating a series of steps for using the biochip shown in FIG. 5 according to an embodiment of the invention.

[0069] FIG. 7 illustrates an integrated biochip that includes sample preparation, target separation, and target detection features according to an embodiment of the invention.

[0070] FIG. 8 is a flowchart illustrating a series of steps for using the biochip shown in FIG. 7 according to an embodiment of the invention.

[0071] FIG. 9 illustrates an integrated biochip that includes universal LUER-LOK® connections integrated into the biochip according to an embodiment of the invention.

[0072] FIG. 10A illustrates a flow gate of an integrated biochip according to an embodiment of the invention.

[0073] FIG. 10B illustrates an open configuration of the flow gate of FIG. 10A according to an embodiment of the invention.

[0074] FIG. 10C illustrates a closed configuration of the flow gate of FIG. 10A according to an embodiment of the invention.

[0075] FIG. 11A illustrates an angled view of an integrated biochip with an integrated cartridge according to an embodiment of the invention.

[0076] FIG. 11B illustrates a bottom view of the integrated biochip of FIG. 10A according to an embodiment of the invention.

[0077] FIG. 12 illustrates an integrated biochip configured to be connected to an integrated cartridge according to an embodiment of the invention.

[0078] FIG. 13 illustrates an integrated cartridge configured to be connected to an integrated biochip according to an embodiment of the invention.

[0079] FIG. 14A illustrates a buffer cartridge assembly with a self-sealing rubber gasket according to an embodiment of the invention.

[0080] FIG. 14B illustrates inserting a sample to a buffer cartridge according to an embodiment of the invention.

[0081] FIG. 15A illustrates a cross-sectional side view of a cartridge, membrane and biochip stack according to an embodiment of the invention.

[0082] FIG. 15B illustrates a cross-sectional view (B-B) of the cartridge of FIG. 15A according to an embodiment of the invention.

[0083] FIG. 15C illustrates a cross-sectional view (C-C) of the membrane of FIG. 15A according to an embodiment of the invention.

[0084] FIG. 15D illustrates a cross-sectional view (DE-DE) of the biochip of FIG. 15A according to an embodiment of the invention.

[0085] FIG. 15E illustrates a cross-sectional view (DE-DE) of the biochip of FIG. 15A according to an embodiment of the invention.

[0086] FIG. 16A illustrates a cross-sectional side view of the stack of FIG. 15A with an open membrane slit according to an embodiment of the invention.

[0087] FIG. 16B illustrates a cross-sectional view (B-B) of the membrane of FIG. 16A with an open slit according to an embodiment of the invention.

[0088] FIG. 17A illustrates a cross-sectional side view of the stack of FIG. 15A with a closed membrane slit according to an embodiment of the invention.

[0089] FIG. 17B illustrates a cross-sectional view (B-B) of the membrane of FIG. 17A with a closed slit according to an embodiment of the invention.

[0090] FIG. 18 illustrates a testing platform according to an embodiment of the invention.

[0091] FIG. 19A lists examples of forward primers, reverse primers, and probe sequences of organisms.

[0092] FIG. 19B lists examples of common quencher sequences with dye-quenching moieties according to an embodiment of the invention.

[0093] FIG. 20 illustrates increases in relative fluorescence for four tested targets according to an embodiment of the invention.

[0094] FIG. 21 illustrates a biochip according to some embodiments;

[0095] FIG. 22 illustrates a separation and detection chamber that has been pre-coated with an immobilized probe according to some embodiments;

[0096] FIG. 23 illustrates a separation and detection chamber that has been pre-coated with different immobilized probes according to some embodiments;

[0097] FIG. 24 illustrates a flowchart of the method according to some embodiments;

[0098] FIG. 25 illustrates a diagram of options for fluorescence-based detection of complementary DNA fragment-probe binding according to some embodiments;

[0099] FIG. 26A illustrates a common quencher with a dye-quencher according to some embodiments;

[0100] FIG. 26B illustrates a probe containing a target specific sequence and a common quencher complementary sequence according to some embodiments;

[0101] FIG. 27A illustrates probes with a common quencher complementary sequence and target DNA fragments according to some embodiments;

[0102] FIG. 27B illustrates target DNA fragments bound to probes according to some embodiments;

[0103] FIG. 27C illustrates common quenchers bound to common quencher complementary sequences of probes according to some embodiments;

[0104] FIG. 28 illustrates an extended common quencher bound to a probe according to some embodiments;

[0105] FIG. 29 illustrates an increase in probe fluorescence for each of the twelve target DNA fragments by using the detection method according to some embodiments;

[0106] FIG. 30A lists forward primers, reverse primers, and probe sequences for the twelve targets; and

[0107] FIG. 30B lists two exemplary common quencher sequences with dye-quenching moieties according to embodiments of the present invention.

## DETAILED DESCRIPTION OF THE EMBODIMENTS

[0108] Embodiments of the invention detect, by fluorescence, multiple DNA targets of similar or different size within a biochip by separating the DNA fragments into designated chambers in the biochip. Herein, this DNA analysis method is termed "space separation and

detection". Using the space separation and detection method offers several key advantages that are not offered by routinely used DNA detection methods such as electrophoresis, mass spectroscopy, qPCR, etc. Embodiments of the invention include an integrated biochip that is capable of accepting a raw sample (such as blood, saliva, urine, swabs) to process via cell lysis, DNA extraction, DNA purification, PCR or isothermal amplification of purified DNA, post-amplification fragment preparation, and, finally, space separation in designated chambers. All processes are performed on a single use disposable integrated biochip capable of "sample-to-results" for research and clinical diagnostics.

**[0109]** FIG. 1 illustrates a biochip 100 that includes separation and detection chambers 105, 110 and a serial flow path 115 according to an embodiment of the invention. The biochip 100 also includes a sample input port 120, a wash buffer input port 125, and a waste output port 130. Channels 135 connect the various features of the biochip 100. Flow gates 140 and vent chambers 145 control fluid flow through the channels 135. The biochip 100 also includes a reference chamber 150, discussed in more detail below.

**[0110]** Biochip 100 is configured to receive a sample that contains a collection of DNA fragments, sequentially capture one or more sets of DNA fragments in the separation and detection chambers 105, 110 (and others), and enable detection of the various fragments. The sample to be analyzed is introduced via the sample input port 120 and flows through one of the channels 135 to a flow gate 140 that controls access to separation and detection chamber 105. Upon opening the flow gate to separation and detection chamber 105, the sample mixture flows into chamber 105 under pressure applied at sample input port 120. The vent chamber 145 immediately downstream of chamber 105 permits gas present in the channels upstream of chamber 105, and in chamber 105 itself, to be vented outside of the biochip. The structure and operation of the flow gates and vent chambers are described in more detail below.

**[0111]** Separation and detection chamber 105 is immediately preceded by a transition channel 155. The transition channel expands the cross-sectional area of the channel exiting the flow gate to more closely match the entrance to the chamber 105. The transition channel 155 may be configured to expand the cross-sectional area in a stepwise manner, as shown in the figure, or it may be configured to do so in a continuous manner. Moreover, although only three distinct sections of the transition channel 155 are shown in the figure, other numbers of sections are within the scope of the invention.

**[0112]** In an illustrative implementation, each sequential section of the transition channel 155 increases the cross-sectional area of the channel about two to four times relative to the

preceding channel. Other ratios may be employed. By gradually increasing the cross-sectional area of the channel leading to the separation and detection chamber 105, a sudden transition from a relatively narrow passage to a relatively large chamber is avoided. This, in turn, reduces or avoids the occurrence of gas bubbles forming in the chamber 105. By reducing or eliminating gas bubbles in chamber 105, the design of the biochip 100 enables detection errors to be reduced or eliminated, thereby providing relatively higher quality results than without such features.

**[0113]** As will be described in more detail in connection with FIG. 2, certain steps are taken to retain one or more of the DNA fragments in the separation and detection chamber 105 before passing the sample into a separation and detection chamber 110. In order to pass the sample mixture from chamber 105 into chamber 110, the flow gates 140 upstream of chamber 110 are opened, and the sample mixture flows, under pressure from the sample input port 120, into chamber 110. As described above, a vent chamber 145 immediately downstream of chamber 110 permits gas in the channels and the chamber 110 to escape. Also as before, certain steps are taken to retain one or more of the DNA fragments in the separation and detection chamber 110 before passing the sample into subsequent separation and detection chambers following the same basic procedure as described above.

**[0114]** After passing the sample mixture into separation and detection chamber 160 and performing the steps needed to retain one or more of the DNA fragments of the sample mixture in chamber 160, the flow gate immediately downstream of the chamber 160 is opened, along with all flow gates preceding chamber 160, to permit introduction of a wash buffer fluid through the wash buffer input port 125. The wash buffer fluid passes through all of the separation and detection chambers and exits via the waste output port 130. The flow of wash buffer fluid through the chambers reduces the amount of sample mixture remaining in the separation and detection chambers with the exception of the DNA fragments that have been bound within the chambers. Although biochip 100 is shown as having six separation and detection chambers, more or fewer chambers may be included and remain within the scope of the invention.

**[0115]** As mentioned above, certain steps are taken to retain one or more DNA fragments in the various separation and detection chambers. In an illustrative implementation of the invention, a set of DNA probes are immobilized within each separation and detection chamber. As used herein, a DNA probe is an agent that binds directly to a predefined sequence of nucleic acids. In general, DNA probes can be labeled or unlabeled, as described herein. FIG. 2 illustrates a single type of probe immobilized within a separation and

detection chamber 200 according to an embodiment of the invention. FIG. 2 shows a cross-section of one of the chambers (*e.g.*, any of chambers 105, 110, or the others shown in FIG. 1). The chamber 200 has a top surface 205, a bottom surface 210 and sidewalls 215 and 220. Techniques for forming the chamber 200 are discussed in greater detail below. In general, however, a void is created in a substrate to form the sidewalls 215, 220.

**[0116]** In some implementations, the top surface 205 is part of the substrate into which the chamber 200 is formed. In other implementations, bottom surface 210 is part of the substrate. In still further implementations, top surface 205 and bottom surface 210 are formed of a plastic and/or glass material. Using glass as an illustrative example, a set of DNA probes 225, all of the same type, are immobilized onto a glass slides (~1 mm thick). One glass slide is mated and sealed above the chamber 200 to form the top surface 205 and another glass slide is mated and sealed below the chamber 200 to form the bottom surface 210, thereby creating a visual detection window. Thus, in the embodiment shown in FIG. 2, the binding locations for the DNA probes 225 are located on the "roof" and "floor" of the chamber. Although not shown, DNA probes can be immobilized on sidewalls 215, 220 in addition to or in place of those immobilized on the top and bottom surfaces.

**[0117]** One of many illustrative techniques for immobilizing the DNA probes onto the glass slide follows. In some embodiments, desalted 3-prime or 5-prime amine-modified oligonucleotide probes are used. The DNA probe material is first mixed in an immobilization buffer (*e.g.*, 300 nM sodium phosphate (pH 8.0), Polysorbate 20 0.005%, and sarkosyl 0.001%) at a concentration of about 20 μM. A drop of the immobilization buffer solution that is about the size of the detection chamber (*e.g.*, 3.5 mm) or several small droplets (*e.g.*, 0.2 mm or smaller) within a space that is roughly the size of the detection chamber, are made on the glass slide using a micropipette or other robotic dispensing instruments. The glass slide is kept in a humidified chamber (*e.g.*, 50% relative humidity) for few to several minutes (*e.g.*, up to 30 minutes) and then soaked in a deactivation buffer (*e.g.*, 50 nM ethanolamine in 50nM sodium borate solution of pH 9.0) for about 30 minutes to 1 hour. The deactivation buffer ensures that any region that does not have an immobilized probe(s) is made relatively chemically inert to reduce the likelihood of DNA material adhering to those regions during the probe-DNA interaction. Finally, the glass is rinsed with deionized water for about 1 minute. The glass slide is then affixed to the substrate above the separation and detection chamber(s).

**[0118]** The above technique is merely illustrative, and other known methods for immobilizing probes may be used. In addition, the process set forth above can be used to

immobilize DNA probes on the sidewalls of the chambers. The probes can be immobilized to particles (e.g., magnetic beads of 10nm to 10μm) and can be placed inside the separation and detection chamber(s) instead of the glass coating method described above. For example, if magnetic particles with immobilized probes were used, a magnet can be energized above and/or below the chamber to retain the magnetic particles during fluid flow.

**[0119]** Referring again to FIG. 1, the biochip 100 also includes a background reference chamber 150. Chamber 150 is formed in the same manner and with the same materials as separation and detection chambers 105, 110, and the other chambers. However, chamber 150 is isolated from the path taken by the sample mixture and, in some embodiments, does not contain any immobilized DNA probes. Thus, any background fluorescence caused by the materials that form the top surface, bottom surface, and sidewalls can be detected. This background fluorescence can then be subtracted from the fluorescence from the separation and detection chambers in order to improve accuracy of the test results.

**[0120]** In other embodiments, one or more DNA probes of the type(s) used in the separation and detection chambers are immobilized in chamber 150. Thus, any background fluorescence caused by the DNA probes, in addition to that contributed by the materials of construction, can also be detected and subtracted from final test results. Further still, other implementations more than one background reference chamber is included in biochip 100. For example, biochip 100 can include one background reference chamber corresponding to each separation and detection chamber (105, 110, and the other) present in biochip 100. In such an embodiment, each of the background reference chambers includes the same type and concentration of immobilized DNA probes present in the corresponding separation and detection chamber. Thus, any variability of background fluorescence due to the different DNA probes can be detected and accounted for.

**[0121]** The visual detection window created by this configuration enables the detection and quantification of fluorescence (for techniques using fluorescent labels or PCR primers) from the DNA probe / target sequence pair from either above or below the surface of the biochip. Furthermore, the use of glass in the structure is advantageous as it is optically clear, has better binding chemistries for the probes, and has low auto-fluorescence compared to most plastics. However, it is understood that the use of plastics in place of glass remains within the scope of the invention.

**[0122]** FIG. 3 Illustrates more than one type of probe immobilized within a separation and detection chamber 300 according to an embodiment of the invention. Chamber 300 shares similar features to the chamber 200 of FIG. 2. However, chamber 300 has two types

of DNA probes immobilized within the chamber. DNA probes 305 and 310 each bind to different predefined sequences of nucleic acids. Thus, during the separation phase, described in more detail below, two different DNA fragment targets are retained in chamber 300.

**[0123]** FIG. 4 is a flowchart illustrating a series of steps for using the biochip shown in FIG. 1 according to an embodiment of the invention. Initially, a sample (*e.g.*, blood, saliva, etc.,) is gathered and prepared to create a collection of target DNA fragments using techniques known to one having ordinary skill in the art. The DNA fragments are then amplified using PCR or other DNA amplification techniques such as isothermal amplification (step 405). The particular amplification process and reagents used are determined in part by the nature of the DNA probes immobilized in the separation and detection chambers (105, 110, and the others) as well as the intended process for detecting the presence of the DNA targets.

**[0124]** For example, the PCR/isothermal process can produce amplified DNA fragments that are fluorescently labeled, which, when captured by the immobilized DNA probes, cause the fluorescence that is ultimately detected. In other implementations, the DNA probes include a fluorescent label and the amplified DNA fragments are not labeled. A quencher compound is included in the sample mixture along with the DNA fragments that are passed to the separation and detection chambers. When the DNA target fragments bind to the probes, the fluorescence of those probes is preserved, while the fluorescence of any DNA probes without bound DNA targets is quenched. Thus, in such embodiments, the probes cause the fluorescence that is ultimately detected. A more detailed description of the various illustrative complementary configurations of DNA probes, PCR/isothermal amplification processes, and detection techniques that can be used in biochip 100 are set forth below.

**[0125]** For illustrative purposes in connection with the process set forth in FIG. 4, six different types of DNA fragments (fragments types 1-6 herein) are amplified, and the DNA fragments produced by the PCR/isothermal amplification process (step 405) are fluorescently labeled with the same fluorescent label (*e.g.*, green dye FAM). Also for the purpose of this example, each of the six separation and detection chambers (105, 110, and the others) has a different DNA probe immobilized therein that are complementary to the six different types of DNA fragments. After the amplification process, the collection of DNA fragments and any reagents or buffers that are added after amplification is complete (collectively called "post-amplification solution") are introduced to biochip 100 via the sample input port 120 (step 410). As described in greater detail above, one or more flow gates are opened to permit the post-amplification solution to pass into the desired features of the biochip 100.

**[0126]** Next, the post-amplification solution, containing the collection of six different types of DNA fragments, is passed to the next available separation and detection chamber (step 415), which at this point in the process is the first chamber 105. The contents of the first chamber 105 are then subject to a thermo-chemical process to bind fragments of type 1 to the immobilized DNA probes in the first separation and detection chamber 105 (step 420). After the binding process is complete, the post-amplification solution is passed to the next available separation and detection chamber (repeat step 415), which is now chamber 110. Again, a thermo-chemical binding process is performed to capture DNA fragments of type 2, which are complementary to the DNA probes in the second separation and detection chamber 110, within the second chamber (repeat step 420).

**[0127]** Steps 415 and 420 are repeated until the post-amplification solution has passed into each of the six separation and detection chambers and each of the six different types of DNA fragments have been bound to the DNA probes to which they correspond. After the final binding step (step 420), the remaining post-amplification solution is passed to waste output port 130 (step 425). The illustrative process next passes a separation wash buffer, which is introduced via wash buffer input port 125, through all of the separation and detection chambers to waste output port 130 (step 430). The wash buffer removes unbound DNA fragments from the separation and detection chambers.

**[0128]** Finally, the fluorescence of the six separation and detection chambers 105, 110, and the others (and, optionally, the background reference chamber 150) are detected and quantified (step 435). Any of the equipment for and methods of detecting and quantifying the fluorescence known to one having ordinary skill in the art can be used during this step. For example, use of fluorometers appropriate for use in Quantitative PCR (qPCR) techniques are within the scope of the invention. Because each separation and detection chamber 105, 110, and the others had a different DNA probe immobilized therein, each chamber captured a different type of DNA fragment. Thus, embodiments of the invention enable one to use the same fluorescent label for each of the plurality of different DNA types in the sample without the problem of being unable to distinguish between the various DNA types.

**[0129]** FIG. 5 illustrates a biochip 500 that includes separation and detection chambers 505 and a serial flow path 515 according to another embodiment of the invention. The biochip 500 also includes a sample input port 520 and channels 535 connect the various features of the biochip 500. Flow gates 540 and vent chambers 545 control fluid flow through the channels 535. The biochip 500 also includes a reference chamber 550, discussed in more detail above.

**[0130]** Biochip 500 is similar in many respects to the biochip 100 described in detail above. However, biochip 500 is configured to receive the post-amplification solution and distribute the solution among six channels that form parallel flow paths 515 in contrast to the serial flow path 115 of biochip 100. In this context, "parallel flow" means that the sample solution passes into a particular separation and detection chamber 505 without having passed through any other separation and detection chamber 505. In this regard, the channels that form the flow paths need not be strictly geometrically parallel (as shown in FIG. 5A and FIG. 5B), however, the paths can be geometrically parallel. In addition, it is understood that the post-amplification solution need not enter the channels or the separation and detection chambers 505 at exactly the same time.

**[0131]** The sample to be analyzed is introduced via the sample input port 520 and flows through the channels 535 to flow gates 540 that control access to separation and detection chambers 505. Upon opening the flow gates to the multiple separation and detection chambers 505, the sample mixture flows into chambers 505 under pressure applied at sample input port 520. The vent chambers 545 immediately downstream of chambers 505 permit gas present in the channels upstream of chambers 505, and in the chambers 505 themselves, to be vented outside of the biochip. The structure and operation of the flow gates and vent chambers are described in more detail below.

**[0132]** Separation and detection chambers 505 include DNA probes therein. However, unlike the serial embodiment of the biochip 100, it is not required that the DNA probes be immobilized in the chambers 505 because the portion of post-amplification solution that enters each of the chambers 505 does not leave the chamber. Although not required, it is understood that in some implementations of the parallel flow embodiment of biochip 500, one or more DNA probes may be immobilized in the chambers 505 using the techniques described above.

**[0133]** Another difference between the serial flow biochip 100 and the parallel flow biochip 500 is that the step of binding the desired DNA fragments of the sample to the probes in the multiple chambers 505 need not happen in sequence. Rather, all of the separation and detection chambers 505 can be subjected to a process for binding the desired DNA fragments to the DNA probes in the same set of steps. In the alternative, one or more of the chambers 505, but less than all chambers, can be subjected to a binding process at the same time. For example, a first set of three chambers can be subjected to a thermo-chemical binding process during a first set of binding steps, while the remaining three chambers can be subjected to the same or similar set of binding steps after the first set of binding steps is completed. Although

biochip 500 is shown as having six separation and detection chambers, more or fewer chambers may be included and remain within the scope of the invention.

**[0134]** FIG. 6 is a flowchart illustrating a series of steps for using the biochip 500 shown in FIG. 5 according to an embodiment of the invention. As with the process for using biochip 100, a sample is gathered and prepared to create a collection of target DNA fragments using techniques known to one having ordinary skill in the art and the DNA fragments are then amplified using PCR or other DNA amplification techniques (e.g., isothermal amplification) (step 605). As before, the particular PCR/isothermal amplification process and reagents used are determined in part by the nature of the DNA probes present in the separation and detection chambers 505 as well as the intended process for detecting the presence of the DNA targets.

**[0135]** For illustrative purposes in connection with the process set forth in FIG. 6, six different types of DNA fragments (fragments types 1-6 herein) are amplified (step 605). However, unlike the process previously described, the DNA fragments produced by the PCR or isothermal amplification process (step 605) are not fluorescently labeled. Rather, the DNA probes present in the chambers 505 are labeled with a fluorescent label at either the 3-prime end or 5-prime end of the probe. Meanwhile, the unlabeled ends of the DNA probes have quenchers thereon. The opposing ends of the DNA probes have a number of bases that are complementary to each other. Also for the purpose of this example, each of the six separation and detection chambers 505 has a different type of DNA probe present therein that is complementary to one of the six different types of DNA fragments. After the amplification process, the collection of DNA fragments and any reagents or buffers that are added after amplification is complete (collectively called "post-amplification solution") are introduced to biochip 500 via the sample input port 520 (step 610). As described in greater detail above, one or more flow gates are opened to permit the post-amplification solution to pass into the desired features of the biochip 500.

**[0136]** Next, the post-amplification solution, containing the collection of six different types of DNA fragments, is passed to the separation and detection chambers 505 (step 615). The contents of the chambers 505 are then subject to a thermo-chemical process to bind each type of DNA fragment to its complementary type of DNA probe (step 620). In this embodiment, when a DNA fragment binds to its complementary DNA probe, the fluorescence of the probe is maintained. Any DNA probes in any of the chambers 505 that are not bound to a complementary DNA fragment will self-quench via the complementary bases at the 3-prime and 5-primes ends binding to each other.

**[0137]** After the binding process is complete, the fluorescence of the six separation and detection chambers 505 (and, optionally, the background reference chamber 550) are detected and quantified (step 625). As mentioned above, any of the equipment for and methods of detecting and quantifying the fluorescence known to one having ordinary skill in the art can be used during this step. Because each of the separation and detection chambers 505 had a different DNA probe therein, each chamber detects a different type of DNA fragment.

**[0138]** FIG. 7 illustrates an integrated biochip 700 that includes sample preparation, target separation, and target detection features according to an embodiment of the invention. The biochip 700 includes a serial flow path 715 leading through separation and detection chambers 705 that is similar to that of the biochip 100. However, other embodiments include a parallel flow path such as the one described above. The biochip 700 includes a wash buffer input port 725, a waste output port 730, connecting channels 735, flow gates 740, vent chambers 745, and a reference chamber 750. These features have similar characteristics and operate in a similar manner to the corresponding features described in connection with the biochip 100 and biochip 500.

**[0139]** Biochip 700 is similar in many respects to the biochip 100 described in detail above. However, biochip 700 has additional features that enable sample preparation and PCR/isothermal amplification to be performed on the biochip 700. Thus, the biochip 700 also includes a sample input port 755, a sample preparation wash buffer input port 760, an elution buffer input port 765, and a sample preparation chamber 770. As described in more detail below, these additional features are used to prepare a sample material for PCR/isothermal amplification. The biochip 700 further includes an amplification chamber 775, a post-amplification buffer input port 780, and a post-amplification vent chamber 785. These later features enable PCR/isothermal amplification to be performed on the biochip 700. Other embodiments of the biochip includes some, but not all, of the components listed above.

**[0140]** The sample input port 755 permits a sample matrix (*e.g.*, blood, saliva, urine, swab material) to be introduced into the sample preparation chamber 770. Similarly, the sample preparation wash buffer input port 760 permits a sample preparation wash buffer to be introduced into the sample preparation chamber 770, and the elution buffer input port 765 permits an elution buffer to added to the mixture in the sample preparation chamber 770. After the steps needed to liberate the target DNA fragments are performed on the sample matrix and reagents in the sample preparation chamber 770, the mixture is introduced into the amplification chamber 775.

**[0141]** After PCR thermocycling is conducted, a post-amplification buffer is introduced to the amplification chamber 775 via the post-amplification buffer input port 780, and the combined solution is passed to the post-amplification vent chamber 785. The post-amplification vent chamber is similar to other vents of the biochips described herein in that it permits gases in the upstream channels as well as the chamber itself to escape while retaining the sample solution. Fluid flow between the sample preparation and PCR amplification features is achieved via the same techniques set forth in more detail above. Generally, flow gates 740 control the flow of fluid between the various features, and vent chambers 745 permit gas to be purged from the chambers and the connecting channels.

**[0142]** The sample to be analyzed is then introduced to the separation and detection chambers 705 in a manner similar to the one set forth in connection with the biochip 100. As mentioned above, alternative embodiments of the biochip 700 include a parallel flow path. In such embodiments, the post-amplification solution generated by the "front-end" of biochip 700 passes into the separation and detection chambers in a manner similar to the one provided above in connection with the biochip 500.

**[0143]** FIG. 8 is a flowchart illustrating a series of steps for using the biochip 700 shown in FIG. 7 according to an embodiment of the invention. Initially, a sample matrix is collected (*e.g.*, a patient's blood) and combined with a lysis buffer and magnetic particles (step 805). The lysis buffer gains access to the DNA material inside the blood cells, and the DNA material binds to the magnetic particles under certain conditions. Next, a magnet is engaged above the sample preparation chamber 770, and the sample mixture created in step 805 is passed into the sample preparation chamber 770 via sample input port 755 (step 810). A sample preparation wash buffer is introduced to the sample preparation chamber 770 via the sample preparation wash buffer input port 760 and passed through the chamber 770 to the waste output port 730 (step 815). This wash step removes unwanted material and contaminants (*e.g.*, cellular debris) from the sample mixture. However, the desired DNA material remains in the sample preparation chamber 770 because the material is bound to the magnetic particles, which are held within the sample preparation chamber 770 via the magnet engaged above.

**[0144]** Next, an elution buffer is added to sample preparation chamber 770 via the elution buffer input port 765 (step 820). The sample mixture and elution buffer are incubated in the sample preparation chamber 770 at the appropriate conditions (*e.g.*, 50 deg C for 3 minutes) (step 825), which would be known to one having ordinary skill in the art. During the incubation step, the elution buffer causes the DNA material to be liberated from the magnetic

particles. The washed and eluted sample mixture is then passed into the amplification chamber 775 (step 830), leaving behind the magnetic particles that are still held in the sample preparation chamber 770 by the magnet. The needed amplification reagents can be included in the elution buffer or present in the amplification chamber 775 (or channels leading to the amplification chamber) in lyophilized form. In embodiments employing reverse-transcriptase-PCR (RT-PCR), the RT-PCR reagents are included in the elution buffer and the RT reaction is performed in the sample preparation chamber 770. Meanwhile, the amplification reagents are present in the amplification chamber 775 (or channels leading to the amplification chamber) in lyophilized form. The sample is then subjected to PCR thermocycling or isothermal amplification (830) to amplify the desired target DNA fragments.

**[0145]** After completion of the amplification reaction by thermocycling in case of PCR amplification or by constant temperature in case of isothermal amplification, the amplified products are passed to the post-amplification vent chamber 785 and a post-amplification buffer is introduced via the post-amplification buffer input port 780 into the post-amplification vent chamber 785 (step 835). The amplified products and post-amplification buffer are mixed in the post-amplification vent chamber 785 to form the "post-amplification solution". At this point, the post-amplification solution is passed into the separation and detection chambers 705 in a serial flow fashion following steps 415-430 of FIG. 4 (step 840). Finally, the fluorescence of the six separation and detection chambers 705 (and, optionally, the background reference chamber 750) are detected and quantified. Any of the equipment for and methods of detecting and quantifying the fluorescence known to one having ordinary skill in the art can be used during this step.

**[0146]** FIG. 9 illustrates an integrated biochip 900 that includes universal LUER-LOK® connections 905 integrated into the biochip according to an embodiment of the invention. In other embodiments, an alternate Luer taper-type connection is used (*e.g.*, a LUER-SLIP®). The LUER-LOK® connectors provide for quick connection between the biochip and the source of materials for use in the biochip while maintaining an air/water-tight seal. Alternatively, a buffer cartridge integrated onto the biochip may be used instead of syringes and LUER-LOK® as shown in FIGS. 14A and 14B.

**[0147]** Certain implementations of the various embodiments of biochips described above include a filter (<50μm) in or connected to the biochip's waste output port. The filter in the waste output port prevents backflow of material from the waste receptacle (*e.g.*, a syringe) to the biochip when liquid is present in the waste receptacle. Alternatively, a check valve is in

or fitted to the waste output port to serve the same purpose of backflow prevention achieved using a filter.

**[0148]** The various implementations of the biochips described herein can be fabricated from a variety of materials including glass, silicone, soft polymers (*e.g.*, PDMS), and plastics. Plastic polymers such as Poly(methyl methacrylate) (PMMA), polypropylene, polycarbonate, polyimide, cyclic olefin copolymers (COC), as well as glass are generally preferred, as those materials have good biocompatibility and are stable at high temperature (>80C) utilized in bioprocesses. Meanwhile, soft polymers are less desirable as they generally exhibit gas and water permeability, especially at elevated temperatures.

**[0149]** In certain embodiments, the features of the biochips (*e.g.*, channels, wells, chambers, etc.) are fabricated in the underside of a plastic substrate via hot embossing, injection molding, laser machining, or micro-machining process. It is understood that the foregoing list is merely illustrative, and other techniques for forming the biochip features are within the scope of the invention. The plastic substrate with the features is then bonded to a plastic film (of , *e.g.*, ~150μm thickness) onto the underside to create enclosed channels, chambers, and other features in the biochip. For example, COC can be used as the substrate and thin film in an embodiment of the biochip. After forming the features in the substrate, the thin film and substrate are thermally bound using heat and pressure (*e.g.*, 135 °C at 200 PSI for about 1 minute). In general, the temperature used is close to the glass-transition temperature of the plastic material used. Thus, other temperatures are within the scope of the invention, depending on the materials of construction. In addition, other pressures and times, *e.g.*, ranging from 20 PSI to 1000 PSI and a few seconds to several minutes, respectively, may be used. A device, such as a Model 4398 laboratory press from Carver, Inc. may be used to achieve these conditions.

**[0150]** Another illustrative technique uses heat, pressure, and a solvent to achieve bonding between the substrate and the thin film. In such a process, cyclohexane is applied to the surface of the substrate and/or thin film. The two components are then brought together under about 100 PSI at roughly 70 °C for about 2 minutes. This process produces a biochip which can withstand internal pressures of at least 100 PSI without delaminating.

**[0151]** As shown in FIG. 7, amplification chamber 775 exhibits a meander. One advantage of this feature is that it reduces or eliminates the tendency of the plastic film to deflect inward into the channel that it forms. By reducing or eliminating this deflection, a relatively flatter surface is available for contacting the heating elements used during the PCR thermocycling or isothermal amplification. This provides better temperature control,

increased efficiency, and improved performance. In addition, the meander provides for relatively better mixing than a chamber with straight channels.

**[0152]** As set forth above, fluid flow control between the chambers, channels, reservoirs, wells, and other features is controlled in part by flow gates (*e.g.*, flow gates 140 of FIG. 1). These flow gates can be any of a number of valves, flow control features, and the like suitable for use in microfluidic service known to one having ordinary skill in the art. In one illustrative implementation, the small chambers at the end of the channels that form the gate function are about 500 μm in diameter; larger and smaller diameters may also be used.

**[0153]** FIG. 10A illustrates a flow gate mechanism in some embodiments. In these embodiments, a flow gate includes a flexible membrane 1042 and an adhesive 1044 on one side of the membrane 1042. The flexible membrane 1042 is affixed to the area circumscribing the openings of channels 1001 and 1002, and the adhesive 1044 initially seals the area between the two disconnected channels.

**[0154]** Without the seal, fluids in the cartridge may leak or flow into other areas of the biochip because flow-gate membrane does not seal airtight when not connected to an instrument. When the adhesive 1044 is applied, it seals the channel leading to the reservoir (upstream) in the cartridge. However, upon the first use of the biochip on the instrument, the fluid pressure will delaminate the adhesive between 1001 and 1002, breaking the adhesive seal. Once the adhesive 1044 is soiled by the fluid at its first use as shown in FIG. 10B and FIG. 10C, the membrane at that flow gate needs a pneumatic pressure to actuate the membrane either to allow fluid flow in FIG. 10B or to prevent fluid flow in FIG. 10C. Therefore, in some embodiments, the adhesive 1044 replaces a typical sealing mechanism (e.g. foil seal) for each reservoir of the cartridge to prevent fluid from moving out of the reservoir and into other areas of the biochip.

**[0155]** FIG. 10B illustrates an open configuration of the flow gate. Applying pressure 1077B to an end of a channel causes fluid pressure to apply upward pressure to the flow gate membrane 1042. For example, air pressure can be applied from the buffer cartridge 1095 to channel 1001 to the membrane 1042. As a result, the flexible flow gate membrane bends and allows the fluid to flow through a next channel. Because the adhesive 1044 circumscribes the area, the fluid is restricted to stay within the area and flows from one channel to another channel.

**[0156]** FIG. 10C illustrates a closed configuration of the flow gate. The flow gate can be closed by applying pressure 1077C (*e.g.*, 20 PSIG) to the thin flexible material adhered to the gating connector ports in the biochip directly above the flow gate junction. By closing the

connection between the two channels of the flow gate, the fluid cannot flow from one channel to another channel. In other embodiments, additional layers of material are disposed above the gating ports to provide channels that pass above the gate junction. Pressure applied to the channels passing above the gate junctions distort the layers of material, thereby closing the flow gate. The fluid gating connectors on the biochip can be controlled by pneumatic pressure between slightly above 0 PSIG and 50 PSIG, but other pressure ranges are within the scope of the invention. In another illustrative implementation, the flow gates are normally closed and are opened by deforming material adjacent to the flow gate in order to open the gate junction.

**[0157]** In another embodiment, pressure applied to the flexible membrane is adjusted to control the flow rate of the fluid. Thus the flexible membrane can be adjusted to a partially open configuration.

**[0158]** Some examples of materials for the flow-gate membrane include PTFE, Teflon, PVC, polyethylene, polypropylene, polyimide, latex, silicone, and nitrile. The thickness can, for example, range from ~5μm to ~2mm. The adhesive on the bottom side can be, for example, acrylic adhesive and silicone adhesive. These examples are two common adhesives on the market, but the scope of the invention covers other types of adhesives. In some embodiments, the adhesive are applied to the membrane prior to assembly onto the biochip by a spray method, film deposition method, laminating method, etc. The thickness of the adhesive is optionally kept as thin as possible and ranges between 5um to 1mm, for example. In some embodiments, a thin adhesive layer and thin (flexible) membrane configuration is preferred.

**[0159]** The scope of the invention is not restricted to the flow gate as described above. Other types of flow gates that controls the fluid flow between two channels are within the scope of the invention.

**[0160]** In one embodiment, the biochip is mated with an interface and control instrument such that the pneumatic controls are disposed atop the flow gate junctions. Each pneumatic control is individually addressable to control flow fluid across a channel on either side of the flow gate. In one embodiment, the interface and control instrument is configured to open the various flow gates according to a programmed sequence at predetermined times. In another embodiment, an individual using the instruction actuated each flow gate manually. The interface and control instrument can also be equipped with the heaters for sample preparation and PCR or isothermal amplification, reagent and sample input mechanisms, and fluorescent detection devices.

**[0161]** In some implementations, the interface and control instrument also controls the introduction and flow of fluids in the biochip. For example, one embodiment of the control instrument actuates (*e.g.*, via a servo-mechanical device) syringes, filled with a predetermined quantity of liquid, that are attached to the LUER-LOK® connections at the sample input port 755, the sample preparation wash buffer input port 760, and the wash buffer input port 725. Alternatively, a cartridge with pre-filled buffers can be assembled or affixed to the biochip such that the input and output ports in the biochip are mated to the designated prefilled buffers in the cartridge. The buffers can then be pneumatically driven from the cartridge into the designated ports in the biochip. Meanwhile, the elution buffer input port 765 and post-amplification buffer input port 780 are supplied with their corresponding fluids by pneumatic pressure applied to a quantity of fluid. In an illustrative embodiment, pressures under 10 PSIG are used to supply the fluid to ports 765 and 780 (*e.g.*, 0.5 PSIG). However, other pressures are within the scope of the invention.

**[0162]** Any of the steps for using the biochips can be performed as part of an automated process. For example, in one process for using the biochip in a semi-automated fashion, an operator connects a prefilled buffer cartridge or the various syringes and/or LUER-LOK® adapters to the various ports on the biochip. The operator then places the biochip into a chamber that is part of the interface and control instrument. The chamber is designed such that individually addressable pneumatic actuators are disposed above the various flow gates of the biochip. The control instrument then conducts each step of the process up to the point of detecting the fluorescence emitted by the separation and detection chambers. At this point, the operator moves the biochip to another chamber of the interface and control instrument that has one or more optical readers to detect the fluorescence signal. One of skill in the art will understand that this semi-automated process is merely illustrative, and that all steps of the process can be performed in a single chamber, which incorporates all controls, fluid interface connections, and optical readers.

**[0163]** Referring again to FIG. 7, the features of an embodiment of biochip 700 have the following illustrative dimension. In one implementation, the overall biochip size is about 9.9 cm (3.9 inches) by about 7 cm (2.75 inches). The channels 735 that carry the sample matrix and lysis buffer also contains nano and microbeads (<10μm), cell debris, and/or other sample related material (*e.g.*, plasma, proteins, and/or debris from swabs used during the sample input). Such channels include, for example, the channel from the sample input port 755 to the sample preparation chamber 770 and the channel leading to the waste output port 730. Hence, the dimensions of these channels are about 800 μm by 800 μm. This relatively large

channel cross section reduces the likelihood of clogging of the channel when micro-beads or debris aggregate. In other implementations, channel dimensions can be 300 μm to 2000 μm to reduce channel clogging. Larger cross-sectional areas are also within the scope of the invention. Channels which are expected to be generally free from debris, magnetic beads, and/or other particles are about 500 μm by 500 μm. However, larger or smaller dimensions are within the scope of the invention (*e.g.*, 50 μm to 2000 μm and beyond). In the illustrative embodiment, all LUER-LOK® ports are standard size, which enable the ports to accommodate reservoirs, syringes, and/or other Luer taper interface connections that have volume storage capacity from, *e.g.*, 50 μL to 50mL.

**[0164]** The separation and detection chambers 705 are about 3.5 mm in diameter with a volume of about 40 μL. Such a size will accommodate an optical sensor above the chamber that has an active capture area of 3 mm diameter. The active capture area of the sensor will determine the size of the detection chamber. Thus, other diameters for the separation and detection chambers are envisioned for larger or smaller optical sensors. Providing a chamber that is about 0.5 mm larger in diameter than the active capture area of the sensor provides alignment tolerance between the biochip and the optical sensor.

**[0165]** In the illustrative embodiment of biochip 700, the volume of the amplification chamber 775 is 20 μL. In other implementations, the amplification chamber can be as small as 10 nL to 50 μL. Meanwhile, the volume of the sample preparation chamber 770 is about 20 μL and has a width of about 1300 μm. In this embodiment, the volume of the sample preparation chamber 770 is about the same size of the amplification chamber 775 so that when the nucleic acids are eluted in the sample preparation chamber 770, that volume can be directly passed into the amplification chamber 775.

**[0166]** The post-amplification vent chamber 785 is about 40 μL. In some implementations, the post-amplification vent chamber 785 volume matches that of the separation and detection chambers 705. Thus, upon completion of amplification, the sample volume moves to the post amplification vent chamber 785 and the addition volume accommodates the post amplification buffer solution, which is mixed with the amplification products inside chamber 785. The total volume of post-amplification solution then directly flows into the separation and detection chambers 705.

**[0167]** In an embodiment of the biochip 700 that includes a parallel flow path for the separation and detection chambers 705, the total volume of the post-amplification vent chamber 785 will be roughly equal to or slightly larger than the volume of all of the separation and detection chambers 705 combined. Thus, the post-amplification vent chamber

785 will accommodate the amplified products as well as the needed amount of post amplified buffer to supply a sufficient amount of post-amplification solution to each of the separation and detection chambers 705.

**[0168]** As set forth above, the various vent chambers in biochip 700 enable gas in the channels and chambers upstream of the corresponding vent chamber to be removed from the biochip. Each vent chamber has a membrane affixed to the top and/or bottom of the chamber. The membrane permits the expulsion of gas while retaining liquid during liquid flow in the biochip. The pore size of the membrane is about 0.45μm. However, the pore size can range from 0.01μm to 2 μm. Non-limiting examples of materials suitable for use as a vent membrane include Millipore 0.22 μm PTFE membrane material and Millipore SUREVENT® 0.45 μm PVDF membrane material. The vent membranes are bonded to the biochip after bonding the thin film to the substrate and creating holes in the thin film corresponding to the vent chambers. The membranes can be affixed using any number of adhesives known in the art, or the membranes can be joined by thermal welding and/or ultrasonic welding. Membranes attached in this way can withstand an internal pressure of at least 45 PSI. Thus, this permits fluids to be loaded into channels and chambers up to a vent membrane by keeping the fluid loading pressures below this amount (*e.g.*, <10 PSI).

**[0169]** FIGS. 11A and 11B illustrate some embodiments of an integrated biochip system, where an integrated biochip 1100 is connected to an integrated cartridge 1195. FIG. 11A shows an angled view, and FIG. 11B illustrates a bottom view of the integrated biochip 1100. The integrated cartridge contains various reservoirs and provide buffer fluids to their corresponding input ports.

**[0170]** FIG. 12 illustrates the integrated biochip without the cartridge. The biochip 1200 uses a parallel detection approach, similar to the biochip 500, and includes 8 detection chambers 1263. In some embodiments, the separation and detection chambers are optionally made to be wide meander channels rather than a circular chamber. Typically, if a circular chamber is several times larger than the size of an incoming channel (e.g. > 2 or 3 times), then fluid will break apart when it flows from the small channel to the large circular chamber. To reduce the improper fillig of fluid, we introduce "a transition channel" as described above. Using the transition channel, the size of the incoming channel gradually increases to finally lead into the circular chamber. This method avoids fluid breaking and bubbles in the chamber when the fluid flows at a slow rate. However, when the fluid moves at a higher flow-rate, the fluid can occasionally break-up and create air bubbles in the circular chamber even with the transition channel. Thus, the circular chamber may not be completely filled with the fluid. In

contrast, a wide meander channels can be more effective for smaller volumes (e.g. less than 20uL).

**[0171]** In some embodiments, the meander channels (i.e. the separation and detection chambers) are designed within a circular periphery. This design enables a detection instrument to read the fluorescence signal for either the 'meander design' or 'circular chamber design'. Thus, in some embodiments the wide meander channel is replaced with a circular chamber.

**[0172]** Similar to the biochip 700, in some embodiments the biochip 1200 includes a sample preparation chamber 1270, an elution vent chamber 1245A, an amplification vent chamber 1245B, an amplification chamber 1275, a post-amplification vent chamber 1285, and flow gates 1240. The biochip 1200 also optionally includes pneumatic/air lines 1233. Other embodiments of the biochip includes some, but not all, of the components listed above.

**[0173]** The elution vent chamber 1245A allows the air in the elution buffer chamber to be vented outside of the biochip, and the amplification vent chamber 1245B allows the air in the amplification chamber to be vented outside of the biochip.

**[0174]** In some embodiments, the pneumatic/air lines 1233 provides connection to the biochip assembly. As shown in FIG. 12, the system interfaces all of the flow-connectors with pneumatic air-lines. Then, the system can use the same interface to deliver air to the cartridge, rather than a separate mechanism on top of the cartridge to supply air as described above. In some embodiments, the pneumatic air lines deliver the air from the biochip to the bottom of the cartridge. Then, the air is routed through a vertical channel from the bottom to the top of the cartridge and is delivered to the top of the fluid reservoirs. Each fluid reservoir has a dedicated pneumatic air line from the biochip to the top of the fluid reservoir in the cartridge.

**[0175]** The biochip 1200 includes various fluid input ports, including waste liquid output ports 1288, a sample and lysis buffer input port 1255, an elution buffer input port 1265, a sample preparation wash buffer input port 1260, a post-amplification buffer input port 1280, and a separation wash buffer input port 1225.

**[0176]** In some embodiments, a pre-filled buffer cartridge is incorporated as part of an assembled biochip. The buffers in the cartridge can be driven pneumatically when the biochip is placed on the instrument and interface with the pneumatic manifold in the instrument. The cartridge also has optional empty chambers to serve as waste chambers for unloading buffers after each bioassay process in the biochip. This cartridge assembly can enable fully automated operation and eliminate the need for the operator to use syringes and

their connection to the biochip. The assembly of the cartridge and biochip is illustrated below in connection with the descriptions of FIGS. 15-17.

**[0177]** FIG. 13 illustrates the integrated cartridge 1195 separate from the integrated biochip 1200. The integrated cartridge 1195 contains a number of reservoirs connected to input ports on the integrated biochip 1200. Exemplary reservoirs are waste reservoirs 1389 (connected to the waste liquid output ports 1288), sample and lysis buffer reservoir 1356 (connected to the sample and lysis buffer input port 1255), an elution buffer reservoir 1366 (connected to the elution buffer input port 1265), a sample preparation wash buffer reservoir 1361 (connected to the sample preparation wash buffer input port 1260), a post-amplification buffer reservoir 1381 (connected to the post-amplification buffer input port 1280), and a separation wash buffer reservoir 1326 (connected to the separation wash buffer input port 1225). In some embodiments, the reservoirs is pre-filled with reagents. The waste liquid output reservoirs 1389 are initially empty, but is filled up with the waste from the separation and detection chambers. There can be multiple waste liquid output reservoirs, or one large reservoir. In some embodiments, the reservoirs have the same size, but the size can be different for different reservoirs.

**[0178]** Some embodiments of the buffer cartridge 1495 have a rubber material between the top cover and the cartridge body. In some embodiments, there exists an opening on the top cover for the sample introduction (for example from a syringe, pipette, or even to introduce a swab top into the cartridge). The rubber directly below this opening has a sealable opening. In some embodiments, the sealable opening is a slit. When a pipette, syringe, or swab, is thrust into the rubber slit, the rubber opens to provide access to the reservoir in the buffer cartridge to enable the user to deposit the sample inside the cartridge. When the pipette/syringe is retracted, the elasticity of the rubber ensures that it closes the slit, shutting-off access to the reservoir. This air tight self-seal created by the rubber ensures that any environmental contaminant is not subsequently introduced into the reservoir and the self-seal also prevents or minimizes pneumatic/air leak from the reservoir when the buffer/sample is driven pneumatically from this reservoir in the cartridge into the biochip.

**[0179]** FIGS. 14A and 14B illustrates embodiments of a buffer cartridge 1495 with a self-sealing rubber gasket. In some embodiments, the cartridge 1495 includes sample reservoirs 1490 and a rubber material 1492 on top of the reservoirs. On top of the rubber material 1492, there is a top cover 1493, which can be plastic. In some embodiments, the top plastic, rubber, cartridge and biochip are affixed/bonded to constitute a fully assembled biochip with the integrated buffer cartridge 1495. The top cover has a hole 1462, to which a pipette 1467,

syringe or swab can be introduced. Immediately below the hole 1462, there is a rubber slit 1468 that can be open to provide access to sample reservoirs 1490. In some embodiments, the slit is a straight line, a 'X' pattern, or a 'star' pattern.

**[0180]** In some embodiments, the thickness of the rubber material 1492 ranges from 0.5mm to 50mm. The material is, for example, silicone, PTFE, neoprene, polyethelene, polymide, polycarbonate, acrylic, cyclo-olyfin polymers, latex, cyclo olefin polymers, or any other material that has elasticity similar to that of rubber or elastomer. The durometer for example is Shore A 50 durometer, as used in one demonstration. The slit 1468 in the rubber that provides access to the sample reservoir below is, for example, between 0.5mm and 25mm, or larger as required. Thicker rubber and/or harder material durometer will require higher mechanical force by the operator to open the slit in the rubber to access the sample reservoir in the cartridge. Nevertheless, thicker rubber and/or harder material durometer can be advantageous as they can provide better self-sealing and can prevent or reduce air leak when pneumatic pressure is applied inside the sample reservoir in the cartridge to drive the sample into the biochip.

**[0181]** In another exemplary configuration, the hole 1462 has a diameter of ~4mm, and the top cover 1493 is ~2mm thick. Also, the slit size is ~2.5mm, and the top cover is an acrylic cover. The rubber material 1492 beneath the top cover 1493 is ~3mm thick and Shore A, 50 durometer.

**[0182]** FIG. 15A illustrates a cartridge, membrane and biochip stack bonded together according to some embodiments. In some embodiments, the biochip, membrane, and cartridge are manufactured separately as independent modules and then be mated and joined/bonded. In alternative embodiments, the biochip and cartridge are fabricated as a single unit. The stack can be bonded by adhesive or clamped by various mechanisms. The cartridge 1595 has a hole 1563 to allow fluid to flow out of the cartridge to the biochip 1500. The membrane 1511 has a slit 1513 that controls the flow of fluid 1503 from the cartridge 1595 to the biochip 1500. In some embodiments, the slit 1513 is located anywhere above the chamber 1517 of the biochip. To provide area for the slit 1513 to open, the chamber 1517 is optionally made larger than input ports of biochip 700. In FIG. 15, by placing the slit 1513 offset from the hole 1563 (i.e., not directly under the hole), leakage through the slit is prevented or minimized when the slit is not open and/or there is backpressure from the fluid/air from the biochip to the reservoir. If the slit was located directly under the hole, there is no object blocking the slit from opening upward when there is backpressure (i.e. fluid trying to enter from the biochip into the cartridge-reservoir). The membrane will stretch

upwards into the hole and eventually open as the fluid pressure from the biochip increases allowing fluid flow in the reverse direction. However, if the slit 1513 is offset from the hole 1563, the slit cannot open because it will be thrust against the body of the cartridge.

**[0183]** Other properties of the membrane and the biochip are described below. Some of the exemplary materials of the membrane are silicone, latex, PTFE, and polyethylene. In some embodiments, thickness ranges from 0.001" to 0.1". The thicker the material, the more pressure required to open the slit 1513 and to enable fluid flow across membrane through the slit. Some embodiments of the chamber 1517 are larger than the size of the hole 1563 of the cartridge. The chamber is connected to a channel 1501 through which the fluid will flow to reach its destination.

**[0184]** FIG. 15B is a B-B cross-sectional view of the cartridge 1595. FIG. 15C is a C-C cross-sectional view of the membrane 1511 with the slit 1513. FIGS. 15D and 15E are DE-DE cross-sectional views of the biochip 1500. Different embodiments of the chamber 1517 of the biochip have different shapes (e.g., slot, circular, oval).

**[0185]** FIG. 16A illustrates a cross-sectional side view of the stack of FIG. 15A with an open membrane slit according to an embodiment of the invention. When pneumatic/air pressure 1677 is applied from the top of the cartridge 1595, the pressure will be applied downward to the slit 1513 and open the slit. Then the fluid flows from the cartridge 1595 to the biochip 1500. FIG. 16B is a B-B cross-sectional view of the membrane 1511 with the open slit 1513.

**[0186]** FIG. 17A illustrates a cross-sectional side view of the stack of FIG. 15A with a closed membrane slit according to an embodiment of the invention. Unlike the downward pressure 1677 of the FIG. 16A, the pressure 1777 from the other side of the channel will create upward pressure on the membrane 1511. As illustrated above, because the bottom portion of the cartridge 1595 is placed against the membrane 1511, the bottom portion of the cartridge prevents the slit 1513 from opening upward. As a result of the closed slit, fluid cannot flow from biochip to the cartridge. FIG. 17B is a B-B cross-sectional view of the membrane 1511 with the closed slit 1513.

**[0187]** In the examples provided above, only one type of DNA probe was included in each of the separation and detection chambers and one type of fluorescent label was used. However, because the separation and detection chambers physically separate the different DNA fragments via binding to the DNA probes, multiple types of DNA probes and multiple types of fluorescent dyes can be used in other implementations. For example, a biochip that is able to separate and detect 30 different types of DNA fragments in six different separation

and detection chambers is within the scope of the invention. In such an embodiment, a collection of 30 different types of amplification primers (either by PCR or other amplification methods) are supplied that include only five different fluorescent labels, each label having a different emission color. Thus, after amplification (assuming each DNA target fragment is present in the sample), the post-amplification solution will contain a collection of 30 different types of DNA fragments, but these fragments will only be labeled with five distinct colors. Using known methods, one would not be able to distinguish the DNA fragments that are labeled with the same color from each other.

**[0188]** In embodiments of the present invention, five different DNA probes can be immobilized in each of the six separation and detection chambers, for a total of 30 different types of DNA probes in the biochip as a whole. Each of the five different types of DNA probes in a particular chamber will correspond to a different label color. In this way, the biochip captures five different DNA fragment types per chamber, each being labeled with a different color fluorescent label. Thus, because each chamber now only has one type of DNA fragment per color in each chamber, the interference between the labels emitting the same color is reduced or eliminated.

**[0189]** Implementations of the invention enable a gas to be introduced into one or more of the separation and detection chambers after the target DNA fragments have been bound to the DNA probes immobilized therein. This enables the removal of substantial amounts of any buffers or other non-bound material from the separation and detection chamber, thereby enabling the fluorescence to be detected on a dry basis.

**[0190]** One having ordinary skill in the art will recognize that the embodiments set forth herein are merely illustrative of the present invention. Thus, the techniques, devices, and system described above can be modified to enable known genetic material amplification methods to be performed while remaining within the scope of the invention.

**Testing Platform**

**[0191]** FIG. 18 depicts a genetic testing platform 1800 including a compact processor unit 1810 and a table-top reader unit 1820 according to embodiments of the present invention. In some embodiments, the units draw power from a standard 110V/220V wall outlet. The processor unit includes a bottom rapid peltier based thermocycler module to provide heat required for cell lysis, DNA elution, reverse-transcriptase and amplification. The processor also includes a top programmable pneumatic (0-100psig) module 1830 for

fluid flow control in the integrated biochip 1801. In alternative embodiments, there are two or more separate units for providing heat, providing pressure, and reading fluorescence.

**[0192]** In some embodiments, the reader unit is FDA classified for *in-vitro* diagnostic use. The 2-unit instrument platform offers significant capital cost savings for customers who desire to simultaneously run several samples on individual biochips (e.g., on-demand, Random Access Testing). For example, customers can easily set up a bench-top work station with a number of processor units (e.g. 2 to 50) and only one reader unit. Multiple samples can be simultaneously prepared on the biochip in less than 5 minutes per sample, tested in individual processor units in about 90 minutes, and sequentially read by the reader unit in less than 2 minutes per chip.

**[0193]** In some embodiments, to perform a diagnostic test, the integrated biochip is placed in the processor unit between the top pneumatic module and bottom peltier module. This stand-alone setup enables the integrated biochip to perform diagnostic test processes such as cell lysis, nucleic acid extraction, nucleic acid capture, nucleic acid purification, reagent mixing, reverse-transcriptase reaction and amplification, sequentially executed in an integrated manner in the disposable biochip. Typical processing time from providing sample to complete multiplex amplification is under 90 minutes. Upon completion, the integrated biochip is transferred to the table-top reader unit for a fluorescence read of amplified DNA fragments, which is typically accomplished in under 2 minutes. The reader unit has a multi-color excitation-emission optical filter system and utilizes highly sensitive photomultipler tube detection. This reader demonstrated to accurately detect fluorescent signal from even a single copy of amplified DNA.

**Experimental data**

**[0194]** This section provides test data for the biochip and method described above. Ultramer/DNA of 4 respiratory pathogens (purchased from IDT Technologies, CA), namely, Influenza-A, Influenza-B, Influenza-A/H1 and Influenza-A/H3, at 100000 copies each were spiked into 10μL of human blood sample. FIG. 19A lists the forward primer, reverse primer, probe sequences of the four respiratory pathogens mentioned above. FIG. 19B lists two common quencher sequences that are used in this experiment. The sample was then used for processing on biochip.

**[0195]** A common quencher oligonucleotide is a random DNA sequence of 8-20bp lengths. A common quencher oligonucleotide with a dye-quencher moiety can be used for

quenching the fluorescence of the probe. For example, in the first sequence of FIG. 19B, the random DNA sequence is 5'-TGTTATTCAGT and the dye-quencher moiety is 3IAbRQSp. The probe includes a gene specific sequence to bind with a target DNA and a common quencher complementary sequence (e.g., complementary sequence to TGTTATTCAGT) to bind with a common quencher. The common quencher complementary sequence of the probe contains fluorescence, and when a common quencher oligonucleotide binds to the probe, the fluorescence of the probe will be quenched. This configuration of probe allows binding of a common quencher oligonucleotide to the probe containing a complementary quencher sequence. Thus, one or few common quencher oligonucleotides can be used for a plurality of probes designed to bind to their corresponding DNA targets. The detailed description of the probe and the common quencher oligonucleotide can be found below.

**[0196]** The sample prep-process utilized the reagents from a commercially available kit, easyMAG™ (bioMérieux, Inc, NC) which includes the lysis buffer, magnetic beads for nucleic acid capture, wash buffer and elution buffer. Briefly, the spiked sample was input into 100µL of lysis buffer containing magnetic beads to capture nucleic acid. The solution was incubated for about 5min.

**[0197]** After incubation, pneumatic pressure provides the flow of such solution through the sample preparation chamber capturing the magnetic beads inside the chamber since a magnet was positioned above (and outside) the sample-prep chamber. The retained magnetic beads would have nucleic acid bound to it from earlier the lysis incubation chemistry. Next, wash buffer 1000µL that flowed through the magnetic beads washed the beads and removed components of the lysis buffer (for example, Chaotropic salts) and other biological elements (e.g. extracellular compounds, proteins). Subsequently, the introduction of the elution buffer 20µL to the magnetic beads and incubation for about 3min released the nucleic acid from the magnetic beads into the elution buffer. Next, pneumatic pressure moved the elution buffer containing the nucleic acid/DNA into the PCR/isothermal amplification chamber. The elution bufferwas mixed with Qiagen multiplex PCR Kit (cat# 206152, Qiagen, CA), HotStar polymerase (1U per reaction) and forward and reverse primers for each of the 4 DNA targets at concentration of 0.2µM each. The designed primers for the 4 targets are listed in FIG. 19 below. Multiplex PCR was then performed in which all for targets are amplified in the same reaction. PCR thermal cycling conditions were, 96°C for 10 min for initial denaturation followed by 40 cycles of 96°C for 1min, 60°C for 1min, 72°C for 1min, and final extension at 72°C for 5min.

[0198] Upon completion of PCR amplification, the PCR amplicons moved to the post-amplification vent chamber where it was mixed with about ~70μL of the post-amplification buffer, in this case deionized water, to a volume of ~90μL including the PCR amplicons from the PCR chamber. Next, pneumatic pressure applied to the input channels moved the solution from the 'post-amplification vent chamber' into 4 detection chambers. Each of the four detection chambers contained probe and quencher for only one targets, all labeled with FAM dye. When the 90μL of reconstituted PCR amplicons flowed into the four detection chambers, it was mixed with the probe and quencher present in its respective chambers. Chamber 1 contained the probe and quencher for Influenza-A, chamber 2 for Influenza-B, chamber 3 for Influenza-A/H1 and chamber 4 Influenza-A/H3. The probe and common quencher sequence for these targets is also listed in FIG. 19. The probe and the common-quencher oligo containing BHQ quencher were at equimolar concentration of 0.2μM contained in ~5μL solution and all probes were labeled with Cy5 dye. With ~25μL capacity in each detection chamber, approximately 20μL of the reconstituted PCR amplicon was filled into each chamber simultaneously. Closing the flow gates leading into an unused detection chamber restricted the flowinto unused detection chambers.

[0199] Once the PCR amplicon was loaded into all 4 detection chambers simultaneously, the temperature was ramped from ~25°C (room temperature) to 94°C at the rate of 1.5°C-2.5°C per second. After the temperature reached 94°C, from the temperature was ramped down from 94°C to 25°C at a ramp down rate of 2°C-4°C per second. This ramp up and ramp down allowed for the different denaturing and annealing interactions between the PCR amplified amplicons, probes and primers, as described below. Finally, a fluorescence reader (FLX800T, BioTek Instruments, VT) as shown below measures the fluorescence of Cy5 in each of the 4 chambers. Presence of Cy5 fluorescence in each chamber indicated the successful binding of a PCR amplicon to the corresponding detection probe present in that chamber. FIG. 20 shows the increases in probe fluorescence [in relative fluorescence units (RFU)] for each of the 4 targets (Influenza-A, Influenza-B, Influenza-A/H1, Influenza-A/H3). Using this detection method of the present invention and using only single color fluorescence, we demonstrated the detection of 4 targets in this example.

**Method of Separating and Detecting DNA Framgments**

[0200] The description above describes the biochip system for multiplex genetic identification along with a simple illustrative use of the biochip. Detailed description of using the biochip to separate and detect DNA fragments is provided below. The method presented below can also be used without the biochip above. A combined use of the biochip

and the method below and an independent use of the biochip or the method are within the scope of the invention.

**[0201]** Embodiments of the invention provide an improved method of detecting and analyzing nucleotide sequences, which overcomes multiple limitations of existing methods by making it possible to differentiate nucleotide molecules or sequences of similar size or with the same fluorescent label. Some embodiments may be used to detect and analyze nucleotide molecules or sequences from the nucleic acids DNA and RNA. Hereinafter, a person of ordinary skill will understand that any references to DNA fragments or sequences would also apply more broadly to nucleotide molecules or sequences of another source.

**[0202]** Biochip technology offers numerous advantages for performing *in vitro* diagnostics, including the ability to integrate multiple biotechnology process steps in a single device, automate preprogrammed assays with minimal to no manual intervention, and enable portable diagnostic tools without the need for a large laboratory setup.

**[0203]** Embodiments of the present invention use fluorescence to detect multiple DNA targets of similar or different size within a biochip by separating the DNA fragments into designated detection chambers in the biochip. More specifically, embodiments include providing a biochip having different separation and detection chambers, each with one or more probes, which have a complementary binding mechanism to a specific target DNA sequence; separating multiple DNA fragments in a sample into the biochip separation and detection chambers so that the DNA fragments bind, if at all, to a complementary probe; and using fluorescence to quantifiably detect the chamber-separated fragments. By separating different DNA fragments into these detection chambers, which are physically spaced apart from each other, embodiments improve the detection and analysis of target DNA sequences. That is, DNA fragments of similar size or with the same fluorescent label can still be differentiated via separation into designated detection chambers in the biochip.

**[0204]** In alternative embodiments, the detection can be made without the biochip. Multiple vials or chambers contain unique probes that are either immobilized within the vial or retained in the vial, *e.g.*, by being immobilized onto magnetic beads. When using the magnetic particles, a magnet can be energized above or below to retain the particles and the probes during fluid flow. For a sequential mechanism, the sample can be pipetted or input into vial 1 to cause binding of specific DNA to corresponding probes in vial 1. Then, the sample can be removed from vial 1, while the bound DNA-probe is retained in vial 1, and put into vial 2. The process can be repeated for the rest of the vials. For a parallel mechanism, the sample can be input into all of the vials and remove as needed.

**[0205]** Further embodiments of the biochip separation and detection method include first selectively amplifying one or more specific DNA sequences. PCR or isothermal amplification may be used to generate additional DNA fragments that are copies of a selected DNA sequence. Multiplex PCR may be used to select and replicate more than one unique DNA sequence at a time. Each amplified DNA fragment is itself a template for subsequent amplification. Thus, the target DNA sequence or sequences may be amplified exponentially, limited only by the available reagents and any feedback inhibition of amplified products, and the amplification process improves detection and analysis of DNA even from very small starting samples. Present embodiments allow these amplified DNA fragments to be of either unique or similar size. In addition, embodiments also allow these amplified DNA fragments to be labeled with either unique or identical fluorescent labels.

**[0206]** A person of ordinary skill will understand that various methods may be employed to amplify target DNA sequences. Preferred embodiments use a PCR or isothermal amplification reaction, combining a DNA sample with one or more DNA primers, nucleotides, a DNA polymerase, and various reagents known to a person of ordinary skill. For example, embodiments may be adjusted for buffers (e.g., Tris, Tricine, and Citrate), pH (e.g., 7 to 9), detergents (e.g., Tween), reducing agents (e.g., DTT), single-strand binding proteins, solvents (e.g., DMSO), salts (e.g., magnesium chloride, potassium chloride, and potassium acetate), derivatising agents (e.g., BSA), and biostabilizers.

**[0207]** A DNA primer or oligonucleotide is a short DNA fragment containing a sequence complementary to the target DNA sequence. Two DNA primers may be used for each target DNA sequence, one primer that is complementary to the 3-prime end of the sense strand and one primer that is complementary to the 3-prime end of the antisense strand. Nucleotides containing triphosphate groups, i.e., deoxynucleoside triphosphates (dNTPs), may be assembled into new DNA fragments. A DNA polymerase enzymatically synthesizes new DNA fragments from dNTP by using each target DNA sequence template and the associated DNA primer. The DNA polymerase may be heat-stable, such as Taq polymerase. Preferred embodiments use native Taq or hot-start Taq polymerase. Also, in preferred embodiments, the target DNA sequence may range from 100 base pairs to 4 kilo base pairs.

**[0208]** A PCR reaction may consist of thermal cycling, that is, alternating cycles of heating and cooling the reaction according to a defined series of temperature steps. Alternatively, if isothermal amplification is used, a constant temperature may be maintained during the amplification process. The temperatures used and the time periods of application depend on, for example, the length of any target DNA sequences, the stability of the DNA

polymerase, the melting temperatures of any DNA primers, and the concentrations of substrates and reagents. Specific embodiments may require additional temperature steps to be included at various points in the thermal cycle. The thermal cycle is repeated as desired or until the substrates and reagents are exhausted.

**[0209]** In embodiments where the DNA polymerase requires heat activation, the PCR reaction is initialized with a temperature of 95 °C for up to five minutes. The PCR reaction is then heated at 95 °C for five to forty-five seconds to disrupt the hydrogen bonds between complementary bases, thus achieving denaturation and physical separation of the two strands in the targeted DNA macromolecule in a process referred to as DNA melting (embodiments may be used to perform DNA melting curve analyses and discriminate between DNA sequences based on melting curve profiling in the presence of intercalating agents). The PCR reaction temperature is then lowered to a temperature ranging from 40 °C and 65 °C for five to forty-five seconds to allow DNA primers to anneal to the single-stranded templates of the target DNA sequence or sequences. In preferred embodiments, the melting temperature (Tm) of a DNA primer is less than 50 °C. The temperature and timing is optimized for the DNA polymerase (e.g., 72 °C to 75 °C for five to forty-five seconds for Taq polymerase) to bind to the primer-template structure and synthesize a new DNA fragment complementary to the DNA template by adding dNTPs in the 5-prime to 3-prime direction. The timing of this DNA amplification step also depends on the length of the target DNA sequence or sequences.

**[0210]** Instead of thermal cycling through denaturation and amplification cycles, a helicase enzyme may be supplied during the PCR reaction to separate the two strands in the targeted DNA sample (or later in the process, to separate the stranded of the amplified DNA fragments for biochip probe binding).

**[0211]** Embodiments may amplify DNA molecules or fragments of similar size concurrently because the sizes of the amplified DNA fragments do not affect detection. Size independence is advantageous over existing DNA detection methods, such as capillary electrophoresis and mass spectroscopy, and enables optimal primer design for multiplex-PCR screening of genes that could result in similarly sized DNA fragments.

**[0212]** FIG. 21 depicts a biochip 2101, which may be used according to some embodiments. Following FIG. 21, a multiplex-PCR sample is introduced to an input port 2102 of a biochip 2101. The multiplex-PCR-amplified DNA fragments are flowed through the biochip 2101. The exemplary biochip in FIG. 21 features a separation wash buffer input port 2103 and seven chambers. Chamber 2110 has neither a probe nor fluid flow, and is used for differential background subtraction of fluorescence during the detection step. On the

other hand, sequential separation and detection chambers 2111 through 2116 have been pre-coated with one or more immobilized probes that are designed to capture specific DNA fragments via complementary DNA-probe thermo-chemical interactions.

**[0213]** The DNA fragments are sequentially flowed through the series of biochip separation and detection chambers, beginning with the chamber 2111. A vent membrane 2104 enables the loading of the sample into each separation and detection chamber. The flow of the sample in the biochip is also aided by individual fluid flow gated controls 2105. Any DNA fragments that are captured by a probe in the chamber 2111 (i.e., bind to a specific complementary DNA sequence) remain bound to the probe while the remaining DNA fragments in the sample flow to the chamber 2112. Likewise, any DNA fragments that are captured by a probe in the chamber 2112 remain bound to the probe while the remaining DNA fragments in the sample flow to the chamber 2113. In a similar manner, the flow continues through all six separation and detection chambers, one chamber at a time, resulting in the extraction and separation of DNA fragments into each designated chamber by a thermo-chemical DNA-probe binding. After passing through all of the separation and detection chambers, what remains of the sample flows to a waste unloading port 2106. Alternatively, the separation and detection chambers can be in parallel such that the fluid can flow into a chamber without first flowing through another chamber.

**[0214]** FIG. 22 illustrates, according to some embodiments, a separation and detection chamber 2201 that has been pre-coated with an immobilized probe 2202, which is designed to complementarily bind via thermo-chemical interaction to any DNA fragments featuring a specific target DNA sequence.

**[0215]** In preferred embodiments, a probe is immobilized directly to a surface of the biochip separation and detection chamber. A glass surface is preferred because glass has better studied binding chemistry and lower auto-fluorescence; however, the surface may be a plastic or similar material. In preferred embodiments, the Tm of an immobilized probe is greater than 75 °C.

**[0216]** According to some embodiments, a probe is immobilized at its 5-prime end to a surface of a biochip separation and detection chamber. The immobilized probe has an amino linker at its 5-prime end and a fluorescent label (i.e., a fluorophore) bound to its free-floating 3-prime end. First, a fluorescence detection system is used to detect and quantify the fluorescence emission of the immobilized probe. Next, multiplex-PCR-amplified DNA fragments are introduced to the separation and detection chamber, and the chamber components are heated to 95 °C (to achieve DNA melting). The temperature is then ramped

down to 45 °C to allow complementary DNA fragments (i.e., fragments featuring the target DNA sequence) to bind to the probe. A quencher oligonucleotide is included in the solution to quench the fluorescence emission of any immobilized probe that did not bind to a complementary DNA fragment by binding to that probe. A quencher oligonucleotide has a sequence that is complementary to the probe with a fluorescent label, so it can bind to the probe and quench the fluorescence. In preferred embodiments, the Tm of a quencher oligonucleotide is less than 45 °C. Preferred embodiments may use one or more of the following quenchers: tetramethylrhodamine (TAMRA) or dihydrocyclopyrroloindole tripeptide (MGB). Following the binding process, at 45 °C, the fluorescence detection system is again used to optically measure and quantify the fluorescence of the immobilized probe. A reduction in the fluorescence emission may indicate the absence or at least a low concentration of bound probe-DNA fragment pairs (and presumably the absence or at least a low concentration of the target DNA sequence in the sample).

**[0217]** According to some embodiments, a probe is immobilized at its 5-prime end to a surface of a biochip separation and detection chamber. The immobilized probe has an amino linker and a quencher oligonucleotide at its 5-prime end while a fluorescent label (i.e., a fluorophore) is bound to its free-floating 3-prime end. The first ten bases from each end of the immobilized probe are complementary to each other, and the immobilized probe is designed to bind complementary DNA fragments in the region between its 5-prime end and 3-prime end (excluding the ten bases from each end). First, a fluorescence detection system is used to detect and quantify the fluorescence emission of the immobilized probe. Next, multiplex-PCR-amplified DNA fragments are introduced to the separation and detection chamber, and the chamber components are heated to 95 °C (to achieve DNA melting). The temperature is then ramped down to 45 °C to allow complementary DNA fragments (i.e., fragments featuring the target DNA sequence) to bind to the probe. If the immobilized probe does not bind to a complementary DNA fragment, then the probe will collapse and bind to itself (i.e., the 3-prime end will bind to the 5-prime end), quenching the fluorescence of the probe. Following the binding process, at 45 °C, the fluorescence detection system is again used to optically measure and quantify the fluorescence of the immobilized probe. A reduction in the fluorescence emission indicates the absence or at least a low concentration of bound probe-DNA fragment pairs (and presumably the absence or at least a low concentration of the target DNA sequence in the sample).

**[0218]** According to some embodiments, a probe is immobilized at its 5-prime end to a surface of a biochip separation and detection chamber while its 3-prime end is free-floating.

The immobilized probe is not fluorescently labeled with a fluorophore. Instead, the DNA primers have fluorescent labels. Thus, the multiplex-PCR-amplified DNA fragments are fluorescently labeled during amplification. Once the DNA fragments are introduced to the separation and detection chamber, complementary DNA fragments (i.e., fragments featuring the target DNA sequence) bind to the probe. The binding process is aided by the presence of a chemical such as 2xSSC (sodium chloride and sodium citrate solution) and a constant temperature between 40 °C and 60 °C. Unbound DNA fragments may be washed out with a separation wash buffer. Alternatively, a quencher oligonucleotide may be included in the solution to quench the fluorescence emission of any DNA fragment that did not bind to the probe. In this case, a quencher oligonucleotide can have a sequence complementary to the DNA fragments, so it can bind to the DNA fragments and quench the fluorescence. A fluorescence detection system is used to optically measure and quantify the fluorescence of the bound probe-DNA fragment pairs. The binding of all probes in the separation and detection chamber will result in a maximal fluorescent signal.

**[0219]** According to some embodiments, a probe is immobilized at its 5-prime end to a surface of a biochip separation and detection chamber while its 3-prime end is free-floating. The immobilized probe has a fluorescent label (i.e., a fluorophore) present in one of its bases (e.g., G or guanine). Meanwhile, in the PCR reaction, dNTPs with a complementary base (e.g., C or cytosine) are also fluorescently labeled. Once the multiplex-PCR-amplified DNA fragments are introduced to the separation and detection chamber, complementary DNA fragments (i.e., fragments featuring the target DNA sequence) bind to the immobilized probe. The fluorescent label in the immobilized probe is quenched if and only if complementary binding occurs (e.g., fluorescently-labeled base G in an immobilized probe is quenched by fluorescently-labeled base C in a DNA fragment). This embodiment is particularly useful for detection of single-nucleotide polymorphism (SNP).

**[0220]** Embodiments may result in double specificity for PCR-amplified DNA fragments. During amplification, DNA primers are designed to be specifically complementary to a target DNA sequence. Then, during space separation, the amplified DNA fragments are bound to specifically complementary probes, if they exist, in the biochip separation and detection chambers. This double specificity increases the ability to discriminate between target and non-target PCR-amplified DNA fragments. Double specificity is advantageous over existing DNA detection methods, such as capillary electrophoresis and methods that rely solely on micro-arrays.

[0221] A person of ordinary skill will understand that various methods may be employed to fluorescently label macromolecules, such as DNA fragments and probes, according to certain embodiments. Preferred embodiments use fluorophores (e.g., TaqMan® probes), which absorb light energy of a specific wavelength and re-emit light at a longer wavelength. In combination with a fluorescence detection system, a fluorophore may indicate the presence (or absence) of a specific nucleotide molecule or sequence and the concentration thereof. For example, an embodiment may be designed to result in fluorescence only when there is a successful DNA fragment-probe complementary binding. Alternatively, a fluorescence reduction method is designed to result in quenching (i.e., loss of fluorescent emission) only when there is a successful DNA fragment-probe complementary binding.

[0222] Fluorophores may differ in their maximum excitation wavelength, maximum emission wavelength, extinction coefficient, quantum yield, lifetime, and other properties. Preferred embodiments may use one or more of the following: EvaGreen® (Biotium, Inc., Hayward, CA), TYE™ (Integrated DNA Technologies, Inc., Coralville, IA), FAM™ (Applera Corp., Norwalk, CT), VIC® (Applera Corp.), TET™ (Applied Biosystems, Inc., Foster City, CA), ROX™ (Applied Biosystems, Inc.), SYBR® Green (Molecular Probes, Inc., Eugene, OR), and Alexa Fluor® dyes (Molecular Probes, Inc.).

[0223] In preferred embodiments, after the different types of DNA fragments have been separated into designated biochip detection chambers according to a specific target DNA sequence, a fluorescence detection system is used to detect the fluorescence of the immobilized probe-DNA fragment pairs in each of the separation and detection chambers.

[0224] Fluorescence detection can be accomplished either with or without a separation wash buffer. However, immobilization of any probes may be necessary if a wash buffer is used; otherwise any probes in the separation and detection chambers may be washed out.

[0225] A person of ordinary skill will understand numerous options for fluorescence detection. For example, a fluorometer or spectrofluorometer may be used to measure the parameters of fluorescence, including the intensity and wavelength distribution of light emission spectra after excitation by a certain spectrum of light. Possible light sources that provide excitation energy capable of inducing fluorescence (usually fluorescent light in the wavelength range of 350 nm to 900 nm, comprising blue, green, and red wavelength spectra) include a laser, a photodiode, a mercury-vapor lamp, and a xenon arc lamp. Thus, these parameters may be used to identify the presence or absence as well as the amount of immobilized probe-DNA fragment pairs in each of the separation and detection chambers.

[0226] In some embodiments, a fluorometer uses two light beams to counteract signal noise produced by radiant power fluctuations. An incident light beam is filtered and passed through the sample, which absorbs the light then emits fluorescence as it returns to a lower energy state. A second beam is attenuated and adjusted to match the intensity of the fluorescence emitted by the sample. Separate transducers detect the second beam and the fluorescent emission from the sample, converting each to electrical signals for interpretation by a computer system. In other embodiments, the fluorescent emission passes through a second filter or monochromator, which is placed at 90° to the incident light beam to minimize the risk of transmitted or reflected incident light reaching the transducer. An additional way to counteract signal noise, according to some embodiments, is to include a base sample for differential background subtraction of fluorescence, such as biochip chamber 2110 in FIG. 21.

[0227] Fluorescence detection is limited by the color detection capability of the fluorescence detection system (i.e., the ability of the instrument to distinguish the different fluorescent colors). For example, a single-color fluorometer can detect only one fluorescent label, while a three-color fluorometer can detect up to three unique fluorescent labels. Typically, when multiple fluorescent labels are used (especially more than the three clearly distinguished blue, green, and red spectra), the light emission spectra may overlap each other, making it difficult to distinguish the unique fluorescent labels. Software algorithms may be employed to compensate for overlapping emission spectra; however, signal sensitivity may nevertheless be compromised. Currently, the best available multi-color fluorescence detection system identifies up to nine colors, with wavelengths ranging from about 350 nm to about 950 nm, a range which includes blue, green, and red light emission spectra.

[0228] In preferred embodiments, because the biochip separation and detection chambers are physically separated, a fluorescence detection system may be used to detect the emission spectra from each of the separation and detection chambers without interference from the emission spectra in other separation and detection chambers. Thus, a single-color fluorometer may be used to detect the fluorescence of the immobilized probe-DNA fragment pairs in each of the separation and detection chambers independent of size and fluorescent label.

[0229] FIG. 23 illustrates, according to some embodiments, a separation and detection chamber 2301 that has been pre-coated with one immobilized probe 2302 and one different immobilized probe 2303, the two of which are designed to bind to two different target DNA sequences. For ease of detection and analysis, the two different immobilized probes are

designed, in some embodiments, to emit different fluorescent colors upon binding to DNA fragments in the sample. However, a multi-color fluorometer must be available.

**[0230]** For example, if a nine-color fluorescence detection system is available, then theoretically the immobilized probe-DNA fragment pairs in each separation and detection chamber may be labeled with as many as nine different colors, representing up to nine different target DNA sequences. If a biochip has six separation and detection chambers, as in FIG. 21, and nine different immobilized probes in each chamber, then an embodiment may be capable of detecting up to fifty-four different target DNA sequences.

**[0231]** FIG. 24 illustrates a flowchart of the method according to some embodiments. In step 2401, target DNA is amplified (e.g., by PCR or isothermal amplification). In step 2402, the resulting DNA fragments are flowed sequentially through N separation and detection chambers of a biochip. In each separation and detection chamber, immobilized probes bind to any complementary DNA fragments, and any unbound DNA fragments flow to the next chamber until the Nth chamber. Following the separation of DNA fragments via DNA fragment-probe thermo-chemical interactions in the detection chambers, in step 2403, the fluorescence emissions, or reduction thereof, is measured and analyzed.

**[0232]** FIG. 25 illustrates a diagram of options for fluorescence-based detection of complementary DNA fragment-probe binding according to some embodiments. In case 2501, a fluorescently-labeled DNA fragment binds with a probe in a separation and detection chamber. In order to detect only the complementary DNA fragment-probe pair and not any unbound DNA fragments, which also may be fluorescently labeled, several steps may be taken. First, according to some embodiments, a quencher oligonucleotide may be included in the solution to quench the fluorescence emission of any unbound DNA fragments. Second, according to embodiments with immobilized probes, the separation and detection chamber may be flushed of any unbound DNA fragments with a wash buffer.

**[0233]** In case 2502, a fluorescently-labeled DNA fragment binds with a fluorescently-labeled probe in a separation and detection chamber. Assuming complementary binding quenches the fluorescence emissions of both the DNA fragment and the probe, a reduction in fluorescence emissions from the chamber may indicate the complementary DNA fragment-probe pair. However, any unbound DNA fragments and any unbound probes still may be fluorescently labeled. Again, according to some embodiments, a quencher oligonucleotide may be included in the solution to quench the fluorescence emission of any unbound DNA fragments or, in this case, any unbound probes. According to embodiments with immobilized probes, the separation and detection chamber may be flushed of any unbound DNA fragments

with a wash buffer; however, any unbound and fluorescently-labeled probes will stay immobilized in the chamber. According to embodiments with probes having a quencher oligonucleotide at the 5-prime end, any unbound probes may self-quench by collapsing and binding 5-prime end to 3-prime end.

**[0234]** In case 2503, a DNA fragment binds with a fluorescently-labeled probe in a separation and detection chamber. In order to detect only the complementary DNA fragment-probe pair and not any unbound probes, according to some embodiments, a quencher oligonucleotide may be included in the solution to quench the fluorescence emission of any unbound probes. Also, according to embodiments with probes having a quencher oligonucleotide at the 5-prime end, any unbound probes may self-quench by collapsing and binding 5-prime end to 3-prime end.

**[0235]** In cases 2502 and 2503, a common quencher method can be used. A set of probes has a specified sequence (a common quencher complementary sequence 2621) in addition to a target specific sequence 2625 capable of binding a target fragment. Each probe of the set of probes has the same common quencher complementary sequence 2621 and a different gene specific sequence 2625 depending on the target sequence. A quenching compound (or a common quencher) has the same common quencher sequence and a dye-quenching moiety attached to the sequence. Because the probes use the same common quencher complementary sequence 2621, quenching compounds having one type of common quencher sequence—complementary to sequence 2621—can quench fluorescence of all of the probes. FIG. 26A shows a common quencher 2601, which contains a 3-prime end quencher with a dye-quencher (or a dye-quenching moiety). FIG. 26B shows a probe 2602 containing a gene specific sequence 2625 and a common quencher complementary sequence 2621 at the 5-prime end. The 5-prime end of the probe is fluorescent labeled. A common quencher sequence is a random DNA sequence of 8-20bp lengths. A non-exhaustive examples of over 300 such sequences designed by NanoMDx are attached in the DNA sequence listing, provided with the application. The sequence listing includes 3 generated lists, each list containing 100 sequences. The list can be combined to one list containing 300 sequences.

**[0236]** Using a common quencher method, each probe 2602 with target specific sequence 2625 used for multiple DNA target detection can have complementary sequence 2621 to the common quencher. This configuration of probe allows binding of a common quencher oligonucleotide to the probe containing a complementary quencher sequence. Thus, one or few common quencher oligonucleotides can be used for a plurality of probes designed to bind to their corresponding DNA targets. When a common quencher binds to the probe, the dye-

quenching moiety of the common quencher will quench the fluorescence of the probe. Similar to target specific quenchers, a common quencher oligonucleotide and probe will bind to each other under certain conditions (e.g., when the temperature is below 45°C).

**[0237]** Some randomly generated common quencher sequences may have sequences complimentary to the target specific sequences 2625 or to the target DNA sequences. To avoid conflicts of sequences (i.e., common quenchers binding to sequences other than complementary sequences 2621), some common quencher sequences are avoided in some embodiments. Alternatively, more than one common quencher sequences are used to quench every unbound probe.

**[0238]** The common quencher oligonucleotide and many target specific probes can be mixed together to form the final probe for the detection process. The probe and the common quencher are bound together to constitute the PCR mix.

**[0239]** This method of using only one or a few 'common quenchers' for all the detection probes in a multiplex-PCR reaction is cost saving and simplifies the overall detection process. Conventionally, a separate quencher is required for each detection probe/PCR product, such as those used in TaqMan® assay and other real-time PCR approaches.

**[0240]** The following is an exemplary, not exhaustive, list of salient features of a common quencher based detection methods. The length of a target specific oligonucleotide ranges from about 15bp to about 45bp. The melting temperature of a target specific oligonucleotide ranges from about 45°C to about 75°C. The guanine-cytosine percentage (GC%) of a target oligonucleotide ranges from about 25% to about 45%. A target specific oligonucleotide has a sequence complementary to the intended target and a sequence complementary to common oligonucleotide. A target specific oligonucleotide can also have 3-terminal modification enabling it to bind to solid surface. The 5-prime end of target specific oligonucleotide will have a fluorescent dye either Cy5, Tye, EvaGreen®, TYE™, FAM™, VIC®, TET™, ROX™, and Alexa Fluor® dyes. The length of common oligonucleotide can range from about 8bp to about 20bp. The melting temperature of common oligonucleotide ranges from about 25°C to about 45°C. The GC% of common oligonucleotide ranges from about 5% to about 30%. The 3-prime end of common oligonucleotide has a quencher dye attached. A single common oligonucleotide quenches fluorescence from all other target specific oligonucleotides. A target specific oligonucleotide binds to a specific target when the temperature is about 45°C to about 75°C. A common oligonucleotide binds to target specific oligonucleotide when the temperature is about 25°C to about 45°C. At this temperature, free unbound target specific oligonucleotides will be

hybridized to common oligonucleotides. Fluorescence from a target specific probe in the presence of intended targets and common oligonucleotides are measured at about 30°C. Binding between target specific oligonucleotides and intended targets and between target specific oligonucleotides and the common oligonucleotides are performed by ramping up the endpoint PCR or isothermal amplification reaction mix from about 25°C to about 95°C, and ramp-down from about 95°C to about 25°C. Binding between target specific oligonucleotides and the intended targets and between target specific oligonucleotides and common oligonucleotides are performed by incubating the PCR or isothermal amplification reaction mix at about 60°C followed by incubation at about 25°C. The common quencher method can be applied to both real-time and end-point PCR amplifications. The design of target specific oligonecleotides and common oligonucleotides can be used as an endpoint detection method after amplification or as a real-time PCR application detection method.

**[0241]** Similar to the use of target specific probes, FIGS. 27A, 27B, and 27C illustrate a fluorescent detection process using the common quenching method according to some embodiments. Amplified DNA fragments 2703 are provided to chambers containing probes 2602 with common quencher complementary sequences of FIG. 27A provided into chambers. After the DNA fragments are provided, the temperature is raised to 95°C to achieve DNA melting and ramped down to 45 °C to allow complementary DNA fragments to bind to the probe. FIG. 27B illustrates the DNA fragments 2703 bound to probes 2602B. For the remaining probes 2602C that do not have binding DNA fragments, common quenchers 2601 quenches the probe fluorescence, as illustrated in FIG. 27C. Then, the fluorescence from the DNA fragments can be observed.

**[0242]** In some embodiments, a common quencher 2801 can be designed slightly longer than a common quencher complementary sequence 2821 as shown in FIG. 28. In this case, after a common quencher 2801 binds to the common quencher complementary sequence 2821 of a probe 2802, the extending portion of the common quencher 2801 increases the steric hindrance, thereby decreasing the likelihood that a target DNA 2803 would bind to the target specific complementary sequence 2825 of the probe 2802.

**[0243]** Four examples are described below. In Examples 1 and 2, separation and detection chambers are in series. Thus, fluid sequentially flows from one channel to another channel. Also, in Examples 1 and 2, probes are immobilized to the surface or beads in the chamber. In Examples 3 and 4, separation and detection chambers are in parallel. Since fluid does not flow sequentially through all of the separation and detection chambers, a wash

buffer is optional. Thus, the probes do not need to be immobilized. Examples 3 and 4 shows the testing results with different numbers of colors and targets.

Example 1

**[0244]** In a first exemplary embodiment, a biochip with six separation and detection chambers is used with a single-color fluorescence detection system to simultaneously detect as many as six target DNA sequences. A multiplex PCR or isothermal amplification process is used to generate a sample comprising six unique types of DNA fragments, which are of similar or different size. All six types of DNA fragments are labeled with an identical fluorescent color.

**[0245]** The sample is introduced to an input port of the biochip and flowed sequentially through a series of six separation and detection chambers, which have each been pre-coated with an immobilized probe designed to bind to a specific target DNA sequence. Chamber 1 has been pre-coated with immobilized probe A, which was designed to complementarily bind via thermo-chemical interaction, to any DNA fragments featuring target DNA sequence A. When the sample comprising unique DNA fragment types 1-6 are first loaded into chamber 1, and if one of the fragment types features target DNA sequence A, all of the DNA fragments of that type are captured, essentially extracted from the sample, and retained in chamber 1. The rest of the sample, including the other DNA fragments, is flowed to chamber 2, thus physically separating any DNA fragments featuring target DNA sequence A from the rest of the sample.

**[0246]** Chamber 2 has been pre-coated with immobilized probe B, which was designed to complementarily bind via thermo-chemical interaction, to any DNA fragments featuring target DNA sequence B. If one of the fragment types features target DNA sequence B, all of the DNA fragments of that type are captured, essentially extracted from the sample, and retained in chamber 2. The rest of the sample is flowed to chamber 3, thus physically separating any DNA fragments featuring target DNA sequence B from the rest of the sample. In a similar manner, the sample is flowed through the remaining four separation and detection chambers, one chamber at a time, resulting in the extraction and physical separation of DNA fragments into the six separation and detection chambers according to the presence of target DNA sequences.

**[0247]** Following space separation, the single-color fluorescence detection system is used to detect the fluorescence emission from each biochip separation and detection chamber, which contains paired immobilized probes and DNA fragments. Because the unique types of

DNA fragments have been physically separated according to target DNA sequence, all six types of DNA fragments may be labeled with an identical fluorescent color and still be detected by a single-color fluorescence detection system.

Example 2

**[0248]** In a second exemplary embodiment, a biochip with six separation and detection chambers is used with a three-color fluorescence detection system to simultaneously detect as many as eighteen target DNA sequences. A multiplex PCR or isothermal amplification process is used to generate a sample comprising eighteen unique types of DNA fragments, which are of similar or different size. Six types of DNA fragments are labeled with a first fluorescent color, six other types of DNA fragments are labeled with a second fluorescent color, and the remaining six types of DNA fragments are labeled with a third fluorescent color.

**[0249]** The sample is introduced to an input port of the biochip and flowed sequentially through a series of six separation and detection chambers, which have each been pre-coated with three different immobilized probes, each designed to bind to specific target DNA sequences. Chamber 1 has been pre-coated with immobilized probes A, B, and C, which are designed to complementarily bind via thermo-chemical interaction, to any DNA fragments featuring, respectively, target DNA sequences A, B, and C. When the sample comprising unique DNA fragment types 1-18 are first loaded into chamber 1, all DNA fragments featuring target DNA sequence A bind to immobilized probe A, all DNA fragments featuring target DNA sequence B bind to immobilized probe B, and all DNA fragments featuring target DNA sequence C bind to immobilized probe C. Thus, any DNA fragments with target DNA sequences A, B, and C are essentially extracted from the sample and retained in chamber 1. The rest of the sample is flowed to chamber 2, thus physically separating those three types of DNA fragments from the rest of the sample.

**[0250]** Each of the remaining separation and detection chambers have also been pre-coated with three different immobilized probes, for a total of eighteen different immobilized probes in the biochip to capture the eighteen different DNA fragment types featuring eighteen target DNA sequences. In a similar manner, the sample is sequentially flowed through the separation and detection chambers, one chamber at a time, resulting in the extraction and physical separation of DNA fragments into the six separation and detection chambers according to the presence of target DNA sequences. The immobilized probes are designed or

chosen for each separation and detection chamber in order to bind three different types of DNA fragments, which are labeled with three different colors.

**[0251]** Following space separation, the three-color fluorescence detection system is used to detect the fluorescence emissions from each biochip separation and detection chamber, which contains paired immobilized probes and DNA fragments that emit three fluorescent colors. Because the unique types of DNA fragments have been physically separated into six separation and detection chambers according to target DNA sequence, up to six types of DNA fragments still may be labeled with an identical fluorescent color and be detected by a three-color fluorescence detection system. In total, all eighteen different target DNA sequences may be detected and analyzed.

Example 3

**[0252]** In a third exemplary embodiment, a biochip with six separation and detection chambers was used with a single-color fluorescence detection system to simultaneously detect as many as six target DNA sequences. A multiplex PCR or isothermal amplification process was used to generate a sample comprising six unique types of DNA fragments, which were of similar or different size. All six types of DNA fragments were labeled with an identical fluorescent color. The sample (e.g., 20μL) might be re-suspended in a buffer (e.g., 30μL) in order to begin with a higher sample volume (e.g., 50μL).

**[0253]** Instead of sequentially flowing the sample through the biochip separation and detection chambers, one chamber at a time, the sample was simultaneously loaded into all six separation and detection chambers (e.g., about 8μL volume per chamber). Each of the six separation and detection chambers had a probe corresponding to one DNA fragment for complementary binding. Hence in chamber 1, DNA fragments with DNA sequence A bound to probe A, while the other types of DNA fragments present in the sample in this chamber had no complementary binding to probe A. Similarly, in chamber 2, DNA fragments with DNA sequence B bound to probe B, while the other types of DNA fragments present in the sample in this chamber had no complementary binding to probe B. The process was similar for the remaining four separation and detection chambers.

**[0254]** One advantage of this approach is that, a probe in a separation and detection chamber does not need to be immobilized to the surface of the chamber. As illustrated above, this is because the amplified DNA fragments does not flow sequentially through all of the separation and detection chambers and a wash buffer is optional. Instead, all of the separation and detection chambers were filled simultaneously. However, a quencher

oligonucleotide might be required to quench the fluorescence emission of any DNA fragment that did not bind to a probe. The absence of flow suggested that complementarily-bound DNA fragment-probe pairs (along with other unbound macromolecules present in the 8μL sample) remained within the separation and detection chamber for subsequent fluorescent detection.

**[0255]** In this example, the fluorescence emission from a separation and detection chamber indicated the presence (or absence in the case of a fluorescence reduction method) of successful DNA fragment-probe complementary binding and the concentration thereof. All six types of DNA fragments was detected in the six independent separation and detection chambers using a single-color fluorescence detection system.

Example 4

**[0256]** To demonstrate the common quencher method for detecting multiplex PCR, we performed the following amplification and detection experiment on a biochip (NanoMDx, MA). For this demonstration, a biochip described in example 3, but with 2 fluorescent colors, was utilized.

**[0257]** Ultramer/DNA of 12 respiratory pathogens (purchased from IDT Technologies, CA) at 100000 copies each were co-amplified in a single PCR reaction of c.a. 25μL. PCR chemistry contained final primers concentration of 0.2μM each for the 12 primers, and the Qiagen multiplex PCR Kit (cat# 206152, Qiagen, CA) and HotStar polymerase (1U per reaction) were utilized to constitute the reaction. PCR thermal cycling conditions were, 96°C for 10 min for initial denaturation followed by 40 cycles of 96°C for 1 min, 60°C for 1 min, 72°C for 1 min, and final extension at 72°C for 5 min. Upon completion of PCR amplification, ~70μL of water was used as buffer to reconstitute the PCR amplicons to a total volume of about 90μL. This volume was then simultaneously input into six detection chambers. Each of the six detection chambers contained probe and quencher for only 2 targets, of which one target was labeled with FAM dye and the other with Cy5 dye. Hence with 2 distinct fluorescence probes/quencher in each of the 6 chambers a total of 12 targets is detected from the multiplex PCR amplification. The primers, probes, and quencher sequences are listed in the table below.

**[0258]** In each of the six chambers, the probe and the common-quencher oligonucleotide containing BHQ quencher was at equimolar concentration of 0.2 μM contained in ~5μL solution. With a detection volume capacity of ~20μL, an c.a. of 15μL of suspended PCR amplicon volume, and 5μL of probe/common-quencher is present in each detection chamber.

**[0259]** Once the PCR amplicon was loaded into all 6 detection chambers simultaneously, the temperature was ramped from ~25°C (room temperature) to 94°C at the rate of 1.5°C - 2.5°C per second. After the temperature reached 94°C, a ramp down was initiated from 94°C to 25°C at a ramp down rate of 2-4°C per second. This ramp up and down allows for the different denaturing and annealing interactions between the PCR amplified amplicons, probes, and primers, as described earlier. Finally, the fluorescence of FAM and Cy5 in each of the 6 chambers was measured on a fluorescence reader (FLX800T, BioTek Instruments, VT), data shown below. Presence of FAM and Cy5 fluorescence in each chamber indicates the successful binding of a PCR amplicon to the corresponding detection probe present in that chamber. As shown in FIG. 29, an increase in probe fluorescence, represented in relative fluorescence units (RFU), is noted for each of the 12 targets. FIG. 30A lists forward primers, reverse primers, and probe sequences for the twelve targets. FIG. 30B lists two exemplary common quenchers with their sequences and dye quenching moieties used in the experiment. The random sequence for both examples is TGTTATTCAGT, and the dye quenching moieties are 3IAbRQSp and 3IABkFQ.

**[0260]** While the above demonstrated the detection of 12 targets in 6 chambers with 2 color detection in each chamber, it is possible to increase the number of detection targets, for example to 4 in each chamber, to achieve a distinct fluorescence detection of 24 targets in 6 chambers. Increasing the number of detection chambers beyond 6 also provides increased target detection ability. The above experiment validated one of our unique simultaneous/parallel detection methods and the unique common-quencher method. Probes that are bound to the DNA strand emits fluorescence. And the remaining probes are bound to the common quencher oligonucleotides, and therefore these remaining probes do not emit fluorescence.

**[0261]** A person of ordinary skill will understand that these different approaches may be combined in different embodiments. For instance, the three-color fluorescence detection system approach in Example 2 can also be applied to the method of Example 3 to detect up to three different target DNA sequences in each separation and detection chamber for a total of up to eighteen different target DNA sequences across a biochip with six separation and detection chambers.

**[0262]** Also, as illustrated above, the scope of the present invention is not limited to a biochip having multiple chambers, but the detection method can use any other multiple enclosures (e.g., vials or chambers).

**[0263]** The following lists provide examples of common quencher sequence listings.

**LIST 1**

>1|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp
aacgtgactttt
>2|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp
attgtatct
>3|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 17 bp
gtcctttattgcaagaa
>4|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp
actggaattc
>5|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp
ttagttcatagcat
>6|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp
atgtattattccg
>7|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp
attatggcac
>8|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 8 bp
catagttt
>9|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp
atttcctgtaatag
>10|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp
gatgtctcatta
>11|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp
ctacgtttttagaa
>12|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp
taagcgtttcat
>13|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp
gaccgtattaattt
>14|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp
attagtttctgac
>15|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 18 bp
gtatccattatgagtatc
>16|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 8 bp
ttttcgaa
>17|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

ttctacgatgctaatg

>18|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp

catgatattctgt

>19|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 19 bp

attaatgtccaagtttgct

>20|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 17 bp

ttgcattagttcaaacg

>21|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp

tacttttag

>22|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

gttgctaaagcattct

>23|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

gagctatgtttcaatc

>24|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp

atccgtagta

>25|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 11 bp

attgacgtcat

>26|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

gctgttcaatat

>27|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp

tacggttacattta

>28|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

tattacgctagagct

>29|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

gtatatccgctgtaa

>30|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 17 bp

cgtctaattaggacatt

>31|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

tcatatattactaggacggc

>32|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp

tatactttg

>33|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

tatgtgactcaatcg

>34|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

atcaagcttcgtagtgctaa

>35|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

tacattagccgtagt

>36|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 11 bp

tatccagtagt

>37|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 11 bp

ttatggcctaa

>38|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp

gattcttta

>39|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

aatcgtatccagtgtt

>40|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

tggtaaaactagactccgtt

>41|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

ttagcgtattatcagc

>42|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

gttgactaatgagcactatc

>43|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp

cttagatagc

>44|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp

aattacagtttctg

>45|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 11 bp

agcatatttgc

>46|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp

atgtcactatgtta

>47|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp

tcacttatgtgta

>48|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 18 bp

ccaagtttttcagagtta

>49|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp

atcagtttt

>50|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 18 bp

tgctttaatatccaggat

>51|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 19 bp

ttttgacaataatgcgtc

>52|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

taatatgcttcg

>53|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 18 bp

aatatgtaccgttgtcat

>54|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp

tgtattcta

>55|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp

acatacttaggttt

>56|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 19 bp

tagccttcagtaagtttat

>57|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

agatctagtgcttcat

>58|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

atttgccttgaa

>59|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp

tgtctgactttaa

>60|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp

gatatacctg

>61|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 18 bp

actgaatgattattcctg

>62|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 19 bp

gtcggtatttaatcactat

>63|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

attcgatcgtcatgat

>64|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp

ttcgtaatt

>65|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp

tcgtttaatgcat

>66|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

tcagatcttatg

>67|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 19 bp

agttcttaaccgtatattg

>68|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

aatctcctaattggg

>69|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp

tctaaggcatttat

>70|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp

atctttagtgact

>71|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 11 bp

aattgcgatct

>72|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

cgatcagtttat

>73|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

gcggtcaatatgctacatta

>74|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

cgctatttagaatgc

>75|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

gtctgatatacagtct

>76|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 9 bp

ttacgattt

>77|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

agagtatcgtcgaattacct

>78|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 8 bp

catgttat

>79|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

acgtatttactg

>80|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

ttcgtacctaagtag

>81|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

cagtgcttaatt

>82|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

cttcacaattgtactgggaa

>83|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

tattactgggcactat

>84|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

acatcttcggcaatttgaag

>85|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 19 bp

agatttccagtctgtatta

>86|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 16 bp

gttacctaatgctagt

>87|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 12 bp

catttaatggtc

>88|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 8 bp

tactttag

>89|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp

cgattcaagt

>90|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp

tatacagtgc

>91|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 15 bp

tccaaggttaatgtc

>92|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 8 bp

actttagt

>93|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 13 bp

tatctcgtaattg

>94|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 20 bp

atatcatacccgagtagttg

>95|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 10 bp

aggtctaact

>96|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 14 bp

tatggttactacta

>97|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 11 bp

gaacttgctta

>98|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 19 bp

cagtggtaatttccatattt

>99|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 8 bp

tacagttt

>100|random sequence|A: 0.3|C: 0.2|G: 0.2|T: 0.23|length: 18 bp

gagttaatttcacattcg

**LIST 2**

>1|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 16 bp

gacctaacttcagggt

>2|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 19 bp

ctaggcagttcatgcttat

>3|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 15 bp

acctgctattaggtt

>4|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 15 bp

ctcttcatggatatg

>5|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 14 bp

cttacttgtggaac

>6|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 11 bp

tttgacacttg

>7|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 20 bp

gccgagtcagaattgttcac

>8|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 12 bp

tctaatggcagc

>9|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 12 bp

cgactatgtcag

>10|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 12 bp

tcgacttcagga

>11|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 20 bp

ctttgtgcaaagcgagtcac

>12|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 15 bp

cgtatgtatcattgc

>13|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 9 bp

ctcggaatt

>14|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 10 bp

ccattttgag

>15|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 11 bp

gcttctagtta

>16|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 20 bp

aattacgccaaccttgggtg

>17|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 16 bp

acctacgtttaggcga

>18|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 18 bp

gcggaattttcactactg

>19|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 17 bp

tggactaatcacggttc

>20|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 8 bp

cgtcaatg

>21|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 16 bp

atctgtgcaggtcaac

>22|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 19 bp

cagtactctagggacttttt

>23|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 8 bp

ttaacgcg

>24|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 13 bp

ttgtcagctagca

>25|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 19 bp

cttctggagcgtctaatat

>26|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 10 bp

gctaagttct

>27|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 8 bp

tcgcagat

>28|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 9 bp

gagtactct

>29|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 8 bp

agtagcct

>30|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 20 bp

tacactggttaggcatcacg

>31|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 19 bp

ccagtgcatacgttttttga

>32|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 15 bp

ctttctcgattagga

>33|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 14 bp

actgactttgcatg

>34|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 17 bp

cggccaattcgaatttg

>35|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 12 bp

tcgcagatacgt

>36|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 12 bp

gaacctgtagtc

>37|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 19 bp

attagtatggtcccctatgc

>38|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 11 bp

gcattgttact

>39|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 17 bp

atggatcgctcttacag

>40|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 19 bp

gcatgtgccgtttctaata

>41|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 17 bp

aacgcatcgtatcggtt

>42|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 14 bp

ttgacgtccatgat

>43|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 12 bp

ccagacttagtg

>44|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 20 bp

atctgaagtggagcactctc

>45|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 13 bp

gatcccgatatgt

>46|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 19 bp

tactgtaggatgctctcta

>47|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 16 bp

caactttcgcaaggtg

>48|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 13 bp

gtctgcataatgc

>49|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 12 bp

caatggtccatg

>50|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 11 bp

tgcaatttcgt

>51|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 15 bp

tatcgctattgctga

>52|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 16 bp

tatgtcgcgagccaat

>53|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 20 bp

ttgacacgtgaatgccactg

>54|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 15 bp

gagcttcttattgac

>55|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 16 bp

acgacttgatgtgcca

>56|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 9 bp

ctatcgtga

>57|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 17 bp

tcacgattagcttcagg

>58|random sequence|A: 0.25|C: 0.25|G: 0.25|T: 0.25|length: 16 bp

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agttaatctt

**[0264]** As will be apparent to one of ordinary skill in the art from a reading of this disclosure, the present disclosure can be embodied in forms other than those specifically disclosed above. The particular embodiments described above are, therefore, to be considered as illustrative and not restrictive. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described herein. The scope of the invention is as set forth in the

appended claims and equivalents thereof, rather than being limited to the examples contained in the foregoing description.

CLAIMS

1. A biochip for multiplex genetic identification, the biochip comprising:

a sample input port to receive a sample containing a plurality of DNA fragments; and

a plurality of separation and detection chambers in fluid communication with the sample input port, each of the plurality of separation and detection chambers being spaced apart from the other chambers, and each chamber containing at least one set of DNA probes immobilized therein, the set of DNA probes in each chamber differing from the sets of DNA probes in the other chambers.

2. The biochip of claim 1, further comprising a wash buffer input port to receive a wash buffer, the wash buffer input port in fluid communication with the plurality of separation and detection chambers.

3. The biochip of claim 1, further comprising an amplification chamber to amplify the plurality of DNA fragments, the amplification chamber in fluid communication with the sample input port and the plurality of separation and detection chambers.

4. The biochip of claim 3, further comprising:

an elution buffer input port to receive an elution buffer; and

a sample preparation chamber in fluid communication with the elution buffer input port, the sample input port, and the amplification chamber.

5. The biochip of claim 3, further comprising:

a sample preparation wash buffer input port to receive a sample preparation wash buffer; and

a sample preparation chamber in fluid communication with the sample preparation wash buffer input port, the sample input port, and the amplification chamber.

6. The biochip of claim 3, further comprising:

a post-amplification buffer input port to receive a post-amplification buffer; and

a post-amplification vent chamber in fluid communication with the post-amplification buffer input port and the amplification chamber.

7. The biochip of claim 3, further comprising a post-amplification vent chamber in fluid communication with the amplification chamber and the plurality of separation and detection chambers.

8. The biochip of claim 3, further comprising a waste output port in fluid communication with the plurality of separation and detection chambers.

9. The biochip of claim 1, further comprising a plurality of vent chambers, each vent chamber in fluid communication with its corresponding chamber of the plurality of separation and detection chambers.

10. The biochip of claim 1, further comprising at least one background reference chamber not in fluid communication with the sample input port and the plurality of separation and detection chambers, wherein the at least one background reference chamber and at least one of the plurality separation and detection chambers are composed of a same material.

11. The biochip of claim 10, wherein each of the at least one background reference chamber contains at least one DNA probe identical to the at least one DNA probe of its corresponding separation and detection chamber.

12. The biochip of claim 1, wherein the plurality of separation and detection chambers are connected in parallel.

13. The biochip of claim 1, wherein the plurality of separation and detection chambers are connected in series.

14. The biochip of claim 1, further comprising at least one flow gate to control fluid flow between the sample input port and at least one of the plurality of separation and detection chambers.

15. The biochip of claim 14, wherein the at least one flow gate has an inlet channel and an outlet channel, the at least one flow gate inhibiting fluid flow from the inlet channel to the outlet channel with an absence of fluid pressure and permitting fluid flow from the inlet channel to the outlet channel with fluid pressure.

16. The biochip of claim 14, wherein the at least one flow gate has an inlet channel and an outlet channel, the at least one flow gate inhibiting fluid flow from the inlet channel to the outlet channel with pneumatic pressure and permitting fluid flow from the inlet channel to the outlet channel with an absence of pneumatic pressure.

17. The biochip of claim 1, further comprising a plurality of transition channels in fluid communication, wherein each of the plurality of transition channel has an increasing cross-sectional area in a direction to its corresponding separation and detection chamber.

18. The biochip of claim 1, further comprising a waste output port in fluid communication with the plurality of separation and detection chambers.

19. The biochip of claim 1, further comprising:

at least one fluid input port in fluid communication with the plurality of separation and detection chambers;

an integrated cartridge comprising at least one reservoir corresponding to the fluid input port, wherein the at least one reservoir has an opening aligned with the corresponding fluid input port to provide fluid from the at least one reservoir to the corresponding fluid input port.

20. The biochip of claim 19, wherein the at least one reservoir comprises at least one of a sample and lysis buffer reservoir, an elution buffer reservoir, a sample preparation wash buffer reservoir, a post-amplification buffer reservoir, and a separation wash buffer reservoir.

21. The biochip of claim 19, further comprising at least one membrane, the at least one membrane having a sealable opening and separating the opening of the at least one reservoir from its corresponding fluid input port.

22. The biochip of claim 21, wherein:

the at least one fluid input port comprising an input chamber;

the sealable opening of the membrane being aligned with its corresponding input chamber to allow the sealable opening to open toward its corresponding input chamber; and

the sealable opening of the membrane being offset from the opening of its corresponding reservoir to prevent the sealable opening from opening toward the reservoir.

23. The biochip of claim 21, wherein the sealable opening is a slit.

24. The biochip of claim 21, further comprising at least one pneumatic line, the at least one pneumatic line in fluid communication with a corresponding fluid input port, the at least one pneumatic line configured to provide pressure to the sealable opening.

25. The biochip of claim 19, further comprising:

a cartridge opening on the integrated cartridge to permit access to the at least one reservoir; and

a membrane affixed to an area in proximity to the cartridge opening, the membrane covering the cartridge opening;

wherein the membrane has a sealable opening aligned with the cartridge opening, the sealable opening being configured to open by external pressure and configured to seal without external pressure.

26. The biochip of claim 25, wherein the external pressure is mechanical.

27. The biochip of claim 25, wherein the membrane is a rubber gasket.

28. The biochip of claim 27, wherein the rubber gasket is one of silicone, PTFE, neoprene, polyethelene, polymide, polycarbonate, acrylic, cyclo-olyfin polymers, latex and cyclo olefin polymers.

29. The biochip of claim 1, further comprising:

at least one waste output port in fluid communication with the plurality of separation and detection chambers;

an integrated cartridge comprising at least one corresponding waste reservoir, wherein the at least one waste reservoir has an opening aligned with the corresponding waste reservoir to receive waste from the at least one waste output port.

30. A device comprising:

a chamber;

an opening to the chamber to receive fluid in the chamber; and

a membrane affixed to an area in proximity to the chamber opening, the membrane covering the chamber opening;

wherein the membrane has a sealable opening aligned with the chamber opening, the sealable opening being configured to open by external pressure and configured to seal without external pressure.

31. A flow gate, comprising:

a first port and a second port separated by a junction, each port being a small chamber at an end of a channel; and

a thin flexible material adhered across the first and second ports,

wherein the flow gate has an open configuration and a closed configuration, wherein pressure applied to the thin flexible material changes between the open configuration and the closed configuration, the open configuration having space between the thin flexible material and the junction and permitting fluid flow between the two channels through the ports, and the closed configuration having the thin flexible material in contact with the junction and inhibiting fluid flow between the two channels.

32. The flow gate of claim 31, wherein the thin flexible material is at least one of plastic and rubber.

33. The flow gate of claim 31, wherein the thin flexible material is at least one of PTFE, Teflon, PVC, polyethylene, polypropylene, polyimide, latex, silicone, and nitrile.

34. The flow gate of claim 31, wherein the pressure applied to the thin flexible material is at least one of pneumatic pressure, thrust, and fluid pressure.

35. The flow gate of claim 31, further comprising at least one layer of material disposed above the ports.

36. The flow gate of claim 35, wherein the flow gate is in the open configuration without the pressure and wherein the pressure changes the flow gate from the open configuration to the closed configuration.

37. The flow gate of claim 35, wherein the flow gate is in the closed configuration without the pressure and wherein the pressure changes the flow gate from the closed configuration to the open configuration.

38. The flow gate of claim 35, wherein the flow gate is in the closed configuration without the pressure and wherein the pressure changes the flow gate from the closed configuration to the open configuration.

39. The flow gate of claim 31, wherein the thin flexible material is adhered to at least a portion of the junction separating the first and second ports to inhibit fluid flow between the two channels, wherein the adhesion is configured to be delaminated by fluid pressure in at least one of the channels above a threshold pressure.

40. The flow gate of claim 39, wherein an adhesive adhering the thin flexible material to the portion of the junction is configured to remain delaminated after delamination by the fluid pressure.

41. A method of detecting nucleic acid fragments, the method comprising:

providing a plurality of chambers, each chamber separated from the other chambers of the plurality, each chamber having at least one set of probes disposed therein, each set of probes being capable of binding to a different target nucleic acid sequence relative to the other sets of probes;

providing a sample comprising a plurality of sets of nucleic acid fragments;

placing at least a portion of the sample into each of the plurality of chambers and causing the at least one set of probes in each chamber to bind with complementary nucleic acid fragments of the sample; and

detecting the binding of the nucleic acid fragments to the sets of probes in each chamber.

42. The method of claim 41, wherein the placing the at least a portion of the sample into each of the plurality of chambers comprises flowing the portions of the sample through a first chamber of the plurality of chambers, the chambers being fluidically coupled in series.

43. The method of claim 41, wherein the placing the at least a portion of the sample into each of the plurality of chambers comprises flowing the portions of the sample into the plurality of chambers, the chambers being fluidically coupled in parallel.

44. The method of claim 41, wherein the nucleic acid fragments are DNA fragments.

45. The method of claim 41, wherein the nucleic acid fragments are RNA fragments.

46. The method of claim 41, wherein the plurality of chambers are disposed in a biochip, the biochip comprising an input port in fluid communication with the plurality of chambers.

47. The method of claim 41, wherein providing the sample comprises performing a nucleic acid amplification.

48. The method of claim 47, wherein the nucleic acid amplification comprises at least one of PCR amplification and isothermal amplification.

49. The method of claim 41, wherein the sets of probes are fluorescently labeled, the binding of the complementary nucleic acid fragments of the sample persevering the fluorescence of the label, and wherein the detecting the binding of the nucleic acid fragments to the sets of probes includes detecting the fluorescence of the labels.

50. The method of claim 49, further comprising quenching the fluorescent labels of unbound probes.

51. The method of claim 49, wherein the sets of probes are immobilized within the chambers.

52. The method of claim 41, wherein providing the sample comprises performing a nucleic acid amplification in which copies of amplified nucleic acid fragments are fluorescently labeled, the binding of the complementary nucleic acid fragments of the sample persevering the fluorescence of the nucleic acid fragments, and wherein the detecting the binding of the nucleic acid fragments to the sets of probes includes detecting the fluorescence of the nucleic acid fragments.

53. The method of claim 52, further comprising quenching the fluorescent labels of unbound nucleic acid fragments.

54. The method of claim 52, further comprising washing unbound nucleic acid fragments out of the plurality of plurality of chambers.

55. The method of claim 52, wherein the sets of probes are immobilized within the chambers.

56. The method of claim 41, wherein:

providing the sample comprises performing a nucleic acid amplification in which copies of amplified nucleic acid fragments are amplified using fluorescently labeled bases of a specified type;

at least one of the sets of probes has fluorescently labeled bases that are complementary to the specified type, the fluorescently labeled bases of the probes being quenched by binding to the fluorescently labeled bases of the nucleic acid fragments; and

the detecting the binding of the nucleic acid fragments to the sets of probes includes detecting when a fluorescently labeled base of the probes is not quenched.

57. A method of detecting nucleic acid fragments, the method comprising:

providing a plurality of sets of probes, each set of probes having a nucleic acid sequence,

a first portion of the sequence being complementary to a target nucleic acid sequence, the target nucleic acid sequence differing for each set of probes relative to the other sets of probes,

a second portion of the sequence being a specified sequence, the second portion of the sequence being the same for each set of probes, and

each probe having a fluorescent label joined to the second portion of the sequence;

providing a sample comprising a plurality of sets of nucleic acid fragments;

causing the first portion of the sequence of at least one set of probes to bind with nucleic acid fragments of the sample having a sequence complementary to said first portion;

providing a quenching compound that has a quenching moiety and a nucleic acid sequence that is complementary to the specified sequence of the second portion of the sets of probes;

causing the quenching compound to bind to the second portions of the sequences of the sets of probes which are not bound to nucleic acid fragments of the sample, thereby causing the quenching moiety to quench the fluorescent labels of probes to which the quenching compounds are bound; and

detecting the binding of the nucleic acid fragments to the sets of probes based on unquenched fluorescent labels of probes.

58. The method of claim 57, wherein the nucleic acid fragments are DNA fragments.

59. The method of claim 57, wherein the nucleic acid fragments are RNA fragments.

60. The method of claim 57, wherein providing the sample comprises performing a nucleic acid amplification.

61. The method of claim 60, wherein the nucleic acid amplification comprises at least one of PCR amplification and isothermal amplification.

62. The method of claim 57, further comprising:

providing a plurality of chambers, each chamber separated from the other chambers of the plurality, each chamber having at least one set of probes of the plurality disposed therein, the sets of probes in each chamber differing from the sets of probes in the other chambers; and

placing at least a portion of the sample into each of the plurality of chambers prior to causing the first portion of the sequence of at least one set of probes to bind with the nucleic acid fragments.

63. The method of claim 62, wherein the placing the at least a portion of the sample into each of the plurality of chambers comprises flowing the portions of the sample through a first chamber of the plurality of chambers, the chambers being fluidically coupled in series.

64. The method of claim 62, wherein the placing the at least a portion of the sample into each of the plurality of chambers comprises flowing the portions of the sample into the plurality of chambers, the chambers being fluidically coupled in parallel.

65. The method of claim 62, wherein the plurality of chambers are disposed in a biochip, the biochip comprising an input port in fluid communication with the plurality of chambers.

66. The method of claim 62, wherein the sets of probes are immobilized within the chambers.

67. The method of claim 62, further comprising washing unbound nucleic acid fragments out of the plurality of plurality of chambers.

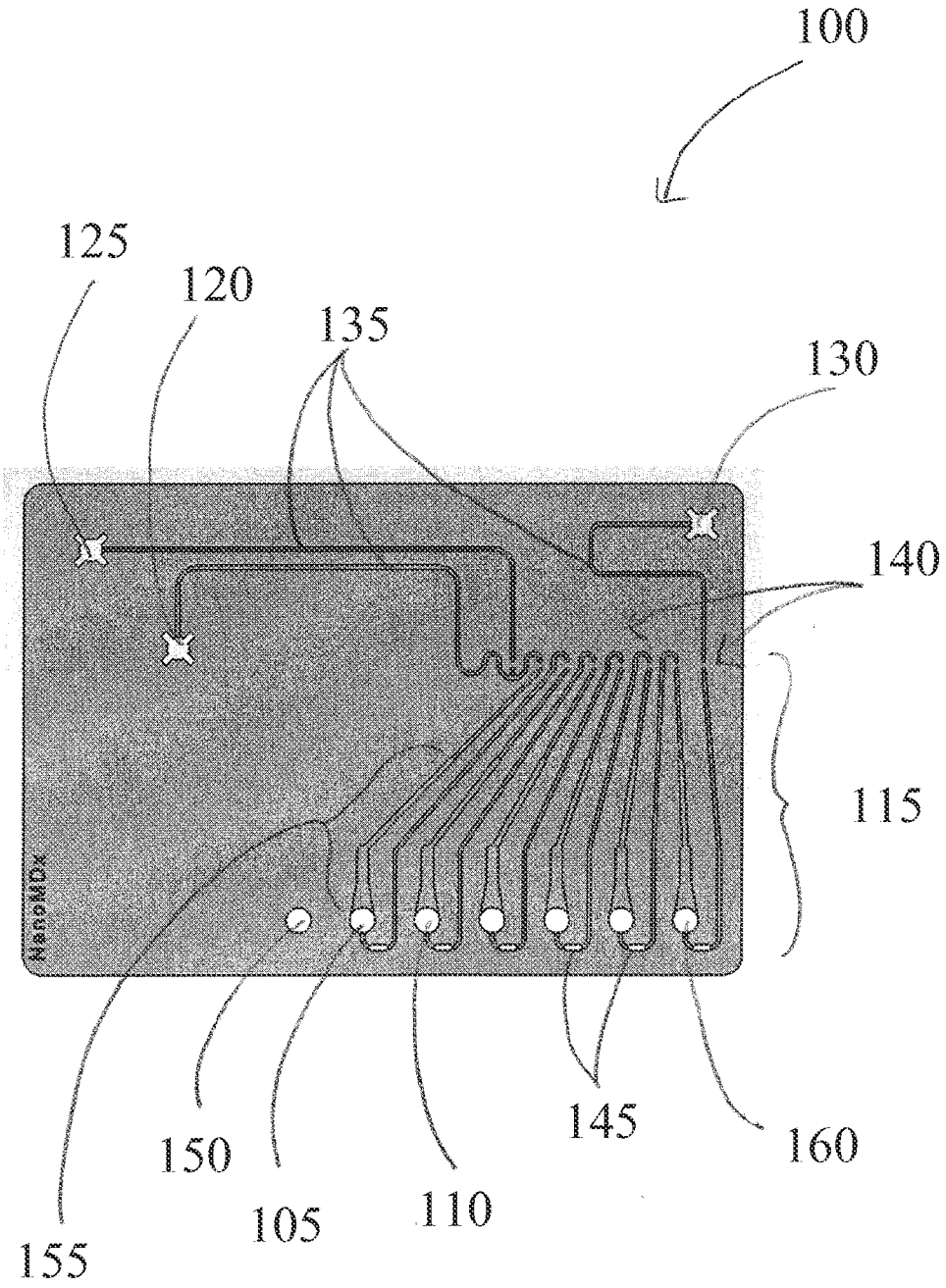



FIG. 1

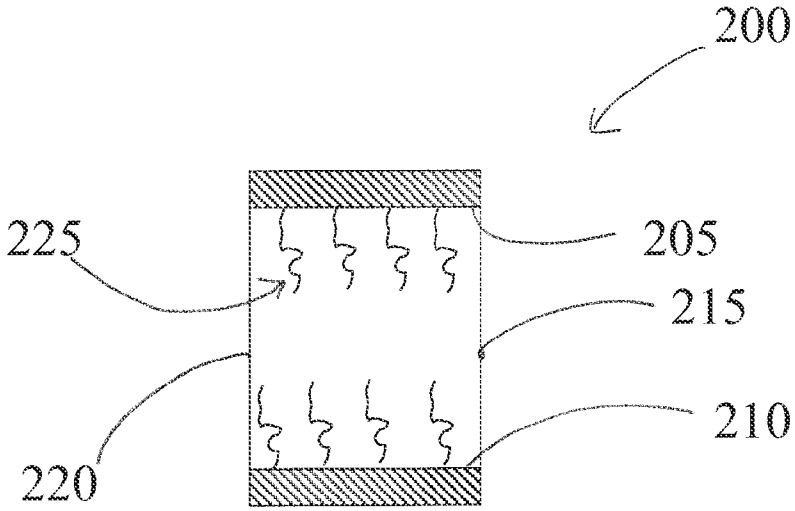



FIG. 2

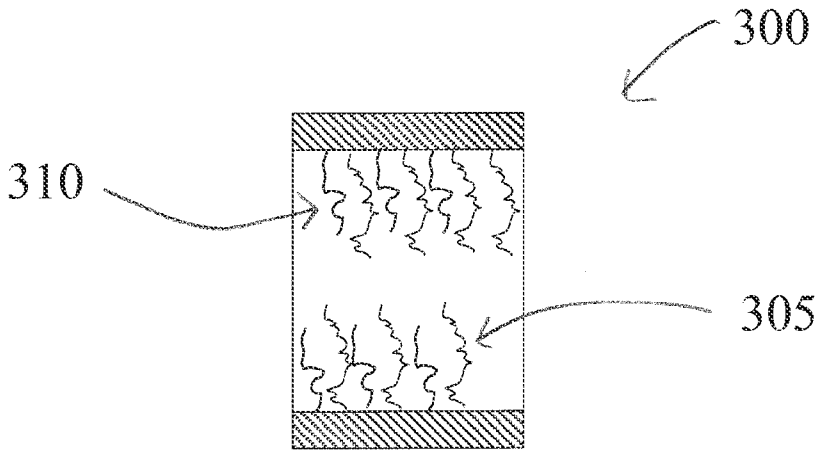



FIG. 3

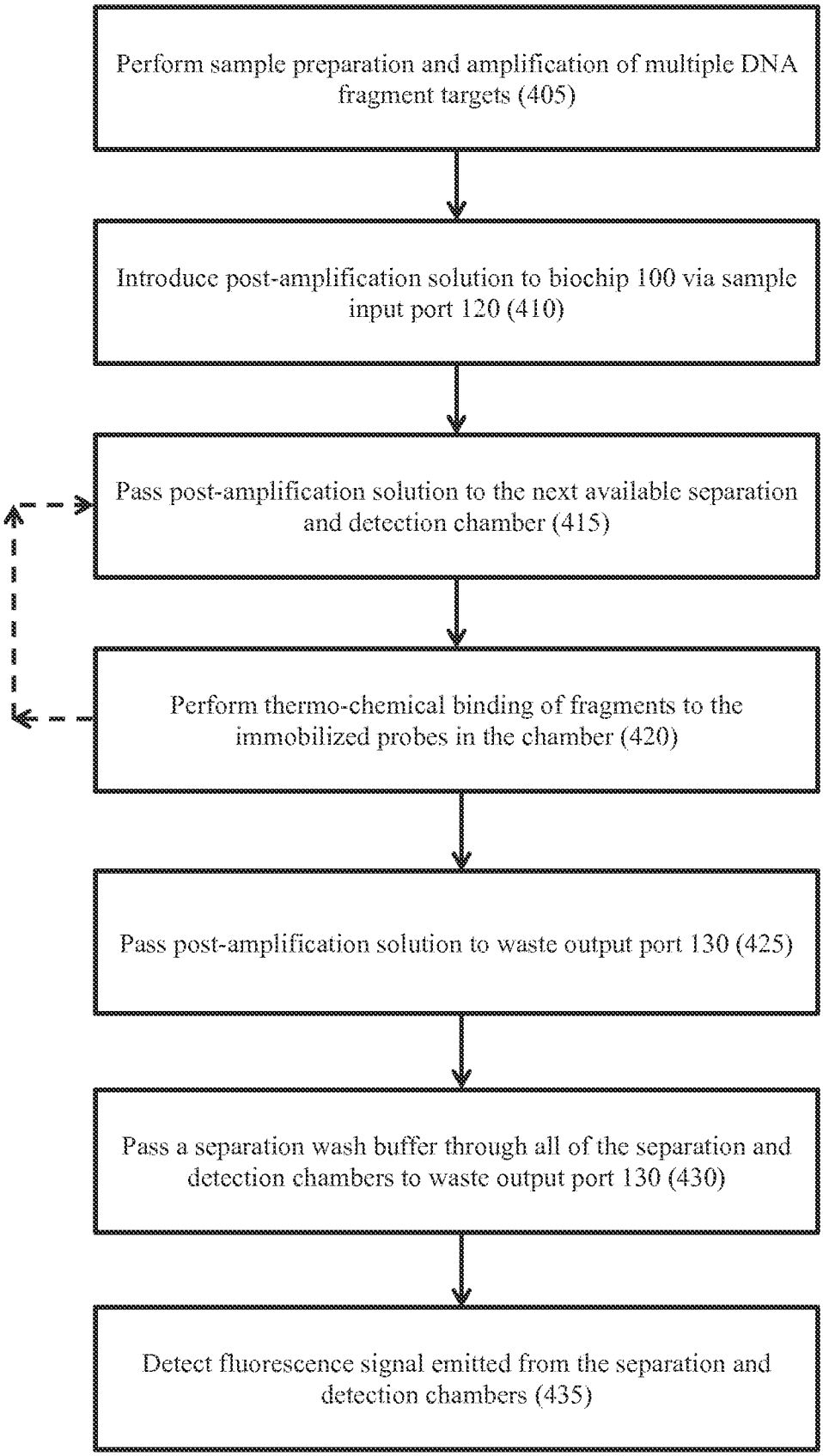

Perform sample preparation and amplification of multiple DNA fragment targets (405)
Introduce post-amplification solution to biochip 100 via sample input port 120 (410)
Pass post-amplification solution to the next available separation and detection chamber (415)
Perform thermo-chemical binding of fragments to the immobilized probes in the chamber (420)
Pass post-amplification solution to waste output port 130 (425)
Pass a separation wash buffer through all of the separation and detection chambers to waste output port 130 (430)
Detect fluorescence signal emitted from the separation and detection chambers (435)

FIG. 4

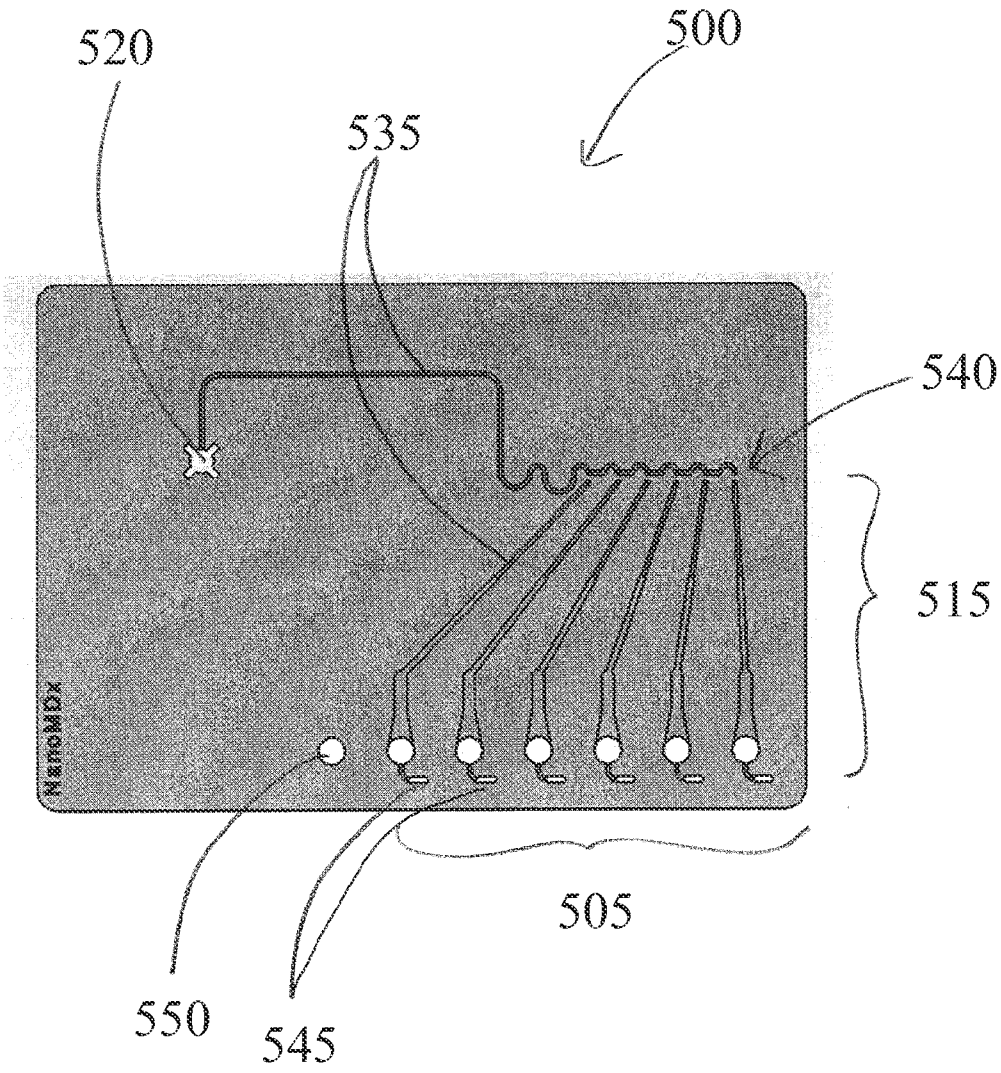



FIG. 5

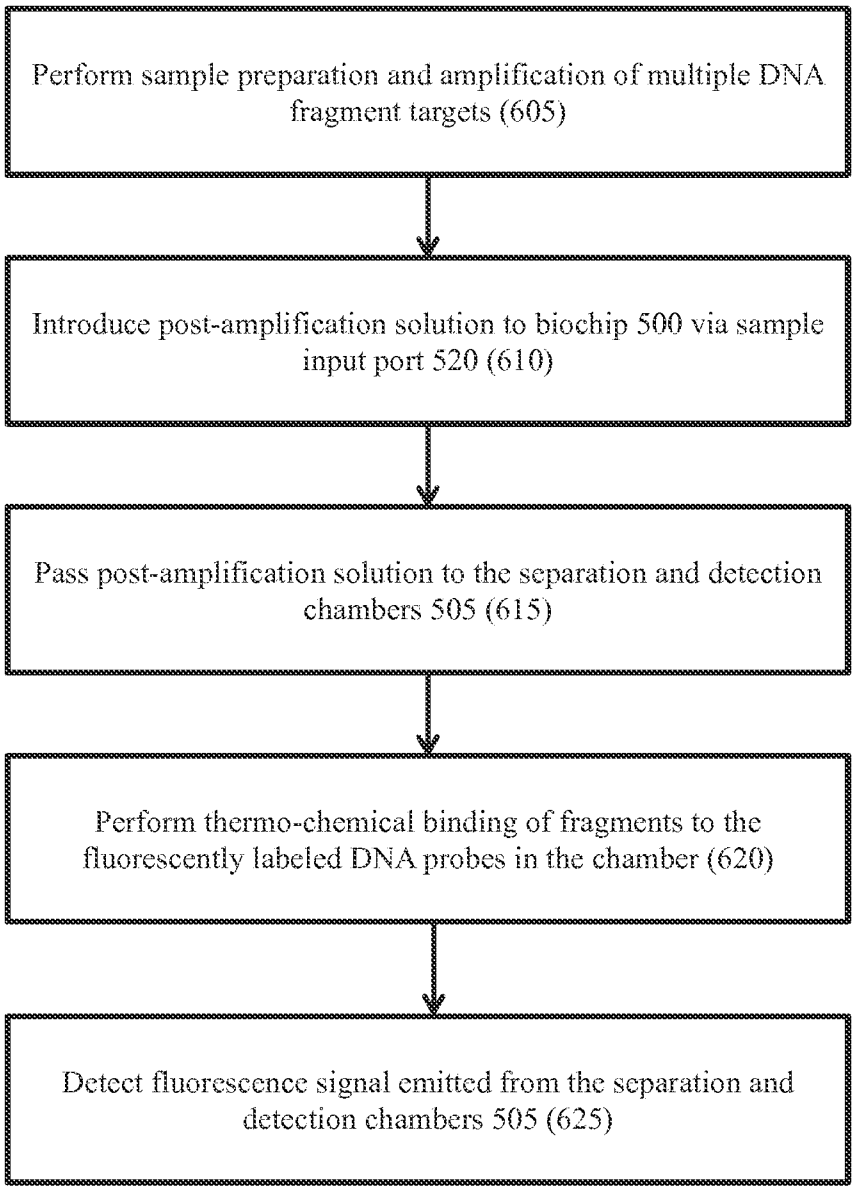

Perform sample preparation and amplification of multiple DNA fragment targets (605)
Introduce post-amplification solution to biochip 500 via sample input port 520 (610)
Pass post-amplification solution to the separation and detection chambers 505 (615)
Perform thermo-chemical binding of fragments to the fluorescently labeled DNA probes in the chamber (620)
Detect fluorescence signal emitted from the separation and detection chambers 505 (625)

FIG. 6

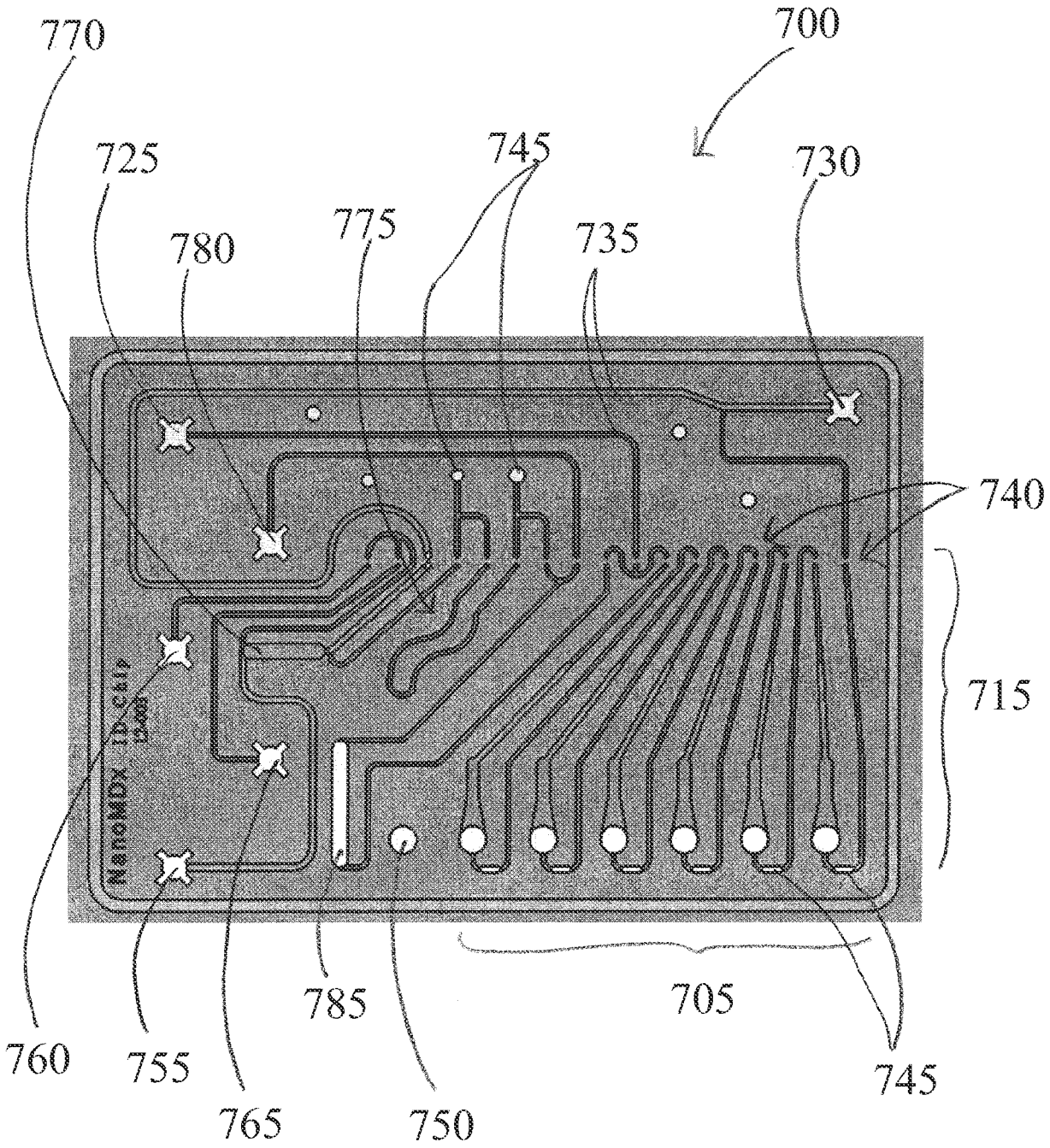

770
700
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715
NanoMDx ID-Chip
760
755
765
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785
705
745

FIG. 7

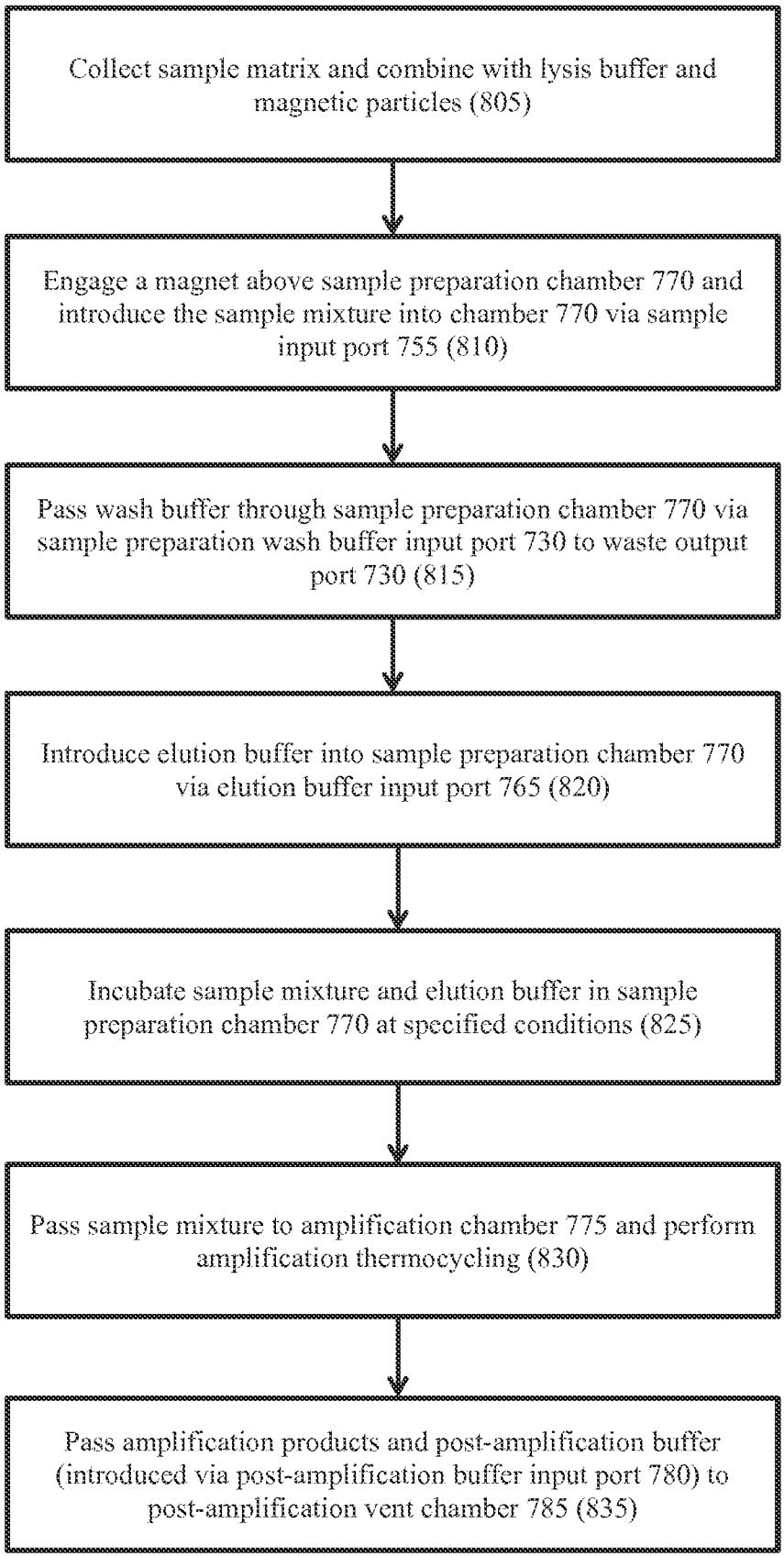

Collect sample matrix and combine with lysis buffer and magnetic particles (805)
Engage a magnet above sample preparation chamber 770 and introduce the sample mixture into chamber 770 via sample input port 755 (810)
Pass wash buffer through sample preparation chamber 770 via sample preparation wash buffer input port 730 to waste output port 730 (815)
Introduce elution buffer into sample preparation chamber 770 via elution buffer input port 765 (820)
Incubate sample mixture and elution buffer in sample preparation chamber 770 at specified conditions (825)
Pass sample mixture to amplification chamber 775 and perform amplification thermocycling (830)
Pass amplification products and post-amplification buffer (introduced via post-amplification buffer input port 780) to post-amplification vent chamber 785 (835)

FIG. 8

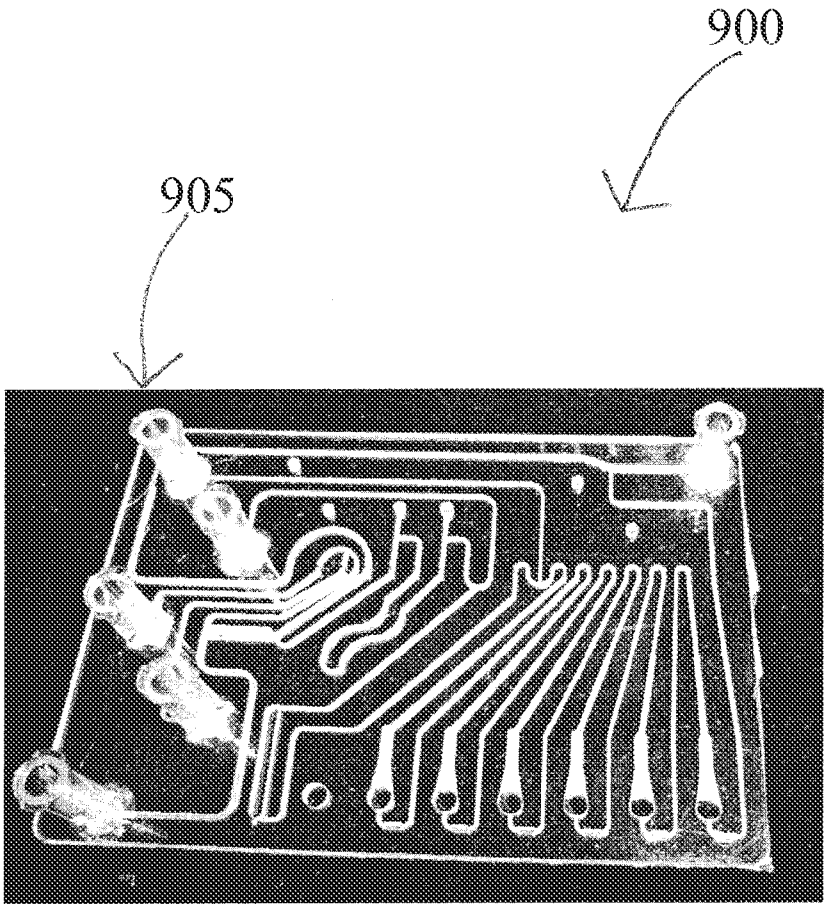



FIG. 9

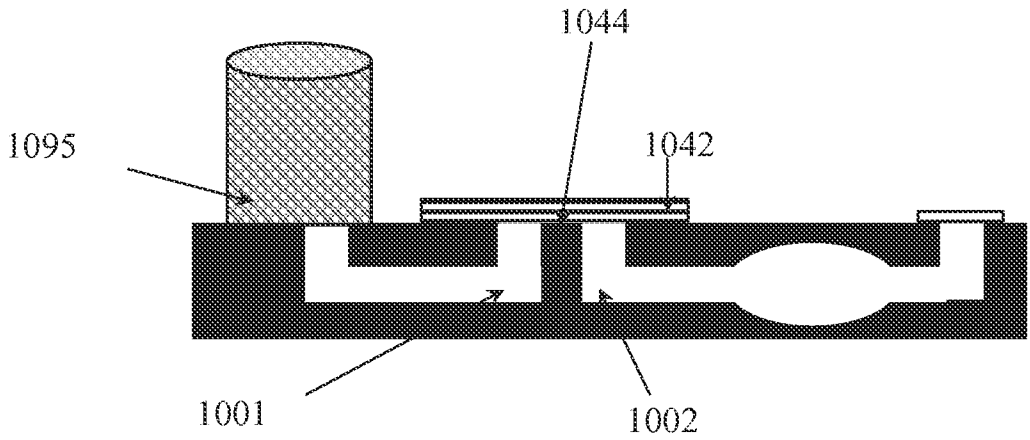



FIG. 10A

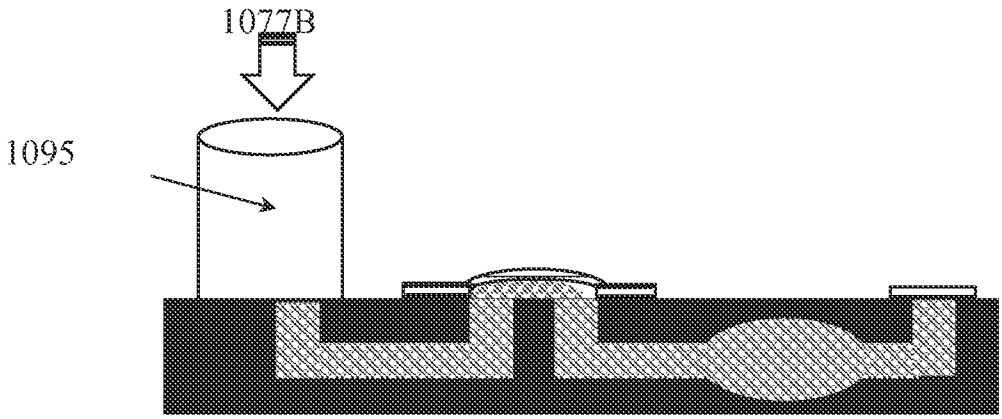



FIG. 10B

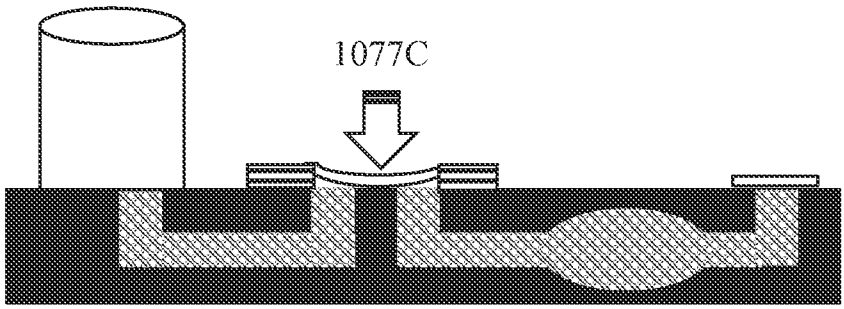



FIG. 10C

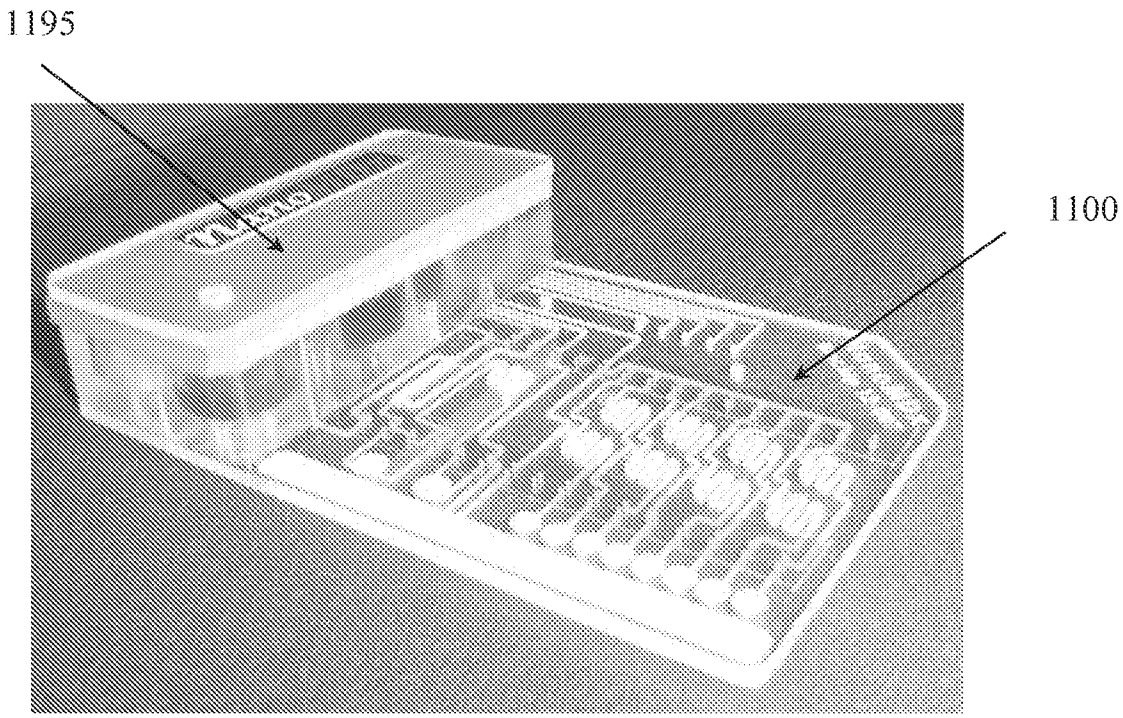



FIG. 11A

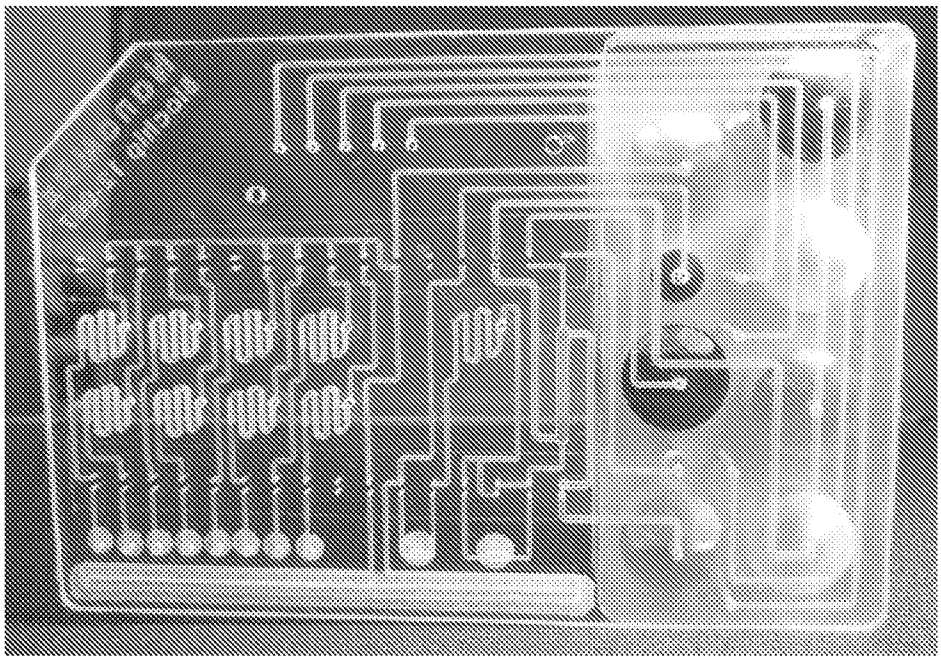


FIG. 11B

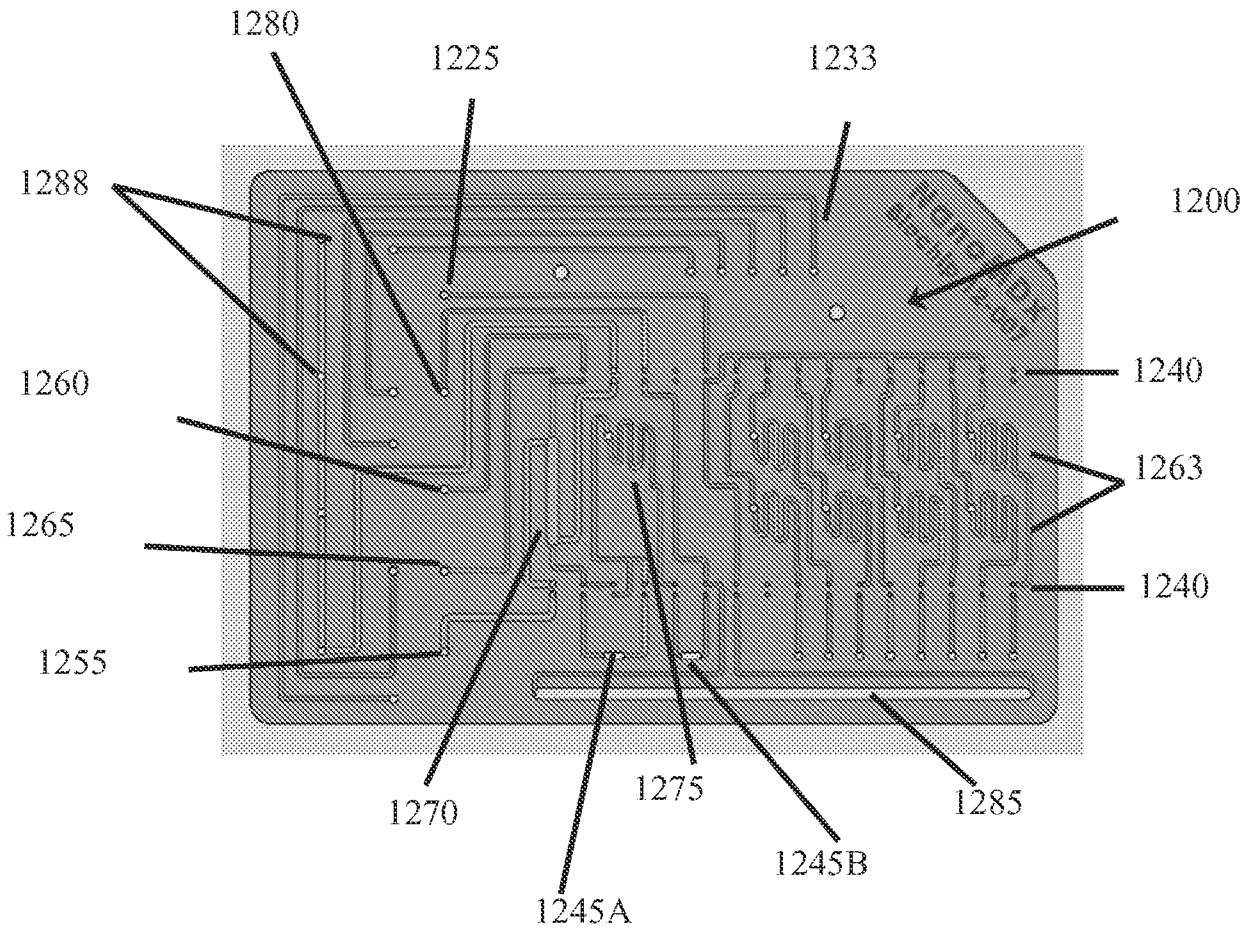

1280
1225
1233
1288
1200
1260
1240
1263
1265
1240
1255
1270
1275
1285
1245B
1245A

FIG. 12

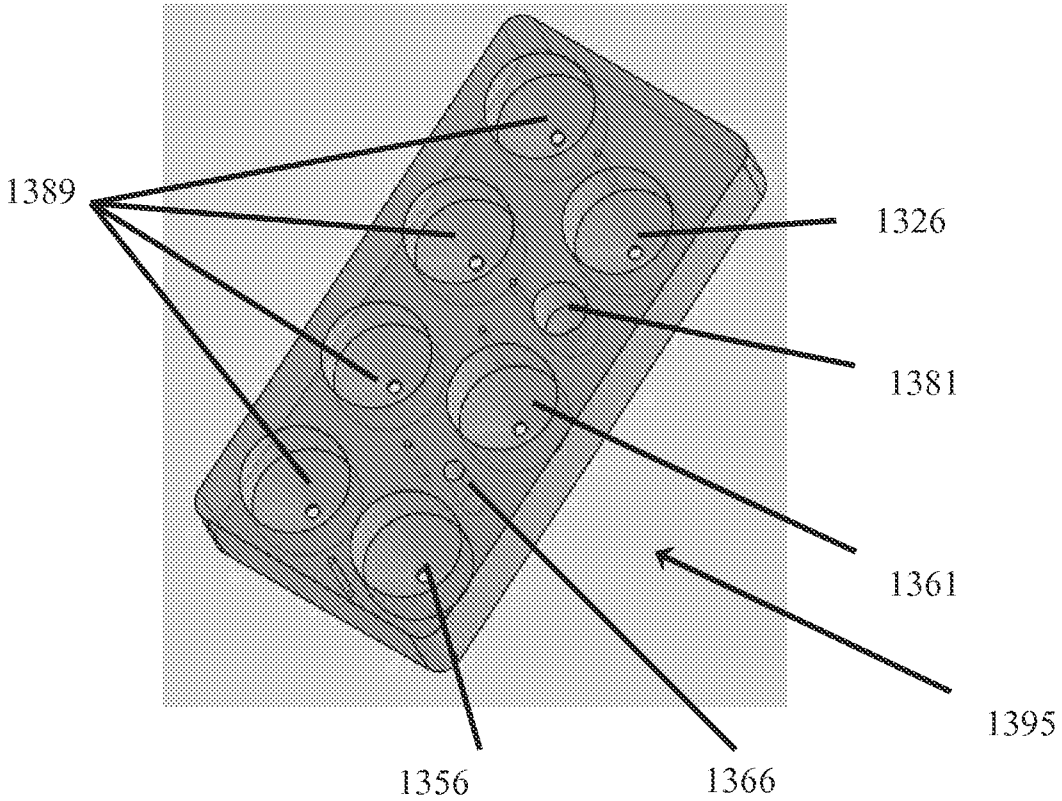



FIG. 13

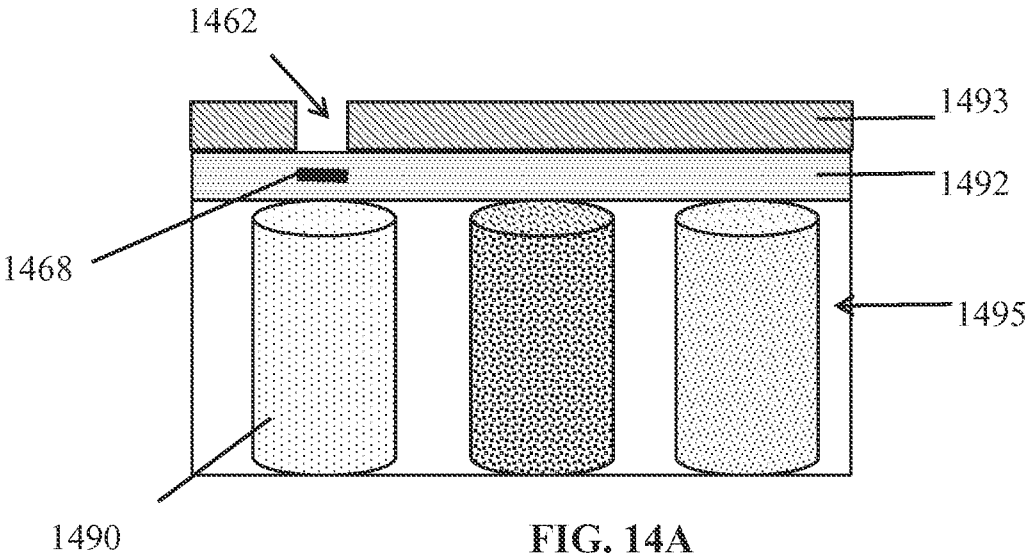



FIG. 14A

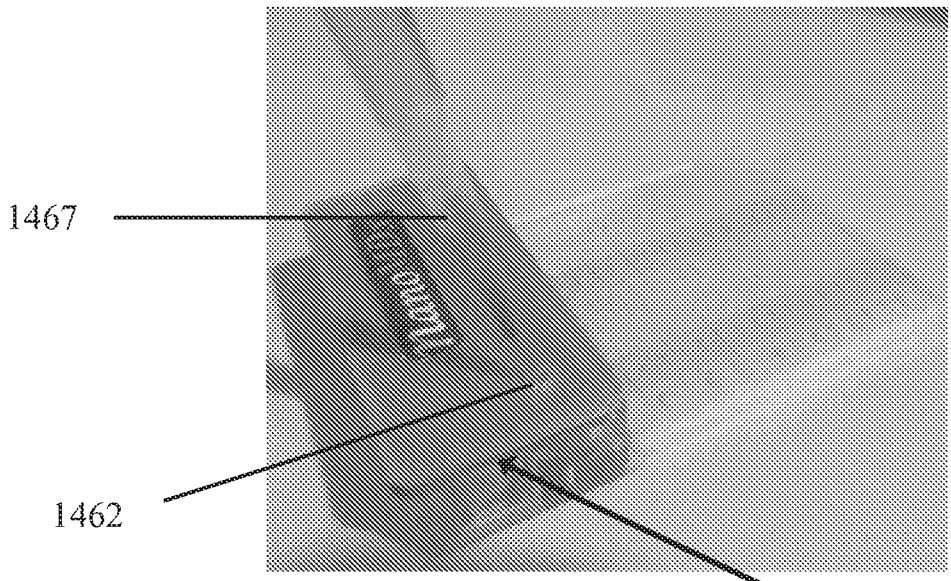



FIG. 14B

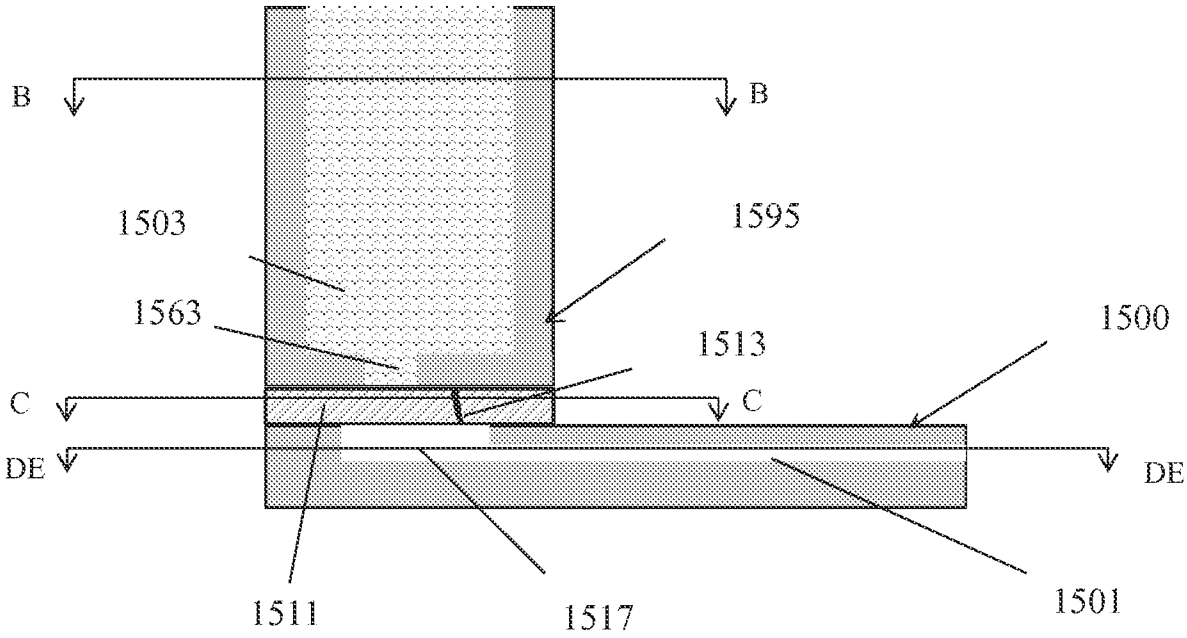



FIG. 15A

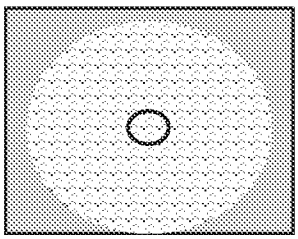


FIG. 15B

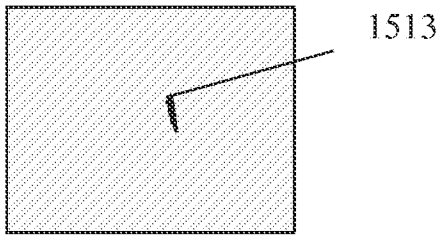



FIG. 15C

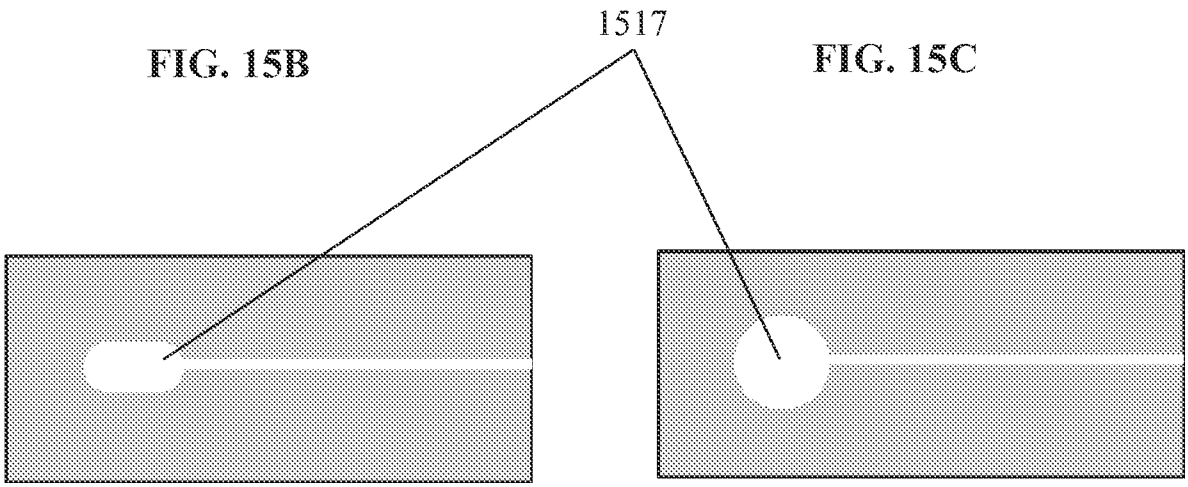



FIG. 15D

FIG. 15E

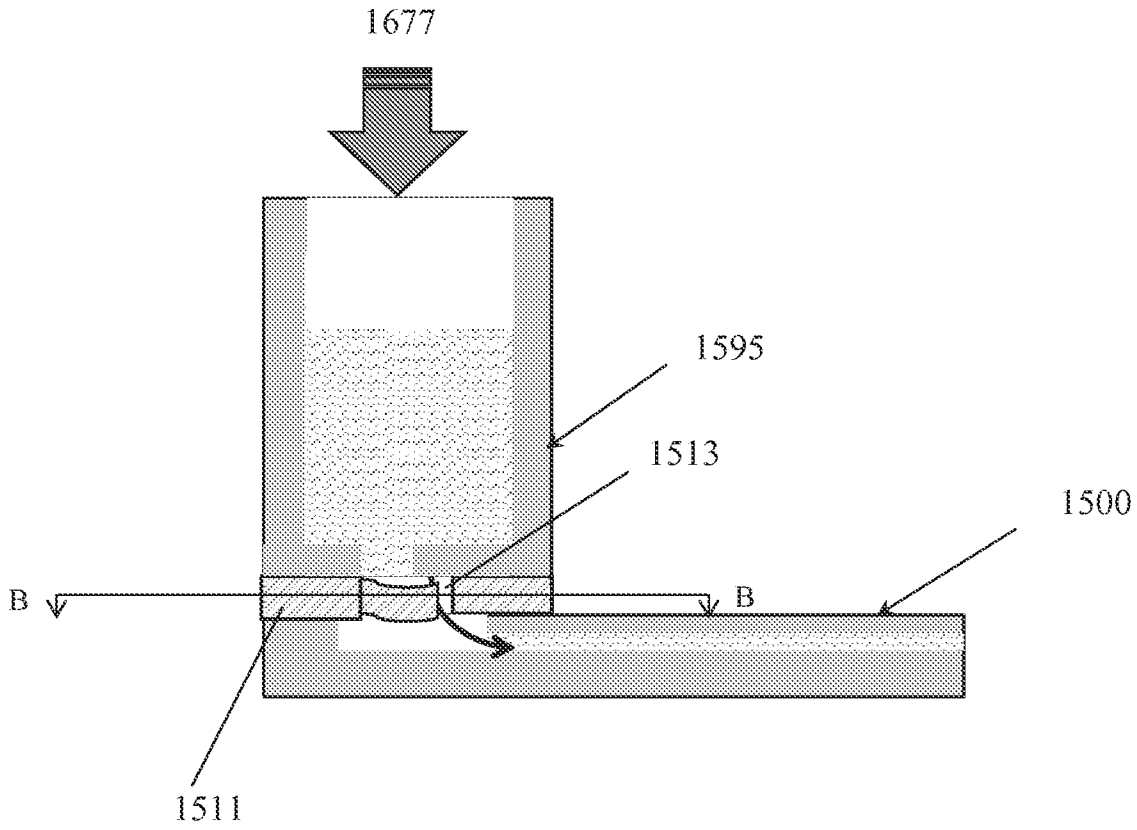



FIG. 16A

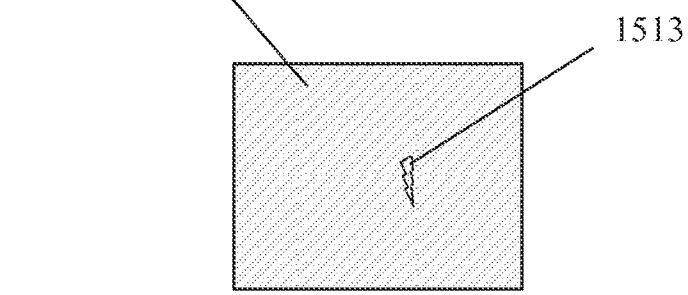



FIG. 16B

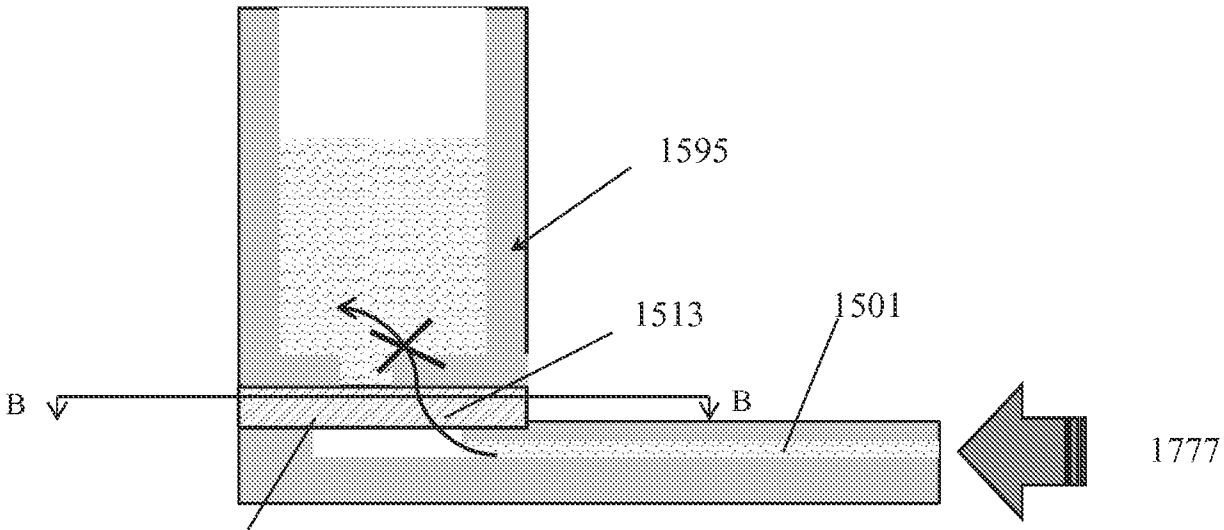



FIG. 17A

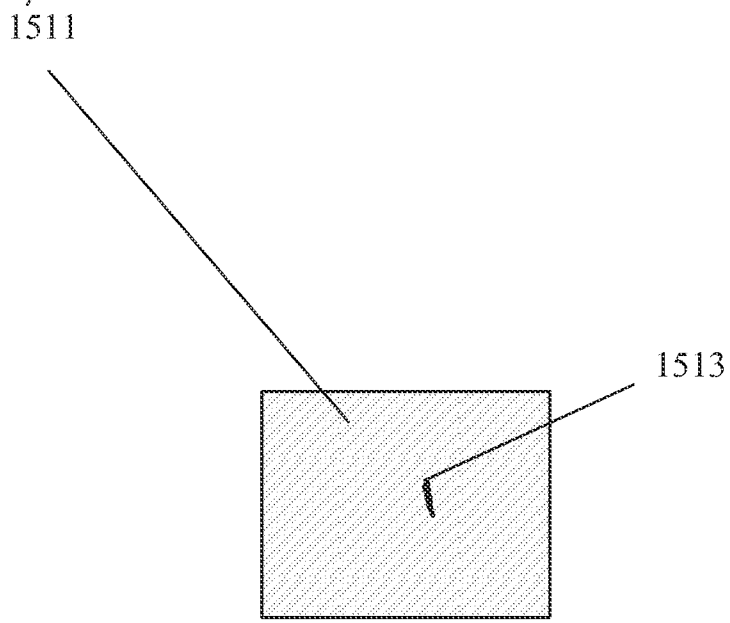



FIG. 17B

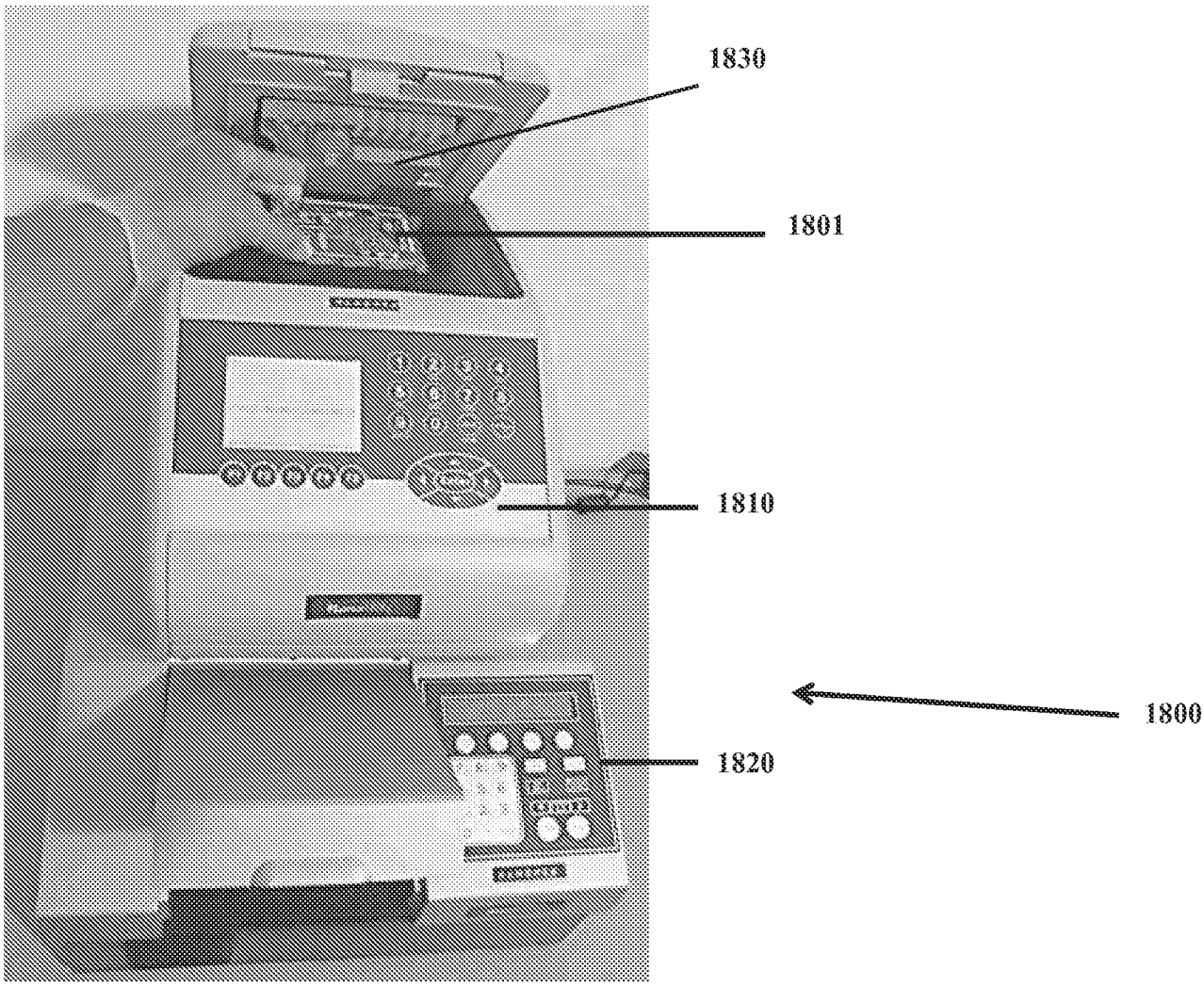



FIG. 18

| Organisms | Forward Primer | Reverse Primer | Probe |
|---|---|---|---|
| Influenza-A/H3 | 5'-GGGCCTGTCCCAGATATGTT-3' | 5'-ATGCCTGAAACCGTACCAAC-3' | /5Cy5/ACTGACTAACAATAGAAAATGGTTGGGAGGGAATGG-3' |
| Influenza-A/H1 | 5'-TATTCCCCAAGCCAAGTTCA-3' | 5'-CAGCACGAGGACTTCTTTCC-3' | /5Cy5/ACTGACTAACATCGAACAAAGGTGTAACGGCAGCATG-3' |
| Influenza-B | 5'-CGGCGAAAGCTTCAATACTC-3' | 5'-TCTGTAGGGTCCTCCTGGTG-3' | /5Cy5/ACTGACTAACAGACCTGTTACATCCGGGTGCTTTCCT-3' |
| Influenza-A | 5'-GCGCAGAGACTGGAAAGTGT-3' | 5'-GCAGTCCTCGCTCACTGG-3' | /5Cy5/ACTGACTAACATTGAGGCTCTCATGGAATGGCTAAAGA-3' |

FIG. 19A

| Common Quencher Sequence |
|---|
| 5'-TGTTATTCAGT/3IAbRQSp/ |
| 5'-TGTTATTCAGT/3IABkFQ/ |

FIG. 19B

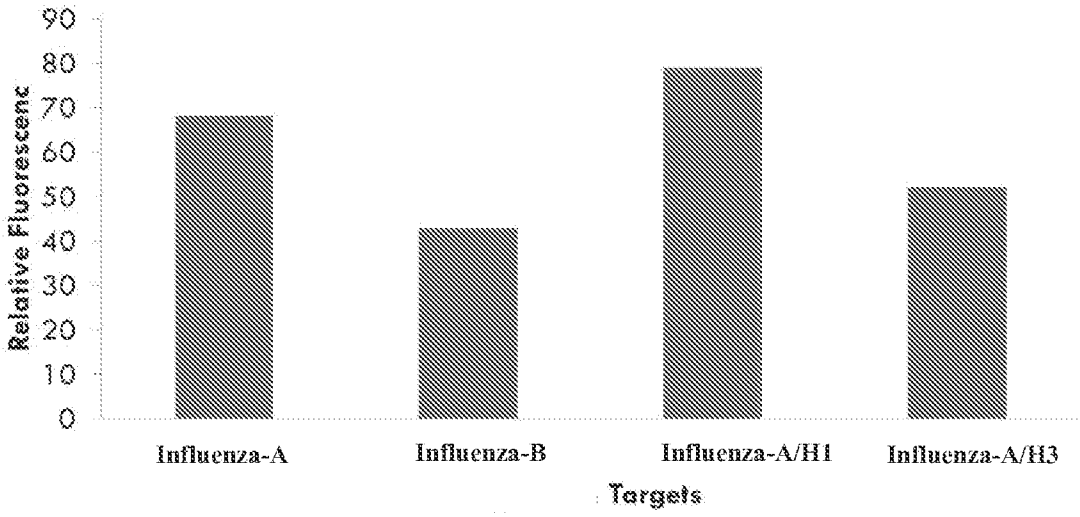

90
80
70
60
50
40
30
20
10
0
Relative Fluorescenc
Influenza-A
Influenza-B
Influenza-A/H1
Influenza-A/H3
Targets

FIG. 20

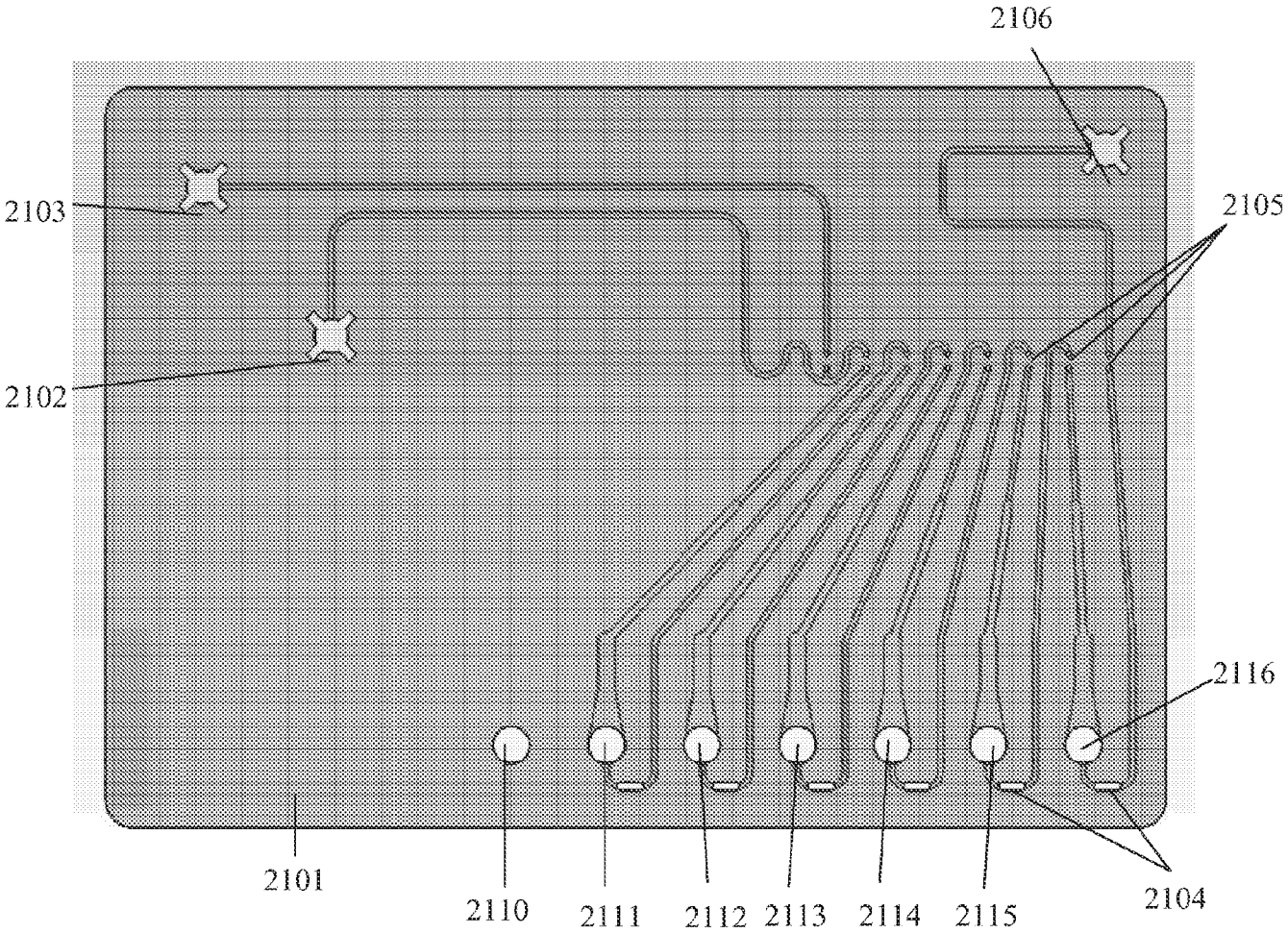

2106
2103
2105
2102
2116
2101
2110
2111
2112
2113
2114
2115
2104

FIG. 21

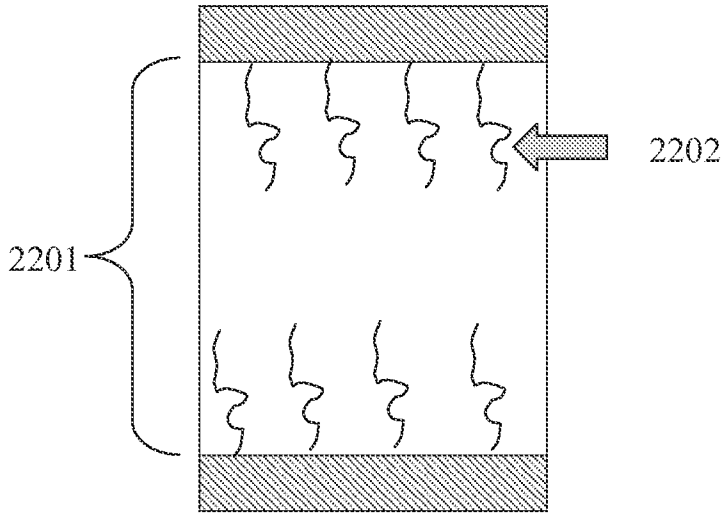



FIG. 22

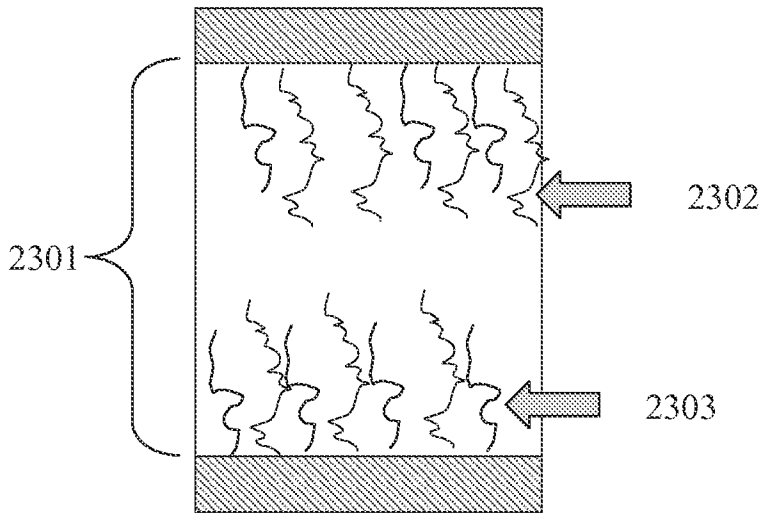



FIG. 23

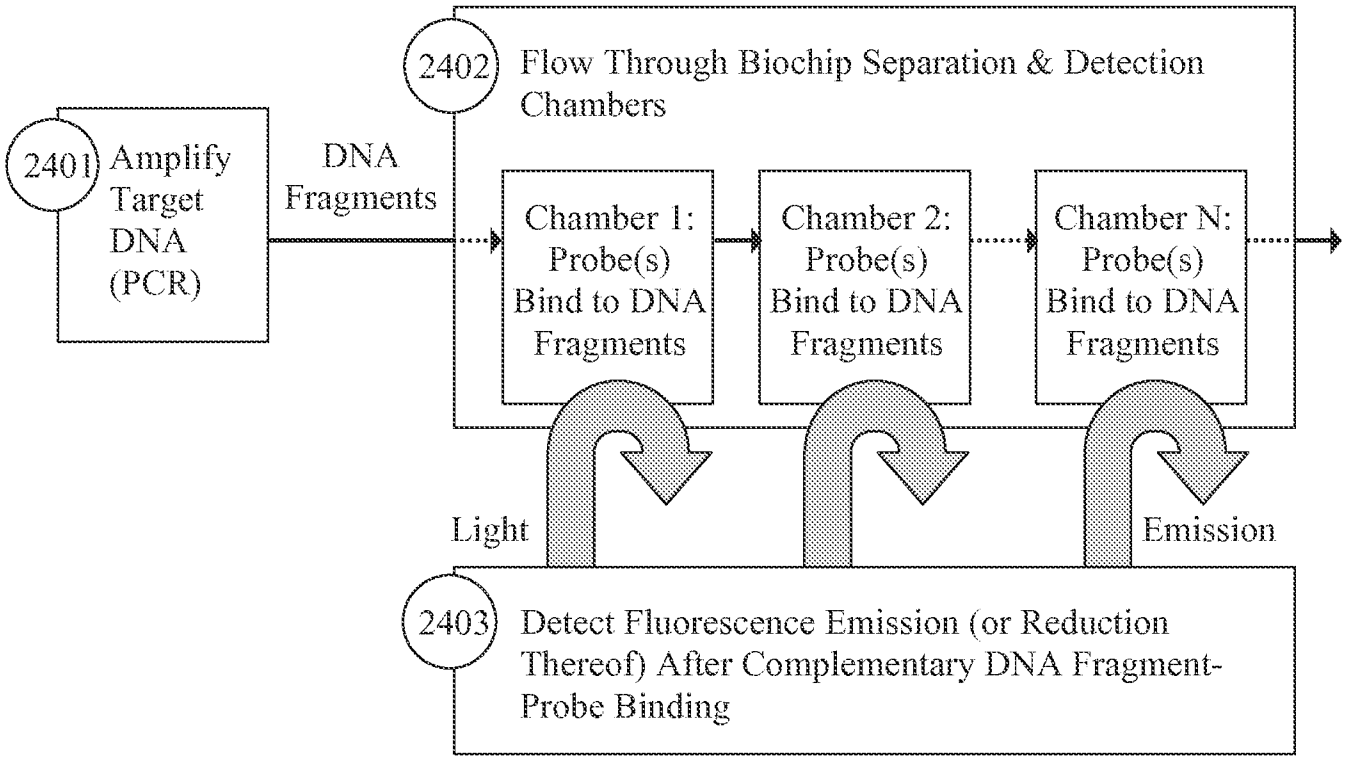

2402
Flow Through Biochip Separation & Detection Chambers
2401
Amplify Target DNA (PCR)
DNA Fragments
Chamber 1: Probe(s) Bind to DNA Fragments
Chamber 2: Probe(s) Bind to DNA Fragments
Chamber N: Probe(s) Bind to DNA Fragments
Light
Emission
2403
Detect Fluorescence Emission (or Reduction Thereof) After Complementary DNA Fragment-Probe Binding

**FIG. 24**

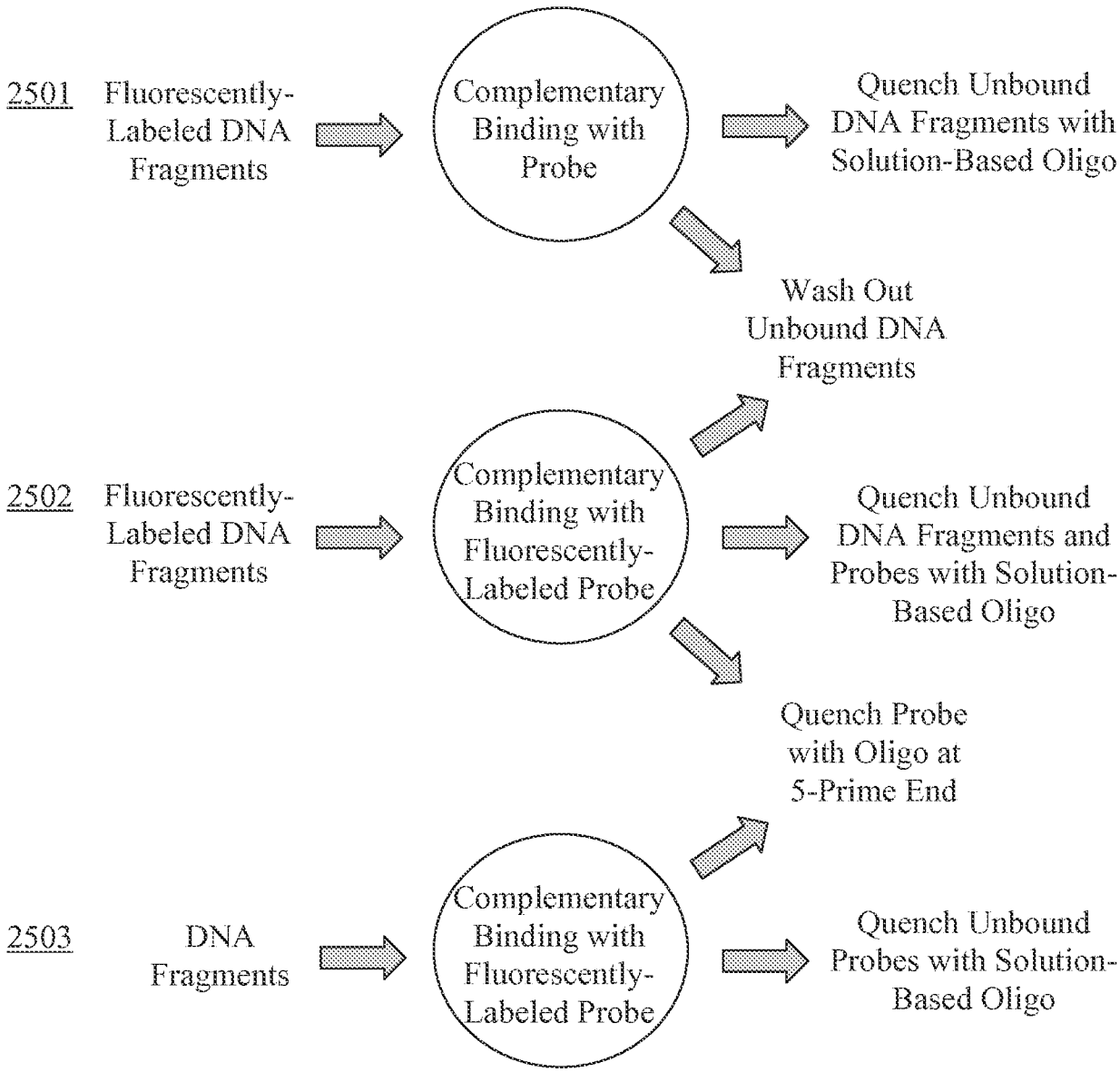

2501
Fluorescently-Labeled DNA Fragments
Complementary Binding with Probe
Quench Unbound DNA Fragments with Solution-Based Oligo
Wash Out Unbound DNA Fragments
2502
Fluorescently-Labeled DNA Fragments
Complementary Binding with Fluorescently-Labeled Probe
Quench Unbound DNA Fragments and Probes with Solution-Based Oligo
Quench Probe with Oligo at 5-Prime End
2503
DNA Fragments
Complementary Binding with Fluorescently-Labeled Probe
Quench Unbound Probes with Solution-Based Oligo

**FIG. 25**




FIG. 26A

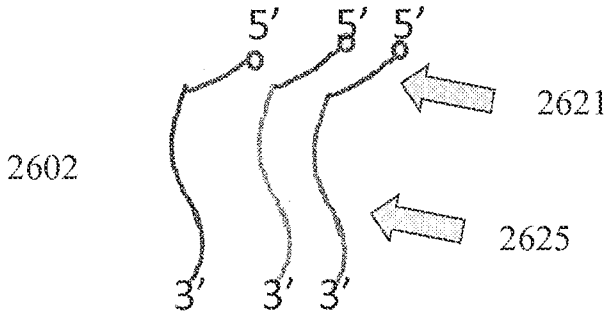



FIG. 26B

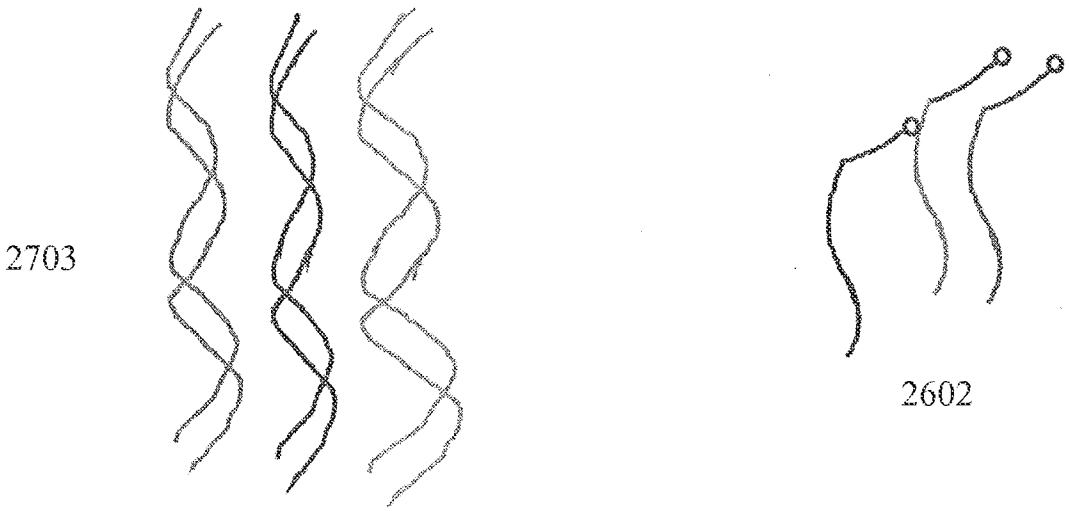



FIG. 27A

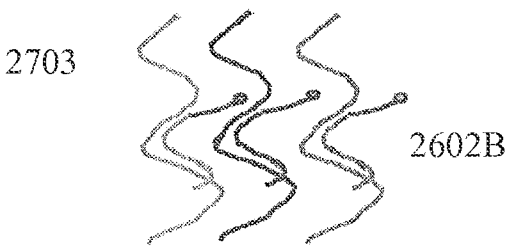



FIG. 27B

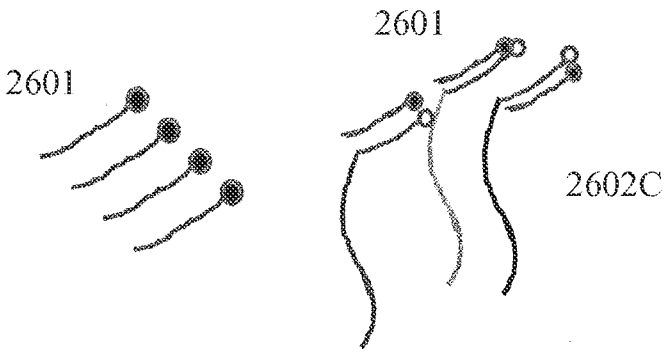



FIG. 27C

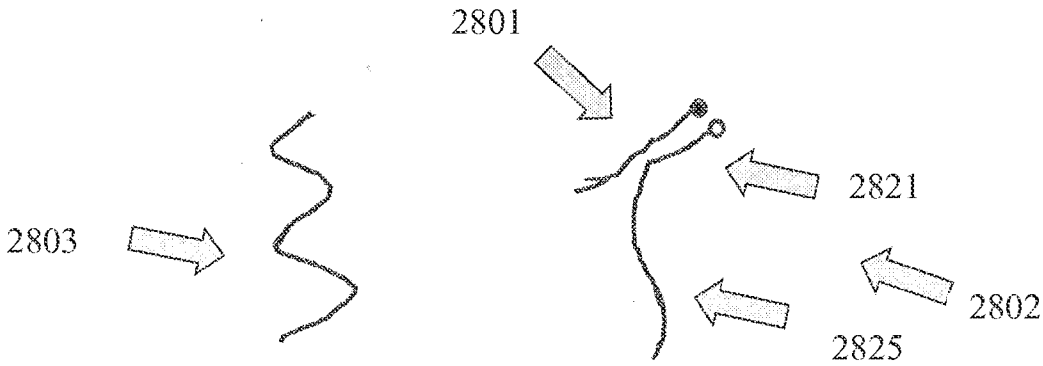



FIG. 28

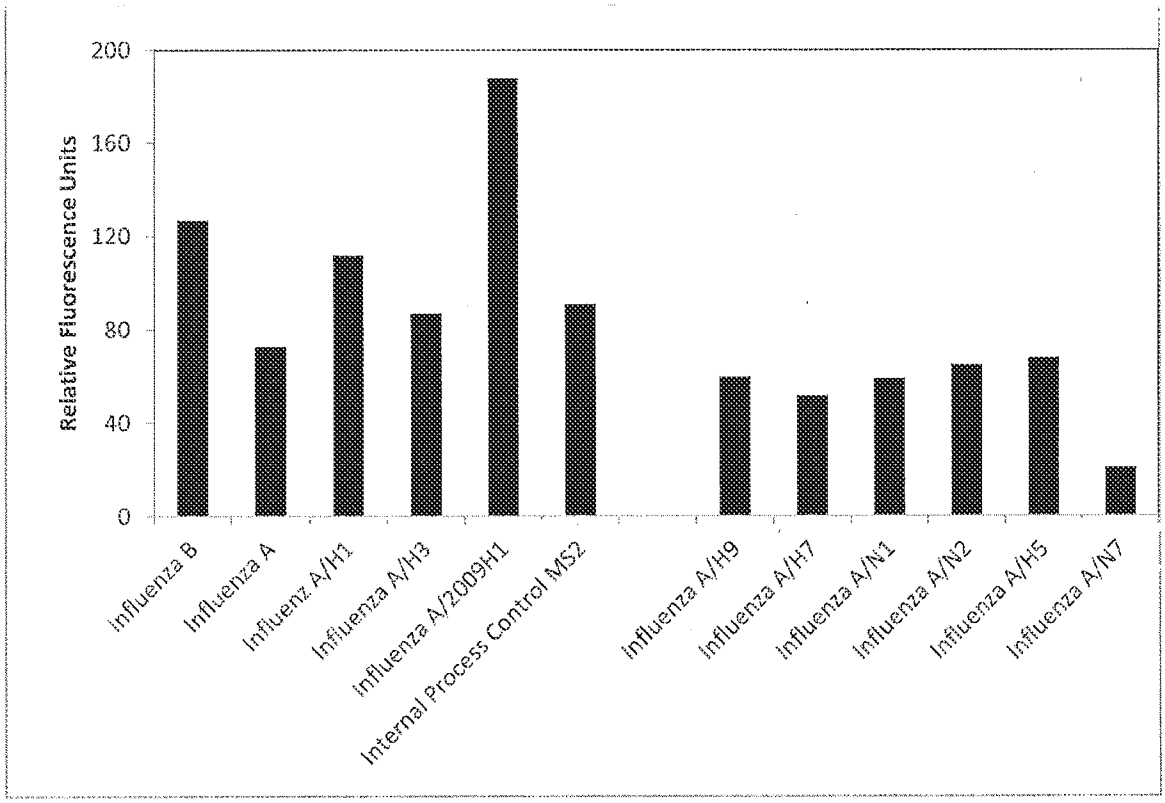

Relative Fluorescence Units
200
160
120
80
40
0
Influenza B
Influenza A
Influenz A/H1
Influenza A/H3
Influenza A/2009H1
Internal Process Control MS2
Influenza A/H9
Influenza A/H7
Influenza A/N1
Influenza A/N2
Influenza A/H5
Influenza A/N7

FIG. 29

| Organisms | Forward Primer | Reverse Primer | Probe |
|---|---|---|---|
| Influenza-A/N7 | 5'-GCAAGGAGCCAATAGACCAG-3' | 5'-GAAGCCGAATCCTTTGACTC-3' | /56-FAM/ACTGAATAACAACTGACACGAGCAGACCATCAGACAAA-3' |
| Influenza-A/N2 | 5'-ATTAGGCTTTCCGCTGGTG-3' | 5'-GGTCCGATAAGGGGTCCTAC-3' | /56-FAM/ACTGACTAACAGTTATCAATTTGCCCTTGGACAAGGAA-3' |
| Influenza A/N1 | 5'-GGCCGCCTCGTACAAAAT-3' | 5'-TGCCAGTGTCTGGGTAACAG-3' | /56-FAM/ACTGACTAACAGAGTTGAATGCACCCAGTTTTCATTAT-3' |
| Influenza-A/H9 | 5'-TTCATGTGGGGCATAAATCA-3' | 5'-CCCTGCTGACCATTGACAAG-3' | /56-FAM/ACTGACTAACAGCCTTCAAACCATTGATAGGGCCAAG-3' |
| Influenza-A/H7 | 5'-AACCCTAGAACTGTGGGGAAG-3' | 5'-GAGGCCCTCCCATCCATT-3' | /56-FAM/ACTGACTAACAGTTCCAGAGAATCCAAAGCCCAGAGG-3' |
| Influenza-A/H5 | 5'-CATACACCCTCTCACCATCG-3' | 5'-ATCTACCATTCCCTGCCATC-3' | /56-FAM/ACTGACTAACAGGAGTGCCCCAAATATGTGAAATCAAA-3' |
| Influenza-A/H3 | 5'-GGGCCTGTCCCAGATATGTT-3' | 5'-ATGCCTGAAACCGTACCAAC-3' | /5Cy5/ACTGACTAACAATAGAAAATGGTTGGGAGGGAATGG-3' |
| Influenza-A/H1 | 5'-TATTCCCCAAGCCAAGTTCA-3' | 5'-CAGCACGAGGACTTCTTTCC-3' | /5Cy5/ACTGACTAACATCGAACAAAGGTGTAACGGCAGCATG-3' |
| Influenza-B | 5'-CGGCGAAAGCTTCAATACTC-3' | 5'-TCTGTAGGGTCCTCCTGGTG-3' | /5Cy5/ACTGACTAACAGACCTGTTACATCCGGGTGCTTTCCT-3' |
| Influenza-A | 5'-GCGCAGAGACTGGAAAGTGT-3' | 5'-GCAGTCCTCGCTCACTGG-3' | /5Cy5/ACTGACTAACATTGAGGCTCTCATGGAATGGCTAAAGA-3' |
| MS2-Phase | 5'-GACGAATTGCTCACAAAGCA-3' | 5'-GACGATCGGTAGCCAGAGAG-3' | /5Cy5/ACTGACTAACACAGCATCCGTAGCCTTATTGGCAAC-3' |
| Pandemic Influenza 2009-H1N1 | 5'-AAGCAACAAAAATGAAGGCAAT-3' | 5'-TCTGTTGAATTGTTCGCATGA-3' | /5Cy5/ACTGACTAACAGCTATACACATTTGCAACCGCAAATG-3' |

FIG. 30A

| Common Quencher Sequence |
|---|
| 5'-TGTTATTCAGT/3IAbRQSp/ |
| 5'-TGTTATTCAGT/3IABkFQ/ |

FIG. 30B