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(54) Title: UNIVERSAL SAMPLE PREPARATION SYSTEM AND USE IN AN INTEGRATED ANALYSIS SYSTEM

(57) Abstract: The invention provides for devices and methods for interfacing microchips to cartridges and pneumatic manifolds. The cartridges, microchips, and pneumatic manifolds can be integrated with downstream preparation devices, such as thermal regulating devices and separation and analysis devices.



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UNIVERSAL SAMPLE PREPARATION SYSTEM AND USE IN AN INTEGRATED ANALYSIS SYSTEM

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/022,722, filed January 22, 2008 and U.S. Provisional Application No. 61/140,602, filed December 23, 2008, which are hereby incorporated by reference in their entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] Aspects of this invention were made with government support under one or more of Project No. W911SR-04-P-0047 awarded by the Department of Defense and Grant No. 5R01HG003583 awarded by the NIH. The government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Sample preparation is a ubiquitous problem in biological analytical systems. The issue of providing sufficiently purified targets from diverse raw sample types to reliably perform downstream analytical assays is pervasive and covers cell biology, genomics, proteomics, metabolomics, food biology, molecular diagnostics, and many other biological and medical assays. While many advances in sample preparation have been made the chief solution has been to develop reagents that are used manually or in robotic systems that use rectilinear stages or multi-axis arms to manipulate samples.

[0004] Microfluidics and nanofluidics allow miniaturized sample volumes to be prepared for analysis. Advantages include the nanoscale consumption of reagents to reduce operating costs and full automation to eliminate operator variances. Microfluidic sample preparation can either interface with existing or future detection methods or be part of a completely integrated system. In the present application, methods and apparatuses are disclosed that integrate full volume sample preparation with volumes over 10 mL with microliter and smaller volumes for sample preparation and analysis.

[0005] Starting from the sample, the present invention can be applied to concentrate, and pre-separate components for further processing to detect and classify organisms in matrices comprising aerosol samples, water, liquids, blood, stools, nasal, buccal and other swabs, bodily fluids, environmental samples with analysis by ELISA, PCR or other nucleic acid amplification techniques, single molecule detection, protein arrays, mass spectroscopy, and other analytical methods well known to one skilled in the art.

[0006] Microfluidic nucleic acid purification can be performed to prepare the sample for nucleic acid assays. For DNA analysis, PCR amplification is one current method. Microarray DNA, RNA and protein analysis also requires extensive sample preparation before the sample can be applied to the microarray for reaction and readout.

[0007] Samples can be obtained by a wide variety of substrates and matrices. The matrix may contain complex mixtures including inhibitory compounds such as hemes, indigo, humic acids, divalent cations, and proteins *etc* that interfere with DNA-based amplification. Aerosols can contain large amounts of molds, metals, and soils humic and other acids that all interfere with PCR amplification—the gold standard.

[0008] Early work showed that as few as three seeded organisms could be detected from diluted samples of soil extracts followed by PCR amplification of two 16S ribosomal gene fragments. Low-melting-temperature agarose has been used to extract DNA from soil samples for 16S and 18S rDNA PCR amplification using universal primers. Spun separation gels in column format can be used, such as Sephadex columns. Multistep purifications such as organic extractions combined with Sephadex columns were developed. Bead beating was found to be an effective way to prepare samples for high numbers of organisms and grinding in liquid nitrogen to detect low numbers of organisms. While these methods are effective they were best suited for research laboratory environments.

[0009] Solid phase extractions to columns, beads, and surfaces can be used to purify DNA before DNA analysis. Proteinase K followed by a Qiagen QIA Amp silica-gel membrane columns and IsoCode Stix, an impregnated membrane-based technology, followed by heating, washing and a brief centrifugation were compared for *B. anthracis* Sterne vegetative cells in buffer, serum, and whole blood and spores in buffer and found to work well.

[0010] A variety of separations can be performed using the devices and methods of the invention. For example, the devices and methods of the invention can be used to perform chromatography, phase-based or magnetic-based separation, electrophoresis, distillation, extraction, and filtration. For example, a microfluidic channel or a capillary can be used for chromatography or electrophoresis. As well, beads, such as magnetic beads can be used for phase-based separations and magnetic-based separations. The beads, or any other surfaces described herein, can be functionalized with binding moieties that exhibit specific or non-specific binding to a target. The binding can be based on electrostatics, van der Waals interactions, hydrophobicity, hydrophilicity, hydrogen bonding, ionic interactions, as well as partially covalent interactions like those exhibited between gold and sulfur. In preferred embodiments, the devices and methods of the invention utilize immunomagnetic separations.

[0011] Immunomagnetic separation (IMS) is a powerful technology that allows targets to be captured and concentrated in a single step using a mechanistically simplified format that employs paramagnetic beads and a magnetic field (see Grodzinski P, Liu R, Yang J, Ward MD. Microfluidic system integration in sample preparation microchip-sets - a summary. Conf Proc IEEE Eng Med Biol Soc. 2004;4:2615-8., Peoples MC, Karnes HT. Microfluidic immunoaffinity separations for bioanalysis. J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Aug 30., and Stevens KA, Jaykus LA. Bacterial separation and concentration from complex sample matrices: a review. Crit Rev Microbiol. 2004; 30(1):7-24.). IMS can be used to capture, concentrate, and

then purify specific target antigens, proteins, toxins, nucleic acids, cells, and spores. While IMS as originally used referred to using an antibody, we generalize its usage to include other specific affinity interactions including lectins, DNA-DNA, DNA-RNA, biotin-streptavidin, and other affinity interactions that are coupled to a solid phase. IMS works by binding a specific affinity reagent, typically an antibody or DNA, to paramagnetic beads which are only magnetic in the presence of an external magnetic field. The beads can be added to complex samples such as aerosols, liquids, bodily fluids, or food. After binding of the target to the affinity reagent (which itself is bound to the paramagnetic bead) the bead is captured by application of a magnetic field. Unbound or loosely bound material is removed by washing with compatible buffers, which purifies the target from other, unwanted materials in the original sample. Because beads are small (nm to μm) and bind high levels of target, when the beads are concentrated by magnetic force they typically form bead beds of just nL-uL volumes, thus concentrating the target at the same time it is purified. The purified and concentrated targets can be conveniently transported, denatured, lysed or analyzed while on-bead, or eluted off bead for further sample preparation, or analysis.

[0012] Immunomagnetic separations are widely used for many applications including the detection of microorganisms in food, bodily fluids, and other matrices. Paramagnetic beads can be mixed and manipulated easily, and are adaptable to microscale and microfluidic applications. This technology provides an excellent solution to the macroscale-to-microscale interface: beads are an almost ideal vehicle to purify samples at the macroscale and then concentrate to the nanoscale (100's of nL) for introduction into microfluidic or nanofluidic platforms. Immunomagnetic separations are commonly used as an upstream purification step before real-time PCR, electrochemiluminescence, and magnetic force discrimination.

[0013] The ability to move fluids on microchips is a quite important. This invention describes technologies in sample capture and purification, micro-separations, micro-valves, -pumps, and -routers, nanofluidic control, and nano-scale biochemistry. A key component of the technology is Micro-robotic On-chip Valves (MOVE) technology (an example of which is shown in **Figure 1**) and its application to miniaturize and automate complex workflows. Collectively the MOVE valves, pumps, and routers and the instrumentation to operate them can be referred to as a microchip fluid processing platform.

[0014] The heart of the microchip fluid processing platform technology are MOVE pumps, valves, and routers that transport, process, and enable analysis of samples. These novel externally actuated, pneumatically-driven, on-chip valves, pumps, and routers, originally developed in the Mathies laboratory at the University of California at Berkeley (U. C. Berkeley) (Grover, W.H. A. M. Skelley, C. N. Liu, E. T. Lagally, and R. M. Mathies. 2003. *Sensors and Actuators* B89:315-323; Richard A. Mathies et al., United States Patent Application, 20040209354 A1 October 21, 2004; all of which are herein incorporated by reference in their entirety) can control fluidic flow at manipulate volumes from 20 nL to 10 μL .

[0015] The MOVE valves and pumps (**Figure 1**) can combine two glass microfluidic layers with a polydimethyl siloxane (PDMS) deformable membrane layer that opens and closes the valve, and a pneumatic layer to deform the membrane and actuate the valve. The microfluidic channel etched in the top glass fluidic wafer is discontinuous and leads to vias through the “via wafer” and microfluidic channels to a valve seat which is normally closed (**Figure 1A**). When a vacuum is applied to the pneumatic displacement chamber by conventional-scale vacuum and pressure sources, the normally closed PDMS membrane lifts from the valve seat to open the valve (**Figure 1B**). The bottom panel of **Figure 1** shows a top view of the valve a similar scale as the other panels.

[0016] Three microvalves can be used to make a micropump on a microchip to move fluids from the Input area to the Output area on Microchip A. The fluids are moved by three or more valves. The valves can be created actuation of a deformable structure. In some implementations a valve seat is created and in other embodiments no valve seat may be needed. **Figure 2** shows MOVE devices from top to bottom: valve, router, mixer, bead capture. Self-priming MOVE pumps (**Figure 2**, top) are made by coordinating the operation of three valves and can create flow in either direction. Routers are made from three or more MOVE valves (**Figure 2**, top middle panel). Mixing has been a holy grail for microfluidics: MOVE mixers (**Figure 2**, bottom middle panel) rapidly mix samples and reagents. MOVE devices work exquisitely with magnetic beads to pump or trap sets of beads (**Figure 2**, bottom panel).

[0017] The normally closed MOVE valves, pumps, and routers are durable, easily fabricated at low cost, can operate in dense arrays, and have low dead volumes. Arrays of MOVE valves, pumps, and routers are readily fabricated on microchips. Significantly, all the MOVE valves, pumps, and routers on a microchip are created at the same time in a simple manufacturing process using a single sheet of PDMS membrane—it costs the same to make 5 MOVE micropumps on a microchip as to create 500. This innovative technology offers for the first time the ability to create complex micro- and nanofluidic circuits on microchips.

[0018] Patents and applications which discuss the use and design of microchips include US 7,312,611, issued on December 25, 2007; US Patent 6,190,616, issued on February 20, 2001; U.S. Patent 6,423,536, issued on July 23, 2002; US patent 10.633,171 March 22, 2005; US Patent 6,870,185, issued on March 22, 2005 US Application No. US 2001-0007641, filed on January 25, 2001; US Application US20020110900, filed on April 18, 2002; US patent application 20070248958, filed September 15, 2005; US patent application US 20040209354, filed on December 29, 2003; US patent application US2006/0073484, filed on December 29, 2003; US20050287572, filed on May 25, 2005; US patent application US20070237686, filed on March 21, 2007; US 20050224352 filed on November 24, 2004; US 20070248958, filed on, September 15, 2005; US 20080014576, filed on February 2, 2007; and, US application US20070175756, filed on July 26, 2006; all of which are herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0019] In one aspect this invention provides a device comprising: a microfluidic microchip comprising at least one port aperture fluidically connected to at least one microfluidic channel in the microfluidic microchip, wherein the channel comprises at least one valve that controls movement of a fluid through the channel; and a cartridge mated to the microchip and comprising a chamber, wherein said chamber comprises two chamber apertures that are each aligned with a port aperture of said microfluidic microchip. In one embodiment said cartridge is adapted to receive at least one sample or one reagent. In another embodiment said cartridge is fluidically connected to another cartridge that is mated to another microchip. In another embodiment said cartridge comprises at least two chambers. In another embodiment at least one of said at least two chambers is adjacent to a movable magnet. In another embodiment at least one of said at least two chambers is temperature controlled. In another embodiment at least one of said at least two chambers comprises a filter. In another embodiment said at least one chamber comprises a fluidic volume greater than or equal to 5 μL . In another embodiment said at least one chamber comprises a fluidic volume greater than or equal to 10 μL . In another embodiment the fluidic volume of said cartridge is a 100 X of the fluidic volume of said microfluidic microchip. In another embodiment one of said at least one chambers further comprises a filter. In another embodiment said device further comprises a magnet for applying a magnetic field to the cartridge or the microfluidic microchip. In another embodiment the valve is pneumatically actuated. In another embodiment said cartridge is adapted to be connected to at least one pressure source for the delivery of said at least one reagent or said at least one sample. In another embodiment said pressure source provides a positive or negative pressure to the cartridge. In another embodiment said at least one pressure source is controlled by a pneumatic solenoid. In another embodiment said at least one pressure source is a pneumatic manifold. In another embodiment the microfluidic microchip comprises a fluidic layer, an elastomeric layer, and a pneumatic layer. In another embodiment said cartridge further comprises at least one input port, wherein said at least one input port is adapted to mate with a delivery device, wherein said delivery device is fluidically connected to the fluidic layer of said microfluidic microchip; and wherein one of said at least one chamber is a closed reaction chamber fluidically connected to the fluidic layer of said microfluidic microchip. In another embodiment said delivery device is thermally coupled to a temperature modulator. In another embodiment said delivery device is a syringe. In another embodiment said cartridge is designed to enrich at least one component from said sample and comprises at least one sample input port, wherein said at least one chamber is a closed reaction chamber comprising beads, and wherein said beads bind to said at least one component. In another embodiment the cartridge further comprises at least one reagent reservoir comprising reagents for amplifying a nucleic acid, wherein the at least one reagent reservoir is fluidically connected to the chamber through the microchip. In another embodiment the cartridge further comprises at least one bead reservoir comprising beads for binding an amplified nucleic acid,

wherein the at least one bead reservoir is fluidically connected to the chamber through the microchip. In another embodiment said beads are paramagnetic beads or glass beads. In another embodiment said binding of at least one component to a bead is reversible. In another embodiment said beads are paramagnetic beads, and wherein said device further comprises a
5 movable magnet that can attract said paramagnetic beads to the wall of said closed reaction chamber. In another embodiment said cartridge is designed to enrich at least one component from a sample, wherein said at least one component is DNA, RNA, microRNA, siRNA, protein, lipid, or polysaccharide.

[0020] In another aspect this invention provides a method for performing biochemical reactions
10 comprising: (a) providing the device comprising a microfluidic microchip comprising at least one port aperture fluidically connected to at least one microfluidic channel in the microfluidic microchip, wherein the channel comprises at least one valve that controls movement of a fluid through the channel; and a cartridge mated to the microchip and comprising a chamber, wherein said chamber comprises two chamber apertures that are each aligned with a port aperture of said
15 microfluidic microchip., and (b) performing at least one enzymatic reaction within said chamber. In one embodiment said at least one enzymatic reaction comprises ligating, blunting, nick repairing, denaturing, polymerizing, hydrolyzing, phosphorylation or any combination thereof. In another embodiment the method further comprises separating a product of said enzymatic reaction using solid-phase particles.

[0021] In another aspect this invention provides a method for enriching at least one component from a
20 sample comprising: (a) mating a delivery device to an input port of a device comprising: a microfluidic microchip comprising at least one port aperture fluidically connected to at least one microfluidic channel in the microfluidic microchip, wherein the channel comprises at least one valve that controls movement of a fluid through the channel; and a cartridge mated to the
25 microchip and comprising a chamber, wherein said chamber comprises two chamber apertures that are each aligned with a port aperture of said microfluidic microchip wherein said cartridge further comprises at least one input port, wherein said at least one input port is adapted to mate with a delivery device, wherein said delivery device is fluidically connected to the fluidic layer of said microfluidic microchip; and wherein one of said at least one chamber is a closed reaction
30 chamber fluidically connected to the fluidic layer of said microfluidic microchip, (b) treating said sample with at least one reagent to increase the availability of said at least one component for enrichment, (c) delivering said at least one component to said at least one reaction chamber of said cartridge, (d) binding said component to one or more particles in said at least one closed reaction chamber, (e) washing said particle bound component to remove waste, and (f) eluting
35 said particle bound component. In one embodiment said delivering comprises pumping said at least one component to said at least one reaction chamber through said at least one valve of the microfluidic microchip. In another embodiment said binding comprises pumping said particles from a reagent port in the cartridge to said at least one reaction chamber through said at least one

valve of the microfluidic microchip. In another embodiment said particle is a paramagnetic bead, a nanoparticle, a resin, or a solid-phase particle. In another embodiment said at least one component is DNA, RNA, microRNA, siRNA, protein, lipid, or polysaccharide. In another embodiment step (b) further comprises thermally modulating said delivery device. In another embodiment step (b) further comprises delivering a lysis reagent or a component isolation reagent from a reagent port on said cartridge into said delivery device to increase the availability of said at least one component for enrichment. In another embodiment the beads of step (d) are paramagnetic beads. In another embodiment said washing step (e) comprises attracting said paramagnetic beads with a movable magnet. In another embodiment said microfluidic microchip directs the flow of said waste in a second direction. In another embodiment step (c) comprises using pneumatically actuated valves in the microfluidic microchip or an external pressure source to deliver said at least one component to said at least one closed reaction chamber of said cartridge. In another embodiment the external pressure source provides a positive or negative pressure to the microfluidic microchip. In another embodiment said sample delivery device is a syringe.

[0022] In another aspect this invention provides a device comprising: (a) a first fluid manipulation module comprising: (i) a first microfluidic microchip comprising a port aperture fluidically connected to a microfluidic channel in the microfluidic microchip, wherein the channel comprises at least one valve that controls movement of a fluid through the channel; and (ii) a cartridge mated to the microfluidic microchip and comprising at least one sample input port, at least one chamber, an exit port, wherein the sample input port is connected to the port aperture, wherein at least one of said at least one exit ports is aligned with an exit port aperture of said first microfluidic microchip, and wherein said at least one chamber is fluidically connected to the fluidic layer of said first microfluidic microchip; (b) a reaction channel, wherein said reaction channel is not contained within said first microchip; (c) a temperature modulator, wherein said reaction channel is fluidically connected to a port on said cartridge that is fluidically connected to said exit port and at least a portion of said reaction channel is in thermal contact with said temperature modulator; and (d) a magnet for applying a magnetic field to the microfluidic microchip, the cartridge, or the reaction channel. In one embodiment said magnet is adjacent to said reaction channel. In another embodiment the device further comprises a second microfluidic microchip that is fluidically connected to said first microfluidic microchip through said reaction channel. In another embodiment said temperature modulator is a Peltier device. In another embodiment the device further comprises a paramagnetic bead.

[0023] In another aspect this invention provides a method comprising: delivering a sample containing a nucleic acid to a device comprising: (a) a first fluid manipulation module comprising: (i) a first microfluidic microchip comprising a port aperture fluidically connected to a microfluidic channel in the microfluidic microchip, wherein the channel comprises at least one valve that controls movement of a fluid through the channel; and (ii) a cartridge mated to the microfluidic microchip

and comprising at least one sample input port, at least one chamber, an exit port, wherein the sample input port is connected to the port aperture, wherein at least one of said at least one exit ports is aligned with an exit port aperture of said first microfluidic microchip, and wherein said at least one chamber is fluidically connected to the fluidic layer of said first microfluidic microchip;

5 (b) a reaction channel, wherein said reaction channel is not contained within said first microchip;

(c) a temperature modulator, wherein said reaction channel is fluidically connected to a port on said cartridge that is fluidically connected to said exit port and at least a portion of said reaction channel is in thermal contact with said temperature modulator; and (d) a magnet for applying a magnetic field to the microfluidic microchip, the cartridge, or the reaction channel.; transporting

10 the nucleic acid and an effective amount of reagents through the portion of the reaction channel in thermal contact with the temperature modulator one or more times; and amplifying the nucleic acid; and analyzing the amplified nucleic acid. In one embodiment the method further comprises using the temperature modulator to perform thermocycling. In another embodiment said reagents are reagents for polymerase chain reaction or cycle sequencing.

15 **[0024]** In another aspect this invention provides a device comprising: (a) a first microfluidic microchip comprising a fluidics layer, an actuation layer, and a pneumatic layer, wherein the fluidics layer comprises one or more microfluidic channels, wherein at least one of said one or more microfluidic channels comprises an exit aperture, (b) a flexible connector fluidically connected to the exit aperture at a first end of the flexible connector; (c) a capillary fluidically connected to

20 said flexible connector; and

(d) a first electrode and a second electrode, wherein the first electrode and second electrode are configured to produce an electric field along a path of the capillary. In one embodiment the flexible connector is surgical, poly(tetrafluoroethylene) or silicon tubing. In another embodiment the flexible connector is elastic tubing. In another embodiment the flexible connector has an

25 outer diameter of about 1.5 to 3 mm and an inner diameter of about 0.25 to 0.5 mm. In another embodiment the flexible connector is also fluidically connected to a second microfluidic microchip or the first microfluidic microchip at a second end of the flexible connector. In another embodiment the flexible connector is fluidically connected to the exit aperture by a cannula, an upfit tubing, a microtubing fitting, or an upchurch tubing adapter. In another embodiment the

30 capillary has an outer diameter of about 150-500 microns and an inner diameter of about 10-100 microns. In another embodiment the capillary is polyamide or poly(tetrafluoroethylene) coated. In another embodiment the capillary comprises a separation gel. In another embodiment the capillary is about 10 to 100 cm long. In another embodiment the first electrode is a forked electrode. In another embodiment said forked electrode comprises one or more conductive

35 channels or one or more metallic conductors. In another embodiment the first electrode and the second electrode produce an electric field that is about 25 to 500 V/cm.

[0025] In another aspect this invention provides a method comprising: providing the composition to a microfluidic microchip, wherein the microfluidic microchip comprises a fluidics layer, a

elastomeric layer, and a pneumatic layer; delivering the composition to a flexible connector that is fluidically connected to the microfluidic microchip; providing a electric field to move the composition into a capillary; and performing capillary electrophoresis on the composition to separate a component based on size or charge. In one embodiment the electric field is about 25 to 500 V/cm. In another embodiment said composition in said tube is adjacent to a first and second bolus of gas, wherein said first bolus of gas is upstream of said composition and said second bolus of gas is downstream of said composition in said tube. In another embodiment said first and second boluses of gas isolates said composition from other compositions. In another embodiment the composition comprises at least one component that is a nucleic acid, protein, fatty acids, or polysaccharides. In another embodiment the nucleic acid is microRNA, DNA, RNA, or siRNA.

[0026] In another aspect this invention provides a device comprising: (a) a separation channel fluidically connected to a loading channel; (b) a forked electrode comprising at least two electrodes that are electrically connected to the loading channel and the separation channel through the loading channel, wherein the fluidic connection between the separation channel and the loading channel is located between the electrical connections of the two electrodes to the loading channel; and (c) a pneumatically actuated valve fluidically connected to the loading channel. In one embodiment the device further comprises a cannular electrode in electrical contact with said forked electrode, wherein the inner diameter of said cannular electrode is at least about 0.2 mm. In another embodiment said cannular electrode is configured to reduce injection of gas into said separation channel. In another embodiment the separation channel is a capillary, and wherein the capillary is fluidically connected to the pneumatically actuated valve using a flexible connection. In another embodiment the separation channel is a microchannel. In another embodiment the separation channel and pneumatically actuated valve are integrated on a microfluidic microchip. In another embodiment the loading channel comprises a loading channel solution and the separation channel comprises a separation channel solution, and wherein the sample solution has a lower electrical conductivity than the separation channel solution. In another embodiment the at least two electrodes comprise at least two microchannels that are on one end fluidically connected to the loading channel on either side of the fluidic connection between the separation channel and the loading channel and on the other end fluidically connected to a base channel. In another embodiment each of the at least two electrodes is a metallic conductors that is electrically connected to a voltage source and the loading channel.

[0027] In another aspect this invention provides a device comprising: a first, a second, and a third microfluidic channel that are joined to form a three-way junction; wherein the first microfluidic channel is electrically connected to a first electrode of a forked electrode, wherein the second microfluidic channel is electrically connected to a second electrode of the forked electrode, and wherein the first, the second, and the third microfluidic channel are each fluidically connected to a pneumatically actuated valve.

[0028] In another aspect this invention provides a method for performing capillary electrophoresis comprising: providing a device comprising: (1) a separation channel fluidically connected to a loading channel; (2) a forked electrode comprising at least two electrodes that are electrically connected to the loading channel and the separation channel through the loading channel, wherein the fluidic connection between the separation channel and the loading channel is located between the electrical connections of the two electrodes to the loading channel; and (3) a pneumatically actuated valve fluidically connected to the loading channel; providing a separation channel solution to the separation channel; delivering a composition to the loading channel, wherein the pneumatically actuated valve is used to control the delivery of the composition to the loading channel; and applying an electric field along the separation channel using the forked electrode; performing capillary electrophoresis on the composition to separate the component based on size or charge. In another embodiment the composition has a lower electrical conductivity than the separation channel solution. In another embodiment the composition is concentrated by said applying the electric field.

[0029] In another aspect this invention provides a microfluidic device comprising: (a) a microfluidic microchip comprising: (1) a first channel comprising a first valve; (2) a second channel that intersects the first channel on one side of the first valve; (3) a third channel that intersects the first channel on the other side of the first valve; wherein at least one of the second or third channel intersect the first channel at a T-valve or at least one of the second or third channel comprise a second valve, and wherein the second and third channel each connect to a port; and (b) a fluid loop that is removably attached to the ports such that fluid can flow from the first channel to the fluid loop.

[0030] In another aspect this invention provides a microfluidic device comprising: (a) a microchip comprising one or more pneumatically actuated valves; and (b) a sample loop, wherein the sample loop is fluidically connected to the one or more pneumatically actuated valves through ports in the microfluidic microchip, and wherein the sample loop has a fixed volume and the sample loop is removable. In one embodiment said pneumatically actuated valves are actuated by one or more pneumatic channels in the microfluidic device. In another embodiment the sample loop comprises a capillary tube. In another embodiment the sample loop is fluidically connected to a pass-through microfluidic channel at a first junction and a second junction, and wherein a pass-through microfluidic pneumatically actuated valve is positioned in the pass-through microfluidic channel between the first and second junctions. In another embodiment at least one junction comprises a T-valve, wherein closure of the T-valve does not prevent passage of fluid through the pass-through microfluidic channel. In another embodiment the sample loop is connected to the pass-through microfluidic channel through first and second channels, and wherein at least one of the first and the second channel comprise a sample loop valve.

[0031] In another aspect this invention provides a method for delivering a fixed volume of fluid to a microfluidic device comprising: configuring a device with a sample loop comprising a desired

volume, wherein the sample loop is removable; using one or more pneumatically actuated valves on a microfluidic device to fill the sample loop with the fixed volume of the fluid; and delivering the fluid to the microfluidic device. In one embodiment the sample loop and a pass-through microfluidic channel are fluidically connected at a first junction and a second junction, and wherein at least one junction comprises a T-valve. In another embodiment the pass-through microfluidic channel comprises a pass-through microfluidic valve positioned between the first junction and the second junction.

[0032] In one aspect, this invention provides a device comprising: (a) a cartridge; (b) a microfluidic microchip having one or more microfluidic diaphragm valves, fluidically interfaced with the cartridge; and (c) a pneumatic manifold interfaced with the microfluidic microchip on a surface of the microfluidic microchip, wherein the pneumatic manifold covers all or only a portion of the surface of the microfluidic microchip. In one embodiment the device further comprises a magnet configured to generate a magnetic field in a chamber of the microfluidic microchip. In one embodiment wherein the pneumatic manifold has an annular space for the magnetic component. In another embodiment the microfluidic microchip comprises a fluidics layer comprising fluidics channels, a pneumatics layer comprising pneumatics channels, and an activation layer sandwiched there between, wherein the cartridge comprises a chamber with an opening, wherein the opening mates with an opening in the fluidics layer that connects to a fluidics channel, and the pneumatic manifold comprises an opening that mates with an opening in the pneumatics layer of the microfluidic microchip that connects with a pneumatic channel.

[0033] In another aspect this invention provides a device comprising: (a) a cartridge; (b) a microfluidic microchip having one or more microfluidic diaphragm valves and interfaced with the cartridge; (c) a pneumatic manifold interfaced with the microfluidic microchip on a surface of the microfluidic microchip; and (d) a temperature controlling block in thermal contact with the cartridge. In one embodiment the microfluidic microchip comprises a fluidics layer comprising fluidics channels, a pneumatics layer comprising pneumatics channels, and an activation layer sandwiched there between, wherein the cartridge comprises a chamber with an opening, wherein the opening mates with an opening in the fluidics layer that connects to a fluidics channel, and the pneumatic manifold comprises an opening that mates with an opening in the pneumatics layer of the microfluidic microchip that connects with a pneumatic channel.

[0034] In another aspect this invention provides a device comprising a microfluidic microchip having one or more microfluidic diaphragm valves and interfaced with a cartridge; wherein the microfluidic microchip has a bead rail and a reagent rail.

[0035] In another aspect this invention provides a method for amplifying mRNA and purifying amplified RNA comprising: (a) providing device comprising: (i) a cartridge; (ii) a microfluidic microchip having one or more microfluidic diaphragm valves, fluidically interfaced with the cartridge; (iii) a pneumatic manifold interfaced with the microfluidic microchip on a surface of the microfluidic microchip, wherein the pneumatic manifold covers all or only a portion of the

surface of the microfluidic microchip and (iv) a magnet configured to generate a magnetic field in a chamber of the microfluidic microchip, wherein the pneumatic manifold has an annular space for the magnetic component; (b) supplying a sample containing mRNA and reagents to the cartridge; (c) mixing the sample and reagents in a well of the microfluidic microchip; (d) amplifying the mRNA to form amplified RNA; (e) capturing the amplified RNA using magnetic beads; and (f) positioning the magnet in the annular space to capture magnetic beads in a reservoir of the microfluidic microchip.

[0036] In another aspect this invention provides a method for amplifying mRNA comprising: (a) providing a device comprising: (i) a cartridge; (ii) a microfluidic microchip having one or more microfluidic diaphragm valves and interfaced with the cartridge; (iii) a pneumatic manifold interfaced with the microfluidic microchip on a surface of the microfluidic microchip; and (iv) a temperature controlling block in thermal contact with the cartridge; (b) supplying a sample containing mRNA and reagents to the cartridge; (c) mixing the sample and reagents in a well of the microfluidic microchip to form a mixture; (d) heating the mixture using the temperature controlling block; and (e) amplifying the mRNA.

[0037] In another aspect this invention provides a method for amplifying mRNA and purifying amplified RNA comprising: (a) providing a device comprising a microfluidic microchip having one or more microfluidic diaphragm valves and interfaced with a cartridge; wherein the microfluidic microchip has a bead rail and a reagent rail; (b) supplying reagents to one or more reagent rail wells; (c) supplying magnetic bead slurry to a bead rail well; (d) supplying a sample containing mRNA to a sample well; (e) pumping the sample and the reagents to an output well of the microfluidic microchip to form a mixture; (f) amplifying the mRNA to form amplified RNA; (g) pumping the magnetic bead slurry to a purification well; (h) contacting the magnetic bead slurry with amplified RNA by pumping the amplified RNA to the purification well; and (i) purifying the amplified RNA.

[0038] In another aspect this invention provides a method for pumping a fluid in a microfluidic device comprising: (a) providing a microfluidic device comprising a pumping valve, a source well, and a mixing well, wherein the pumping valve, the source well, and the mixing well are fluidically connected by a channel; (b) pumping the fluid in a first direction through the channel from the source well to the pumping valve; and (c) pumping the fluid in a second direction through the channel from the pumping valve to the mixing well, wherein the second direction is opposite of the first direction.

[0039] Sample preparation is a challenging area of the bioanalytical process. In one aspect, a method is disclosed for the preparation of samples from many different sample types. In another aspect, an apparatus is disclosed that can prepare samples from many different sample types. In one embodiment, the apparatus operates a cartridge with microscale valves that direct fluid flow in a microchip component that can be fabricated separately or as an integral part of the cartridge. In another embodiment, the apparatus can move samples into the cartridge using pressure-driven

flow or vacuum modulated by the microvalves on the cartridge. In another embodiment, the apparatus can manipulate paramagnetic beads for magnetic separation of components of the sample to purify desired analytes with fluid flow directed by the microvalves.

[0040] In one embodiment, the apparatus is a universal sample preparation system that can process biological or chemical samples. Samples can be loaded in liquid, swabs, swipes, solids, gases, or other matrices into the cartridge. The apparatus is controlled by electronics which may include a computer to select the proper reagents and direct the fluids using the microscale valves to open and close circuitry that is formed by the cartridge and by the microchip component. The sample can be processed to extract nucleic acids, including DNA, RNA, microRNAs, proteins, lipids, polysaccharides, cell walls, small molecules, and all other biological components of a sample. Similarly the sample can also be processed to extract or purify chemical components. For example, DNA can be processed onto microbeads.

[0041] In one embodiment, a sample can be moved into a reaction chamber in a cartridge comprising one or more chambers, channels, tubing, or capillaries that may be permanently attached to the cartridge or may be reversibly joined to the cartridge. Samples may be reproducibly positioned in the channels, tubing or capillaries using vacuum or pressure modulated or created by micropumps that may be located on the cartridge, on the apparatus, on an external device, or other configurations.

[0042] In one embodiment, for DNA, the processed sample can be amplified by PCR, rolling circle, branched DNA, EXPAR, LAMP, and other DNA amplification methods well known to one skilled in the art or analyzed by mass spectroscopy or single molecule detection methods. RNA may be processed by Reverse Transcriptase real time-PCR, or samples prepared for DNA microarrays, or other analytical methods. Real time or end point analyzes can be performed with the apparatus. For proteins, assays may be performed in the cartridge including enzymatic assays, sandwich immunoassays, antibody precipitation, protein digestion, protein and peptide labeling, and other commonly used protein analysis methods. Similarly, other cellular components or chemicals can be extracted or purified using standard methods in the apparatus. Molecular biology methods are readily adapted to the apparatus. Samples can be completely analyzed on the apparatus in a single cartridge, moved to a separate cartridge, or analyzed or further processed in a separate instrument comprising a capillary electrophoresis system or microchip capillary electrophoresis ;multidimensional gel and capillary electrophoresis; mass spectroscopy, multidimensional mass spectroscopy with HPLC, ICP, Raman spectroscopy, particle, nanoparticles, and bead based detection, imaging, comprising fluorescence, IR, optical, or any other analytical systems well know to one in the art.

[0043] In one embodiment, the integration of a complete sample-to-answer instrument incorporating the cartridge to prepare DNA samples from many inputs and sample types and a microchip-based capillary electrophoresis device for separation of DNA fragments is used for analysis, such as DNA sequencing, fragment sizing, and forensics.

INCORPORATION BY REFERENCE

[0044] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0046] Figure 1 depicts an example of a microscale on-chip valve (MOVE).

[0047] Figure 2 shows a MOVE microvalve, a microrouter, a MOVE mixer, and bead capture on microchips.

[0048] Figure 3 shows a fluidic cartridge with MOVE microvalves.

[0049] Figure 4 shows a fluidic cartridge with ports to a microfluidic microchip with microvalves.

[0050] Figure 5 shows a microfluidic microchip with MOVE valves that controls flows in a cartridge.

[0051] Figure 6 shows a cartridge connected to reaction chamber and detector with downstream MOVE pumps and reagents.

[0052] Figure 7 shows a temperature control device that can thermal cycle and incorporates magnetic capture, pinch clamps and the capability of cycling seven reactions simultaneously.

[0053] Figure 8 shows a temperature control device that can thermal cycle and incorporates magnetic capture, pinch clamps and the capability of cycling seven reactions simultaneously.

[0054] Figure 9 shows PowerPlex16 STR (Single tandem repeat) amplification reaction performed in a passive, Teflon (PTFE) based Tube reaction chamber.

[0055] Figure 10 shows purification of DNA from 25 uL of blood at 69', 23.5', 10.5', and 4.5'; yield in ng is shown on the bars.

[0056] Figure 11 shows a schematic of using microvalves to capture beads on a microchip.

[0057] Figure 12 shows bead capture from a cartridge on a microchip using a MOVE microvalve.

[0058] Figure 13 shows bead capture from a cartridge on a microchip using a MOVE microvalve.

[0059] Figure 14 shows a capture and reaction microchip using MOVE microvalves.

[0060] Figure 15 shows a capture and reaction microchip using MOVE microvalves.

[0061] Figure 16 shows a four cartridge assembly.

[0062] Figure 17 shows an example of STR reactions on microchips.

[0063] Figure 18 shows a universal sample preparation workflow to prepare nucleic acids and toxins.

[0064] Figure 19 shows purification of samples in a cartridge using paramagnetic beads.

[0065] Figure 20 shows an integrated pneumatic manifold to operate the MOVE microvalves in cartridge.

[0066] Figure 21 shows a cartridge mounted on a computer controlled apparatus.

[0067] Figure 22 shows a cartridge mounted on a computer controlled apparatus.

- [0068] Figure 23 shows a reagent distribution manifold based on MOVE technology that can distribute five reagents to five extraction / isolation or other devices.
- [0069] Figure 24 shows a reagent distribution manifold based on MOVE technology that can distribute five reagents to five extraction / isolation or other devices.
- 5 [0070] Figure 25 shows a distribution manifold with sample loops and MOVE microvalves.
- [0071] Figure 26 shows a pneumatic manifold, top panel shows the top side and the lower panel the bottom side.
- [0072] Figure 27 shows detection of *E. coli* by immunomagnetic separation, followed by alkaline lysis and PEG-facilitated capture on magnetic beads, and analyzed by real-time PCR.
- 10 [0073] Figure 28 shows application of a cartridge with three chambers that can be used to construct genomic libraries and other applications.
- [0074] Figure 29 shows the workflow to prepare genomic libraries using the cartridge.
- [0075] Figure 30 shows a forked injector for microchip based electrophoresis.
- [0076] Figure 31 shows sample stacking with a forked injector.
- 15 [0077] Figure 32 shows a forked injector coupled to MOVE microvalves.
- [0078] Figure 33 shows a forked cathode injector coupled with a MOVE microchip.
- [0079] Figure 34 shows a photograph of a microchip with the forked injector.
- [0080] Figure 35 shows a photograph of a microchip with the forked injector.
- [0081] Figure 36 shows an electropherogram of a single color from a DNA sequencing trace from a
20 forked cathode injector.
- [0082] Figure 37 shows STR separations on a forked cathode injection system.
- [0083] Figure 38 shows a forked cathode with MOVE microfluidics for shuttle loading.
- [0084] Figure 39 shows an integrated system for nucleic acid isolation, amplification(s), separation and detection.
- 25 [0085] Figure 40 depicts a device with a cartridge, microfluidic microchip, and a magnet.
- [0086] Figure 41 depicts a microfluidic microchip with a fluidics layer, an elastomeric layer, and a pneumatics layer.
- [0087] Figure 42 depicts a fluidics layer made of two layers of material.
- [0088] Figure 43 depicts a fluidics layer made of a single layer of material.
- 30 [0089] Figure 44 depicts a reaction scheme for amplifying mRNA.
- [0090] Figure 45 depicts an expanded view of a heat block 509, a cartridge 50, a microfluidic microchip 519 and a pneumatics manifold 507.
- [0091] Figure 46 depicts a heat block, a cartridge, a microfluidic microchip and a pneumatics manifold in an assembled form.
- 35 [0092] Figure 47 depicts fluidics and pneumatic layers of a microfluidic microchip with four sets of pumps.
- [0093] Figure 48 depicts a cartridge for interfacing with a fluidics layer of a microfluidic microchip.
- [0094] Figure 49 depicts a block for holding tips that interface with a cartridge.

[0095] Figure 50 depicts results of reverse transcription reactions of an mRNA amplification scheme.

[0096] Figure 51 depicts an expanded view of a heat block with a heat distributing element, a cartridge, a microfluidic microchip and a pneumatics manifold.

[0097] Figure 52 depicts a heat block with a heat distributing element, a cartridge, a microfluidic microchip and a pneumatics manifold in an assembled form.

[0098] Figure 53 depicts a pneumatics manifold.

[0099] Figure 54 depicts a pneumatics manifold.

[00100] Figure 55 depicts a pneumatics manifold.

[00101] Figure 56 depicts a pneumatics manifold.

[00102] Figure 57 depicts fluidics and pneumatic layers of a microfluidic microchip with a reagent and bead rail with the fluidic layer shown in solid lines and the pneumatics layer shown in dashed lines.

[00103] Figure 58 depicts fluidics layers of a microfluidic microchip with a reagent and bead rail.

[00104] Figure 59 shows a microfluidic microchip with MOVE valves that controls flows in a cartridge.

[00105] Figure 60 shows a forked electrode.

[00106] Figure 61 shows a forked electrode, a forked electrode with a wire run electrode, and a forked electrode with a cannular electrode.

[00107] Figure 62 shows sample injection into a separation channel.

[00108] Figure 63 shows a device for mating a separation capillary with an injection tubing.

[00109] Figure 64 shows a device for mating separation capillaries with four injection tubings.

[00110] Figure 65 shows a thermocycler with an Ultem pinch clamp.

[00111] Figure 66 shows a diagram indicating movement of reagents between components of a four channel parallel processing device.

[00112] Figure 67 shows a four-channel parallel reagent delivery device: the Chip C microchip design is shown on the top left, a fluidic manifold is shown on the bottom left, and the fabricated and assembled device is shown on the right.

[00113] Figure 68 shows a four-channel sample preparation device on the left and a four-channel sample preparation device mounted on a monolithic pneumatic manifold on the right.

[00114] Figure 69 shows MOVE microchip designs of the four-channel sample preparation device: the Chip D microchip design is shown on the left with flow through valves that form a T-junction between two bisecting channels shown in top panel; the Chip D microchip design with flow-through valves is shown on the right top; the Chip F microchip design with in-line valves that have only one channel passing through the middle of the valve is shown on the right bottom.

[00115] Figure 70 shows IdentiFiler STR profiles of DNA samples prepared on the four-channel sample preparation device, where STR amplifications were performed using fast protocols (1.5 hrs) on a STR Reaction subsystem thermocycler.

[00116] Figure 71 shows a four-channel post amplification device combined with an Chip A microchip with a fluidics manifold: the Chip A microchip design is shown on the left, the fabricated

microchip is shown in the center, and the assembled fluidic manifold and microchip is shown on the right.

[00117] Figure 72 shows a post-amplification STR clean-up subsystem with the post-amplification device.

[00118] Figure 73 shows the Chip E microchip design, which can be used a post-amplification device.

5 [00119] Figure 74 shows a diagram of a mixer.

[00120] Figure 75 shows a diagram of a mixer.

[00121] Figure 76 shows results of using a mixer to lyse cells.

DETAILED DESCRIPTION OF THE INVENTION

10 [00122] This invention includes devices that incorporate valves, such as microvalves (including but not limited to pneumatically actuated valves and microscale on-chip valves), into their design in order to control the movement of fluid. These devices can be used for the enrichment of a component, for sample preparation, and/or for analysis of one or more components in or from a sample.

[00123] The invention also provides devices for fluid and analyte processing and methods of use thereof. The devices of the invention can be used to perform a variety of actions on the fluid and analyte. 15 These actions can include moving, mixing, separating, heating, cooling, and analyzing. The devices can include multiple components, such as a cartridge, a microfluidic microchip, and a pneumatic manifold. **Figure 40** shows an exemplary device having a cartridge (101), microfluidic microchip (103), and pneumatic manifold (113).

I. Sample preparation device

20 [00124] In one aspect a sample preparation device as shown in device 800 in **Figure 16**, device 1000 in **Figure 21** and **Figure 22**, and device 1000 in **Figure 68** comprises a cartridge integrated with a microfluidic microchip that controls movement of the fluid in the cartridge through microvalves and the components to operate the cartridge. The cartridge and/or the compartments therein can be of sufficient size to process one or more milliliter of an input sample in an automated device. 25 The cartridge can process a sample to output a component that can be moved using pressure-driven flow or vacuum modulated by microvalves. The cartridge can provide an interface with a delivery device comprising macroscale samples, such as blood, aerosol liquids, swabs, bodily fluids, swipes, and other liquid, solid, and gas samples. The cartridge can process macroscale sample volumes using microscale sample preparation and analysis. The cartridge can allow for 30 processing of macroscale or large volume samples using microfluidic devices and components have reduced void volumes that allow for reduced loss of materials.

A. Cartridges

[00125] A cartridge, also referred to as a fluidic manifold herein, can be used for a number of purposes. In general, a cartridge can have ports that are sized to interface with large scale devices as well as 35 microfluidic devices. Cartridges or fluidic manifolds have been described in U.S. Patent Application No. 61/022,722, which is hereby incorporated by reference in its entirety. The cartridge can be used to receive materials, such as samples, reagents, or solid particles, from a source and deliver them to the microfluidic microchip. The materials can be transferred between

the cartridge and the microfluidic microchip through mated openings of the cartridge and the microfluidic microchip. For example, a pipette can be used to transfer materials to the cartridge, which in turn, can then deliver the materials to the microfluidic device. In another embodiment, tubing can transfer the materials to the cartridge. In another embodiment, a syringe can transfer material to the cartridge. In addition, a cartridge can have reservoirs with volumes capable of holding nanoliters, microliters, milliliters, or liters of fluid. The reservoirs can be used as holding chambers, reaction chambers (e.g., that comprise reagents for carrying out a reaction), chambers for providing heating or cooling (e.g., that contain thermal control elements or that are thermally connected to thermal control devices), or separation chambers (e.g. paramagnetic bead separations, affinity capture matrices, and chromatography). Any type of chamber can be used in the devices described herein, e.g. those described in U.S. Patent Publication Number 2007/0248958, which is hereby incorporated by reference. A reservoir can be used to provide heating or cooling by having inlets and outlets for the movement of temperature controlled fluids in and out of the cartridge, which then can provide temperature control to the microfluidic microchip. Alternatively, a reservoir can house Peltier elements, or any other heating or cooling elements known to those skilled in the art, that provide a heat sink or heat source. A cartridge reservoir or chamber can have a volume of at least about 0.1, 0.5, 1, 5, 10, 50, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 2000, 3000, 4000, 5000 or more μL . The relative volume of a chamber or reservoir can be about 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10000 or more greater than a channel or valve within the microfluidic microchip. The size of the chambers and reservoirs of the cartridge, which can be mated to the microfluidic microchip, can be chosen such that a large volume of sample, such as a sample greater than about 1, 5, 10, 50, 100, 500, 1000, 5000, 10000, 50000 or more μL , can be processed, wherein the flow of fluids for processing the sample is controlled by valves in the microfluidic microchip. This can allow for a reduced amount of sample and reagent loss due to the reduced void volumes in the microfluidic microchip compared to other flow control devices, such as pipettes and large scale valves. The void volume within a microfluidic microchip can be less than 1000, 500, 100, 50, 10, 5, 1, 0.5, 0.1, or 0.05 μL . This can allow for the amount of sample or reagent loss during processing of a sample to be less than 20, 15, 10, 7, 5, 3, 2, 1, 0.5, 0.05 percent.

[00126] For example, **Figure 40** shows cartridge (101) with a reservoir with a port (115) opening to a side of the cartridge that can be used to receive materials from a pipette or any other large scale device. The port can also be adapted with fitting to receive tubing or a capillary to connect the cartridge to upstream fluidics. The reservoir can taper down to form a cartridge reservoir opening (117) that interfaces, aligns, or mates with an opening 105 in the fluidics layer of the microfluidic microchip.

[00127] A cartridge can be constructed of any material known to those skilled in the art. For example, the cartridge can be constructed of a plastic, glass, or metal. A plastic material may include any plastic known to those skilled in the art, such as polypropylene, polystyrene, polyethylene,

polyethylene terephthalate, polyester, polyamide, poly(vinylchloride), polycarbonate, polyurethane, polyvinylidene chloride, cyclic olefin copolymer, or any combination thereof. The cartridge can be formed using any technique known to those skilled in the art, such as soft-lithography, hard-lithography, milling, embossing, ablating, drilling, etching, injection molding, or any combination thereof.

[00128] As exemplified in **Figure 3** and **Figure 4**, a cartridge (1) can comprise a rectilinear configuration with flat sides. In another embodiment, a cartridge comprises a surface that is curved, rounded, indented or comprises a protrusion. In one embodiment a cartridge has at least one substantially flat surface which is adjacent to a microfluidic microchip. The cartridge is adapted to be fluidically connected with ports in the microchip. For example, openings in the surface of the cartridge can be aligned with ports in the microchip. When the cartridge and microchip are mated to one another, the openings align to create the fluidic connections allowing liquids to pass from the cartridge into the ports of the microchip, which are connected to channels typically having valves that form fluidic circuits. Another embodiment of a cartridge is shown in **Figure 59**.

Figure 59 shows a cartridge with multiple ports that mate with a microchip and external components, such as syringes. The compartment in the cartridge can be shaped to allow insertion of the syringe and its protrusion. The cartridge can also include a vent port to vent gases in chambers of the cartridge or chambers of the microchip. As well, **Figure 59** shows the position where an actuated magnet can be used to apply a magnetic field to a mix chamber. Additionally, **Figure 59** shows a cap that can be used to close the mix chamber.

[00129] In one embodiment a cartridge contains one or more features, including but not limited to a chamber, a port, a channel or a magnet. In one embodiment, microvalves, such as pneumatically actuated valves are combined with the microfluidic cartridge. In some embodiments the microvalves are active mechanical microvalves (such as magnetic, electrical, or piezoelectric thermal microvalves), non-mechanical microvalves (such as bistable electromechanical phase change or rheological microvalves), external microvalves (such as modular or pneumatic), passive mechanical (such as check microvalves or passive non-mechanical (such as capillary microvalves) (Oh et al., A review of microvalves, J. Micromech Microeng. 16 (2006) R13-R39, herein incorporated by reference in its entirety)).

[00130] In another embodiment, pneumatically actuated valves, such as MOVE valves modulate the flow of air pressure, vacuum, or fluids in a microfluidic microchip 2 or multiple microfluidic microchips. MOVE valves can be microscale on-chip valves, microfluidic on-chip valves or micro-robotic on-chip valves. In one embodiment the flow of air pressure, vacuum, or fluids is regulated by one or more variable pressure pumps, such as solenoid valves or solenoid pumps. In one embodiment, a microfluidic microchip is a structure that contains microchannels and/or microtrenches, where a microchannel is a closed structure and a microtrench is an open structure. In one embodiment a microfluidic microchip is a planar structure. In a related embodiment a microfluidic device comprises a microfluidic microchip with microvalves clustered on one side of

a cartridge. In one embodiment (**Figure 3** and **Figure 4**) the cartridge (1) can comprise one or more ports (4, 5, 6, 7, 8, 9) to external fluids, air, or vacuum. Functions of the ports can be for waste (4), reagent entry (5), vent (6), sample input (7), product output (8). The cartridge (1) can contain one or more sample input or reaction chambers, (7) and (3).

5 [00131] A single chamber within the cartridge, such as a reaction chamber, can have one or more, or at least one, two, or three fluidic connections to a microchip. For example, reaction chamber (3) can have a fluidic connection to the microchip through connection 120, which is at the base of the chamber, and another fluidic connection to the microchip through port (9), which is connected to chamber (3) through a passageway located at the top of the chamber. The top of chamber (3),
10 port (9), and the passageway between chamber (3) and port (9) can be closed from the exterior environment such that fluids in chamber (3) necessarily are pumped into port (9) when chamber (3) is full and vice versa. Such a chamber or combination of chamber and port can be referred to as a closed chamber. The positioning of the fluidic connections need not necessarily be at the base and top of the chamber, however, fluidic connections at the base and top positions of the
15 chamber allow for reduced trapping of gas in the chamber. Alternatively, reaction chamber (3) can be viewed as a combination of two chambers that are fluidically connected to each other at a top position, which can be within the cartridge, and, where each chamber also has an opening at a base location. The openings at the base locations, also called chamber apertures, can be fluidically connected to port apertures on the microchip. The two fluidic connections can allow
20 for fluids to be directed into and out of the chamber through the microfluidic microchip.

[00132] In another embodiment a device comprises a cartridge comprising at least one pneumatically actuated valve, such as a MOVE valve, located on one or more surfaces or structures in a non-linear manner. A cartridge can comprise one or more pneumatically actuated valves that are located within the cartridge, in a location other than the base of the cartridge.

25 [00133] Functional elements of a cartridge can include ports, channels, chambers, filters, magnets, or vents, chambers can be collectively referred to as functional elements. In one embodiment, **Figure 4**, the functional elements connect to the microfluidic microchip containing microvalves at junctions 100, 120, 140, 160, and 230. The functional elements can connect with tubing or capillaries inserted into the ports, by a flush connection, or by fittings. In one embodiment a flush
30 connection can comprise a port of a cartridge aligned directly with an aperture of a microfluidic microchip. In one embodiment the cartridge and microfluidic microchip form an integrated module. In another embodiment the cartridge and microfluidic microchip are two separate pieces which are attached together, prior to use.

[00134] A cartridge can comprise at least one chamber, a sample input port, a reagent port, an exit port, a
35 waste port and a magnet. The magnet can be located adjacent to the chamber, so that the magnet force generated by the magnet can attract paramagnetic particles in said chamber to a wall of the chamber. In one embodiment the paramagnetic particles are beads or cells rendered magnetically

responsive (e.g., cells comprising hemoglobin that are treated with sodium nitrate). The magnet can be an electric magnet or a permanent magnet, such as a rare earth metal magnet.

[00135] In one embodiment, **Figure 4**, connections or ports (4, 5, 6, 7, 8, and 9) lead to channels in the cartridge (14, 15, 16, 17, 18, and 19) respectively. Ports (4, 5, 6, 7, and 8) show indents to reliably attach a connector or tubing to the indent, such as the indent shown for connection (7) (see the difference in diameter of connection (7) with channel (17)). In one embodiment, the ports or ports can interface with a variety of connector or tubes, such as the capillaries as described in U. S. Patent 6,190,616, U.S. Patent 6,423,536, US Application No. 09/770,412, 1-25-01, US Application No. 60/402,959 or one or more microchips with modular microfluidic ports as described in US Patent 6,870,185 and US application 11/229,065; all of which are herein incorporated by reference in their entirety. In one embodiment, the modular microfluidic ports enable microchips or capillaries to be reversibly joined without dead volumes or leakage.

[00136] In another embodiment chamber (3) is connected to passageway (9) and to cone (13), leading to junction (120). Chamber (3) can be used for reactions as may any of the channels. In **Figure 4** the cartridge channels lead directly to the apertures of ports on the microchip (2). The channels of the cartridge can interconnect with each other as needed. In some embodiments, at least one channel in a cartridge does not physically connect to a microfluidic microchip. In another embodiment at least one channel in a cartridge is fluidically connected to at least one microchannel in a microfluidic microchip. The connection may or may not utilize an aperture on the microfluidic microchip. An aperture can be an opening or a fitting designed to mate between the microchip and the cartridge. In some embodiments of the invention, the fitting comprises a seal such as a gasket or an o-ring.

B. Microchips

[00137] In one embodiment a cartridge and a microfluidic microchip are integrated together to form a single modular device. The cartridge and a microfluidic microchip can be attached by a fluid or solid adhesive or mechanically. In one embodiment the adhesive is a polyacrylate, adhesive tape, double-sided tape, or any other adhesive known to one skilled in the art. A cartridge can comprise a feature (12) that is capable of wicking a fluid-based adhesive into the junction between a microfluidic microchip and a cartridge. In another embodiment a cartridge is attached to a microfluidic microchip with a non-fluidic adhesive layer. Alternatively, the cartridge and microchip can be held together by clips, clamps, or another holding device. The cartridge and microchip can be aligned prior to integration by visual cues, with or without a microscope, or by physical guiding features. Visual cues can include lines or features that are drawn, etched, or otherwise present on the cartridge, the microchip, or both. Physical guiding features include indentations, protrusions, and edges that can be 'keyed' to aid or insure proper assembly.

[00138] In some instances, the microfluidic microchip has diaphragm valves for the control of fluid flow. Microfluidic devices with diaphragm valves that control fluid flow have been described in U.S. Patent No. 7,445,926, U.S. Patent Publication Nos. 2006/0073484, 2006/0073484, 2007/0248958,

and 2008/0014576, and PCT Publication No. WO 2008/115626, which are hereby incorporated by reference in their entirety. The valves can be controlled by applying positive or negative pressure to a pneumatics layer of the microchip through a pneumatic manifold.

[00139] In one embodiment, the microchip is a “MOVE” microchip. Such microchips comprise three functional layers – a fluidics layer that comprises microfluidic channels; a pneumatics layer that comprises pneumatics channels and an actuation layer sandwiched between the two other layers. In certain embodiments, the fluidics layer is comprised of two layers. One layer can comprise grooves that provide the microfluidics channels, and vias, or holes that pass from the outside surface to a fluidics channel. A second layer can comprise vias that pass from a surface that is in contact with the actuation layer to the surface in contact with the pneumatic channels on the other layer. When contacted together, these two layers form a single fluidics layer that comprises internal channels and vias that open out to connect a channel with the fluidics manifold or in to connect a channel with the activation layer, to form a valve, chamber or other functional item. The actuation layer typically is formed of a deformable substance, e.g., an elastomeric substance, that can deform when vacuum or pressure is exerted on it. At points where the fluidic channels or pneumatic channels open onto or are otherwise in contact with the actuation layer, functional devices such as valves can be formed. Such a valve is depicted in cross section in **Figure 1**. Both the fluidics layer and the pneumatics layer can comprise ports that connect channels to the outside surface as ports. Such ports can be adapted to engage fluidics manifolds, e.g., cartridges, or pneumatics manifolds. .

[00140] As shown in **Figure 40**, the microfluidic microchip (103) can be interfaced with the cartridge (101). The microfluidic microchip can have a chamber (105) with an opening that is mated to an opening (117) of the cartridge (101). The chamber can be used for a variety of purposes. For example, the chamber can be used as a reaction chamber, a mixing chamber, or a capture chamber. The chamber can be used to capture magnetic particles such as magnetic beads, paramagnetic beads, solid phase extraction material, monoliths, or chromatography matrices.

[00141] A magnetic component (109) can be positioned such that magnetic particles in the cartridge reservoir (107) and/or the microfluidic chamber (105) are captured against a surface of the microfluidic chamber (105). The magnetic component can generate a magnetic and/or electromagnetic field using a permanent magnet and/or an electromagnet. If a permanent magnet is used, the magnet can be actuated in one or more directions to bring the magnet into proximity of the microfluidic microchip to apply a magnetic field to the microfluidic chamber. In some embodiments of the invention, the magnet is actuated in the direction (111) indicated in **Figure 40**.

[00142] Alternatively, any of a variety of devices can be interfaced with the microfluidic microchip. For example detectors, separation devices (e.g. gas chromatographs, liquid chromatographs, capillary electrophoresis, mass spectrometers, etc), light sources, or temperature control devices can be positioned next to the microfluidic microchip or used in conjunction with the microfluidic

microchip. These devices can allow for detection of analytes by detecting resistance, capacitance, light absorbance or emission, fluorescence, or temperature or other chemical or physical measurements. Alternatively, these devices can allow for light to be introduced to a region or area of the microfluidic microchip.

5 [00143] A microfluidic device can be designed with multiple chambers that are configured for capture of magnetic particles. The multiple chambers and magnetic component can be arranged such that a magnetic field can be applied simultaneously to all chambers, or be applied to each or some chambers independent of other chambers. The arrangement of chambers and magnetic components can facilitate faster or more efficient recovery of magnetic particles. In particular, 10 the arrangement can facilitate recovery of magnetic particles in multiple chambers.

[00144] As shown in **Figure 41**, the microfluidic microchip (103) can be formed of a fluidics layer (203), an elastomeric layer (205), and a pneumatic layer (207). The fluidics layer can contain features such as a chamber (105), as well as channels, valves, and ports. The channels can be microfluidic channels used for the transfer of fluids between chambers and/or ports. The valves can be any 15 type of valve used in microfluidic devices. In preferred embodiments of the invention, a valve includes a microscale on-chip valve (MOVE), also referred to as a microfluidic diaphragm valve herein. A series of three MOVEs can form a MOVE pump. The MOVEs and MOVE pumps can be actuated using pneumatics. Pneumatic sources can be internal or external to the microfluidic microchip.

20 [00145] An example of a MOVE valve is shown in **Figure 1**. A cross-sectional view of a closed MOVE valve is shown in **Figure 1A**. A cross-sectional view of an open MOVE valve is shown in **Figure 1B**. **Figure 1C** shows a top-down view of the MOVE valve. A channel (251) that originates from a fluidic layer can interface with an elastomeric layer (259) by one or more vias (257). The channel can have one or more seats (255) to obstruct flow through the channel when the 25 elastomeric layer (259) is in contact with the seat (255). The elastomeric layer can either be normally in contact with the seat, or normally not in contact with the seat. Application of positive or negative pressure through a pneumatic line (261) to increase or decrease the pressure in a pneumatic chamber (253) relative to the fluidic channel (251) can deform the elastomeric layer, such that the elastomeric layer is pushed against the seat or pulled away from the seat. In some 30 embodiments of the invention, a MOVE does not have a seat, and fluid flow through the fluidic channel is not completely obstructed under application of positive or negative pressure. The vacuum that can be applied include extremely high vacuum, medium vacuum, low vacuum, house vacuum, and pressures such as 5 psi, 10 psi, 15 psi, 25 psi, 30 psi, 40 psi, 45 psi, and 50 psi.

35 [00146] Three MOVE valves in series can form a pump through the use of a first MOVE as an inlet valve, a second MOVE as a pumping valve, and a third MOVE as an outlet valve. Fluid can be moved through the series of MOVEs by sequential opening and closing of the MOVEs. For a fluid being supplied to an inlet valve, an exemplary sequence can include, starting from a state where all three MOVEs are closed, (a) opening the inlet valve, (b) opening the pumping valve, (c) closing

the inlet valve and opening the outlet valve, (d) closing the pumping valve, and (e) closing the outlet valve. Since the inlet and outlet valve can have the same structure, a MOVE pump can move fluids in either direction by reprogramming of the sequence of opening inlet or outlet valves.

5 [00147] The fluidic layer (203) can be constructed of one or more layers of material. As shown in **Figure 42**, the fluidic layer (203) can be constructed of two layers of material. Channels (301, 303, 305) can be formed at the interface between the two layers of material, and a chamber (105) can be formed by complete removal of a portion of one layer of material. The channels can have any shape, e.g., rounded and on one side (301), rectangular (303), or circular (305). The channel can
10 be formed by recesses in only one layer (301, 303) or by recesses in both layers (305). The channels and chambers can be connected by fluidic channels that traverse the channels and chambers shown. Multidimensional microchips are also within the scope of the instant invention where fluidic channels and connections are made between multiple fluidic layers.

[00148] The thickness (307) of the second layer of material can be of any thickness. In some
15 embodiments of the invention, the second layer has a thickness that minimizes reduction of a magnetic field in the chamber (105) that is applied across the second layer from an external magnetic component or minimizes reductions in heat transfer.

[00149] As shown in **Figure 43**, the fluidic layer (203) can be constructed of a single layer of material. The single layer is then interfaced with an elastomeric layer, such that channels (305, 303) and
20 chambers (305) are formed between the fluidic layer and the elastomeric layer (205).

[00150] The microfluidic microchip can be constructed from any material known to those skilled in the art. In some embodiments of the invention, the fluidics and pneumatic layer are constructed from glass and the elastomeric layer is formed from PDMS. In alternative embodiments, the elastomer can be replaced by a thin membrane of deformable material such as Teflon (PTFE), silicon, or
25 other membrane. The features of the fluidics and pneumatic layer can be formed using any microfabrication technique known to those skilled in the art, such as patterning, etching, milling, molding, embossing, screen printing, laser ablation, substrate deposition, chemical vapor deposition, or any combination thereof.

[00151] In one embodiment, microchannel circuits are formed on a microfluidic microchip 2, as shown in
30 **Figure 5**, linking sets of microvalves with microchannels. In one embodiment the microvalves are pneumatically actuated valves. In one embodiment the pneumatically actuated valves are MOVE microvalves. In one embodiment, the fluidic path between a cartridge and a microfluidic microchip, such as between chambers, ports, channels, microchannels, and other functional elements can be controlled by opening or closing at least one microvalve. In one embodiment the
35 microvalve is controlled by a microprocessor control such as a computer. A computer can include an input/output controller, or any other components known to one skilled in the art such as memory storage and a processor. In one embodiment, a microvalve is a MOVE valve that is actuated by a pneumatic source, such as through pneumatic ports 10, 20, 30, 40, 50, 60, or 70. In

one embodiment the pneumatic source is controlled by at least one solenoid. In one embodiment the solenoid is miniaturized and can be connected to vacuum or pressure sources. In one embodiment the pneumatic source is connected to a pneumatic port using a force such as clamping, springs, pneumatics, or a screw force, optionally with sealing provided by an o-ring.

5 [00152] In one embodiment **Figure 5** shows a view of the top of a microfluidic microchip (2), this side makes contact with the bottom of cartridge (1). A microvalve 110 controls the fluidic path between microchannels 101 and 121. A microvalve 130 controls the fluidic path between microchannels 131 and 141. Microvalve (150) controls the fluidic path between microchannels 151 and 152. Microvalve 180 controls the fluidic path between microchannels 181 and 191.
10 Microvalve 200 controls the fluidic path between microchannels 201 and 212. Microvalve 220 controls the fluidic path between microchannels 221 and 231.

[00153] In one embodiment junctions can connect one or more microchannels. **Figure 5** shows the schematic for a microchip that can be mated with the cartridge shown in **Figure 4**. In **Figure 5**, junction 100 connects to single microchannel 101, junction 140 connects to single microchannel
15 141, junction 160 connects to single microchannel 161, and junction 230 connects to single microchannel 231. Junction 190 connects to two microchannels 191 and 201. Junction 120 connects to three microchannels 121, 131, and 151. In one embodiment more than three microchannels can be connected to a single junction.

[00154] The microchannels can be fabricated by one or more techniques such as photolithography,
20 molding, embossing, casting, or milling. The microchannels can be manufactured in a material such as glass, plastic, polymer, ceramic, gel, metal, or another suitable solid.

[00155] In another embodiment a device comprises a cartridge comprising at least three chambers, more than one input port and more than one output port (**Figure 28**). The cartridge can be adapted to process a nucleic acid sample for analysis. The cartridge can be adapted to receive one or more
25 reagents from an external reagent source. The reagents can be a paramagnetic bead, a non-paramagnetic bead, an enzyme, a dNTP, a buffer solution, a salt solution, an alcohol solution, an solution comprising EDTA or an oligonucleotide or other reagents. The enzymes can be a ligase, a restriction enzyme, a polymerase, or a kinase or any other enzyme or catalytic biomaterials including RNAs. The device can comprise a magnet which can attract paramagnetic beads to a
30 wall of one or more chambers. In another embodiment at least one chamber comprises a filter to capture beads, such as non-paramagnetic beads.

[00156] In one embodiment the cartridge is used in a method of sample enrichment comprising: delivery of a sample to a chamber by a sample port and delivery of paramagnetic particles to a chamber by a reagent port. The paramagnetic particles (e.g. paramagnetic beads) bind to at least one
35 component in the sample (such as DNA, RNA, micro RNA, a protein, a lipid, a polysaccharide or other ligand). The paramagnetic particles are attracted to a wall of a chamber by virtue of the magnetic force exerted by a magnet located outside the chamber. The paramagnetic particles are washed with a wash solution delivered to the chamber comprising the paramagnetic particles by a

reagent port, and the wash solution is removed by a waste port. A reagent can be added to elute the component of the sample from the paramagnetic particles and output the sample component to another device for further processing or analysis. A preferred embodiment is to output the component of the sample on the paramagnetic particles.

5 [00157] In one embodiment a device comprising a microfluidic microchip is used in a method of diagnosis. In one embodiment the diagnosis comprises the detection of an infectious agent in a sample. In one embodiment the infectious agent is a bacteria, virus, fungi, mycoplasma or prion. In another embodiment a device comprising a microfluidic microchip is used in a method of diagnosis of a hereditary disease. In one embodiment the hereditary disease is caused by one or
10 more DNA mutations, such mutations include but are not limited, triplet base expansions, base substitution mutations, deletion mutations, addition mutations, nonsense mutations, premature stop codons, chromosomal deletions, chromosomal duplications, aneuploidy, partial aneuploidy or monosomy. In another embodiment a device comprising a microfluidic microchip is used in a method to diagnose cancer or a predisposition to cancer. In another embodiment a device
15 comprising a microfluidic microchip is used in a method to diagnose a hereditary disease such as autism, downs syndrome, trisomy, Tay-sachs, or other hereditary diseases. In some embodiments a sample used for diagnosis in a device comprising a microfluidic microchip is a blood sample, a mucus sample, a lung lavage sample, a urine sample, a fecal sample, a skin sample, a hair sample, a semen sample, a vaginal sample, or an amniotic sample.

20 [00158] In another embodiment a device comprising a microfluidic microchip is used to identify the presence of environmental contamination of an agent. In one embodiment the agent is a biological agent such as bacteria, virus, fungi, or mycoplasma in an environmental sample. In another embodiment the agent is a contaminant agent, such as a pesticide, an herbicide, or a fertilizer. In one embodiment the environmental sample is a soil sample, a water sample, an air
25 sample, a meat sample, a vegetable sample or a fruit sample. In another embodiment, the agent is a genetically modified organism.

[00159] In another embodiment a device comprising a microfluidic microchip is used for genotyping, identification of an individual mammal (such as a human), forensics, gene expression, gene modification, microRNA analysis, or ribotyping.

30 [00160] In another embodiment a microfluidic microchip is used in a method comprising molecular biological analysis, including but not limited to polymerase chain reaction (PCR) amplification of nucleic acids in a sample (such as Allele-specific PCR, Assembly PCR, Asymmetric PCR, Colony PCR, Helicase-dependent amplification, Hot-start PCR, Intersequence-specific (ISSR) PCR, Inverse PCR, Ligation-mediated PCR, Methylation-specific PCR Multiplex Ligation-
35 dependent Probe Amplification, Multiplex-PCR, Nested PCR, Overlap-extension PCR, Quantitative PCR Reverse Transcription PCR -PCR, Thermal asymmetric interlaced -PCR, Touchdown PCR, or PAN-AC PCR), isothermal nucleic acid amplifications, (such as Loop-mediated Isothermal Amplification (LAMP); nick displacement amplification; Helicase

Dependant Amplification platform (HDA); and the primase-based Whole Genome Amplification platform (pWGA); single primer isothermal amplification (SPIA) and Ribo-SPIA for RNA; strand displacement amplification (SDA); EXPAR [Van Ness J, Van Ness LK, Galas DJ. (2003) Isothermal reactions for the amplification of oligonucleotides. Proc Natl Acad Sci U S A. 100:4504-9.]; rolling circle amplification (RCA); transcription-based amplification system (TAS) and its derivatives include self-sustaining sequence replication (3SR), isothermal nucleic acid sequence-based amplification (NASBA), and transcription-mediated amplification (TMA); ligase chain reaction (LCR)), sequencing reactions of DNA or RNA (such as Maxam-Gilbert sequencing, Sanger chain-termination method, Dye-terminator sequencing Emulsion PCR sequencing, massively parallel sequencing, polony sequencing, sequencing by ligation, sequencing by synthesis, or sequencing by hybridization), restriction fragment length polymorphism (RFLP) analysis, single nucleotide polymorphism (SNP) analysis, short tandem repeat (STR) analysis, microsatellite analysis, DNA fingerprint analysis, DNA footprint analysis, or DNA methylation analysis.

[00161] In one embodiment a cartridge employs beads coupled to a binding moiety, including but not limited to a binding receptor, transferrin, an antibody or a fragment thereof (such as an Fc fragment or an Fab fragment), a lectin, or a DNA or RNA sequence. In another embodiment a cartridge comprises a reagent such as an anti-coagulant, a fixative, a stabilization reagent, a preservative or precipitation reagent.

C. Pneumatic Manifold

[00162] A pneumatic manifold can be integrated with any microchip and/or cartridge described herein to facilitate distribution of air pressure or vacuum. The air pressure or vacuum can be used to actuate valves on the microchip. Alternatively, air pressure or vacuum can be supplied to a cartridge such that air pressure or vacuum is provided to microchannels within the fluidics layer of a microchip which can be used to move fluids or gases within the fluidics layer. A pneumatic manifold provides the air pressure or vacuum to operate microvalves on microchip (2) on cartridge (1) of **Figure 3** or operate microvalves in other devices.

[00163] A pneumatic manifold can be used to mate the pneumatic lines of a microfluidic microchip to external pressure sources. The pneumatic manifold can have ports that align with ports on the pneumatics layer of the microfluidic microchip and ports that can be connected to tubing that connect to the external pressure sources. The ports can be connected by one or more channels that allow for fluid communication of a liquid or gas, or other material between the ports.

[00164] The pneumatic manifold can be interfaced with the microfluidic microchip on any surface of the microchip. The pneumatic manifold can be on the same or different side of the microfluidic microchip as the cartridge. As shown in **Figure 40**, a pneumatic manifold (113) can be placed on a surface of the microfluidic microchip opposite to the cartridge. As well, the pneumatic manifold can be designed such that it only occupies a portion of the surface of microfluidic microchip. The positioning, design, and/or shape of the pneumatic manifold can allow access of

other components to the microfluidic microchip. The pneumatic manifold can have a cut-out or annular space that allows other components to be positioned adjacent or proximal to the microfluidic microchip. This can allow, for example, a magnetic component (109) to be placed in proximity of a chamber within the microfluidic microchip.

5 [00165] A pneumatic manifold can be constructed of any material known to those skilled in the art. For example, the cartridge can be constructed of a plastic, glass, or metal. A plastic material includes any plastic known to those skilled in the art, such as polypropylene, polystyrene, polyethylene, polyethylene terephthalate, polyester, polyamide, poly(vinylchloride), polycarbonate, polyurethane, polyvinylidene chloride, cyclic olefin copolymer, or any combination thereof. The
10 pneumatic manifold can be formed using any technique known to those skilled in the art, such as soft-lithography, conventional lithography, milling, molding, embossing, drilling, etching, or any combination thereof.

[00166] A pneumatic manifold (370) was designed (**Figure 20**) that eliminates over twenty tubing ports and provides a robust, reproducible interface between the control system, the pneumatic solenoids
15 and the MOVE input ports. The manifold (370) has a gasket (380) and a bottom plate (390) that are fastened together. The cartridge (1) is held on the plate (370) by a bracket in position to align the pneumatic ports 10, 20, 30, 40, 50, 60, and 70 on microfluidic microchip (2), shown in **Figure 5**, with the pneumatic lines shown of the reverse side of 370 in the **Figure 20** insert. The external pneumatics are controlled by a solenoid valve bank that can be miniaturized and can be connected
20 to vacuum or pressure sources.

[00167] The apparatus shown in **Figure 21** and **Figure 22** can incorporate the pneumatic manifold shown in **Figure 20**. The apparatus can be used for sample preparation, as described herein, and can incorporate a cartridge. Cartridge (1), labeled 'cube', is attached to manifold (370) with solenoids (1819). The assembly of the cartridge and manifold is mounted on a base plate of the
25 apparatus. The pneumatic manifold can be controlled by an IO controller (1803).

[00168] A gas supply, such as a reservoir that can be maintained at a desired pressure or vacuum, can supply gas to the manifold. The gas supply can be connected to an outside pressure or vacuum source. The gas supply feeding the gas supply manifold can have a pressure gauge to monitor the inlet pressure. The gas supply can supply gas to multiple components of the system through a gas
30 supply manifold (1821). The gas supply manifold can supply gas to the pneumatic manifold (370) and to individual reagent containers, (1809) and (1807). The line supplying the distribution valve (390) with gas can be regulated by a regulator (1815).

[00169] Reagents and/or sample can also be supplied to the cartridge through the reagent distribution valve (390) that is connected to containers (1809) in a reagent storage region (380) and a bead
35 solution container (1807) that is mounted on a bead mixer (1805). Adapter (1817) can be mounted and/or aligned with the cartridge such that a delivery device, such as a syringe, can deliver a material to the cartridge. The adapter (1817) can be thermally regulated by a heater control (1801). The adapter can have a thermal conductor, such as brass, to distribute heat

generated by heater coil or a Peltier device. The adapter can maintain temperature between about 20 to 100, 20 to 75, or 50 to 60 degrees Celsius.

[00170] A magnet assembly (1811) can be positioned adjacent to the cartridge. A magnet (300) of the magnet assembly can be positioned adjacent to the cartridge (1) and moved by an actuator, such that the magnet can exert a magnetic field within the cartridge, or a microchip integrated, mated, or interfaced with the cartridge. The magnetic field can be used to capture paramagnetic or magnetic particles, such as beads, within the cartridge or microchip and separate material bound to the particles from waste materials. Waste from the cartridge and/or microchip can be delivered to a waste container (1813).

[00171] The apparatus shown in **Figure 21** and **Figure 22** can use seven solenoid valves to operate the cartridge (1). The size and complexity of the apparatus can be further reduced with MOVE microvalves. **Figure 23** and **Figure 24** shows a reagent distribution device that contains microfluidic microchip 600, which is approximately two inches wide. Solenoid banks 680 and 684 provide connection to full scale external vacuum and pressure through connectors 681, 682, 685, and 686. The solenoids are controlled through electrical junctions 689 and 687. The microfluidic microchip 600, which has MOVE valves, is held in contact with the manifold 700 by attachment 711 using clamp 710. Other methods known to one skilled in the art can be used to connect the microchip to the pneumatics manifold 700.

[00172] As shown in **Figure 25**, microchip 600 connects five reagent sources, 621, 622, 623, 624, and 625 with two sample loops 630 and 631 and five devices 634, 635, 636, 637, and 638 which may be microfluidic devices such as cartridge (1). Sample loops 630 and 631 can be configured to have predetermined volumes. The sample loops can have a portion which is removable. Thus, they can be removably connectable to ports in the fluidic manipulation module. The sample loop can be removed to allow for adjustment of the volume of the sample loop. A portion of the sample loop can be capillary tubing, any other type of tubing, or a microfluidic channel. The sample loop can be connected to the microchip using any type of junction described herein. For example, a junction can connect to a cannula, an upfit tubing, a microtubing fitting, an Upchurch tubing adapter, or a FROLC connector [Jovanovich, S. B., G. Ronan, D. Roach and R. Johnston. Capillary valve, connector, and router. February 20, 2001. U. S. Patent 6,190,616.]. It is apparent that the number of reagent sources, microfluidic devices, and sample loops can be increased or decreased. Each microfluidic device can perform the same function, different, or complementary functions. The devices can be connected through modular microfluidic ports.

[00173] In an aspect of this invention shown in **Figure 25**, a microchip (600) comprises a main microfluidic channel that intersects with two other second microfluidic channels. The main microfluidic channel can be a channel that connects the reagent source 625 with device 634, as shown in **Figure 25**. The second channels can be the channel that connect valve 606 to the sample loop 630 and the channel that connects valve 608 to sample 630. At least one, and optionally both, of these second channels connects with the main channel through a flow-through

valve (606 and 608) that allow a fluid to flow through the main channel but only into or out of the second channel with the flow through valve is open. The flow-through valve can be redesigned as an in-line valve. The main channel also comprises an intermediate valve (674) between the points of intersection of the two second channels. Each second channel opens from the microchip at an entry port. A sample loop (630) having a channel of defined volume is removably attached to each of the entry ports. Thus, a specific volume of fluid in the sample loop can be injected into the main channel by closing the intermediate valve (674), opening the flow through valves (606 and 608) and applying pressure to the main channel. The sample loop can also be referred to as a fluid loop or reagent loop.

[00174] The microchip 600 of the distribution manifold uses eighteen microvalves 601 to 618 to direct flow through the manifold. The microvalves are operated through pneumatic ports with o-rings or other connectors including modular microfluidic ports to pneumatic manifold 700. For example, connection 671 provides pressure or vacuum to microvalve 641 and connection 673 provides pressure or vacuum to microvalve 642. The flow of reagents from reagent sources, 621, 622, 623, 624, and 625 can be directed to fill the sample loops individually or to move samples to devices 634, 635, 636, 637, and 638.

[00175] The pneumatics on manifold 700, as shown in **Figure 26**, connects pressure sources 685 and vacuum sources 686 through solenoid banks 680 and 681 to pneumatic channels 683 leading to the array of ports 684 that includes ports 671 and 673. The top portion of **Figure 26** shows the pneumatic lines of the pneumatic manifold that lead into the pneumatic layer of a microchip from the solenoids. The bottom portion of **Figure 26** shows the solenoids and vacuum (685) and pressure source (686) that are connected to each solenoid. The solenoid banks are controlled by electronics to open and close each individual solenoid to the common vacuum or pressure sources. The individual vacuum or pressure control is also envisioned.

[00176] The pneumatic manifold 700, shown in **Figure 23**, can operate the microvalves 601 to 618 on microfluidic microchip 600, shown in **Figure 25**. For example, to move a reagent from reagent source 622 to sample loop 630, microvalves 602, 606, and 608 are opened and pass-through valve 658 is closed. Alternatively, reagent can by-pass sample loop 630 by closure of T-valves 606 and 608 and opening pass-through valve 658. The valves can be controlled by pneumatic lines that are fluidically connected to the pneumatic manifold. For example, pneumatic line 672 is controlled by a solenoid on pneumatic manifold 700, shown in **Figure 23**, to open or close the microvalve 642. Valves 603, 604, 605, 609, 610, are always open to flow through the microfluidic circuit containing microchannels 641 to 644 and 656 to 668. The reagent is moved into sample loop 630.

[00177] The circuit between 622 and 612 can be overfilled if desired or precisely controlled by MOVE microvalves to modulate flow or control of timing. Once the sample loop 630 is filled, a defined volume has been selected. The microfluidic circuit can be cleaned by flushing cleaning solutions or air or gas through the main channel to further define the reagent volume. If reagent source 621

was a compressed air or gas source (pressure and vacuum are types of reagents in a pressurized flow system), opening microvalve 601 and microvalves 606, 610, and 616 creates the circuit to move the measured reagent in sample loop 630 to device 636. In one embodiment a means to connect any number of reagent sources to a microdevice such as cartridge (1) is provided.

5 **II. Parallel Processing of Samples**

[00178] In some embodiments of the invention, one or more cartridges can be operated simultaneously to allow for parallel processing of samples. **Figure 16** illustrates parallel or ganged operation of multiple cartridges with microvalves on a single pneumatic manifold in swab extraction assembly (800). The manifold (370) distributes regulated vacuum and pressure to operate four cartridges (1), indicated in the figure, using solenoids (680). Solenoids (680) control pressure to the pneumatic layer of a microchip integrated with each cartridge through the pneumatic manifold (370, 380, 390). The pneumatic manifold is formed by a top plate (370), a gasket (380) and a bottom plate (390). The top plate can have channels etched into it. The channels can be sealed by the gasket, which is sandwiched against the top plate by the bottom plate (390). Actuator 310 moves rod 810 to move magnets (320) close to or away from the cartridges (1). Clamps 805 hold cartridges (1) in place.

[00179] In other embodiments of the invention, a single cartridge integrated with a microchip can process multiple samples at one time using parallel channels. **Figure 14** and **Figure 15** shows an assembled capture and reaction microchip with capillary feed and magnets. This microchip can capture bead solutions and perform four STR-PCR reactions simultaneously. **Figure 14** shows a microchip (1201) with a cartridge (1203) adhered to the microchip and tubes (1205, 1207, 1209, 1211, 1213, 1215, 1217, and 1219) leading into and out of the microchip. A total of eight tubes are shown and two tubes are used per parallel reaction. For example, one unit of the parallel processing device is served by tubes 1205 and 1213.

[00180] In another embodiment, a four-channel sample preparation device (Figure 66) combines a four-channel parallel reagent delivery device (Figure 67) that meters and delivers reagents simultaneously to all four channels of a single integrated cartridge (Figure 68) enabling four samples to be processed simultaneously and rapidly.

[00181] The four-channel parallel reagent delivery device combines an Chip C microchip (see **Figure 67**) with a fluidics manifold mounted on a pneumatics control manifold. Reagents are metered, using one of the two different size reagent loops, which can be similar to the sample loops described herein, for each channel, and delivered in parallel to all four channels of the sample preparation device. Delivering reagents simultaneously to all four channels of the sample preparation device using the parallel reagent delivery device can takes <4 minutes, representing a process time saving of >11 minutes as compared to the first generation serial reagent delivery device that took ~15 minutes per four samples processed.

[00182] Bonded pneumatics manifolds can be used to control both the reagent delivery and sample preparation devices by fabricating the manifolds using an adhesive bonding approach; however,

these may be prone to delamination over time due to the pneumatic pressures used in the subsystem, and the size and complexity of the manifold. Thermally bonded manifolds can mitigate delamination issues, but may only be a viable approach for relatively small and low complexity manifold designs such as the reagent delivery device. A monolithic manifold made from a single piece of polycarbonate with tubing connecting pneumatic ports to the solenoid control valves can operate the four-channel sample preparation cartridge and has proved to be a viable alternative to bonded pneumatic manifolds, see Figure 45 and Figure 46 for examples. This pneumatic manifold design concept is also being utilized for control of the Chip A microchip on the Post-amplification STR (Single Tandem Repeat) clean-up subsystem.

[00183] Assembly processes for the microchip and fluidic manifold of the four-channel sample preparation cartridge have also been improved. Historically, silicon epoxy can be used to attach the cartridge to its associated MOVE microchip by wicking the adhesive between the microchip and the cube. An inherent lack of control of the movement of the epoxy can allow it to occasionally wick into the ports on either the microchip or the cube creating a blockage in the fluidic pathway rendering the device unusable. This process has been improved by using a double-sided adhesive tape (Adhesives Research ARcare90106) to assemble the fluidic cubes and microchips; this is now the preferred assembly method used for the four-channel reagent delivery cartridge, the sample preparation device, and the post amplification device in the Post-amplification STR clean-up subsystem described below.

[00184] The integrated four-channel sample preparation cartridge with the Chip D microchip (see Figure 69) was tested. The Chip D microchip, shown in Figure 69 on the left panel, highlighted an issue with the design wherein the PDMS membrane inadvertently closed off fluidic channels adjacent to flow through valves on the MOVE microchip. Without being limited to theory or conjecture, it is thought that this effect is due to a combination of variables including minor differences in alignment during microchip assembly, the etch depth of the microchip's fluidic layer, and the pneumatic pressure used to operate the microchip on the sample preparation device. A microchip, Chip E, shown in Figure 69 right panel, was been designed to convert all flow through valves that form a T-junction between two bisecting channels in the Chip D microchip, to in-line channel that have only one channel passing through the middle of the channel. The Chip E microchip can reduce the occurrence of inadvertent channel closure during valve closure. In Figure 69, left panel, the four circles in that are positioned along the middle of the microchip can be operated independently and are each fluidically connected to a swab extraction device that can be used to extract analytes from a swab. Other ports in the microchip can connect to chambers in a cartridge (similar to the ports and chambers described for Figure 3 and Figure 4) that are mated to the microchip (shown in Figure 68) for performing reactions, such as nucleotide binding. Operation of the integrated four-channel sample preparation cartridge with microchip is similar to the operation of the device shown in Figure 3 and Figure 4.

[00185] Microchip blockages due to the inadvertent introduction of fibers into the systems and devices described herein can be problematic in microfluidics. To minimize blockages, all reagents with the exception of paramagnetic bead solutions, can be filtered prior to loading and in-line filters used to minimize microchip blockages.

5 [00186] Subsystem testing of the second-generation sample preparation device focused on characterization of system reproducibility and failure modes. A total of 80 samples were processed on the subsystem with a success rate of 63%. Failure modes included Reagents line accidentally becoming disconnected (2.5%), chip blockages (3.75%), no STR profiling observed (9%), and DNA yield <0.08 ng (22%), which is the limit of detection of the downstream system.
10 The average yield of purified DNA yield was found to be 0.26 ng. Approximately half of the samples tested in STR reactions gave full profiles and half gave partial profiles (see Figure 70). A number of blank samples were processed on the system using cleaned and recycled cartridges, and run-to-run cross contamination was found to be negligible.

III. Integrated Sample Preparation and Polymerase Chain Reaction

15 [00187] In some embodiments of the invention, a cartridge can be integrated with devices for performing polymerase chain reaction and product analysis. Such a device is shown in **Figure 6**. **Figure 6** shows a cartridge with integrated microchip (1), temperature modulating device (400), and downstream analysis device (500). In certain embodiments the device comprises a fluid preparation module comprising a cartridge mated or otherwise fluidically connected to a
20 microchip; an off-chip thermal modulation module connected to the fluid preparation module through a fluid transporter with a fluidic channel, such as a tube, through the cartridge, and configured to modulate the temperature in the fluid transporter, wherein the fluid transporter is further fluidically connected to a second microchip with valves and fluidic channels that can selectively route fluid to one or more subsequent devices. This device can be used for thermal
25 cycling or isothermal reactions.

[00188] The cartridge with integrated microchip can be formed of any cartridge and microchip described herein. For example, the cartridge and microchip shown in **Figure 3**, **Figure 4**, and **Figure 5**. A movable magnet (300) can be positioned adjacent to the cartridge. The movable magnet can be moved by an actuator (310). The movable magnet can be used to apply a magnetic field within
30 the cartridge or the microchip. In some embodiments, the movable magnet can be used to facilitate gathering or collecting of beads against a wall of a chamber within the cartridge or the microchip.

[00189] A temperature modulator can be fluidically connected to the cartridge and microchip through reaction channel (250). The reaction chamber (250) can be connected at an end (251) to the
35 cartridge. The temperature modulator can be used for thermal cycling the temperature of a reaction channel (250) containing a reaction mixture and a nucleic acid enriched from a sample (collectively referred to as the PCR reaction sample). A control mechanism can be used for controlling the operation of the temperature modulator. An optical assembly can be used to

monitor or control the reaction. The optical assembly can introduce or detect light. For example, an optical assembly 410 can be used for performing Real-time PCR or other real-time or end point measurements. In certain embodiments the temperature modulator employs a thermocoupled Peltier thermoelectric module, a conventional thermoelectric module, hot air, infrared
5 light or microwave. In one embodiment the temperature modulator uses a Peltier thermoelectric module external to the reaction channel to heat and cool the PCR reaction sample as desired. The heating and cooling of the thermoelectric module can be distributed over a region 350.

Additional views of the temperature modulator 400 are shown in **Figure 7** and **Figure 8**. **Figure 7** shows the reaction channel 250 in contact with a temperature controlled region 350. The
10 temperature modulator can also include a movable magnet 320 that is positioned by an actuator 330. The movable magnet can be used to capture magnetic particles at position 340, as shown in **Figure 6**. In some embodiments of the invention, the temperature controlled region comprises two parts. The two parts can be parts of a clamshell that are clamped, locked, or held together to maintain thermal contact with the reaction channel 250. One portion of the temperature
15 controlled region, portion 711 of **Figure 8**, can be hinged to the second portion of the temperature controlled region. The temperature controlled regions can have grooved channels for positioning of one or more reaction channels, as shown on the right side of **Figure 7** and in **Figure 8**. The left side of **Figure 7** shows the temperature controlled region in a closed configuration.

Additionally, the temperature controlled region can comprise one or more constriction
20 components, shown as 709 and 701 in **Figure 8**. The constricting points can pinch the reaction channel such that a portion of the reaction channel is isolated from another portion of the reaction channel. In some embodiments of the invention, the reaction channel is pinched in two locations such that a body of fluid, such as a reaction mixture, is isolated. Constriction components 709 and 701 can mate with additional constriction components 707 and 705 to facilitate pinching of
25 the reaction channel.

[00190] Alternatively the temperature modulator can constrict the reaction tubing using an pinch clamp, as shown in Figure 65. Use of the pinch clamp, which can be formed of a plastic such as Ultem, can reduce heat transfer to the reaction channel. The reduction in heat transfer can reduce the likelihood that the reaction channel has for being welded closed during thermocycling or
30 temperature regulation. Alternatively, different material tubing can be used as the reaction channel to ensure that the reaction channel can maintains its shape before and after the thermocycling or temperature regulation process. Different material tubing can also be used to reduce rate of evaporation during the temperature modulating process. Example materials include ethylvinyl acetate, silicone, and silanized c-flex tubing.

[00191] The temperature modulating device can modulate temperatures at a rate of 0.5 to over 3 degrees Celsius per second. The heater can utilize about 25 to 100 Watts and a fan, which can be used to cool the temperature modulating device, can produce an air flow rate of at least about 75, 100, 130, 150, 200, 250, or 300 cfm.

[00192] In one embodiment a sample preparation device comprising a cartridge integrated with a microfluidic microchip 1, which can be used to control the movement of fluid in the cartridge, can be used in conjunction with a temperature modulator 400 as a flow-through PCR thermal cycler. Driving force for moving the fluid can be an external pressure source or an internal pressure source, such as a MOVE valves within the microchip. A flow-through PCR thermal cycler can be used when highly sensitive or high throughput PCR is desired. There are many situations in which one might want to sample air, blood, water, saliva, a cellular sample, or other medium in a sensitive PCR assay. This can be used to look for a variety of biological contaminants including influenza, bacterial pathogens, and any number of viral or bacterial pathogens. Flow-through PCR can allow PCR to be practiced in an automated manner without the need for human interaction. A flow-through PCR system can also serve as an early warning system in HVAC systems of buildings, airplanes, busses, and other vehicles, and can be used in the monitoring of blood, water, or other sample sources for the presence of an infectious agent or a contaminant.

[00193] As shown in **Figure 6**, the flow-through PCR device takes a sample from a collection device, such as a buccal swab, a syringe, an air sampler, fluid sampler or other sampler and delivers it to a sample preparation device 1. **Figure 6** is not necessarily drawn to scale. The sample is prepared in the preparation device 1, which in some embodiments may include cell lysis, DNA, RNA, or micro RNA enrichment or purification, filtration, or reverse transcription. In one embodiment at least one nucleic acid is enriched. In another embodiment at least one enriched nucleic acid is prepared for PCR by adding the nucleic acid to PCR reagents (such as at least one DNA polymerase, RNA polymerase, dNTPs, buffer or a salt) and primers, (such as assay-specific primers or broadly applicable primer sets for multiple target pathogens). These primers may be chosen to selectively amplify at least one nucleic acid isolated from a specific pathogen (such as a mold, virus, bacteria, parasite or amoeba), gene, other desired nucleic acid, or any combination thereof. The composition comprising at least one nucleic acid enriched from a sample, PCR reagents and primers is called a PCR reaction sample. In one embodiment, the flowthrough PCR can be used as a continuous flow device while in other embodiments samples are moved into the thermal cycling region and stopped.

[00194] The PCR reaction sample then flows through a reaction channel (250) to a temperature controlled device or region (350). In some embodiments the reaction channel is clear or transparent. In another embodiment the reaction channel is opaque. In one embodiment the reaction channel is a cylinder. In another embodiment the reaction channel's cross section comprises one or more planes forming a shape such as a triangle, square, rectangle, pentagon, hexagon, heptagon, octagon, nonagon, decagon, or other polygon. In one embodiment the volume of PCR reaction sample is such that it takes up a small discrete length of space in the reaction channel, the rest of which is occupied by air, gas, or a non-reactive liquid, such as mineral oil. Air, gas, or a non-reactive liquid can be used to separate individual PCR reaction samples from each other. In one

embodiment the temperature controlled region (350) is thermally modulated by one or more modules, including but not limited to thermo-coupled Peltier thermoelectric module, a conventional thermoelectric module, hot air, microwave, or infrared light. In one embodiment the thermal cycler uses Peltier thermoelectric modules external to the tube to heat and cool the sample as desired. In one embodiment a detection module (410) measures fluorescence, luminescence, absorbance or other optical properties to detect a signal emitted from a PCR reaction sample while it is located with a temperature control region, or after it has left a temperature control region. A detection module can comprise a light source (such as a coherent light source or incoherent light source) used to excite a fluorescent dye (such as an intercalating dye, including but not limited to ethidium bromide or Syber green) in a PCR reaction sample, and the excitation light is sensed with a photodetector (such as a CCD, CMOS, PMT, or other optical detector). Detection electronics can evaluate the signal sent from the detection module (410).

[00195] In one embodiment, after the desired number of thermal cycles are complete, the PCR reaction sample is pumped or pushed further down the reaction channel, using pressure or vacuum, exiting the temperature controlled region and passing into a second microfluidic microchip (500). The second microchip (500) can be attached at end (252) to the reaction channel (250). Microfluidic microchip (500) can comprise microvalves (510, 520, 530, and 545). Any three microvalves such as 510, 520, and 530 or 510, 520, and 545 can form a pump. Microchannels 505, 515, 525, and 540 can connect the pumps on the microchip. Downstream devices 535 and 550 can be connected to the microchip. Flow of material to devices (535 and 550) can be controlled by the microvalves, for example, by keeping either valve 530 or 545 closed while pumping or moving fluid. In one preferred embodiment, the downstream device are analytical devices that can be used for performing electrophoresis, mass spectroscopy, or other analytical techniques known to one skilled in the art.

[00196] In one embodiment the second microfluidic microchip can deliver the PCR reaction sample to a module or region for further processing or analysis. In another embodiment multiple reaction channels may be used in parallel to increase sample throughput. In yet another embodiment the system may alert the user when amplification has occurred (a positive result), indicating that the target sequence is present. In one embodiment a reaction channel is used for a single use only, then disposed of. In an alternative embodiment a reaction channels can be used to amplify and detect the presence or absence of PCR amplification products in multiple samples. More than one PCR reaction samples can be loaded at intervals and interspaced with a barrier bolus of gas or liquid to prevent intermixing. In one embodiment samples are spaced apart in a manner so that as one is undergoing thermal cycling another sample is in the detection region undergoing interrogation. It will be obvious to one skilled in the art that the PCR amplification can be replaced by other nucleic acid amplification technologies which may use thermal cycling or be isothermal reactions.

[00197] In other embodiments, the device can perform isothermal reactions such as sandwich assays using affinity reagents such as antibodies or aptamers to determine if cells, proteins, toxins, or other targets are present with the detection module (410) providing a reading of the amount of target present. In these applications, the cartridge 1 may perform an affinity purification such as an IMS
5 purification and then add a secondary antibody that may have a fluorescent label attached. The sample can then move into region 350 where the thermal control is set to optimize the reaction. Detection module (410) can then monitor the reaction. In one embodiment, a plurality of cartridges are ganged to reaction channel (250) and a series of boluses can be readout with detector 410.

10 **IV. Device for Capillary Electrophoresis**

[00198] In one embodiment a complete sample-to-answer system is used, which can comprises microfluidics, requiring coupling all steps together to match volumes and concentrations. Sample analysis using capillary electrophoresis is a standard analytical method that can be used with microfluidic sample preparation methods as described above. Capillary electrophoresis is readily
15 adaptable to microfluidic microchips. In the instant invention, capillary electrophoresis on microchips is combined with MOVE valves to provide control of samples, process beads to concentrate the samples, and improve the loading and separations.

[00199] In one embodiment the Twin-T injection system is used in the design of the microfluidic injector for separations. In an alternative embodiment a design is used for the Forked Cathode injector
20 (**Figure 30**). The layout is similar to the Twin-T in that the sample plug is described by a section of channel adjacent to the separation channel but key differences exist. First, the cathode channel is divided into two parts, this splits the injection electrically into two parts and thus doubles the quantity of material injected for a given sample plug dimension. Second, the sample channel and separation channel are at right angles to one another. This allows the sample channel to be
25 straight and filled with buffer (rather than separation polymer), which facilitates manipulating the contents of this channel with pumps and fluid flows, and allows the separation polymer interface to be sharp. Lastly, the injector can be run in a mode that allows Field Amplified Sample Stacking (FASS).

[00200] **Figure 30** shows an example of a forked cathode injector that utilizes microchannels as the
30 forked cathode. As shown in **Figure 30**, a sample is moved electrokinetically across a sample loading channel (shown in the drawing on the lower left with the arrow through it). Then the sample is driven into the separation channel (the vertical channel) by applying a field between it and the cathode arms (the two channel dropping down) while pull back is applied to the sample and waste. The initial sample plug dimension is defined by the distance between the cathode
35 arms. The configuration of the channels allows for a more reproducible plug and better integration with MOVE microfluidic systems.

[00201] In an aspect of the invention shown in **Figure 30**, fluidic channel 3003 is in electrical contact with forked electrodes 3001 and 3002. The points of contact of the electrodes with the channel

are spaced apart, thereby creating a segment in the channel in which there is an electric field. Separation channel 3004 intersects fluidic channel 3003 at a point in the segment between the points of contact of the forked electrodes. Another electrode of opposite charge is put in electrical contact with the separation channel. In this way, a voltage is applied through the separation channel.

[00202] Figure 62 show a sample source 6009 connected to a sample channel 6005, also referred to as a loading channel, that is mated with a separation channel 6011. Two electrodes, 6003 and 6001, can be used to apply an electric field to the separation channel. In some embodiments of the invention, the sample source can pass through a MOVE pump in a microchip used to drive fluid flow within the sample channel. The sample channel can be a microfluidic channel or an injection tubing. The injection tubing can be flexible tubing or another flexible connector. Examples of flexible tubing include polytetrafluoroethylene tubing or silicon tubing. The flexible connector can also connect to another cartridge interfaced with a microchip. Alternatively, the flexible connector can return to the cartridge that it originated from. The separation channel can be a microfluidic channel, capillary tubing, or capillary electrophoresis tubing. The capillary tubing can have an outer diameter of about 150 to 500 microns and an inner diameter of about 10 to 100 microns. The capillary can be polyimide or polytetrafluoroethylene clad. The capillary can be about 2 to 100 cm long. The capillary can be mated to the injection tubing or flexible tubing by first drilling a hole into the injection tubing and then inserting the capillary into the flexible tubing. Alternatively, the capillary can be inserted into the flexible tubing without having to pre-drill the flexible tubing.

[00203] One of the two electrodes, for example electrode 6003, can be a cathode and the other electrode, for example 6001, can be an anode. The cathode can be any cathode, such as a forked cathode, described herein. The anode can be connected to the separation channel using any devices known to those skilled in the art. For example, the separation channel can be joined to a reservoir by an Upchurch fitting, which is in electrical contact with the anode, which can be a metallic electrode.

[00204] In some embodiments of the invention, a stabilizing component, shown at the intersection of a separation capillary and injection tubing in Figure 63, can be used to align, seal, and/or protect the connection between the separation capillary and the injection tubing. In some embodiments of the invention, multiple injection tubings are aligned with multiple separation capillaries using a stabilizing component. As shown in Figure 64, the stabilizing component can hold four injection tubings, shown as the vertical tubings in the figure, and stabilize the connection with four separation capillaries (not shown).

[00205] Panels 1-6 of Figure 62 show a process for injecting a sample into a separation channel. In panel 1, no sample is present in the sample channel 6005. In panel 2, sample entering the sample channel from the sample source (6009) is shown. As sample is moved down the sample channel, the sample intersects the separation capillary, as shown in panel 3. The sample can be isolated by boluses of gas upstream and downstream to the sample. Once sample is adjacent to the

separation channel, an electric field, which can be between 25 and 500 V/cm, is applied between a first electrode 6003, which can be a cathode or a forked cathode, and a second electrode 6001, which can be an anode. Electrophoresis buffer, shown entering into the sample channel from the sample source, can also enter the sample channel, as shown in panel 3. The voltage potential and/or current between the anode and cathode can drop when an air bolus passes by the junction between the sample channel and the separation channel, reducing or preventing the injection of air into the separation channel. The voltage potential and/or current drop can be detected to ascertain when the sample and/or electrophoresis buffer is adjacent to the separation channel. Once the electrophoresis buffer is adjacent to the separation channel, as shown in panel 5, the current and/or voltage drop between the anode and cathode can be increased. This can allow for the separation of the analyte in the separation channel, as shown in panel 6, as the electrophoresis buffer provides ions for a high performance separation.

[00206] FASS is a chromatographic technique that uses the increase in the electric field caused by areas of low conductivity to increase analyte mobility in the sample area and concentrate the analyte at an interface of an area of lower mobility, i.e., at the separation matrix. The net effect of running the injector in this manner can be seen in **Figure 31**. Significant decrease of the sample plug length, herein referred to as stacking, can be observed.

[00207] The injector is filled with buffer (dashed line) then the separation polymer is loaded (solid line) while the interface is swept. The sample channel (horizontal channel) is filled with sample reaction products in low ionic strength media. This allows sample stacking and significantly decreases the sample injection plug size. This is shown in the five frames on the right of **Figure 31** versus the all polymer injection in the left panel. The effect of ionic strength and stacking is seen in the images from second left to right as the buffer dilution increases and the ionic strength decreases. The sample plug narrows from approximately 300 microns to less than 100 microns.

[00208] In one embodiment for STR analysis the injection process is as follows:

[00209] The microfluidic channels can be filled with buffer.

[00210] The separation channel can be filled with gel while buffer is pulled across the sample channel, thus sweeping the separation polymer from the cross section formed by the separation and sample channels.

[00211] The STR amplified sample (desalted and captured on beads) can be captured on microchip 500, eluted in a low conductivity fluid (water) containing the size standard, and pumped into the sample channel with MOVE technology.

[00212] A field can be applied across the cathode and anode, with "pull back" voltage on the sample and waste arms, to drive the sample into the separation channel where it stacks at the head of the separation polymer.

[00213] As the sample is injected the conductivity of the sample channel can quickly equilibrate with the buffer in the cathode arms providing a single step injection.

[00214] The MOVE controlled Forked Cathode injector design (**Figure 32**) can be optimized for DNA separations in microchip channels. In addition to the FASS described above, the unique integrated injector design also incorporates the MOVE pumping system which facilitates the use of magnetic bead technology to desalt and concentrate samples.

5 [00215] Purified STR amplification products are eluted from magnetic beads, heat denatured and pumped through the loading channel of the Forked Cathode injector. A voltage regime is applied to facilitate an FASS injection at the head of the polymer column, and DNA separation is performed in a polymer filled micro-channel (**Figure 33**). In **Figure 33**, the photos show the movement of dye in the injector in order to illustrate the STR sample injection mechanism. Field amplified
10 stacking occurs at the polymer head when injection is initiated.

[00216] Alternatively, the forked electrode or cathode can be two metallic conductors, as shown in **Figure 60**. The fluid path for a sample to be analyzed, as shown in **Figure 60**, can be along a loading channel. When the location of the sample is adjacent to the separation channel, the forked electrode can be used to inject the sample into the separation channel, as described herein. The
15 conductance of the material in the sample channel can be lower than the conductance of the material in the separation channel, which can be a separation polymer. The difference in conductance can cause sample stacking when an electric field is applied through the forked electrode, which can be a cathode, and a downstream electrode, which can be an anode. The polarity of the forked electrode and the downstream electrode can be reversed such that the
20 forked cathode is the anode and the downstream electrode is the cathode.

[00217] In some embodiments of the invention, an additional electrode can be used to reduce injection of gas into the separation channel or formation of bubbles within the sample loading channel which can lead to loss of the applied field on the separation channel. Injection of gas into the separation channel or formation of bubbles within the sample loading channel can cause inconsistent
25 separation of analytes and can be detected by inconsistent current between the anode and cathode used to apply an electric field to the separation channel. Use of an additional electrode to circumvent or reduce injection of gas or bubbles into the separation channel is shown in **Figure 61**. The additional electrode can be a single wire run electrode or a cannular run electrode. The increased surface area and/or larger internal diameter of the cannular run electrode can allow for a
30 significant reduction in bubble formation or blockage and/or injection into the separation channel. In some embodiments of the invention, the cannula used for the cannular run electrode and has an inner diameter of at least about 1/64, 1/32, 1/16, 1/8, or 1/4 inches.

V. mRNA amplification

35 [00218] The devices of the invention can be utilized for microarray sample preparation processes. Gene expression microarrays monitor cellular messenger RNA (mRNA) levels. However, mRNA can constitute only 1-3% of cellular total RNA. The vast majority of cellular RNA is ribosomal RNA (rRNA), and these molecules may interfere with mRNA analysis by competing with mRNA for

hybridization to microarray probes. Any mRNA amplification method can be performed by the devices described herein, for example LAMP, TLAD (Eberwine), and MDA. In some embodiments of the invention, isothermal mRNA amplification methods can be performed using the devices described herein. In other embodiments, thermal cycling can be performed to accomplish PCR or cycle sequencing.

[00219] The Eberwine mRNA amplification procedure specifically targets polyadenylated mRNA (polyA+ mRNA) for amplification, virtually eliminating rRNA interference. This characteristic removes any need to pre-purify mRNA from total RNA, which can be an inefficient, time-consuming, and expensive process. In addition, by greatly increasing the amount of target RNA (that is, amplified mRNA or aRNA) available for microarray hybridization, mRNA amplification can allow much smaller samples (fewer numbers of cells) to be analyzed. This can be helpful because the relatively large amount of target RNA required for microarray analysis (typically 15 ug) is frequently difficult to obtain. Moreover, it can be essential in many important clinical diagnostic applications analyzing samples containing few cells, for example, samples derived from fine needle aspirates (FNA) or laser capture microdissection (LCM).

[00220] Any process that alters relative mRNA abundance levels may potentially interfere with accurate gene expression profiling. An important aspect of the Eberwine amplification procedure is that it employs a linear amplification reaction that can be less prone to bias mRNA populations than exponential amplification methods such as PCR.

[00221] The original Eberwine protocol has been streamlined and simplified by commercial vendors such as Ambion. As shown in **Figure 44**, the Ambion procedure comprises three binary (two component) additions followed by an RNA purification process. Each binary addition can be followed by incubation(s) at specific temperatures, as indicated in **Figure 44**. The initial reverse transcription (RT) reaction can have three inputs (primer, total RNA, and reverse transcriptase [RT] Mix); however, total RNA and primer can conveniently be premixed. Typical volumes for this first reaction can be 5 ul RNA+Primer 5 ul RT Mix. Only mRNA hybridizes to the oligo dT primer and is transcribed into DNA. The second-strand reaction can be initiated by addition of 20 ul of a Second-Strand Mix, and the final T7 amplification reaction can be initiated by addition of 30 ul of a T7 Mix. Synthesized RNA can be labeled at this stage by incorporation of biotin-labeled ribonucleotides. Mixes contain buffers (Tris), monovalent and divalent salts (KCl, NaCl, MgCl₂), nucleotides, and DTT, along with enzymes as indicated. Typically, enzymes can be premixed with concentrated mixes just prior to use.

[00222] After synthesis, aRNA can be purified to remove enzymes, buffers, salts, unincorporated nucleotides, pyrophosphate, etc. Purification typically relies on commercial kits exploiting the association of aRNA with silica membranes or beads in the presence of chaotropic salts such as guanidinium hydrochloride (GuHCl) or thiocyanate (GuSCN). After binding, the silica is washed with 70% ethanol (EtOH), dried, and aRNA is eluted with water.

[00223] Each of these steps can be carried out on the devices described herein (See U.S. Provisional Patent Application No. 61/140,602). For example, reagents and sample can be supplied through ports in the cartridge and then delivered to the microfluidic microchip. The on-chip valves can be used to pump the reagents and samples to chambers and reservoirs in the cartridge and the microfluidic microchip through channels. Temperature control can be accomplished using internal or external heating and cooling devices. The reaction products can be moved to product outlet ports of the cartridge for further handling. Alternatively, the reaction products can be purified or separated using the devices of the invention.

VI. Separation and Cleanup

[00224] A variety of separations can be performed using the devices described herein. These separations include chromatographic, affinity, electrostatic, hydrophobic, ion-exchange, magnetic, drag-based, and density-based separations. In some embodiments of the invention, affinity or ion-exchange interactions are utilized to bind materials to solid-phase materials, such as beads. The beads can be separated from fluid solutions using any method known to those skilled in the art.

[00225] Magnetic separation can be used to capture and concentrate materials in a single step using a mechanistically simplified format that employs paramagnetic beads and a magnetic field. The beads can be used to capture, concentrate, and then purify specific target antigens, proteins, carbohydrates, toxins, nucleic acids, cells, viruses, and spores. The beads can have a specific affinity reagent, typically an antibody, aptamer, or DNA that binds to a target. Alternatively electrostatic or ion-pairing or salt-bridge interactions can bind to a target. The beads can be paramagnetic beads that are only magnetic in the presence of an external magnetic field. Alternatively, the beads can contain permanent magnets. The beads can be added to complex samples such as aerosols, liquids, bodily fluids, extracts, or food. After (or before) binding of a target material, such as DNA, the bead can be captured by application of a magnetic field. Unbound or loosely bound material is removed by washing with compatible buffers, which purifies the target from other, unwanted materials in the original sample. Beads can be small (nm to μm) and can bind high amounts of target. When the beads are concentrated by magnetic force they can form bead beds of just nL- μL volumes, thus concentrating the target at the same time it is purified. The purified and concentrated targets can be conveniently transported, denatured, lysed or analyzed while on-bead, or eluted off the bead for further sample preparation, or analysis.

[00226] Separations are widely used for many applications including the detection of microorganisms in food, bodily fluids, and other matrices. Paramagnetic beads can be mixed and manipulated easily, and are adaptable to microscale and microfluidic applications. This technology provides an excellent solution to the macroscale-to-microscale interface: beads can purify samples at the macroscale and then concentrate to the nanoscale (100's of nL) for introduction into microfluidic or nanofluidic platforms. Magnetic separations can be used as an upstream purification step before real-time PCR, electrochemiluminescence, magnetic force discrimination,

magnetophoretic, capillary electrophoresis, field-flow separations, or other separation methods well known to one skilled in the art.

[00227] The devices of the invention can accommodate the use of magnetic beads. For example, beads or bead slurry can be supplied to a port of a cartridge. The beads can be mixed or suspended in solution within the cartridge using pumping, magnetic fields, or external mixers. The beads can then be pumped to desired chambers or reservoirs within the microfluidic device or cartridge. Beads can be captured within a chamber using a magnetic field. Beads in a solution can be captured as the solution travels through the magnetic field, or beads can be captured in a stagnant solution.

[00228] To illustrate methods of use of the cartridge, several examples are described below. The first example describes processing of nucleic acid from a buccal swab with paramagnetic beads to purify the sample followed by PCR amplification and bead purification of the PCR products. A second example describes performing immunomagnetic separations to purify cells, proteins, or other antigenic material using a binding moiety coupled to beads. A third example describes performing molecular biology to prepare samples for sequencing technologies such as sequencing by synthesis, sequencing by hybridization, or sequencing by ligation. It would be known to one skilled in the art that many different chemistries and biochemistries can be used with the instant invention. These include, but are not limited to, enzymatic reactions, purifications on gels, monoliths, beads, packed beds, surface reactions, molecular biology, and other chemical and biochemical reactions.

EXAMPLES

Example 1: Operation of a Cartridge for Nucleic Acid Purification

[00229] This example refers to the use of a device comprising a cartridge mated to a microchip. The numbers refer to the cartridge of **Figure 3** and **Figure 4** mated to a microchip with the circuit architecture of **Figure 5**. This sub-assembly also can be fluidically connected other sub-assemblies in the instrument of **Figure 6**. For reference, a cartridge mated with a microchip also is shown in **Figure 40** and **Figure 59**.

[00230] Nucleic acids can be purified from a wide variety of matrices for many purposes including, but not limited to, genotyping, identification, forensics, gene expression, gene modification, microRNA analysis, ribotyping, diagnostics, or therapeutics. The input sample can be a solid, swab, liquid, slurry, aerosol or a gas.

[00231] For molecular diagnostics and forensics, swabs are commonly used. A buccal swab can be taken using a swab with an ejectable tip and the swab ejected into a syringe attached to connection 7 of **Figure 4**. Connection 5 of **Figure 4** leads by tubing or capillary to a reagent manifold that can select a single reagent from multiple reagents by opening a full scale valve or by opening a MOVE valve with the reagents either under pressure or moved by vacuum. MOVE or other micropumps on microchip 2 of **Figure 4** can also move the fluids or gases.

[00232] In one embodiment, human and other cells in a swab are first lysed using a buffer with a heated chaotropic agent and/or other commercial-off-the shelf (COTS) chemistries in a syringe inserted into port 7. The lysate is transported to a DNA isolation chamber (**Figure 4 #3**) where paramagnetic beads have been added from a reservoir to adsorb nucleic acids onto the beads. A moveable magnet is then actuated to capture the beads onto the side of the isolation chamber where they are washed automatically using a buffer. The purified DNA, still bound to beads, is then pumped through a small diameter tube 250 where multiplexed PCR is performed. Pre-scripted DevLink™ software automates the complete process. The DevLink software defines a set of communication and command protocols in a standardized automation architecture that is simpler, more flexible, and quicker to implement than other software development approaches. The DevLink implementation framework is based on core technologies that span multiple operating systems, development languages, and communication protocols. Software drivers wrap individual smart components of the system, greatly reducing the time needed for typical de novo system software development. This makes it relatively straightforward to integrate the operation of multiple system modules (pumps, valves, temperature controllers, I/O controllers, etc.) that are either COM- or .NET-based. DevLink provides a professional quality software development system for prototyping through product release and maintenance.

[00233] While DNA amplification is useful for positive identification of microorganisms, samples can be obtained from a wide variety of substrates and matrices that contain compounds that are inhibitory to DNA amplification reactions. Raw samples are most often complex mixtures that can include inhibitors such as hemes, metal ions, humic and fulvic acids, chelators, DNases, proteases, and molds. While the initial isolation of target organisms and toxins from the sample matrix by IMS should remove most of these inhibitors, lysed cell components and lysis agents can also need to be removed or diluted from nucleic acid samples so that they do not interfere with successful amplification.

[00234] In one embodiment, a small volume nucleic acid purification is used. These purification methods can be used with a wide range of samples, such as blood, to aerosols, to buccal swabs. Paramagnetic beads can be used in a disclosed device to purify DNA from various sample sources. In one embodiment a microfluidic microchip can be used to sequence a nucleic acid using magnetic beads and reagents to purify nucleic acid products for sequencing in microscale reactions. In one embodiment, the microfluidic microchip is a 24-channel microfluidic microchip.

[00235] In one embodiment, polyethylene glycol (PEG)-based nucleic acid purification is used on carboxylated magnetic beads. This PEG-facilitated process can produce yields of over 80% from upstream immunomagnetic separations (IMS) captured samples. Development of a universal sample preparation module (USPM) can partly involve porting the PEG-based nucleic acid purification onto a device containing a cartridge such as the devices shown in **Figure 21** or

Figure 16. In another embodiment, Agencourt Orapure or Promega DNA IQ chemistries are used in conjunction with a device of the present invention.

[00236] Bead dispensation and delivery.

[00237] To purify nucleic acids, paramagnetic beads with different surface chemistries can be mixed in a reagent container. Pressure is then applied to send the reagents to connection 5. MOVE microvalves or other valves may be closed unless referred to as open. To move the paramagnetic beads into the reaction chamber (3), microvalves 180 and 150 are opened. The beads are moved through connection 5 into channel 15 which leads to junction 190 and microchannel 191. Because microvalves 180 and 150 are open and microvalves 200 and 170, and the other microvalves, are closed, an open microfluidic connection is from microchannel 191 through microvalve 180 to microchannel 181 through microchip 152 to open microvalve 150 and microchip 151 to junction 120. Junction 120 leads to cone 13 and chamber 3, which can be filled with beads. The volume of beads supplied to chamber 3 can be controlled by timing the opening of the reagent valves and the microvalves or by filling and emptying a sample loop connected to the microchip or the cartridge.

[00238] Commercial bead based chemistries can be used in the disclosed system, including but not limited to Orapure from Agencourt (Waltham MA) and DNA IQ from Promega (Madison, WI). Orapure uses a carboxylated bead surface and SPRI chemistry while DNA IQ is an example of a silica bead and chaotrophic chemistry. Other embodiments of paramagnetic beads or chemistries to process nucleic acids can be used in conjunction with the disclosed system, including but not limited to beads with oligonucleotides, locked nucleic acids, degenerate bases, synthetic bases, conformation, nucleic acid structures, or other hybridization and specific capture methods.

[00239] Filling chamber (3) with beads.

[00240] For Orapure or DNA IQ beads, 450 microliters can be moved into chamber (3) using three fills of a 150 microliter sample loop 630 or 631. A movable magnet 300 attached to actuator 310 can then be moved towards cartridge (1) near the side of 3 to pull the beads to the side of chamber (3). Magnet size and orientation can be adjusted to generate magnetic fields appropriate to specific applications. Pressurized air can then be applied through the reagent manifold with microvalve 180, 150, and 110 open. The opening of microvalve 110 connects from junction 190 which connects to the reagent manifold through junction 120 and microchannels 121 and 101 to connection 100 which leads through channel 14 to connection (4) and to waste. The air can move any remaining liquid through the circuit. Air or other gases can also be used to dry beads, volatilize solvents, or for bubble-enabled mixing (described herein).

[00241] Bubbling of gas through chamber (3).

[00242] If microvalves 180, 150, and 220 are open, and all other microvalves closed, the pressure can force air through chamber (3) to channel 9 and down channel 19 to junction 210 through

microchannels 211 and 221, through open microvalve 220 and microchannel 231 to junction 230, through channel 16 to connection 6 which can be a vent. This sequence can bubble air or other gases through chamber (3) and can be used to mix reactions in chamber (3) or to change the gas phase.

5 **[00243] Moving liquids and beads from chamber (3) to waste.**

[00244] Liquids and beads can be moved from reaction chamber (3) or any other location to waste. This can be used to wash beads, flush channels, move liquids or beads to waste. When pressure is applied to connection 6 with microvalves 220 and 110 open, and all other microvalves closed, the pressure can force air through channel 16 to junction 230 to microchannel 231, through open
10 microvalve 220 and microchannels 222 and 221, through junction 210, and channels 19 and 9 into reaction chamber (3) and through junction 120 through microchannel 121, open microvalve 110, microchannel 101, channel 14 and to connection 4.

[00245] The equivalent effect can be obtained by applying vacuum to connection (4) if connection 6 is a vent without any additional control of air pressure. The air pressure or vacuum can move any
15 liquids in chamber (3) to the waste connection 4. When magnet 300 is close to chamber (3), paramagnetic beads can remain on the side of chamber (3) and the result is that the liquid is removed. When magnet 300 is far enough from chamber (3), paramagnetic beads can not remain on the side of chamber (3) and the result is that the liquid and beads are removed.

[00246] To clean paramagnetic beads, the beads are pulled to the side of chamber (3) with magnet 300
20 (see **Figure 6**) and the liquid removed to waste. 450 microliters of buffer can be dispensed from the reagent manifold and added to chamber (3) by opening microvalves 180 and 150. The beads can be released if desired and then recaptured by moving the magnet 300 and the liquid then removed. This is repeated for a total of three times to produce beads ready to process samples.

[00247] Lysis and extraction of nucleic acids from cells on the swab.

25 [00248] A swab can be loaded into a syringe barrel inserted into connection 7 and then be lysed by addition of lysis buffer through reagent connection 5 with microvalves 180 and 170 opened. In some embodiments Orapure or DNA IQ chemistries are used.

[00249] Movement of the lysed cellular material to chamber (3) and mixing with beads.

[00250] The material in the syringe connected to connection 7 can be moved into chamber (3) by applying
30 pressure to the syringe or by applying vacuum to vent 6. When vacuum is used, microvalves 170, 150, and 220 are opened. The vacuum connects through microchannels 231, 221, 211, and channels 9 and 19 through chamber (3), microchannels 151, 152, 171, and 161 to pull material from connection 7 into chamber (3). When paramagnetic beads are loaded and cleaned in chamber (3), the lysed sample material mixes with the beads in chamber (3) with the magnet is
35 the far position.

[00251] Purification of nucleic acids on the beads.

[00252] The paramagnetic beads are then incubated with the lysed sample. Continued air or gas flow can aid mixing. The magnet 300 is then moved to the closed position and the beads are captured on

the wall of chamber (3). The sample lysate can then be removed from chamber (3) to waste and multiple volumes of wash solution added according to manufacturers' specifications for the Orapure chemistry or DNA IQ chemistry. The sample components on the beads have now been purified and are ready for reactions in the cartridge or exporting to the sample product connection. In one embodiment the beads are used to enrich a nucleic acid component from a sample.

[00253] Exporting samples through the sample product connection 8.

[00254] The purified sample components on the beads can be moved to connection 8 by applying pressures on reagent connection 5 with microvalves 180, 150, and 130 open. In one embodiment, connection 8 is connected with reaction channel 250 such as C-flex tubing (Cole Parmer) and additional reactions are performed in the reaction channel.

[00255] Multiplexed PCR amplification of STR markers.

[00256] DNA amplification can be performed by PCR amplification. The present invention enables PCR reactions as well as many other DNA amplification and modification reactions. The reactions can be performed in chamber (3), in reaction channel 250 attached to connection 8 which can be a tube 250 (**Figure 3, Figure 4, Figure 6**), or in another device or microdevice connected to tube 250. This demonstrates the utility of the sample preparation for DNA reactions including thermal cycling.

[00257] Capture of nucleic acid containing beads in a reaction channel.

[00258] The purified DNA output through the sample product connection 8 is moved into a reaction channel 250 at end 251 by applied pressure or alternatively through vacuum applied to end 252. An actuator 330 moves a magnet 320 under software control into a position close to bead capture region 340. Fixed magnets of different sizes and shapes (such as rare earth magnets) as well as electromagnets or superconducting magnets can be used. As the solution containing the beads moves through region 340, the magnetic field attracts the beads to the side of the reaction channel and holds them in place. The fluid is then followed by air pressure through reagent connection 5 leaving the beads region 340 in air.

[00259] Addition of reagents and movement of samples into reaction region.

[00260] Reagents can be added from the reagent manifold as described. In one embodiment, reagents are added from end 252 of reaction channel 250. End 252 is attached to a microfluidic microchip 500 comprising microvalves 510, 520, 530, and 540. Any three microvalves such as 510, 520, and 530 or 510, 520, and 540 can form a pump. Microvalve 530 connects through a microchannel to a downstream device 535, which can connect to tubing leading to a reagent reservoir. Microvalve 540 connects through a microchannel to downstream device 545, which can connect to tubing that leads to a reagent reservoir.

[00261] Reaction mixes (such as at least one DNA polymerase, dNTPs, buffer and a salt) including but not limited to master mixes and primers, (such as assay-specific primers or broadly applicable primer sets for multiple target pathogens), or complete PCR master mixes such as PowerPlex 16

from Promega (Madison, WI) or IdentiFiler or MiniFiler from Applied Biosystems (Foster City, CA) in reagent reservoir 600 can be delivered by a micropump formed by microvalves 530, 520, and 510 through tubing 610 and microchannels 531, 521, 511, and 512, into end 252 of reaction channel 250, as shown in Figure 6. MOVE microvalves can precisely position fluids and move the fluid to region 340 where the reaction mix encounters the beads comprising nucleic acids. Magnet 320 is moved away from reaction channel 250 by actuator 330 which releases the beads from the inner surface of the reaction channel 250. The MOVE microvalves on microchip 500 pump the beads into device 400 with an area of reaction channel 250 forming temperature controlled region 350. The region 350 can be held at isothermal temperatures or thermal cycled or other varied as is well known to one skilled in the art. The region 350 can be a temperature modulator or thermally coupled to a temperature modulator.

[00262] Figure 7 shows a temperature control device 400 that is capable of thermal modulation using a temperature modulator for heating and cooling to thermocycle the reaction channel. In one embodiment the temperature modulator comprises a Peltier module, infra-red module, microwave module, a hot air module or a light module. In another embodiment a PCR reaction sample is moved inside the reaction channel past one or more constant temperature zones.

[00263] Figure 9 shows the amplification of PowerPlex 16 STR reactions that have been prepared in a cartridge (1) from buccal swab samples and processed in reaction channel 250 using the temperature control device 400 in Figure 7. The STR markers are amplified from standard conditions with Mg optimized for the apparatus 1000.

[00264] The temperature control device 400 can also have a detector 410. The detector can detect optical detection such as absorbance, fluorescence, chemiluminescence, imaging, and other modalities well known to one skilled in the art or measurement such as IR, NMR, or Raman spectroscopy. The detector can comprise a light source is used to excite a fluorescent or luminescent dye in the PCR reaction sample, and the excitation light is sensed with a photodetector (such as a CCD, CMOS, PMT, or other optical detector). In one embodiment the light source is a coherent light source, such as a laser or a laser diode. In another embodiment the light source is not a coherent light source, such as a light emitting diode (LED) or a halogen light source or mercury lamp.

[00265] For nucleic acid amplification, real-time PCR is one example of a nucleic acid assay method that can be performed in tube 250 in temperature controlled region 350 and detected with detector 410.

[00266] On microchip reactions

[00267] In addition to transfer to tubing, apparatus 1000, as shown in Figure 6, can transfer material to microchips. To facilitate the movement of this solution onto a microfluidic device for processing a microchip was specifically designed with large MOVE valves for high volume pumping and bead capture, stepped ports for interface with input and output capillaries, side ports for reagent introduction and a 1 μ L reaction chamber. Refer to Figure 11, Figure 12 and Figure 14 for microchip details.

[00268] **Figure 11** shows a microchip schematic. The left depiction in **Figure 11** diagrams the introduction and capture of beads from the cartridge and apparatus device. The large pump 101 and the magnetic bead capture chamber 103 are fed by a capillary from the cartridge. Input capillary indicates where sample is added through the cartridge. Output capillary indicates where sample is removed through the cartridge. Beads can be placed in the input capillary and then moved into the magnetic bead capture chamber by pumping of valves between the input capillary and the magnetic bead capture chamber 103. Valves are indicated by the dark circles and opposing triangles. A movable magnet can be positioned adjacent to the magnetic bead capture chamber to capture magnetic beads as solution carrying the beads flow through the magnetic bead capture chamber. The diagram on the right illustrates the resuspension of the beads and DNA in the STR pre-mix as the sample is moved into the reaction chamber. The two arrows in the diagram on the right indicate where STR pre-mix and DNA can be added to the microchip. Valves between the location indicated by the arrows and the reaction chamber can be used to pump the DNA or STR pre-mix into the magnetic bead capture chamber for resuspension of beads, and then into the reaction chamber.

[00269] **Figure 12** and **Figure 13** show bead capture on microchip after transfer from cartridge device. The bead capture can be performed by using a magnet positioned adjacent to the microchip such that a magnetic field is applied within a chamber of the microchip. Shown in **Figure 12** and **Figure 13** is a large capacity (500 nL) MOVE valve, which can be utilized for pumping and capture of beads. The capture of beads is shown in the inset with a captured bead bed. As shown in **Figure 13**, the magnet 1103 is positioned over valve 1101 of the microchip. Beads, which can flow into or out of the valve through fluidic channels 1111 and 1107, are captured against a wall of the valve due to the magnetic field exerted by the magnet. The valve can be actuated by a pneumatic channel 1109 that can deliver a positive pressure or negative pressure, relative to the fluidic chamber of the valve, causing the elastomeric layer of the valve to raise or lower.

[00270] One microliter on microchip reactions has been successfully run with good signal strength and relatively good loci balance. **Figure 17** shows the results of reactions with using one microliter on microchip reactions as compared to equivalent off microchip reactions and no template controls (NTC) that contain only size standards. Peaks represent detection of nucleotide base pairs.

[00271] **Purification of reaction products on beads.**

[00272] In one embodiment, the reaction products on the beads can then be moved into cartridge (1) using vacuum applied to the reagent connection 5 with microvalves 200 and 130 open with the path connecting through 201, 212, 211, to junction 210 and channel 19 and 9 to chamber (3) through 131, 141, and 140 to reaction channel 250. The microvalves on microchip 500 can modulate the vacuum and flow. The reaction products can be moved into chamber (3) which can be loaded with beads that are cleaned in place as described above.

[00273] The beads can capture many types of biomolecules using affinity or other interactions well known to one skilled in the art using bead purifications, immunomagnetic separations, and reactions with beads, nanoparticles, quantum dots or other types of particles.

[00274] Continuing the STR example, after STR amplification is complete, the reaction products are transferred back to the cartridge (1) using vacuum. Amplified STR products are purified, desalted and concentrated prior to injection using the same Orapure magnetic bead beads present on the device for the isolation of DNA from the buccal swab. This time the beads are used with only ethanol; no PEG/NaCl solution as described previously for the swab extraction is used.

[00275] The beads are loaded into the cube mix chamber, captured and cleaned with 70% ethanol. Then the 5-10 μ L of STR reaction is pulled back into the chamber from the cycling zone and into contact with the beads. A 20 μ L chase solution of electrophoresis run buffer (chosen because of its availability on the separation subsystem) is pulled through to scavenge any remaining STR reaction solution in the reaction channel and 100% ethanol is added to take the solution up to a 95% total ethanol concentration.

[00276] **Figure 19** shows data for standard material prepared in this manner on the swab extractor and analyzed on a MegaBACE. The products were cleaned with Orapure beads in the sample preparation device configured as a swab extractor using cartridge (1) and compared to products that were cleaned using a manually prepared control that had been processed with CleanSeq (Agencourt). About ~60% recovery was observed in comparison with the same process performed off device. It should be noted that the swab extractor cleaned material yields significantly more efficient injections than the commonly used process which dilutes the sample by 1:50 to 1:100.

[00277] In another embodiment, a Post-amplification STR clean-up device delivers the STR reaction premix to the thermocycler; meters the sample during the isolated DNA bead capture; performs a bead cleanup on the STR amplified products; delivers the eluted products to the cathode; and provides reagents to the cathode assembly during preparation of the separation and detection device and sample injection processes.

[00278] The four-channel Post-amplification STR clean-up device combines an Chip A microchip, shown in Figure 47, with an enlarged fluidics manifold with cleanup chambers (Figure 71) and mounted on a pneumatics control manifold (**Figure 72**). Agencourt CleanSeq beads are delivered to the clean-up chamber, the sample is pumped through the reaction tubing 250 from the thermocycler to the clean-up chamber and ethanol is added. The sample is mixed by air bubbling to facilitate DNA capture onto the beads. A magnet is actuated at the base of the device cause the DNA and beads to be captured against the bottom of the clean-up chamber; the remaining liquid is then pumped to waste. The magnet is moved away from the device and eluent containing fluorescently labeled DNA size standard in a formamide solution is pumped into the clean-up chamber. The STR amplification products are eluted in this solution and the magnet is once

again actuated to capture the beads before the purified and concentrated sample and size standard are pumped to the cathode, ready for injection into a separation capillary.

[00279] Testing of the post amplification device using Chip A highlighted issues with priming and pumping ethanol through the Chip A microchip due to the high level of resistance in the reagent pathway of this microchip. The microchip design, Chip E, (Figure 73) significantly improves functionality and robustness of the post amplification device by widening channels and replacing three way MOVE routers with a pair of MOVE microvalves.

Example 2: Universal sample preparation

[00280] The previous example illustrated one embodiment in which the disclosed apparatus can be used to prepare samples for analysis and showed one example of STR amplification. Another embodiment involves the use of a Universal Sample Preparation Module (USPM). The USPM device can consist of a sample processing cartridge (1), accompanying apparatus to operate the cartridge, a microprocessor, and software that can readily be interfaced to downstream analytical devices. In one embodiment the USPM can be tightly integrated with analytical devices to form a modular sample-to-answer system. The cartridge can be configured as a disposable single-use device that can process swabs or liquids (including aerosol samples) for field monitoring processes, or as a reusable, flow-through format for remote operations with rare positives. Target specificity of the USPM is imparted through the use of specific antibodies (that bind selected targets) attached to paramagnetic beads; different cartridges can be supplied with various mixtures of targets.

[00281] A USPM can use a multistep fully automated process to prepare biological samples for downstream analysis. One example in **Figure 18** can use swabs or liquids; the operator can select the sample type and then insert samples into input port(s). The first step can apply immunomagnetic separations (IMS) to capture, concentrate, and purify target molecules from solution onto paramagnetic beads. Targets already tested include cells, spores, viruses, proteins, or toxins. For toxin and protein detection, or for use as a triggering device, the captured targets from the IMS can be exported directly to the downstream analytical device. For nucleic acid detection, the second step can lyse the cells or spores to release the DNA and/or RNA using mechanical or other lysis techniques. The third step, nucleic acid purification, can adsorb, concentrate, and purify the nucleic acids onto a second set of paramagnetic beads and output the beads with nucleic acid, or purified desorbed nucleic acid, for downstream analysis.

[00282] Referring to cartridge (1), the immunomagnetic separation can be performed by using reagent beads that have antibodies or other immunomagnetic, affinity magnetic, or surface chemistry magnetic separations. For example, immunomagnetic beads with antibodies can be added to cartridge (1) to capture, purify, and concentrate cells, viruses, spores, toxins and other biomolecules onto bead.

[00283] Upstream sample processing for the USPM can be done in the sample preparation devices, which can process samples over 0.6 mL in a microfluidic cartridge (1) (Figure 21). The sample

processing cartridge, about 1 in cubed dimension, (**Figure 3, Figure 21**) was developed to automatically remove collected buccal cells from a swab, lyses the cells, and purifies released cellular DNA on magnetic beads. The bead beds are typically 100 nL and can be used for downstream STR analysis with microfluidics devices or full scale qPCR reactions.

5 [00284] The sample preparation device uses a MOVE microvalve microchip interfaced with the bottom of the cube (**Figure 3**, arrow labeled 2) to direct pressure-driven flows consisting of fluids, beads, and samples among the reagent and reaction reservoirs. The MOVE microvalves replace conventional valves and tubing between the reservoirs, thereby providing a non-leakable, directable fluid transport and enable miniaturization of the entire cube and sample preparation device.

10 [00285] This sample preparation device technology has been used to automate DNA extraction from buccal swabs as described above. **Figure 10** shows automated preparation of DNA from 25 uL of blood in the automated sample preparation device using pressure driven flows, vibrational mixing, MOVE valves, actuated magnets, and magnetic beads. The fully automated process produced DNA ready for STR analysis in less than five minutes.

15 [00286] We have developed an automated system for capturing, concentrating, and purifying cells, viruses, and toxins from liquid samples (1-10 mL) using magnetic beads coated with antibodies specific to targets of interest. Thus, a variety of targets have been concentrated and purified with this automated system. Using this approach, *E. coli* cells were captured and detected at cell concentrations as low as 15 cells/mL/sample (**Figure 27**). Similar results of greater than 90% capture efficiency were obtained using *Bacillus* spores, Gm⁺ and Gm⁻ vegetative cells, a model virus (bacteriophage fd), SEB, and ovalbumin as targets. Purified samples can be further processed in the sample preparation device (e.g., lysis and nucleic acid purification), moved onto a microchip for analysis, or used with an off-chip PCR/qPCR device.

20 [00287] We have shown that IMS capture works well in complex samples such as aerosols and in the presence of biological clutter (See U.S. Patent Publication No. 20080014576, herein incorporated by reference in its entirety). For clutter, we showed that up to 10⁵-fold levels of added bacteria produced only a two-fold reduction in capture efficiency. For complex samples, add-back experiments using many different aerosol samples established that aerosol samples reduce the binding of *B. cereus* spores to IMS beads by less than 50%. Therefore, there is less than a two-fold loss of sensitivity in complex, real-world samples.

25 [00288] We have used IMS to capture, concentrate, and detect toxins. We have developed IMS assays for ovalbumin and SEB, multiplexed the assays, and developed two generations of completely integrated microfluidic systems that automate the IMS assays. Less than 10 ng of SEB can be reliably detected in a one mL samples with no interference from closely related *Staphylococcal* enterotoxins.

30 [00289] We have shown that IMS can:

- Select target organisms from samples with high backgrounds of interferents (selectivity),

- Discriminate between two different strains or species of bacteria (specificity),
- Effectively capture cells and toxins across a wide range of concentrations from a wide range of samples (sensitivity, robustness)
- Reduce target sample volume significantly, from mL to nL volume

5 [00290] The instant invention and the apparatus and methods are capable of implementing IMS and coupling it to nucleic acid extractions.

[00291] The next step in the USPM is the lysis of the captured target when it is a cell, virus, prion, or spore. Lysis of spores is particularly challenging. A MagMill or magnetically driven lysis or homogenizing device has been developed for efficient lysis of Bacillus and other spores, as well as vegetative cells. The MagMill consists of a rapidly rotating magnet 2000 actuated by a motor 2001 (*Figure 74*) that drives rotation of another magnet 2002 contained within a sample-containing vessel 2003 or compartment (*Figure 75*). The magnet 2002 contained within the sample-containing vessel can have any shape. For example, the magnet can have a bar, spherical, cylindrical, rectangular, oval, hexagonal, or propeller shape. Alternatively, the magnet can have holes through it, such that liquid may be forced through the holes and increase the shear force applied to the sample when the magnet is rotated by a magnetic field. The same basic components can be miniaturized, incorporated into a microfluidic format, or connected to a microfluidic format. The overall effect is analogous to a magnetic stir plate, with the sample being rapidly vortexed within the sample tube. Using magnetically driven sample agitation by MagMill treatment, spore lysis is achieved without silica, zirconia or other beads. Lysis may be accomplished by shear forces generated as the spore passes between the magnet and the vessel walls. The magnet can rotate at a rate of greater than about 10, 50, 100, 150, 200, 250, 500, 750, 1000, 1500, 2000, 2500, 5000, or 10000 rpm.

25 [00292] This device disrupts spores with similar efficiency as traditional bead beating that employs silica/zirconia beads (*Figure 76*). Spores (3.2×10^7) were lysed in a volume of 1 ml with viability was determined by plating on Tryptic Soy Agar; results are an average of two separate experiments each run with duplicate samples (total n=4). The non-viability of magnetically-driven spore lysates was 93% compared to traditional bead beating (BioSpec beater) lysates using either Zirconia/silica which was 96% or silica beads which was 80%. The same pattern was confirmed by qPCR

30 [00293] The advantage of using the MagMill (versus traditional bead beating) is that the design is more mechanically robust and thus able to withstand many cycles of use without failure, and samples can be lysed using just the agitation of the magnet in the sample, without the need for inclusion of silica/zirconia beads that have been shown to bind released DNA causing a loss in follow-on detection sensitivity. The basic features of the MagMill can be reconfigured in a miniaturized format that can be integrated into a sample preparation device. The system can potentially be down-sized to fit into a microfluidic microchip. Despite changes in configuration, however, the principle driving lysis, that of a rapidly rotating magnet contained within a sample vessel, remains the same.

Example 3: Sample preparation for library construction

[00294] The cartridge (1) technology and the sample preparation device can be further used to perform a series of complex molecular biology reactions in small volumes with bead-based manipulation of DNA samples. The DNA can be processed to prepare genomic libraries for next generation sequencing systems (i.e., Roche 454 system, or the Applied Biosystems SOLiD), Real-time PCR, or other DNA assay systems.

[00295] By incorporating a reverse transcriptase step, RNA libraries can also be converted into DNA libraries by essentially the same method. The advantage of building an RNA library is that the representation in the final amplified library will directly mirror the original starting material since the amplification will be based upon single molecule amplification.

[00296] The design concept for the Library Construction Module (LCM) is an instrument that holds bulk reagents and provides fluidic control to the small disposable Library Construction Module cartridges that processes individual libraries. The LCM instrument can control fluid flow, mixing, temperature, and bead manipulation in LCM cartridges through DevLink software. An array of reagents stored in temperature controlled reservoirs can be accessible through a MOVE block of valves (actuated by computer-directed pneumatics 700). Workflow for producing beads with amplified DNA attached from single molecules is shown in Figure 29. The Library Construction Module uses the cartridge as a preferred implementation.

[00297] MOVE valves are extremely durable, compact, inexpensive compared to conventional valves, have dead volumes of about 10 nL, and are compatible with dispensing volumes as low as 100 nL. The instrument can use pressure-driven flows to move fluids from the reagent reservoirs through the MOVE valve block, to the reaction chambers in the LCM disposable cartridge, and out to final sample output vessels (Figure 25). Reagents and substrates can be mixed in sample preparation devices using pressurized flow, vibrational mixing, and pneumatic-driven MOVE valves; magnetic beads are captured and released using DevLink-controlled actuated magnets.

[00298] Each disposable LCM cartridge can contain embedded MOVE valves (Figure 28). The MOVE valves direct fluids from reagent reservoirs and connect the three reaction chambers. One chamber, the processing chamber A, can carry out the sequential solution-based molecular biology reactions. The second, the purification chamber B, can be for bead-based purification of intermediate products. The third chamber, the annealing chamber C, can perform the final annealing to glass beads. The annealing chamber can comprise a filter to separate the beads from surrounding solution. Pneumatic lines (P1, P2, P3, P4, P5, P6, P7, and P8) can control the valves of the microchip that control and/or force flow to the chambers.

[00299] Pressure driven flow through the MOVE valves can move fluids between chambers. The processing and annealing chambers can be capable of mixing contents and incorporate thermal control.

[00300] In the next sections, the workflow to prepare DNA library samples using the LCM is described as an example. It is readily apparent that RNA libraries can be prepared after a reverse transcriptase step.

[00301] **Demonstration of microfluidic reactions preceding emPCR**

5 [00302] The starting input for the Library Construction Module can be nebulized, sheared DNA that has been size fractionated to 800-1,000 bp, purified, and has already had small fragments (<500 bp) removed. Size fractionation can also be carried out on the LCM by selective binding to magnetic beads, e.g., AMPure,(Agencourt). Input DNA can be assessed for size and concentration on a BioAnalyzer 2100 (Agilent) and by dye binding (Pico-Green, Quant-it, Invitrogen). The sequence
10 of reactions is shown schematically in **Figure 29** in the Library Construction Module box.

[00303] **Fragment end polishing**

[00304] In one embodiment DNA nebulization generates fragments with a preponderance of frayed ends that require filling in/blunt-ending before further manipulations can take place (Bankier 1987). Phosphorylation of 5' hydroxyl termini is also required for subsequent ligation of adapters. This
15 can be accomplished through the successive activities of T4 DNA polymerase and T4 polynucleotide kinase. Substrates are combined with reaction buffer, BSA, ATP, dNTPs and the two enzymes in a small reaction volume, initially 25 uL, incubated first at 12 degrees Celsius for 15 min., then at 25 degrees Celsius for 15 min. Temperature control can be through Peltier
20 devices mounted on the hard instrument interface. Controls for the polishing reactions can rely on incorporation of fluorescently labeled dNTPs, assayed using fluorescent imaging and quantification or using radiolabeled dNTPs.

[00305] **Microfluidic bead-based purification**

[00306] Following polishing, the fragments are purified by precipitation onto beads. The LCM instrument can move fluids to the LCM cartridge (see **Figure 28**) to force DNA onto beads, actuate a magnet
25 to concentrate beads onto a wall of the purification chamber, and then wash the beads. As needed the DNA can be eluted from the beads, the DNA can be moved to the processing chamber A and the beads discarded, or the beads moved into another chamber for further processing. This method can be reused multiple times throughout the process. Column-based purifications are thus replaced with bead-based purification. The sample preparation device routinely purifies
30 DNA in cartridges using magnetic SPRI beads (Agencourt Biosciences), DNA IQ(Promega), and carboxylated beads (Invitrogen) for DNA sequencing, forensics, and biodefense applications respectively. Purified products can be assayed on a BioAnalyzer (Agilent) and by dye binding (Pico-Green, Quant-it, Invitrogen).

[00307] **Ligation of Adaptors**

35 [00308] Next, adaptors are ligated to the purified DNA. The DNA in the purification chamber can first be eluted from the beads into ligation buffer, which is moved to the reaction chamber by pressure driven flow, and adaptors and ligase added. The adaptors can contain nested PCR and sequencing priming sites, blunt 3' ends and 5' overhangs; one adaptor of each pair can have a biotin on the 5'

end. Each adaptor can incorporate a nucleotide-based 'key' that serves to identify the sample and the processing steps and a 'sequencing key' to assist in base-calling. Ligation and further selection selects for fragments with different adaptor (one with biotin, one without) on each end. At this point before being bound to streptavidin beads the ligation products again can need to be purified on beads in the purification chamber and the library moved back to the processing chamber.

[00309] Ligations can be conducted with ligase such as quick ligase (New England Biolabs), buffer and adaptors and incubated for 15 min at 25 degrees Celsius. Products can be assayed for the ligation of adaptors by Q-PCR, using the PCR priming sites and 5' sequencing priming site for probe. Efficiencies of ligation and recovery of target can be calculated by comparing the amount of starting materials and yields after relevant steps. Also, pre-binding one adaptor via a biotin-streptavidin linkage to a paramagnetic bead will result in bead-bound fragments that can then be subjected to a second round of ligation with the second adaptor. This approach can prevent the generation of fragments with no biotinylated adaptors and those with two biotinylated adaptors, which are both lost during processing. This could significantly improve the final yield of template. After ligation, the products are again bead-purified.

[00310] Library immobilization

[00311] Pre-washed streptavidin-coated beads resuspended in Library Binding Buffer can be added from a reagent reservoir to the purified ligation reaction products that have been moved back to the processing chamber and incubated for 20 min with mixing at RT. The material is then bead purified on the streptavidin bead in the purification chamber to remove adaptor dimers.

[00312] Nick repair

[00313] Ligation results in 3' nicks, which can be repaired by Bst DNA polymerase (Large Fragment). Fill-in Buffer, and dNTPs, can be added directly to the beads and incubated at 65degrees Celsius for 30 min. The beads are then purified in the purification chamber.

[00314] u

[00315] 25 uL of premixed Melt Solution (125 mM NaOH) can be added directly to washed beads in the purification chamber, mixed, the bead pelleted using a magnet, and the resulting supernatant moved to the processing chamber where 62.5uL Neutralization Solution (0.15% acetic acid) can be added. A second round of treating the beads with denaturant results in a total volume of 113 uL of single stranded template. These would again be purified before eluting in a volume of 10 uL.

[00316] Automation, upstream normalization of the input material, or using limiting amounts of beads are various steps that can greatly aid in uniform production and amplification of libraries, which can lead to the elimination of the quality check at this point.

[00317] In one embodiment, continued quality assessment and functional quantification can be performed. The quality of the SS library can be assessed off-line, if required, using an Agilent 2100 Bioanalyzer, which can provide the size range of fragments; yields can also be assessed using Q-

PCR, or dye binding (RiboGreen, Quant-iT, Invitrogen). The library can further be functionally tested by forming a dilution series, performing emulsion PCR (emPCR), and pyrosequencing to determine the working dilution for the emPCR. Automated fragment separation can also be incorporated into the device .

5 **[00318] Binding to capture beads**

[00319] The SS DNA prepared in prior steps can be annealed to controlled pore glass (CPG) or styrene beads with bound DNA capture primers complementary to the ends of the ligated adapters. Handling of non-magnetic beads, such as these, can require the use of pumped filtration technology (to avoid the use of centrifugation). Because the reagents used can be purified of any
10 particulates and the beads can be pre-washed and quantified using a Coulter Counter (Beckman Coulter), filter clogging should not be an issue.

[00320] The beads can be added to the template library, now single stranded, and mixed to favor annealing of a single template/bead in hybridization solution in the annealing chamber. Initially, the bead-template mixture can be divided into aliquots for subsequent emulsion generation and
15 annealed by ramping from 80 degrees Celsius with holds at 10 degrees Celsius intervals in a standard thermal cycler. Temperature-ramping capabilities can also be incorporated into the LCM device. Resuspension of filtrates (i.e., washed beads) can be accomplished by back washing the filters. Hybridization of the SS library can be assessed by assaying for unhybridized SS DNA in the supernatant by Q-PCR on aliquots before and after bead hybridization. At this stage of
20 processing the single stranded template on beads can be transferred to the emPCR Production Module or similar device for further processing.

Example 4: Coupling of a sample preparation device with a microchip-based sample cleanup and separation

[00321] Figure 34 and Figure 35 show a device with a cartridge (2907) and microchip (2901) that was
25 designed to incorporate the Forked Injector design, as shown in **Figure 32**, a gel filling manifold (2903), and associated components. The cartridge is fluidically connected to a pneumatics manifold and tubing (2905). Different configurations of the injector design, separation channel length and separation polymer were tested. Figure 36 show an electropherogram of an M13 T track injected and separated on a microchip channel using the Forked Cathode injector, with
30 sample detection on a confocal microscope breadboard system. The sample was injected uniformly with short and long DNA fragments represented equally. The results show that an M13 T track DNA ladder can be uniformly injected and single base pair resolution can be obtained out to approximately 330 base pairs in less than 20 minutes. Higher sample signal strengths were obtained compared to injections using a conventional twin T design. When
35 integrated with a detection system, the microchip is held at a constant 50°C in order to obtain separations with good resolution.

[00322] Using these processes, excellent results were obtained for MOVE integrated, field amplified stacking injections of liquid samples (Figure 37). This data was generated with all sample

loading, manipulation and injection processes carried out under software control using MOVE microvalves. The data has been minimally processed, color corrected from a detector that uses eight diode channels to four dye traces.

[00323] One embodiment of a microchip that combines the forked cathode with a MOVE sample

5 preparation device is shown in Figure 38. This device comprised additional processes that enable integration with the rest of the system, i.e., the sample preparation device (1000 shown in **Figure 22**), the reaction channel (250 shown in Figure 6), and the output of the STR purification as described in the STR example. Figure 38 shows a forked cathode with MOVE fluidics and shuttle sample loading for integration with post amplification STR purification system. The parts are: 1 -
10 Reagent input port, 2- Reagent pump head, 3 - Sample input port, 4 - Size Standard / eluent input port, 5 - Capture valve, 6 - Waste port, 7 - Elution valve, 8 - Sample waste port, 9 - Cathode, 10 - Cathode port, 11 - Sample valve, 12 - Sample port, and 13 - Separation channel. The anode port, which is downstream of the channel, is not shown.

[00324] The sample to be separated is introduced as a bead solution in ethanol. This can be the purified

15 reaction products on beads output as described above. In one embodiment, the sample is an STR reaction. In other embodiments, the sample can be nucleic acid fragments of different lengths produced by other reaction chemistries including DNA sequencing by Sanger chemistry. The solution containing the sample is flowed from the Sample input port to the Sample waste port with the Capture valve and other intervening valves open. The open Capture valve facilitates a
20 slowing of the stream flow and bead capture by a fixed magnet placed above or below the valve. The ethanol solution is completely run through the system followed by air yielding a relatively dry and clean bead bed, with purified products, in the valve. At this point the valve is closed and reopened (in coordination with other valves) to fill it eluent solution from the associated port. For an STR analysis or other analyzes where an internal size standard is needed, the eluent can
25 contain a size standard. The solution is moved between the Elution valve and the Capture valve to facilitate mixing, ending with the solution in the Elution valve. The Sample valve is then opened in coordination with the Elution valve closing to “shuttle” the sample through the sample channel leaving it filled. The sample FASS injection is carried out as previously described. An additional noteworthy function of the device is that in one embodiment the Reagent input port and Reagent pump are used to provide metered STR reaction premix to the reaction channel (250
30 shown in Figure 6) after the swab extraction of DNA on the sample preparation device; in other embodiments, the device can provide other nucleic acid reaction reagents such as cycle sequencing mixture or provide PCR reagents to perform a PCR amplification followed by providing cycle sequencing reagents to perform cycle sequencing with bead-based cleanup
35 reactions integrated as needed. Other chemistries will be apparent to one skilled in the art.

Example 5 : Integrated nucleic acid isolation, amplification, separation and detection system.

[00325] A sample preparation device with cartridge(s) and thermal regulating device can be integrated with downstream detection systems to produce a sample-to-answer fully integrated system. The

system can be fashioned into a compact format that is compatible with laboratory, clinical, and field operation as a benchtop or portable device, as shown in Figure 39. Figure 39 shows one embodiment of a system that can extract swabs or other materials using a five channel swab extraction assembly 800 using cartridges (1) to purify nucleic acids from input buccal swabs, liquids, solids, and other materials. The purified nucleic acids on beads is PCR amplified in Thermal Cycling Module 400. In one embodiment, the samples are bead purified in swab extraction assembly 800 using cartridges (1) to purify the desired products onto beads. The beads are moved to Separation and Detection Module 900 to receive the products on beads, elute the sample and separate it by capillary electrophoresis or microchip capillary electrophoresis or other separation methods with detection methods such as Laser Induced Fluorescence or mass spectroscopy. For capillary electrophoresis or microchip capillary electrophoresis, Gel Injection Module 850 pumps separation matrix or gels or other materials to provide the separation columns and regenerate them. In another embodiment, the Gel Injection Module 850 could pump chromatography media if the separation was by HPLC or other liquid chromatography methods. Electronics and Power Supply Module 860 provides control and power function. Pneumatics Module 870 supplies regulated air and vacuum to operate the swab extraction assembly 800. Reagents are stored in reagent storage 880. Reagents can be stored in solution or dehydrated or stabilized forms such as Ready-to-Go (GE Healthcare) and lyophilized forms.

[00326] In one embodiment, the system is configured to perform STR analysis of buccal swabs. Buccal swabs are extracted in assembly 800 and the extracted samples amplified in Thermal Cycling Module 400 using reagents for STR amplification. The amplified samples are purified using nucleic acid extractions onto beads, for example using Orapure or DNA IQ chemistries and beads. The purified STR products on beads are then moved to Separation and Detection Module 900 and the beads are captured and the DNA eluted, preferably on a microchip with MOVE microvalves. The samples are then injected preferably into a Forked Cathode Injector on a microchip or a capillary electrophoresis capillary coupled to a microchip and using capillary gel electrophoresis separations with gels such as dynamic coating gels, V2E (GE Healthcare), polydimethyl acrylamide, the POP family of matrices (Applied Biosystems), hydroxymethylcellulose, guarin, and linear polyacrylamide. The detected products with fluorescent labels pass a Laser Induced Fluorescent detector which detects the peaks as they move by.

[00327] In another embodiment, the system is configured as an integrated DNA sequencer. The sample is extracted in assembly 800 and the extracted samples amplified in Thermal Cycling Module 400 using reagents for PCR amplification. The samples can be whole organisms, tissues, cell, viruses, air, liquid, or solid without limitation. The DNA extraction can be non-specific using a bead-based purification method such as Orapure (Agencourt) or can use hybridization or other methods to select one or more regions from the input sample to produce a less complex sample. It will be apparent to one skilled in the art that the USPM workflow with IMS followed by the nucleic acid

purification can also be adapted to the sample preparation device 800 and the swab extraction replaced by many other initial purification workflows. The PCR amplification can be of a single region or multiplexed to target multiple regions. The amplified PCR samples are purified using nucleic acid extractions onto beads, for example using Orapure or DNA IQ chemistries and beads. The purified PCR products on beads are then moved to the Thermal Cycling Module 400 and cycle sequencing master mix added and the samples cycle sequenced. This can be with fluorescent labels or as four sets of unlabeled primers for label-less detection by UV or other methods. The cycle sequenced samples are then moved to assembly 800 and bead purified to remove unwanted ions and labels, and other material. The beads are then moved to Separation and Detection Module 900 and the beads are captured and the DNA eluted, preferably on a microchip with MOVE microvalves or into capillaries. The samples are then injected preferably into a Forked Cathode Injector on a microchip or a capillary electrophoresis capillary coupled to a microchip and using capillary gel electrophoresis separations with gels such as dynamic coating gels, V2E (GE Healthcare), polydimethyl acrylamide, the POP family of matrices (Applied Biosystems) hydroxymethylcellulose, guarin, , and linear polyacrylamide. The detected products with fluorescent labels pass a Laser Induced Fluorescent detector which detects the peaks as they move by.

[00328] In other embodiments the protein, carbohydrate, or other assays are performed and the detection is by mass spectrometry, imaging, HPLC, GC, or other analytic methods well known to one skilled in the art.

[00329] For DNA, the processed sample can be amplified by PCR, rolling circle, branched DNA, EXPAR, and other DNA amplification methods well known to one skilled in the art or analyzed by mass spectroscopy or single molecule detection methods. RNA can be processed by Reverse Transcriptase real time-PCR, or samples prepared for DNA microarrays, or other analytical methods. Real time or end point analyzes can be performed with the apparatus. For proteins, assays can be performed in the cartridge including enzymatic assays, sandwich immunoassays, antibody precipitation, protein digestion, protein and peptide labeling, and other commonly used protein analysis methods. Similarly, other cellular components or chemicals can be extracted or purified using standard methods in the apparatus. Molecular biology methods are readily adapted to the apparatus. Samples can be completely analyzed on the apparatus in a single cartridge, moved to a separate cartridge, or analyzed or further processed in a separate instrument comprising a capillary electrophoresis system or microchip capillary electrophoresis; multidimensional gel and capillary electrophoresis; mass spectroscopy, multidimensional mass spectroscopy with HPLC, ICP, Raman spectroscopy, particle, nanoparticles, and bead based detection, imaging, comprising fluorescence, IR, optical, or any other analytical systems well known to one in the art.

[00330] The integration of a complete sample-to-answer instrument incorporating the cartridge to prepare DNA samples from many inputs and sample types and a microchip-based capillary

electrophoresis device for separation of DNA fragments is taught for DNA sequencing, fragment sizing, and forensics.

Example 6: Device with four processing channels

[00331] A microchip or microfluidic microchip can be used to amplify mRNA, concentrate nucleic acids on magnetic beads and inject purified samples into electrophoretic separation capillaries. As shown in Figure 45 and Figure 46, a microchip (505) can be interfaced with a cartridge (503) and a pneumatic manifold (507). Figure 45 shows an expanded view of the microchip, cartridge, and pneumatic manifold. Figure 46 shows a view of the cartridge interfaced with the microchip, which is interfaced with the pneumatic manifold. The cartridge can completely cover the surface of the microchip. Additionally, a block (501) has holes (509) that help to hold for incubation materials delivered to or from ports (511) of the cartridge (503). The block can be a heat block or a temperature controlling block. The holes (509) can be used to hold to hold pipette tips or be used as a large volume reactor or processor. The block (509) can be heated or cooled to control the temperature of material being delivered to cartridge and microchip or removed from the cartridge and microchip. The block can be in thermal contact with the cartridge. The ports of the cartridge can lead to reservoirs that are fluidically connected to ports that mate with ports (515) on the microchip (505). The microchip (505) can have pneumatic line ports (519) that mate with ports (517) of the pneumatic manifold. The ports of the pneumatic manifold can have o-ring gaskets that seal the pneumatic manifold to the microchip, allowing for high and low pressures to be delivered without leaking or with a reduced loss of pressure or vacuum. The cartridge, microchip, and pneumatic manifold can be held together using bolts or other securing objects that pass through openings (513, 501) of the cartridge and the pneumatic manifold.

[00332] A diagram of the Chip A microchip is shown in Figure 47. The microchip comprises three layers: (i) a top fluidics layer (e.g., glass) carrying fluidic channels and wells, (ii) a bottom pneumatics layer (e.g., glass) carrying pneumatic channels and wells, and (iii) a middle flexible membrane, (e.g., 250 μm thick, PDMS layer) (not shown). The PDMS membrane can be featureless. The PDMS membrane can deflect in response to positive or negative pressure applied to localized areas defined by the pneumatic channel system (dashed lines in Figure 47). Pneumatic and fluidic channel etch depths are typically 50 μm and can be designed to offer minimal hydraulic resistance. Valves in the microfluidic system can include pump valves. Pump valves can be larger than other valves, depending on the desired pump capacity, and can lack a valve seat. Removal of the valve seat can increase the pumping capacity, or the volume of fluid that is pumped per pump stroke. Other valves can be smaller, and have a valve seat that allows them to close firmly. Such reduced volume on/off valves can reduce the overall void space within the microfluidic microchip. Inlet and outlet valves can be such on/off valves.

[00333] As shown in Figure 47, the microchip has four identical processing channels, each of which can control reagent flows for off-microchip or on-microchip magnetic-bead-based nucleic acid concentration and capillary electrophoresis sample injection. The four processing channels are

fed by a common “reagent rail” which can select input reagents from four input wells (the fifth well can be used as a waste port). Individual samples can be fed into each processing channel from channel-specific sample wells, and can be processed in parallel (the microchip shown is a 4-plex processing device). Each processing channel can have a 0.8 μ L pump, sample input well, two output wells, and a waste well. Beads, such as magnetic beads, can be captured in any of the wells, e.g. one or both of the output wells. This output well can interface with the cartridge or fluidic manifold.

[00334] A photograph of the Chip A microchip, is shown in Figure 48. The microchip can be mounted between a top fluidic manifold, mating fluid reservoirs to wells on the top surface of the microchip, and a bottom pneumatic manifold, mating pneumatic control lines to wells on the bottom surface of the microchip via o-ring seals. Microchip valves and pumps can be actuated by a pneumatic control system driven by computer scripts, such as DevLink scripts, or other software. The system can supply positive pressure (approximately +10 psi) to close valves, and/or negative pressure (approximately -20 psi or vacuum) to open them. The same pneumatic system can operate microchip pumps, with negative pressure acting to fill the pump bodies (on the fluidic channel side of an elastomeric layer), and positive pressure acting to empty them. As described herein, pumping action depends on the coordinated actions of pumps and flanking inlet and outlet valves.

Example 7: mRNA amplification using device with four processing channels

[00335] Chip A was used to perform the first, reverse transcription, reaction of the Eberwine protocol. Effective mixing and incubation methods were developed.

[00336] In order to provide temperature control for the approximately 10 μ L reaction, a simple heated aluminum block (509), carrying eight 200 μ L pipette tips, was fabricated and mated to the fluidic manifold as shown in Figure 45, Figure 46, and Figure 49. Each pipette tip connected to a microchip output. In the experiment, only 4 tips, connected to microchip Output-1 wells (as indicated in Figure 47), were actually used. Heating (50 $^{\circ}$ C) was accomplished with a thin-film heater attached to the outer surface of the block with adhesive. A thermocouple was inserted into the center of the block, and the heater was controlled with a DevLink PID control loop.

[00337] For mixing, a DevLink script was developed to mix samples and a reagent in a 1:1 ratio by alternately pumping approximately 0.6 μ L from each respective reservoir into a pipette tip mated to Output-1. Experiments with food dye confirmed that alternate pumping effectively mixed the two components in the tip.

[00338] To perform the reaction, a mixture of total RNA and T7 Promoter-Primer (in water) was pipetted into sample well reservoirs, and a two-fold concentrated RT Mix, containing Superscript III reverse transcriptase, was pipetted into a reagent reservoir. After 15 μ L had been loaded into the tips (12 pump cycles), the script terminated, and the reactions continued incubating for 15 minutes at 50 $^{\circ}$ C. At this point the tips were removed, emptied into 0.2 mL PCR tubes, and the reactions terminated by incubation at 85 $^{\circ}$ C for 5 minutes in a thermocycler.

[00339] Positive control reactions were treated identically, except that these reactions were performed entirely at the bench and incubated 15 min at 50 °C in a thermocycler. Negative control reactions were mixed (at the bench) and immediately terminated by incubation for 5 min at 85 °C.

[00340] Reactions were analyzed by TaqMan real-time quantitative PCR using a Gusb primer and probe set (ABI). The results are shown in Figure 50 for 15 µL reactions containing approximately 500 ng total RNA (Rat Liver) and 10 U/µL Superscript III RT. Results from channels 1-4 are shown with results on the performed entirely at the bench indicated by iK. In Figure 50, the pair of bars shown for each of channels 1, 2, 3, 4, and iK represent replicate reactions. The results showed that microchip and bench reactions were identical in their yield of first-strand Gusb cDNA. Both reactions produced Ct's between 26 and 27. Negative control reactions produced Ct > 35 (data not shown).

Example 8: Device with bead clean-up chambers

[00341] A microchip or microfluidic microchip can be used to amplify mRNA, and to concentrate and purify nucleic acids on magnetic beads. Figure 51 shows an expanded view of a device that has a microchip 1307 that can be interfaced with a cartridge made of two pieces 1313, 1315 and a pneumatic manifold 1301. The cartridge is made of a first piece that has serpentine channels 1311, wells, reservoirs, and chambers, and a second piece (1315) that has wells and chambers. The serpentine channels can be used to increase heat transfer between the heat distributing piece and a fluid contained within the serpentine channels. The second piece and first piece can be bonded together such that the serpentine channels are enclosed on a top side. The cartridge can be overlayed or in thermal contact with a heat distributing piece (1317) that distributes heat from thermal control blocks (1323, 1321). The thermal control blocks can be thermo electric coolers (TECs or Peltier devices), thin-film heaters, or other thermal control devices. The heat distributing piece and heat blocks may or may not be bonded or secured to the cartridge. Screws, bolts, and/or hinges may facilitate the securing of the heating distributing piece and/or heat blocks to the cartridge. Figure 52 shows a view of the cartridge interfaced with the microchip, which is interfaced with the pneumatic manifold. The pneumatic block may have annular spaces (1305, 1309) for bolts and/or screws and ports (1303) that interface between pneumatic lines and the pneumatic layer of the microfluidic microchip. Additional views of the pneumatic layer are shown in Figure 53, Figure 54, Figure 55, and Figure 56. Figure 53 and Figure 55 show views with dashed lines indicating edges that are hidden from view. Figure 53 and Figure 54 show three dimensional views of the pneumatic layer. Figure 55 and Figure 56 show top views of the pneumatic layer.

[00342] Figure 57 and Figure 58 show diagrams of a microfluidic microchip, the Chip B microchip, with bead clean-up chambers. Referring to Figure 58, the microchip has four main sections: Reagent Rail, Bead Rail, Processor 1, and Processor 2. The two rails and the two processors have mirrored geometries. The microchip is configured so that either reagent rail may feed either processor. Access to the processors is controlled by valves Vr and Vb for the top processor and

by valves Vrb and Vbb for the bottom processor. During reagent processing and enzymatic reactions of the top processor, Vr is opened and Vb is closed. During reagent processing and enzymatic reactions of the bottom processor, Vrb is opened and Vbb is closed. During clean-up, the reverse applies, that is, for the top processor Vr is closed and Vb is opened and for the bottom processor Vrb is closed and Vbb opened. It can be seen that the top and bottom processor can operate either in parallel or separately. Reagent and Bead Rail design closely follows the Chip A design. Each rail can access four different reagents (R1-4 and B1-4), via valves Vr1-4 and Vb1-4 respectively, and each rail has waste wells (RW and BW), accessed by valves Vrw and Vbw, respectively. Each processor has a sample input well (S), two output wells (Oa, Ob), and a bead side channel accessed by valves Vs, Voa, Vob, Vbs respectively. The bead side channel has a bead reservoir (R), and two valves (Vw and Ve) accessing waste (W) and elution (E) wells, respectively. Pneumatic lines and ports for control of the valves are shown as dashed in Figure 57.

[00343] For purposes of explanation below, it is assumed that Processor 1 and Processor 2 are operated identically in parallel, and that Chip B is a duplex device, processing two samples simultaneously. For clarity, the operation of only the top processor is detailed. However, non-parallel operation is also possible. It is also assumed that wells Oa and Ob are connected to appropriate capacity reservoirs in a fluidic manifold or tubing. Reservoirs can be pipette tips, a reservoir in a cartridge, or connected by tubing to larger volumes. The microfluidic microchip can have 75 μm channel depth, 250 μm (final) fluid channel width, and 0.6 μl (estimated) pumping stroke volume.

[00344] Referring to Figure 58, a reaction comprising Reagent 1 and Sample may be assembled in well Oa by alternate 4-cycle pumping (A, B, C, D). Assume all valves are initially closed. In cycle A, valves Vr1 and Vr open, allowing pump P to draw Reagent 1 from well R1 with a down-stroke (negative pressure applied to pump P to open the valve). In cycle B, valves Vr1 and Vr close and valve Voa opens, allowing pump P to expel its contents (from R1 in this example) into well Oa with an up-stroke (positive pressure applied to pump P to close it). In cycle C, valve Vs opens and valve Voa closes, allowing pump P to draw Sample from well S with a down-stroke. In cycle D, valve Vs closes and Voa opens, allowing pump P to expel its contents into well Oa with an up-stroke. These four cycles are repeated until a sufficient volume has been pushed into Oa. The mixing ratio between Sample and Reagent 1 is determined by the ratio of cycles AB:CD. In the process described above, the mixing ratio is 1:1, but it can in principle be any value. Finally, similar procedures can be used to mix any of the reagents (R1-4) with sample S, by substituting the appropriate valve for Vr1. Referring to Figure 58, in 4-cycle pumping, the fluid can be pumped in a first direction from a first source well to a space within a pumping valve in the first step. In the second step, the fluid can be pumped in a direction opposite to the first direction by moving the fluid from the pumping valve to a mixing well. The third and fourth steps can be repeated with a second source well instead of the first source well. The pumping in opposite

directions to obtain mixing in the mixing well can be a result of having the source well, mixing well, and pumping valve positioned along a channel such that the pumping valve is not located between the source well and the mixing well. This configuration can reduce the dead space within the microfluidic microchip, improve mixing, or improve uniformity of reagent and sample handling. As well, this configuration can allow for a central pump to move liquid between many different wells on a microfluidic microchip through the opening and closing of appropriate valves.

[00345] The valves shown in Figure 57 and Figure 58 and any other valve shown herein sometimes are placed at T-shaped junctions. The valves can close off flow from one channel of the T to the other two channels leading into the T, while continuing to allow flow between the other two channels. For example, closing valve V_{oa} prevents fluid from flowing from pump P to O_a, but does not prevent fluid from flowing from pump P to S if valve V_s is open. Alternatively, a valve can obstruct flow between all channels leading into the T. The same can be applied to valves that are placed at junctions of 4, 5, 6, or more channels. The valves can also be replaced by valves that are only in the reagent or bead channel as needed.

Example 9: mRNA amplification using device with bead clean-up chambers

[00346] As described above, the Eberwine mRNA amplification procedure is a cascade of three binary additions. To execute the Eberwine sequence, R1 contains RT Mix, R2 contains 2S (second-strand) Mix, and R3 contains T7 Mix, as shown in Figure 58. A two-fold (2X) volume of 2S Mix can be added to the RT reaction, and a one-fold volume of T7 Mix can be added to the 2S reaction, as shown in Figure 44. This requires a 2:1 pumping ratio (AB:CD) for the 2S Mix addition, and a 1:1 ratio for the T7 Mix addition.

[00347] The first (RT) reaction with a 1:1 mixture of total RNA+Primer, from well S, and 2X RT Mix, from well R1, can be formed using the methods described herein. After an appropriate incubation period, the second-strand reaction may be assembled in well O_b by drawing from well O_a (rather than well S), and drawing from well R2 (rather than from well R1). A four-cycle pumping scheme (A, B, C, D) similar to that described in Example 8 can be used. In cycle A, V_{r2} opens rather than V_{r1}; in cycle B, V_{ob} opens rather than V_{oa}; in cycle C, V_{oa} opens rather than V_s; and in cycle D, V_{ob} opens rather than V_{oa}. To obtain the required 2:1 mixing ratio, two cycles can draw from R2 for every cycle drawing from O_a.

[00348] After another appropriate incubation period, the third (T7) reaction may be assembled in well O_a with a similar process (drawing from R3 and O_b, 1:1 ratio). Thus the final T7 reaction can reside in O_a. After an appropriate incubation period, aRNA can be ready for purification.

[00349] Purification involves operation of the Bead Rail rather than the Reagent Rail. Thus, during this phase of microchip operation, valve V_r remains closed and V_b can open.

[00350] To purify aRNA, reservoir R must first be loaded with magnetic beads. This may be accomplished with a 2-cycle procedure, similar to that of cycles A and B of Example 8, except that input to pump P can be via valves V_{b4} and V_b (cycle A), and output from pump P can be via

valves Vbs and Vw (cycle B). This sequence can draw bead slurry from well B4 into pump P, and expel bead slurry from pump P through reservoir R into waste well W. As the slurry passes through reservoir R, beads can be captured by a magnet placed below reservoir R.

[00351] Before aRNA (in well Oa) can be captured, it must be mixed with Binding Buffer. This can be accomplished with another 4-cycle procedure, similar to that in Example 8, except that Binding Buffer can be drawn from well B1 in the Bead Rail, and the mixture can accumulate in well Ob.

[00352] aRNA in well Ob can then be captured by beads in reservoir R by pumping the contents of well Ob through reservoir R out into waste well W. This can be accomplished with a 2-cycle procedure in which pump P is filled via valve Vob (cycle A) and emptied via valves Vbs and Vw (cycle B).

[00353] Loaded beads can then be washed with ethanol pumped from well B2. This can be accomplished with a 2-cycle procedure in which pump P is filled via valves Vb2 and Vb (cycle A) and emptied via valves Vbs and Vw (cycle B). After ethanol from well B2 has been exhausted, pumping can continue to draw air over the beads to dry them.

[00354] Finally, aRNA can be eluted from the beads into well E by pumping water through reservoir R with a 2-cycle procedure in which pump P is filled via valves Vb3 and Vb (cycle A) and emptied via valves Vbs and Ve (cycle B).

Example 10: Short RNA amplification using device with real-time PCR detection

[00355] MicroRNA (miRNA) are short (19-25 nucleotide) single-stranded RNAs that are produced by processing larger RNAs, while siRNA are short (20-25 nucleotide) double-stranded RNAs that are also produced by processing larger RNAs. miRNAs have been implicated in regulating translation of mRNA while siRNAs can silence or activate transcription of genes. Both miRNA and siRNA, collectively small RNAs, can be assayed using the devices described herein. To assay either small RNA, the device in Figure 58 would be reconfigured with 1) R3 containing a mixture to polyadenylate the short RNA, 2) R4 containing real-time PCR primers and real-time master mix, and 3) the RT mixture in R1 can contain a polyT sequence with a 5' sequence for real-time PCR amplification instead of a T7 promoter. The reaction would proceed as described in Example 9 except first a polyA tail would be added to the small RNAs to produce polyadenylated small RNAs using R3 as the reagent source. The reverse transcription and second strand synthesis can operate as described in Example 9. The final step of T7 transcription is then replaced by mixing the real time PCR primers and master mix from R4 with the second strand product. The real time PCR primers are then amplified using PCR with real time detection. The amplification can occur off-microchip or a detector and thermal cycling can be incorporated on the microchip or in heating block (509). Real time PCR on microchips was previously described, e.g. Jovanovich, S., I. Blaga, and D. Rank. Microfluidic Devices. US Patent Publication No. 2007/0248958 and PCT Publication No. WO/2006/032044, which are hereby incorporated by reference. It will be obvious to one skilled in the art that other mRNAs can be processed as described in this example by omitting the polyadenylation step.

[00356] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. For example, any MOVE valve, pump, router, or other
5 MOVE device described herein can be replaced with any pneumatically actuated valve, pump router or other device. It should be understood that various alternatives to the embodiments of the invention described herein can be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A device comprising:
5 a microfluidic microchip comprising at least one port aperture fluidically connected to at least one microfluidic channel in the microfluidic microchip, wherein the channel comprises at least one valve that controls movement of a fluid through the channel; and
a cartridge mated to the microchip and comprising a chamber,
wherein said chamber comprises two chamber apertures that are each aligned with a port
10 aperture of said microfluidic microchip.
2. The device of claim 1, wherein said cartridge is adapted to receive at least one sample or one reagent.
3. The device of claim 1, wherein said cartridge is fluidically connected to another cartridge that is mated to another microchip.
- 15 4. The device of claim 1, wherein said cartridge comprises at least two chambers.
5. The device of claim 4, wherein at least one of said at least two chambers is adjacent to a movable magnet.
6. The device of claim 4, wherein at least one of said at least two chambers is temperature controlled.
- 20 7. The device of claim 4, wherein at least one of said at least two chambers comprises a filter.
8. The device of claim 1, wherein said at least one chamber comprises a fluidic volume greater than or equal to 5 μL .
9. The device of claim 1, wherein said at least one chamber comprises a fluidic volume greater than or equal to 10 μL .
- 25 10. The device of claim 1, wherein the fluidic volume of said cartridge is a 100 X of the fluidic volume of said microfluidic microchip.
11. The device of claim 1, wherein one of said at least one chambers further comprises a filter.
12. The device of claim 1, wherein said device further comprises a magnet for applying a magnetic field to the cartridge or the microfluidic microchip.
- 30 13. The device of claim 1, wherein the valve is pneumatically actuated.

14. The device of claim 1, wherein said cartridge is adapted to be connected to at least one pressure source for the delivery of said at least one reagent or said at least one sample.

15. The device of claim 1, wherein said pressure source provides a positive or negative pressure to the cartridge.

5 16. The device of claim 14, wherein said at least one pressure source is controlled by a pneumatic solenoid.

17. The device of claim 14, wherein said at least one pressure source is a pneumatic manifold.

18. The device of claim 1, wherein the microfluidic microchip comprises a fluidic layer, an elastomeric layer, and a pneumatic layer.

10 19. The device of claim 1, wherein said cartridge further comprises at least one input port, wherein said at least one input port is adapted to mate with a delivery device, wherein said delivery device is fluidically connected to the fluidic layer of said microfluidic microchip; and wherein one of said at least one chamber is a closed reaction chamber fluidically connected to
15 the fluidic layer of said microfluidic microchip.

20. The device of claim 19, wherein said delivery device is thermally coupled to a temperature modulator.

21. The device of claim 19, wherein said delivery device is a syringe.

22. The device of claim 1, wherein said cartridge is designed to
20 enrich at least one component from said sample and comprises at least one sample input port, wherein said at least one chamber is a closed reaction chamber comprising beads, and wherein said beads bind to said at least one component.

23. The device of claim 1, wherein the cartridge further comprises at least one reagent reservoir
25 comprising reagents for amplifying a nucleic acid, wherein the at least one reagent reservoir is fluidically connected to the chamber through the microchip.

24. The device of claim 23, wherein the cartridge further comprises at least one bead reservoir comprising beads for binding an amplified nucleic acid, wherein the at least one bead reservoir is fluidically connected to the chamber through the microchip.

30 25. The device of claim 22, wherein said beads are paramagnetic beads or glass beads.

26. The device of claim 22, wherein said binding of at least one component to a bead is reversible.

27. The device of claim 22, wherein said beads are paramagnetic beads, and
wherein said device further comprises a movable magnet that can attract said paramagnetic beads to the wall of said closed reaction chamber.
28. The device of claim 22, wherein said cartridge is designed to enrich at least one component from a sample, wherein said at least one component is DNA, RNA, microRNA, siRNA, protein, lipid, or polysaccharide.
29. A method for performing biochemical reactions comprising:
(a) providing the device of claim 1, and
(b) performing at least one enzymatic reaction within said chamber.
30. The method of claim 29, wherein said at least one enzymatic reaction comprises ligating, blunting, nick repairing, denaturing, polymerizing, hydrolyzing, phosphorylation or any combination thereof.
31. The method of claim 29, further comprising separating a product of said enzymatic reaction using solid-phase particles.
32. A method for enriching at least one component from a sample comprising:
(a) mating a delivery device to said input port of the device of claim 19,
(b) treating said sample with at least one reagent to increase the availability of said at least one component for enrichment,
(c) delivering said at least one component to said at least one reaction chamber of said cartridge,
(d) binding said component to one or more particles in said at least one closed reaction chamber,
(e) washing said particle bound component to remove waste, and
(f) eluting said particle bound component.
33. The method of claim 32, wherein said delivering comprises pumping said at least one component to said at least one reaction chamber through said at least one valve of the microfluidic microchip.
34. The method of claim 32, wherein said binding comprises pumping said particles from a reagent port in the cartridge to said at least one reaction chamber through said at least one valve of the microfluidic microchip.
35. The method of claim 29, wherein said particle is a paramagnetic bead, a nanoparticle, a resin, or a solid-phase particle.
36. The method of claim 29, wherein said at least one component is DNA, RNA, microRNA, siRNA, protein, lipid, or polysaccharide.

37. The method of claim 29, wherein step (b) further comprises thermally modulating said delivery device.
38. The method of claim 29, wherein step (b) further comprises delivering a lysis reagent or a component isolation reagent from a reagent port on said cartridge into said delivery device to increase the availability of said at least one component for enrichment.
39. The method of claim 29, wherein the beads of step (d) are paramagnetic beads.
40. The method of claim 39, wherein said washing step (e) comprises attracting said paramagnetic beads with a movable magnet.
41. The method of claim 29, wherein said microfluidic microchip directs the flow of said waste in a second direction.
42. The method of claim 29, wherein step (c) comprises using pneumatically actuated valves in the microfluidic microchip or an external pressure source to deliver said at least one component to said at least one closed reaction chamber of said cartridge.
43. The method of claim 29, wherein the external pressure source provides a positive or negative pressure to the microfluidic microchip.
44. The method of claim 29, wherein said sample delivery device is a syringe.
45. A device comprising:
- (a) a first fluid manipulation module comprising:
 - (i) a first microfluidic microchip comprising a port aperture fluidically connected to a microfluidic channel in the microfluidic microchip,
 - wherein the channel comprises at least one valve that controls movement of a fluid through the channel; and
 - (ii) a cartridge mated to the microfluidic microchip and comprising at least one sample input port, at least one chamber, an exit port,
 - wherein the sample input port is connected to the port aperture,
 - wherein at least one of said at least one exit ports is aligned with an exit port aperture of said first microfluidic microchip, and
 - wherein said at least one chamber is fluidically connected to the fluidic layer of said first microfluidic microchip;
 - (b) a reaction channel, wherein said reaction channel is not contained within said first microchip;
 - (c) a temperature modulator,
 - wherein said reaction channel is fluidically connected to a port on said cartridge that is

fluidically connected to said exit port and at least a portion of said reaction channel is in thermal contact with said temperature modulator; and

(d) a magnet for applying a magnetic field to the microfluidic microchip, the cartridge, or the reaction channel.

5 46. The device of claim 45, wherein said magnet is adjacent to said reaction channel.

47. The device of claim 45, further comprising

a second microfluidic microchip that is fluidically connected to said first microfluidic microchip through said reaction channel.

48. The device of claim 45, wherein said temperature modulator is a Peltier device.

10 49. The device of claim 45, further comprising a paramagnetic bead.

50. A method comprising:

delivering a sample containing a nucleic acid to the device of claim 45;

transporting the nucleic acid and an effective amount of reagents through the portion of the reaction channel in thermal contact with the temperature modulator one or more times; and

15 amplifying the nucleic acid; and

analyzing the amplified nucleic acid.

51. The method of claim 50, further comprising using the temperature modulator to perform thermocycling.

20 52. The method of claim 50, wherein said reagents are reagents for polymerase chain reaction or cycle sequencing.

53. A device comprising:

(a) a first microfluidic microchip comprising a fluidics layer, an actuation layer, and a pneumatic layer,

25 wherein the fluidics layer comprises one or more microfluidic channels,

wherein at least one of said one or more microfluidic channels comprises an exit aperture,

(b) a flexible connector fluidically connected to the exit aperture at a first end of the flexible connector;

(c) a capillary fluidically connected to said flexible connector; and

30 (d) a first electrode and a second electrode,

wherein the first electrode and second electrode are configured to produce an electric field along a path of the capillary.

54. The device of claim 53, wherein the flexible connector is surgical, poly(tetrafluoroethylene) or silicon tubing.

55. The device of claim 53, wherein the flexible connector is elastic tubing.

56. The device of claim 53, wherein the flexible connector has an outer diameter of about 1.5 to 3 mm and an inner diameter of about 0.25 to 0.5 mm.

57. The device of claim 53, wherein the flexible connector is also fluidically connected to a second microfluidic microchip or the first microfluidic microchip at a second end of the flexible connector.

58. The device of claim 53, wherein the flexible connector is fluidically connected to the exit aperture by a cannula, an upfit tubing, a microtubing fitting, or an upchurch tubing adapter.

59. The device of claim 53, wherein the capillary has an outer diameter of about 150-500 microns and an inner diameter of about 10-100 microns.

60. The device of claim 53, wherein the capillary is polyamide or poly(tetrafluoroethylene) coated.

61. The device of claim 53, wherein the capillary comprises a separation gel.

62. The device of claim 53, wherein the capillary is about 10 to 100 cm long.

63. The device of claim 53, wherein the first electrode is a forked electrode.

64. The device of claim 63, wherein said forked electrode comprises one or more conductive channels or one or more metallic conductors.

65. The device of claim 53, wherein the first electrode and the second electrode produce an electric field that is about 25 to 500 V/cm.

66. A method comprising:

providing the composition to a microfluidic microchip,

wherein the microfluidic microchip comprises a fluidics layer, an elastomeric layer, and a pneumatic layer;

delivering the composition to a flexible connector that is fluidically connected to the microfluidic microchip;

providing an electric field to move the composition into a capillary; and

performing capillary electrophoresis on the composition to separate a component based on size or charge.

67. The method of claim 66, wherein the electric field is about 25 to 500 V/cm.

68. The method of claim 66, wherein said composition in said tube is adjacent to a first and second bolus of gas, wherein said first bolus of gas is upstream of said composition and said second bolus of gas is downstream of said composition in said tube.

69. The method of claim 67, wherein said first and second boluses of gas isolates said composition from other compositions.

70. The method of claim 66, wherein the composition comprises at least one component that is a nucleic acid, protein, fatty acids, or polysaccharides.

71. The method of claim 70, wherein the nucleic acid is microRNA, DNA, RNA, or siRNA.

72. A device comprising:

(a) a separation channel fluidically connected to a loading channel;

(b) a forked electrode comprising at least two electrodes that are electrically connected to the loading channel and the separation channel through the loading channel,

wherein the fluidic connection between the separation channel and the loading channel is located between the electrical connections of the two electrodes to the loading channel; and

(c) a pneumatically actuated valve fluidically connected to the loading channel.

73. The device of claim 72, further comprising a cannular electrode in electrical contact with said forked electrode, wherein the inner diameter of said cannular electrode is at least about 0.2 mm.

74. The device of claim 72, wherein said cannular electrode is configured to reduce injection of gas into said separation channel.

75. The device of claim 72, wherein the separation channel is a capillary, and wherein the capillary is fluidically connected to the pneumatically actuated valve using a flexible connection.

76. The device of claim 72, wherein the separation channel is a microchannel.

77. The device of claim 72, wherein the separation channel and pneumatically actuated valve are integrated on a microfluidic microchip.

78. The device of claim 72, wherein the loading channel comprises a loading channel solution and the separation channel comprises a separation channel solution, and wherein the sample solution has a lower electrical conductivity than the separation channel solution.

79. The device of claim 72, wherein the at least two electrodes comprise at least two microchannels that are on one end fluidically connected to the loading channel on either side of the fluidic connection between the separation channel and the loading channel and on the other end fluidically connected to a base channel.

80. The device of claim 72, wherein each of the at least two electrodes is a metallic conductors that is electrically connected to a voltage source and the loading channel.

81. A device comprising:

a first, a second, and a third microfluidic channel that are joined to form a three-way junction;

wherein the first microfluidic channel is electrically connected to a first electrode of a forked electrode,

wherein the second microfluidic channel is electrically connected to a second electrode of the forked electrode, and

wherein the first, the second, and the third microfluidic channel are each fluidically connected to a pneumatically actuated valve.

82. A method for performing capillary electrophoresis comprising:

providing the device of claim 72;

providing a separation channel solution to the separation channel;

delivering a composition to the loading channel, wherein the pneumatically actuated valve is used to control the delivery of the composition to the loading channel; and

applying an electric field along the separation channel using the forked electrode;

performing capillary electrophoresis on the composition to separate the component based on size or charge.

83. The method of claim 82, wherein the composition has a lower electrical conductivity than the separation channel solution.

84. The method of claim 82, wherein the composition is concentrated by said applying the electric field.

85. A microfluidic device comprising:

(a) a microfluidic microchip comprising:

(1) a first channel comprising a first valve;

(2) a second channel that intersects the first channel on one side of the first valve;

(3) a third channel that intersects the first channel on the other side of the first valve;

wherein at least one of the second or third channel intersect the first channel at a T-valve or at least one of the second or third channel comprise a second valve, and

wherein the second and third channel each connect to a port; and

(b) a fluid loop that is removably attached to the ports such that fluid can flow from the first channel to the fluid loop.

86. A microfluidic device comprising:

(a) a microchip comprising one or more pneumatically actuated valves; and

(b) a sample loop, wherein the sample loop is fluidically connected to the one or more pneumatically actuated valves through ports in the microfluidic microchip, and wherein the sample loop has a fixed volume and the sample loop is removable.

5 87. The device of claim 85, wherein said pneumatically actuated valves are actuated by one or more pneumatic channels in the microfluidic device.

88. The device of claim 85, wherein the sample loop comprises a capillary tube.

89. The device of claim 85, wherein the sample loop is fluidically connected to a pass-through microfluidic channel at a first junction and a second junction, and wherein a pass-through microfluidic pneumatically actuated valve is positioned in the pass-through microfluidic channel
10 between the first and second junctions.

90. The device of claim 89, wherein at least one junction comprises a T-valve, wherein closure of the T-valve does not prevent passage of fluid through the pass-through microfluidic channel.

91. The device of claim 89, wherein the sample loop is connected to the pass-through microfluidic channel through first and second channels, and wherein at least one of the first and the second
15 channel comprise a sample loop valve.

92. A method for delivering a fixed volume of fluid to a microfluidic device comprising:
configuring a device with a sample loop comprising a desired volume, wherein the sample loop is removable;
using one or more pneumatically actuated valves on a microfluidic device to fill the sample loop
20 with the fixed volume of the fluid; and
delivering the fluid to the microfluidic device.

93. The method of claim 92, wherein the sample loop and a pass-through microfluidic channel are fluidically connected at a first junction and a second junction, and wherein at least one junction comprises a T-valve.

25 94. The method of claim 93, wherein the pass-through microfluidic channel comprises a pass-through microfluidic valve positioned between the first junction and the second junction.

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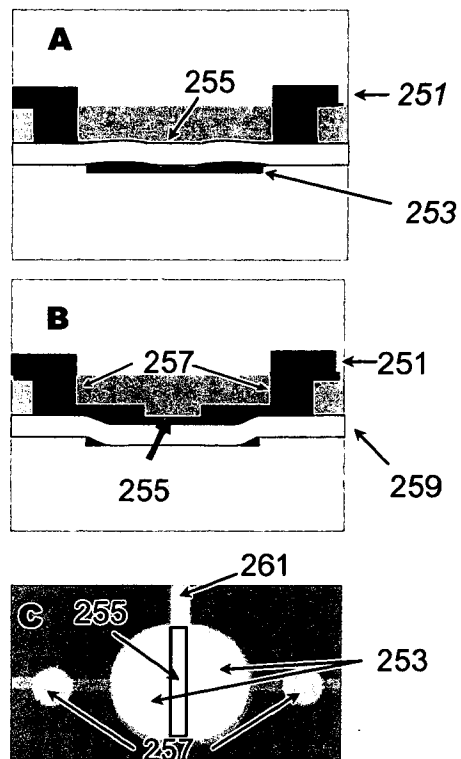


Figure 1

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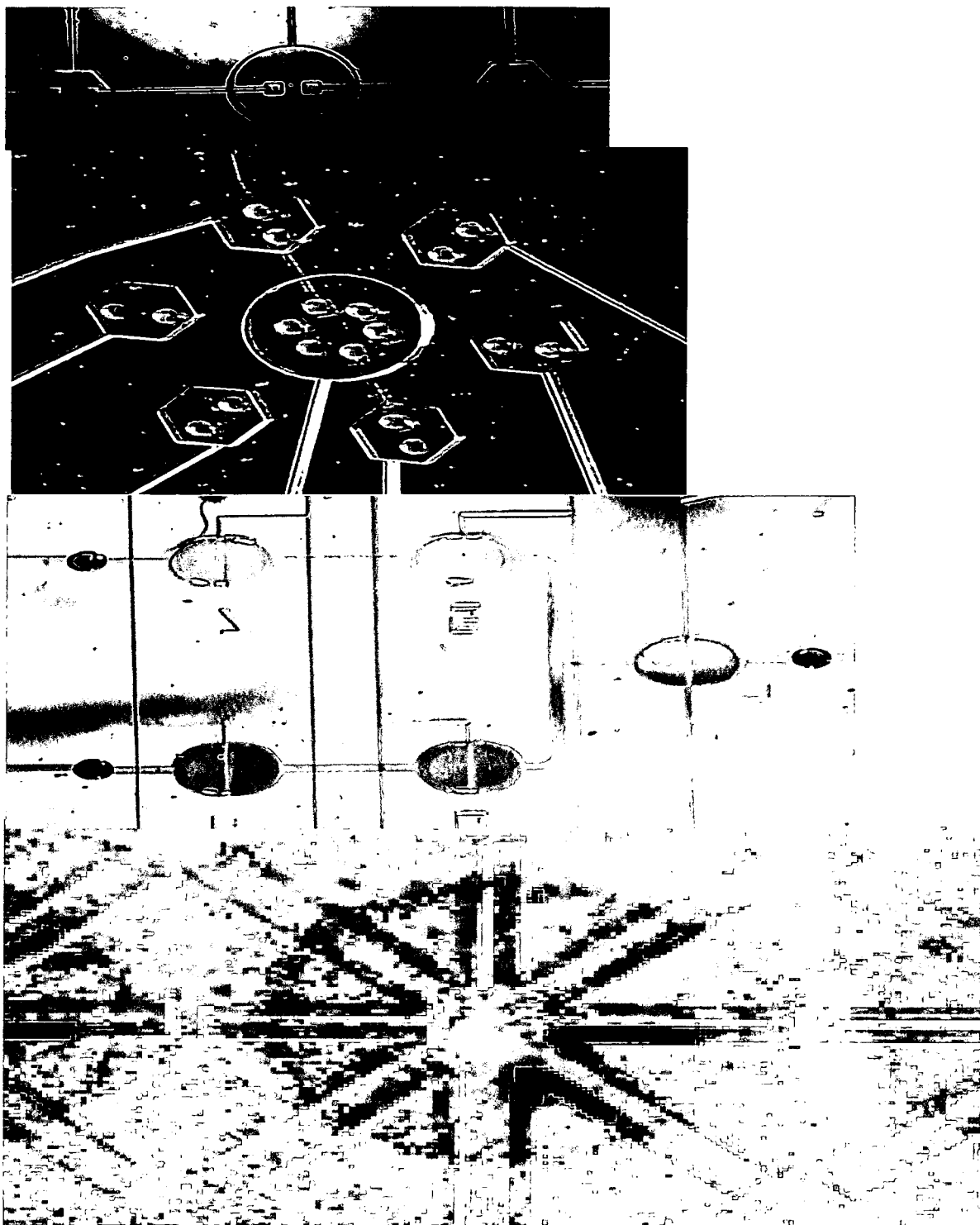


Figure 2

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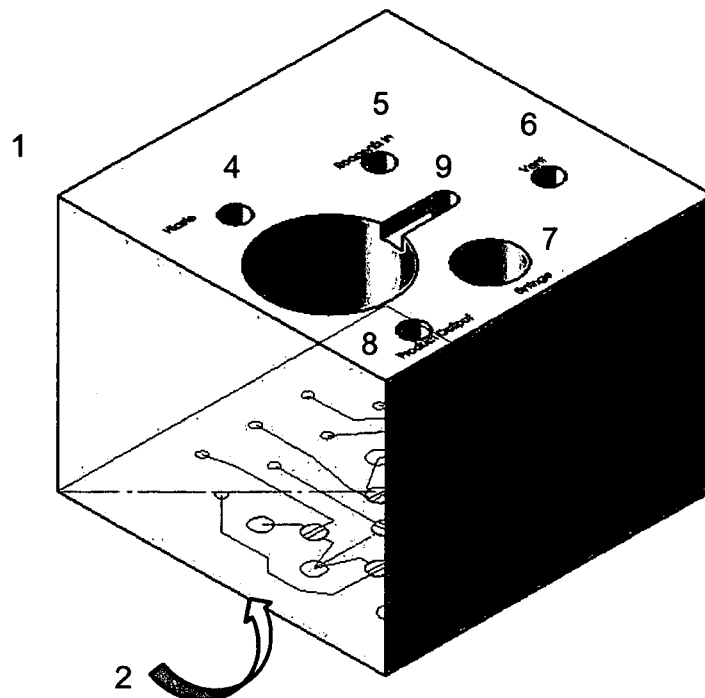


Figure 3

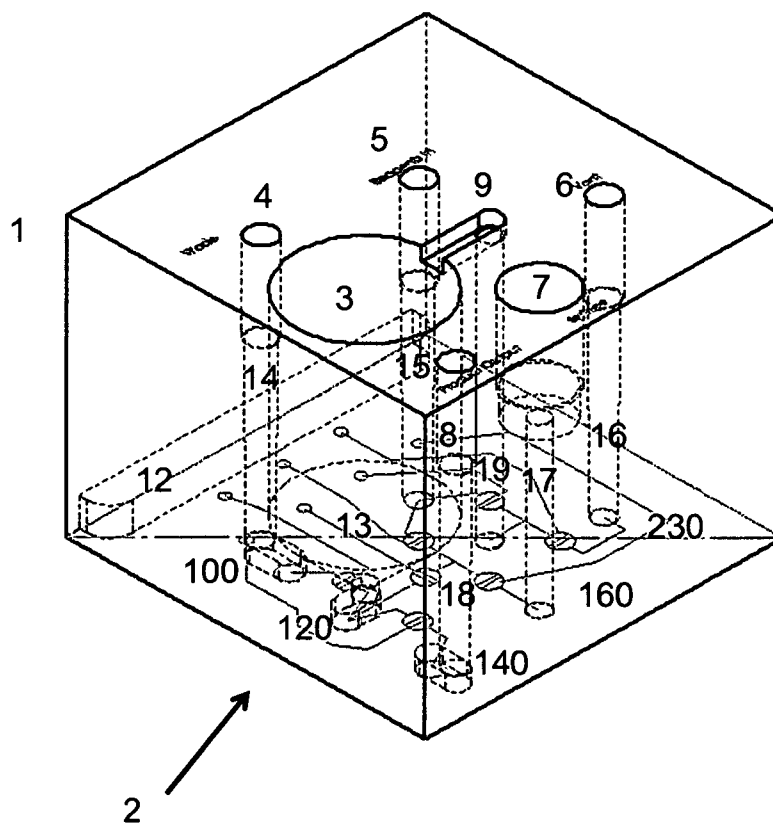


Figure 4

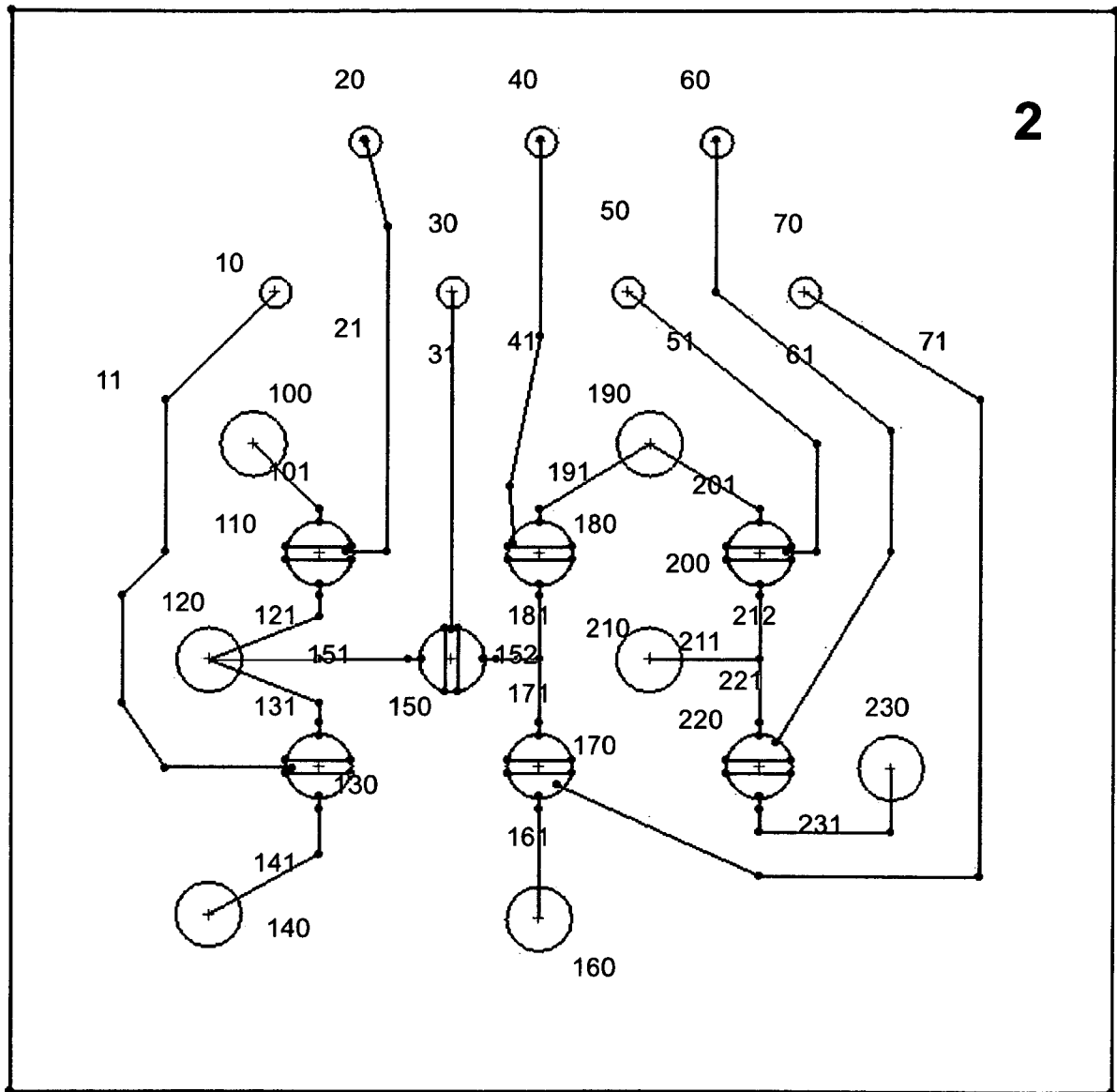


Figure 5

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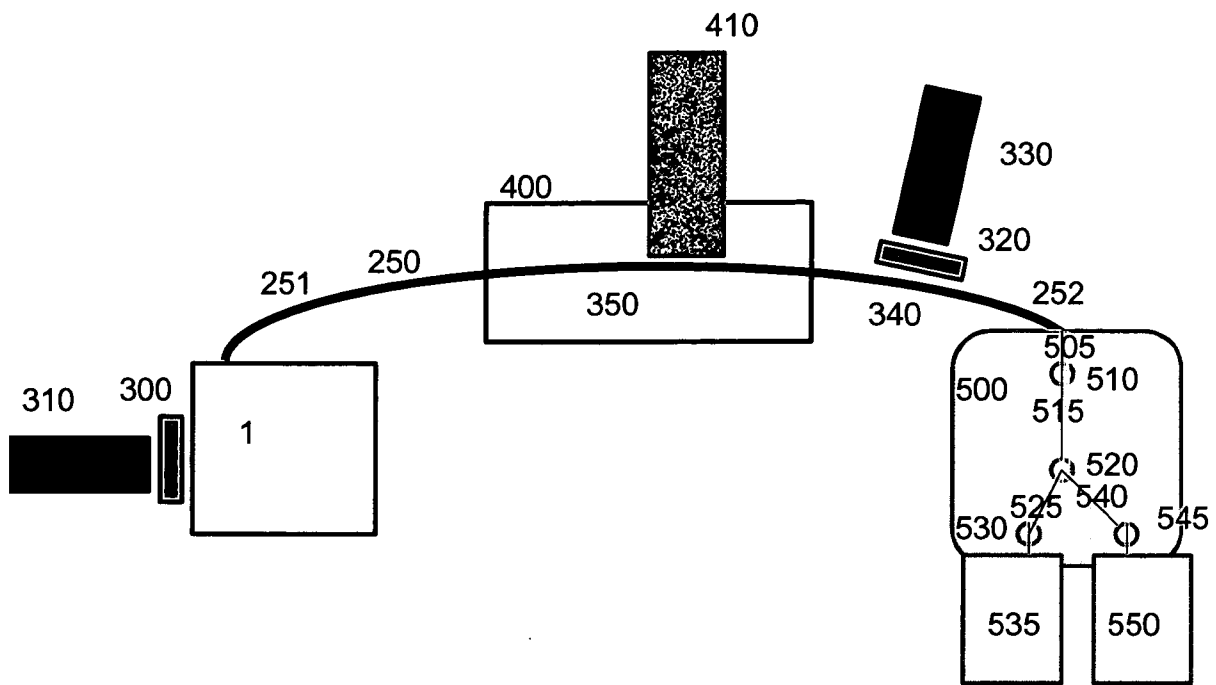


Figure 6

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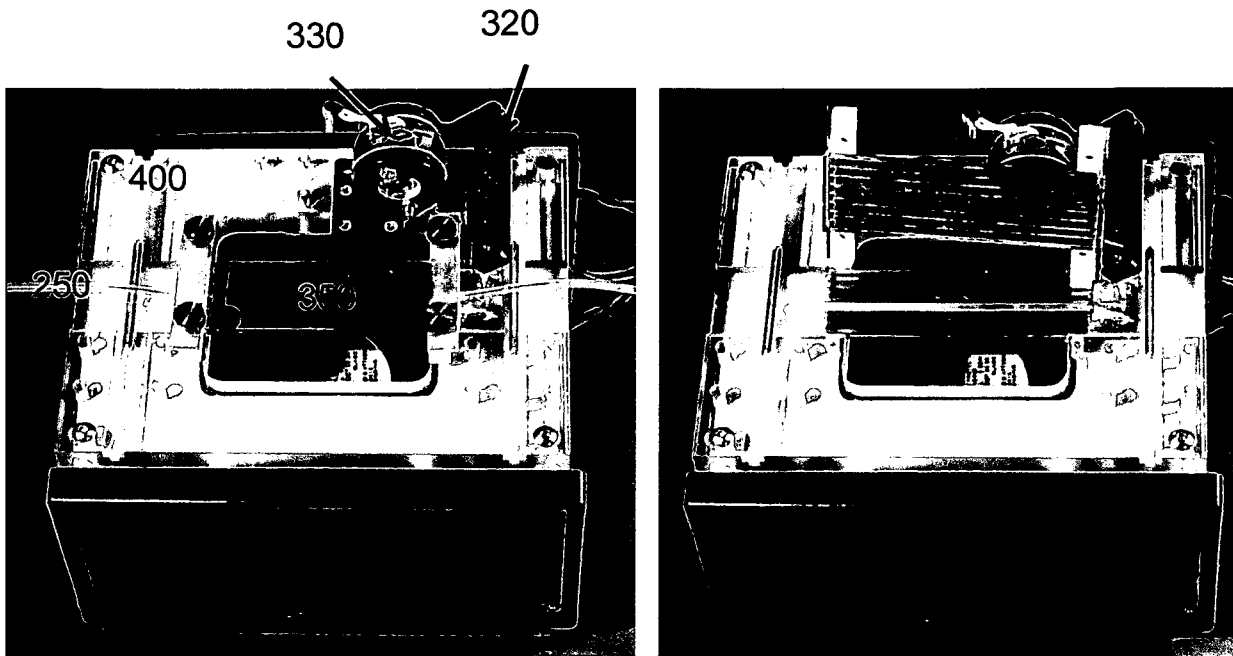


Figure 7

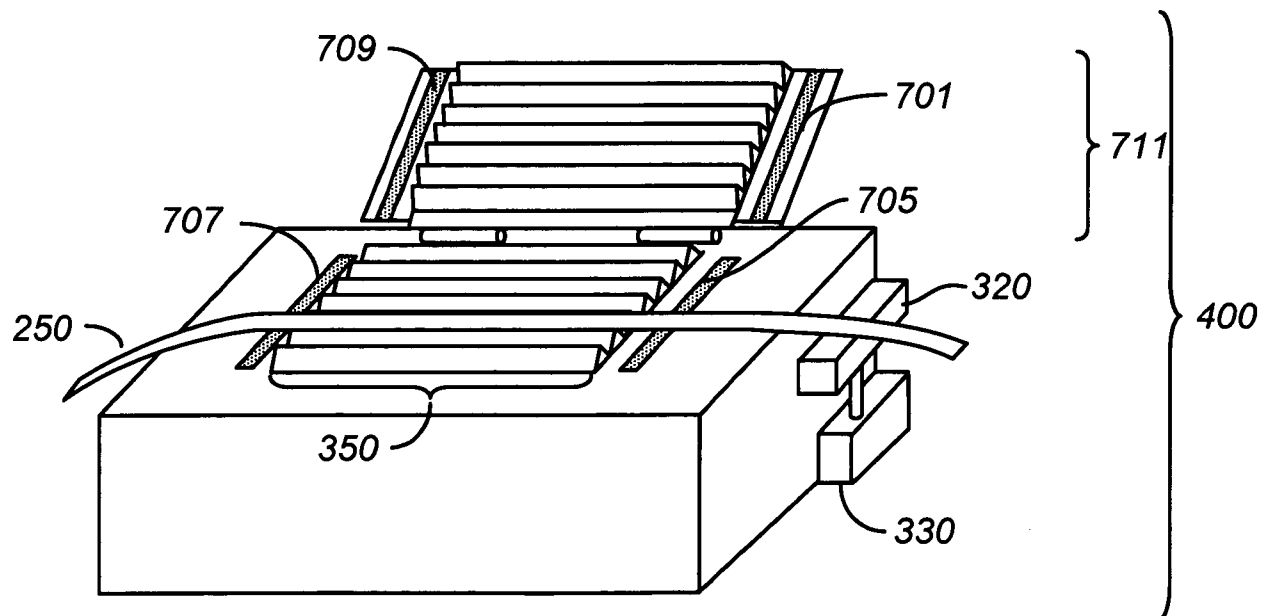


Figure 8

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PowerPlex16 reaction amplified in Teflon based Reaction Chambers on an MBI Thermocycling Subsystem device with separation and detection on the MegaBACE1000

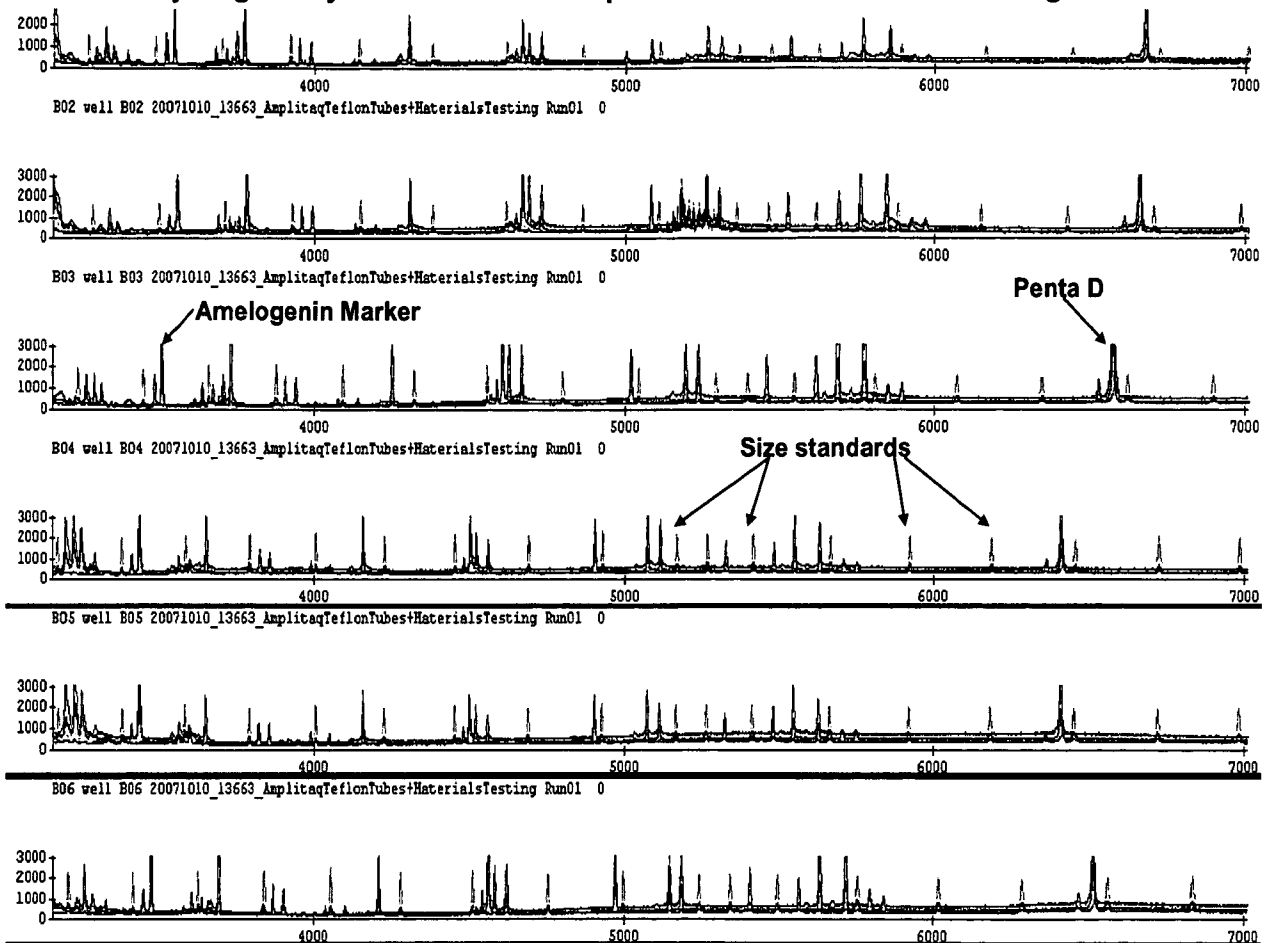


Figure 9

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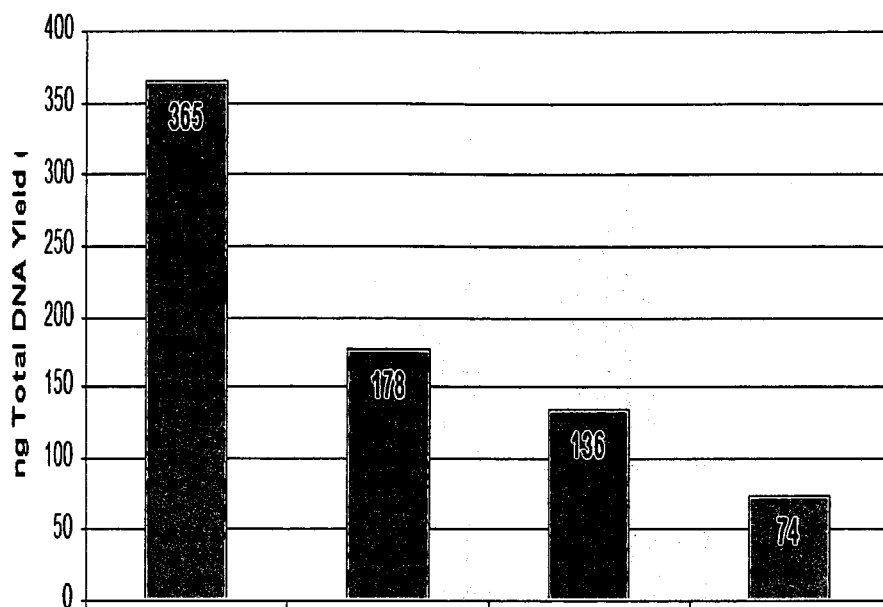


Figure 10

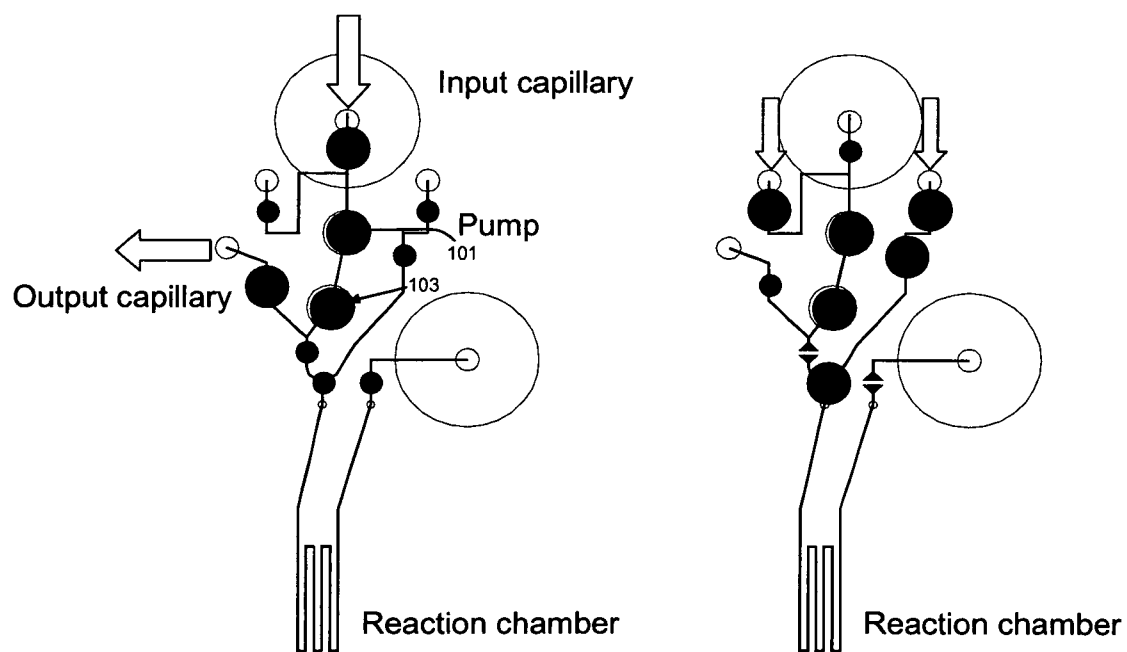


Figure 11

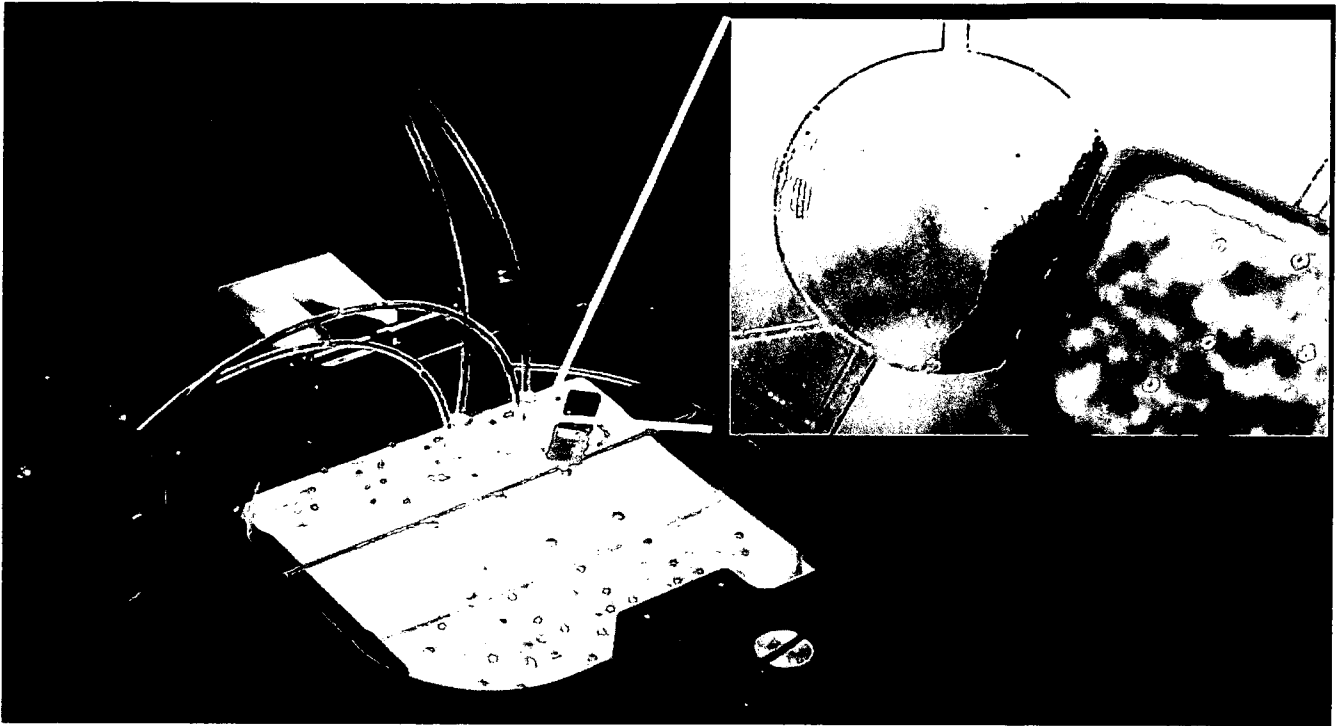


Figure 12

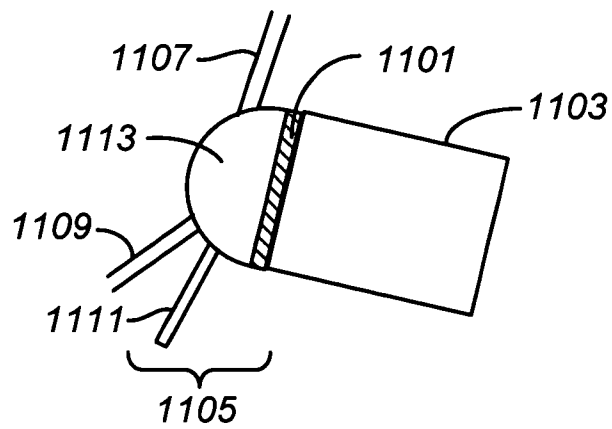


Figure 13

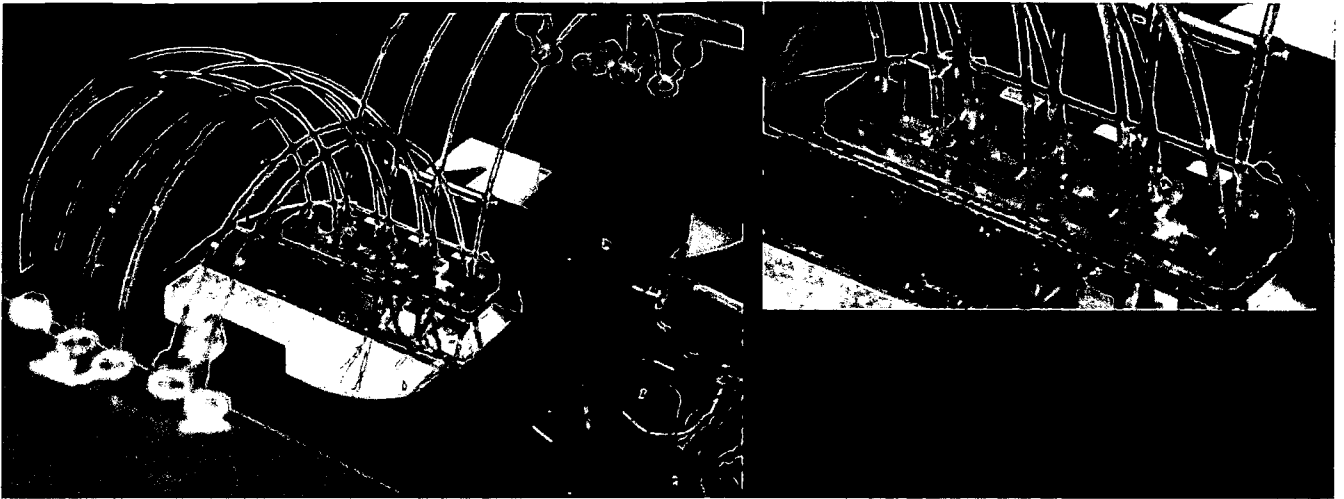


Figure 14.

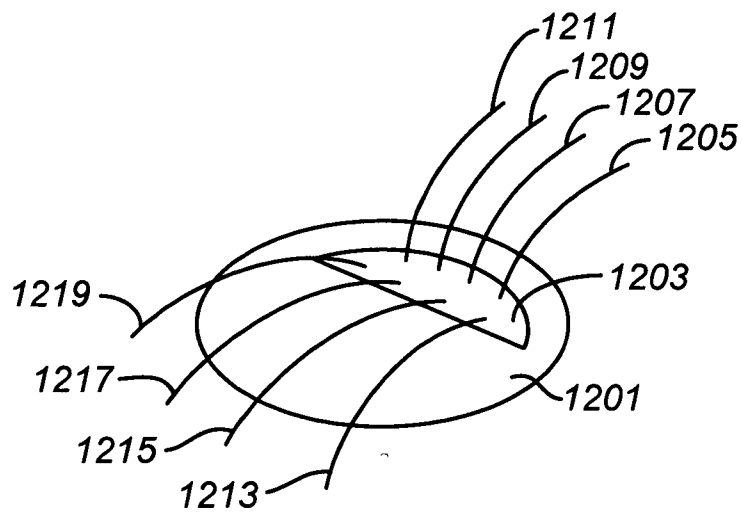


Figure 15.

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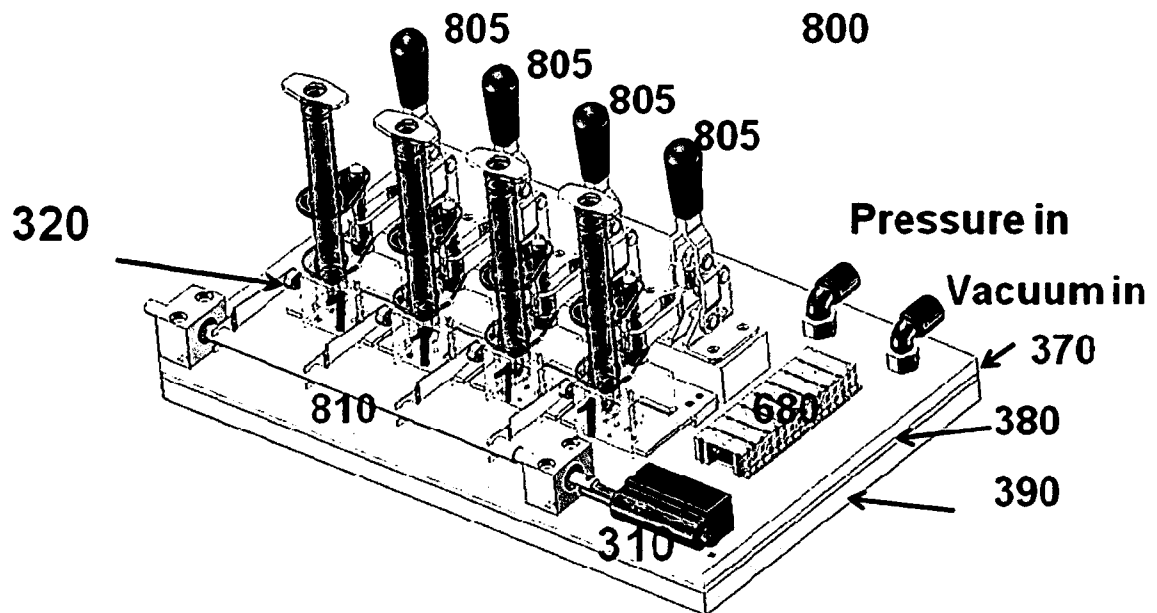


Figure 16

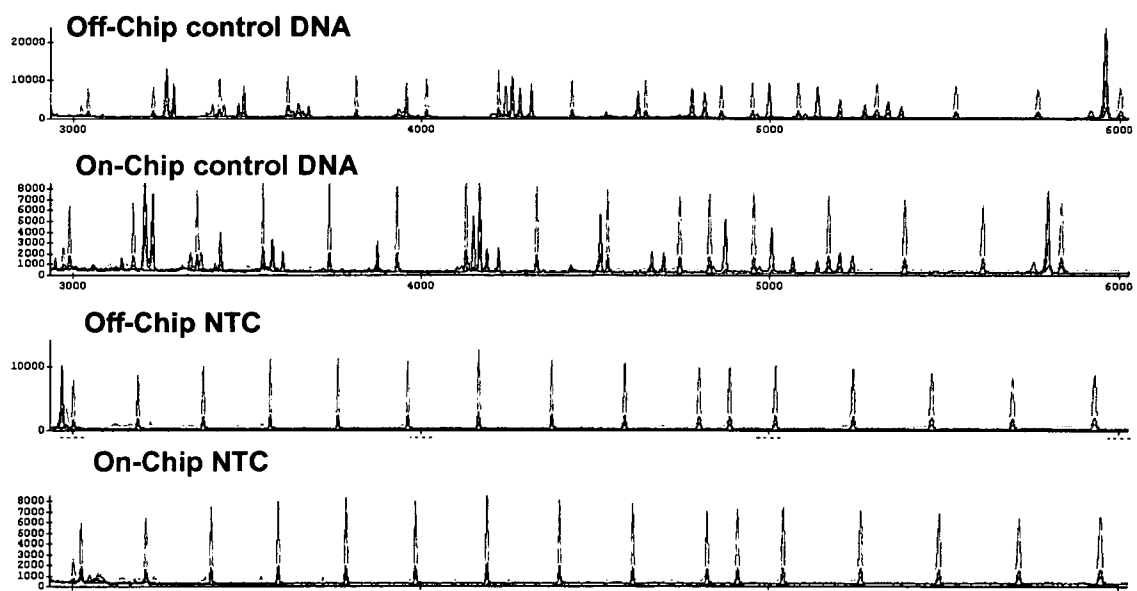


Figure 17

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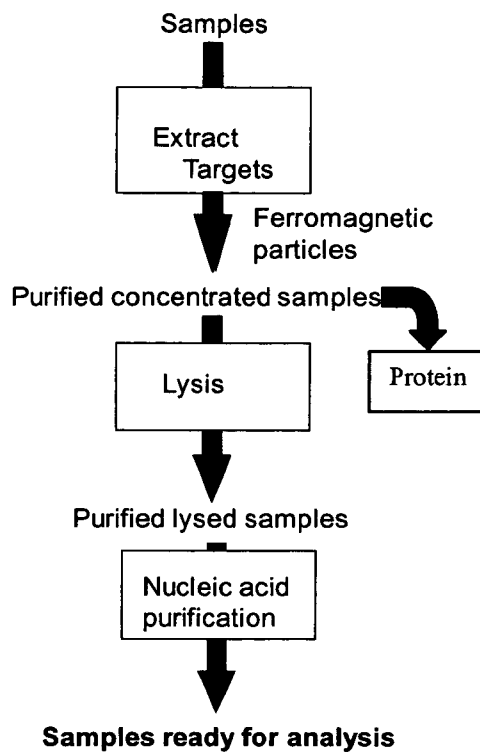


Figure 18

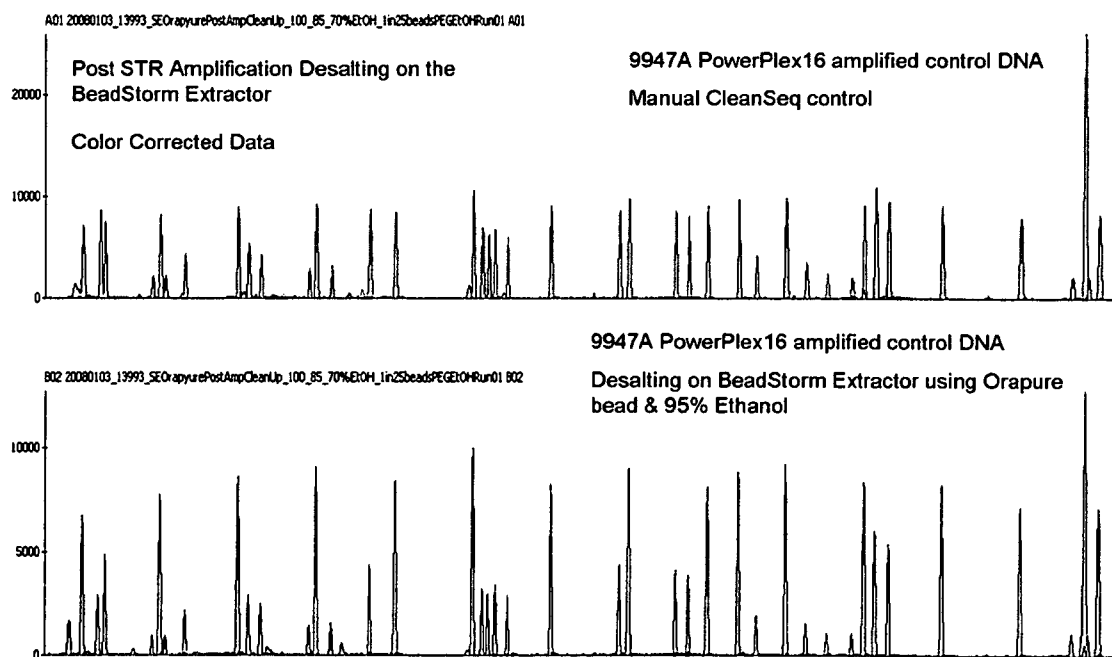


Figure 19

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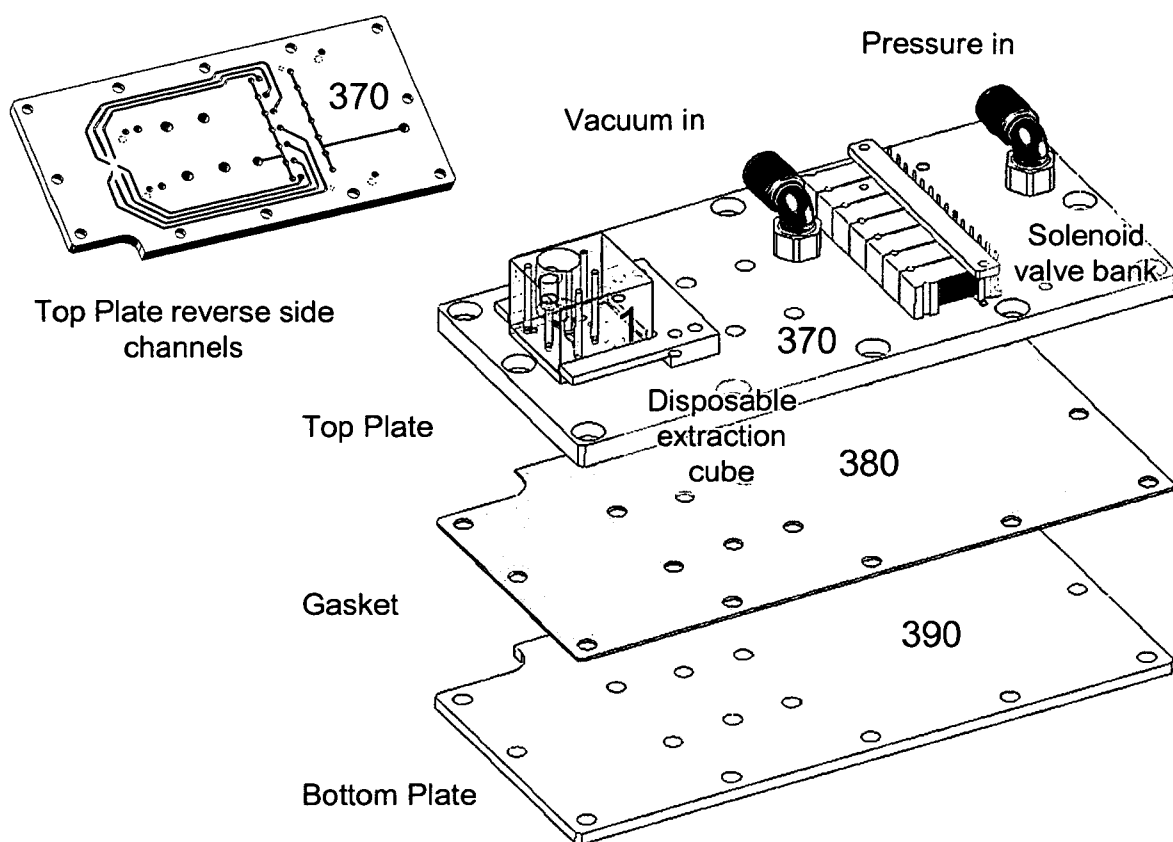


Figure 20

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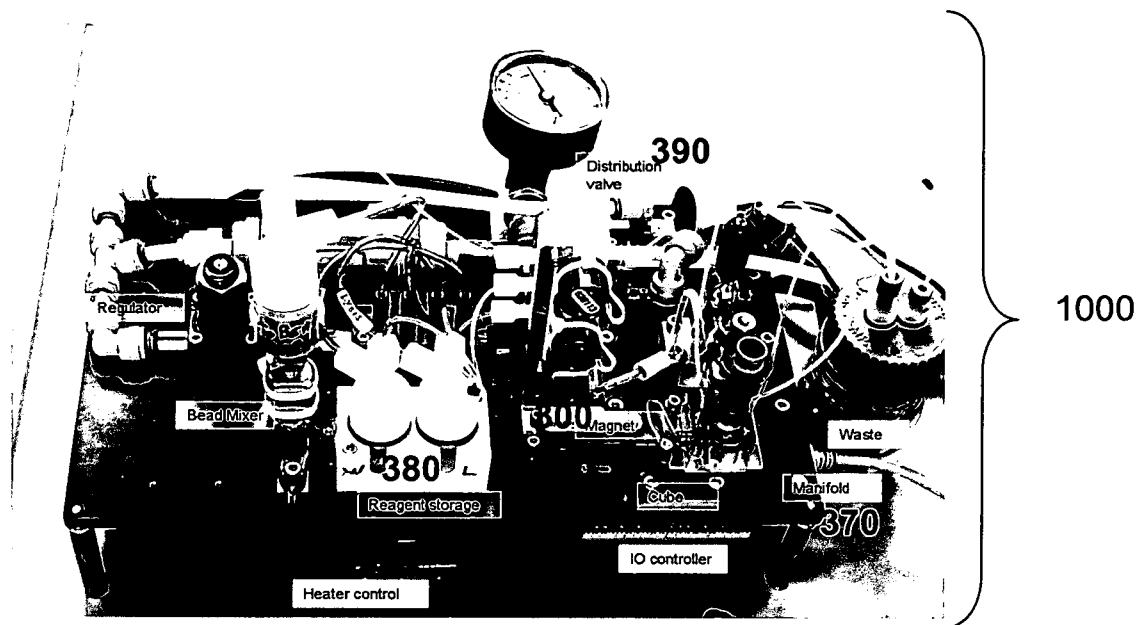


Figure 21

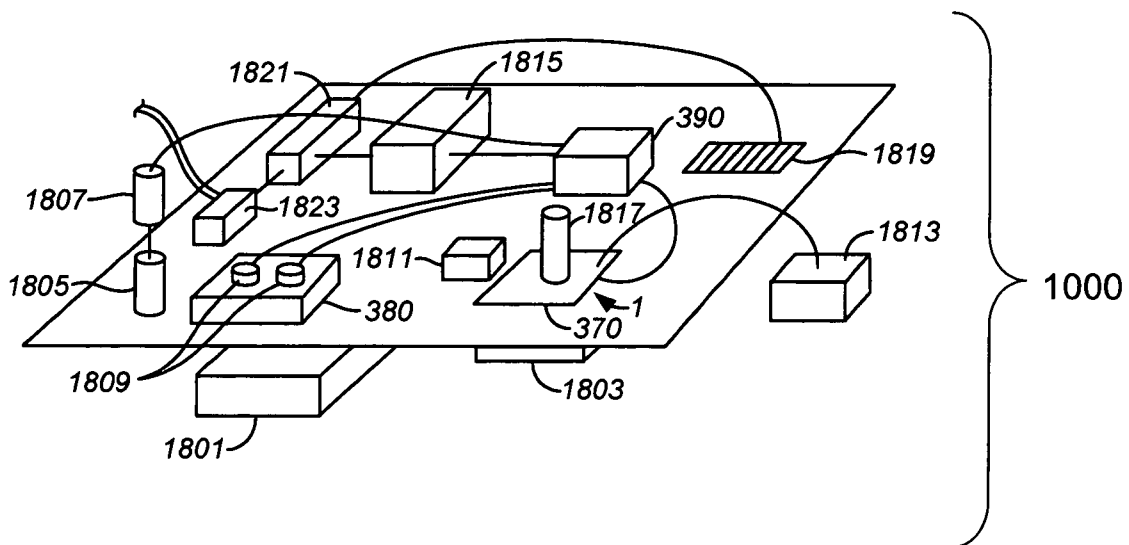


Figure 22

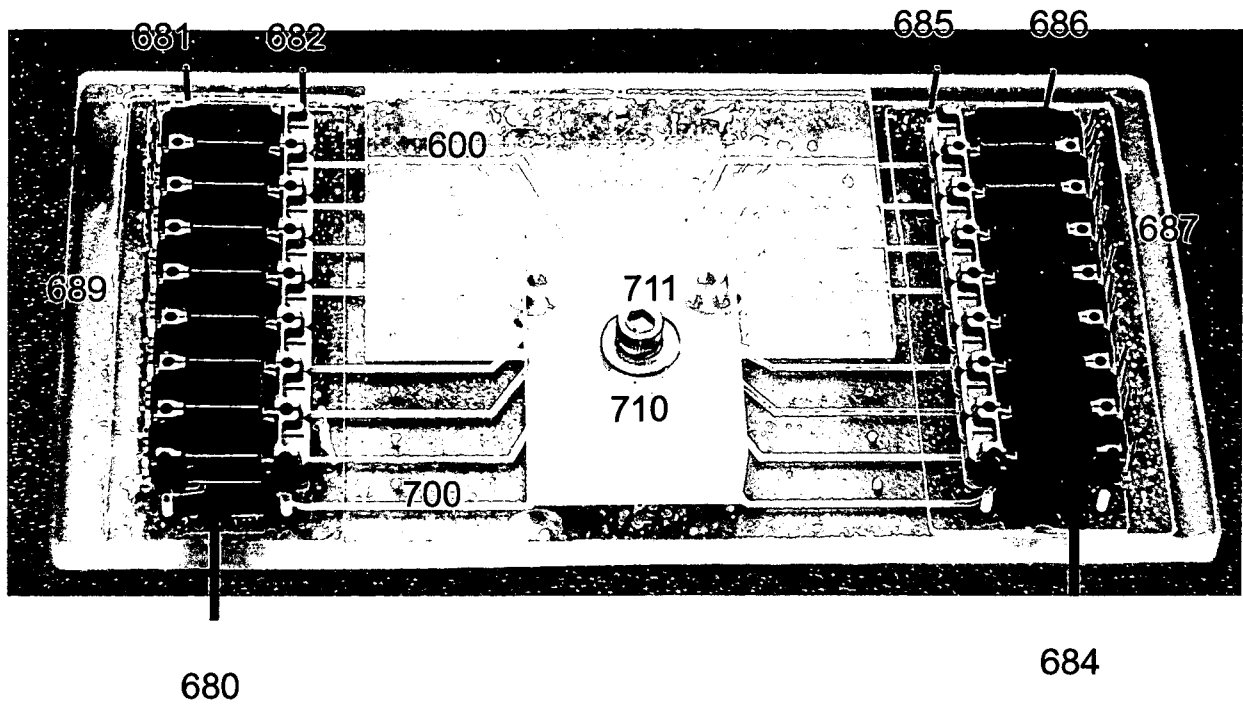


Figure 23

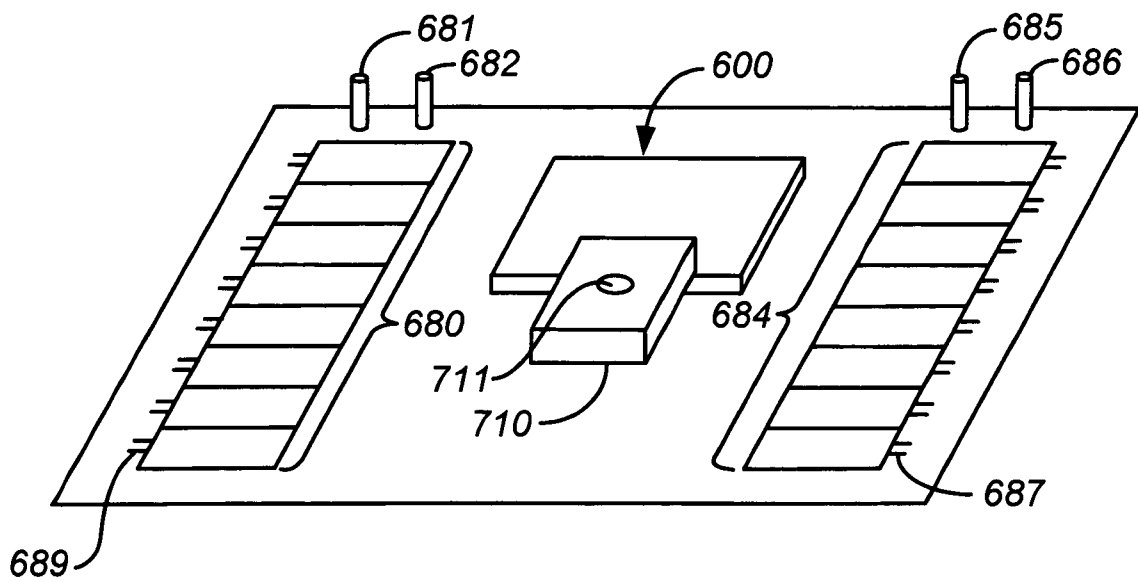


Figure 24

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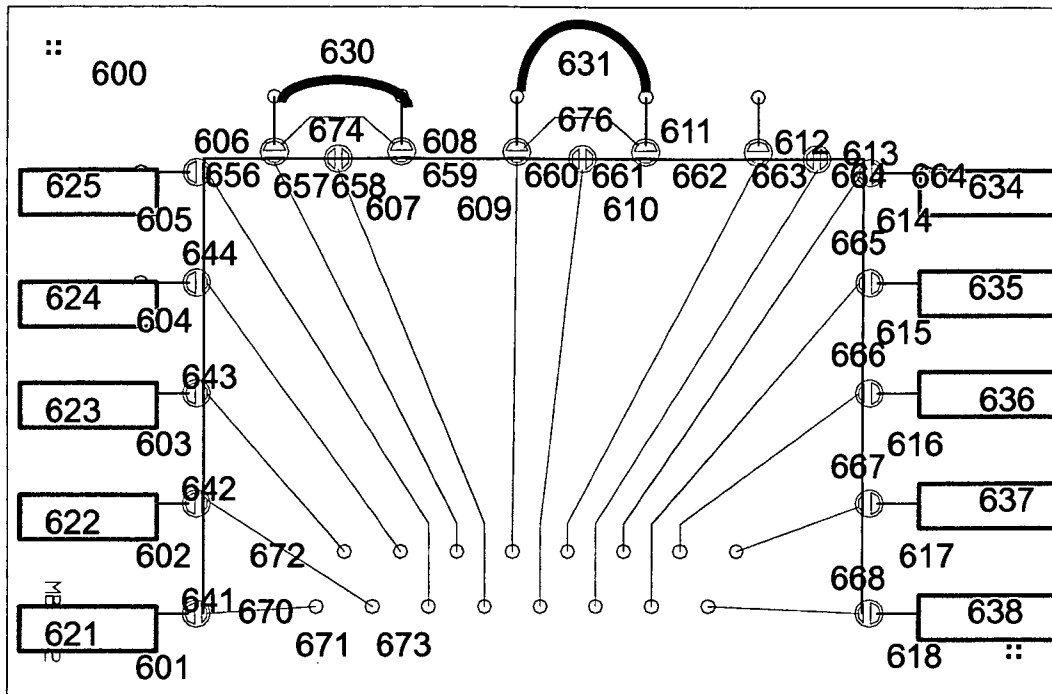


Figure 25

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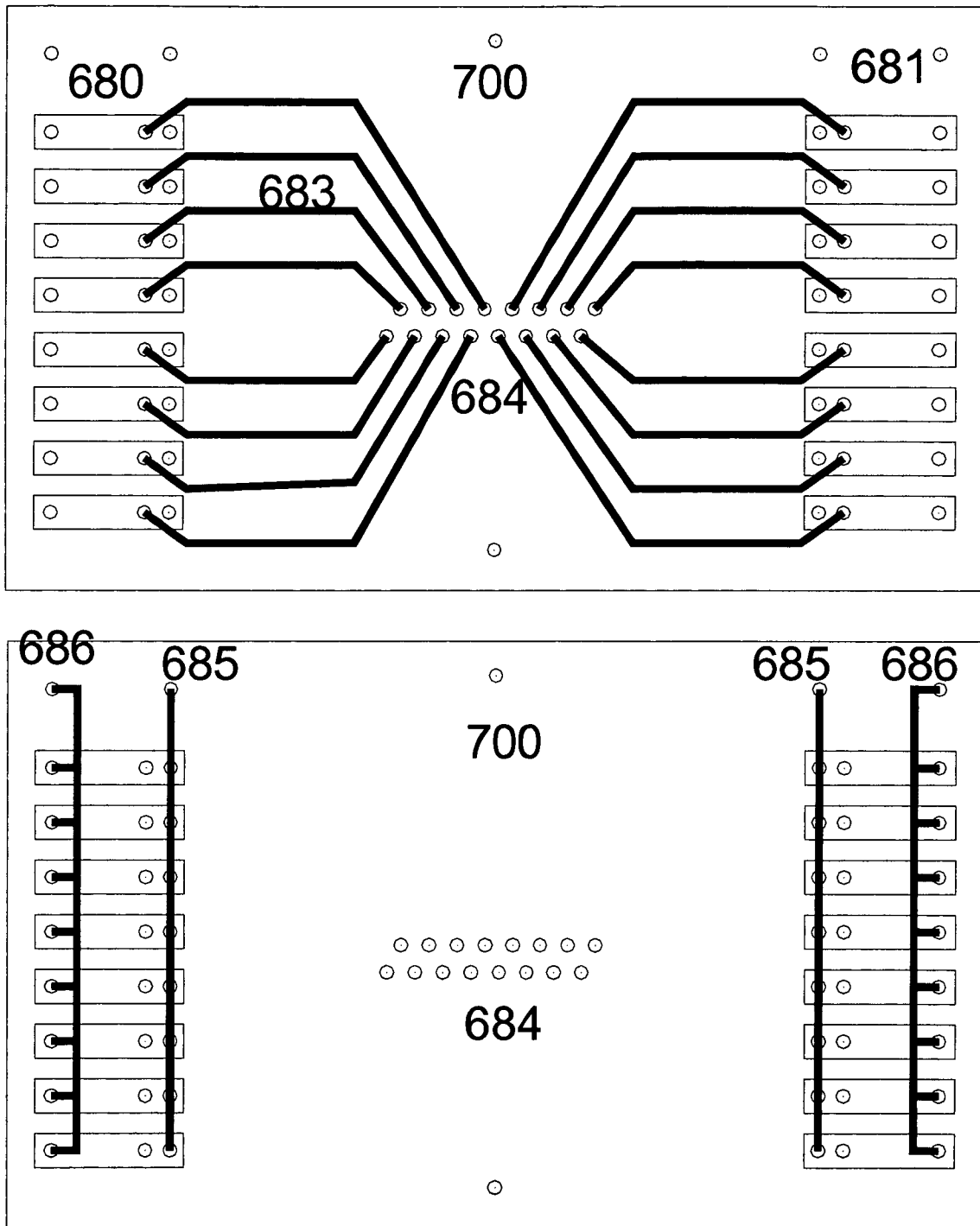


Figure 26

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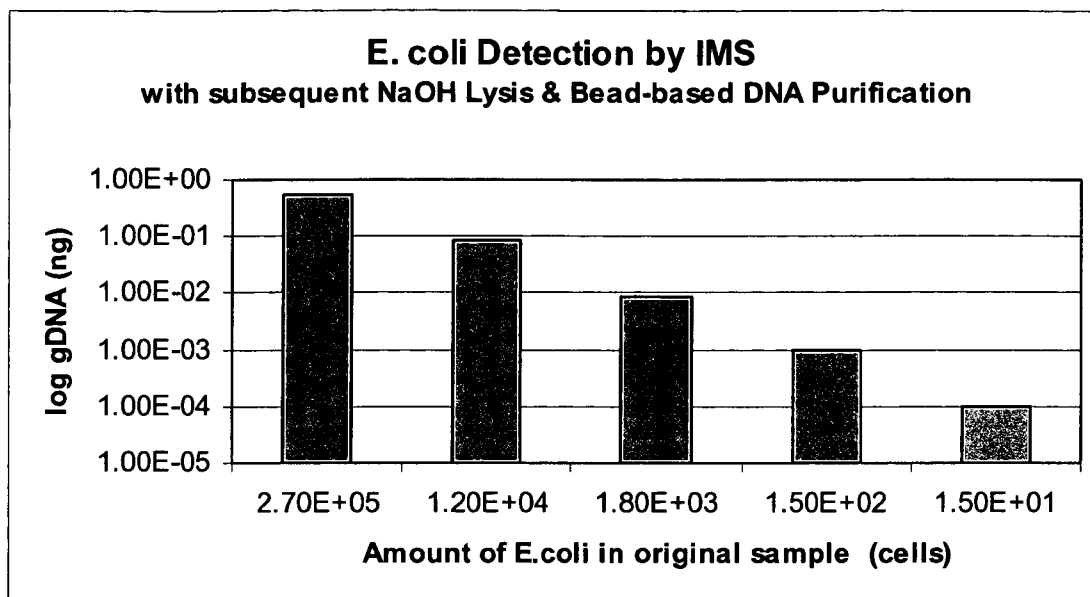


Figure 27

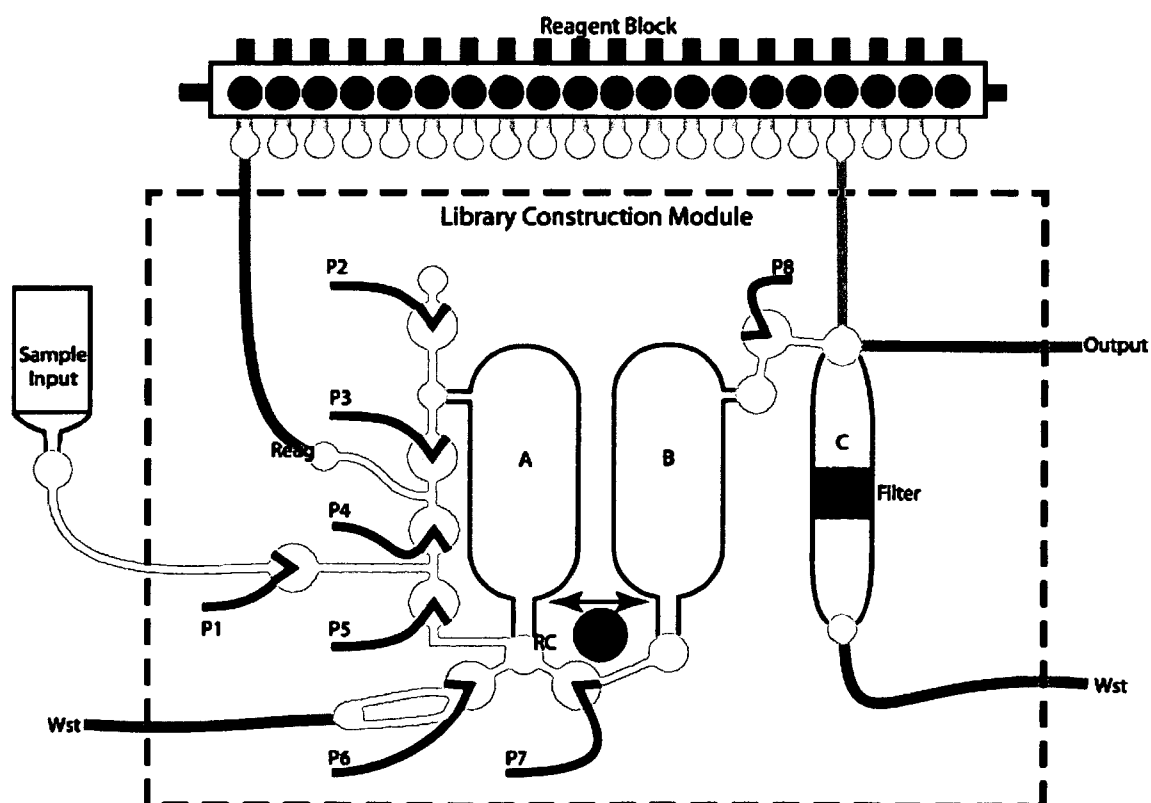


Figure 28

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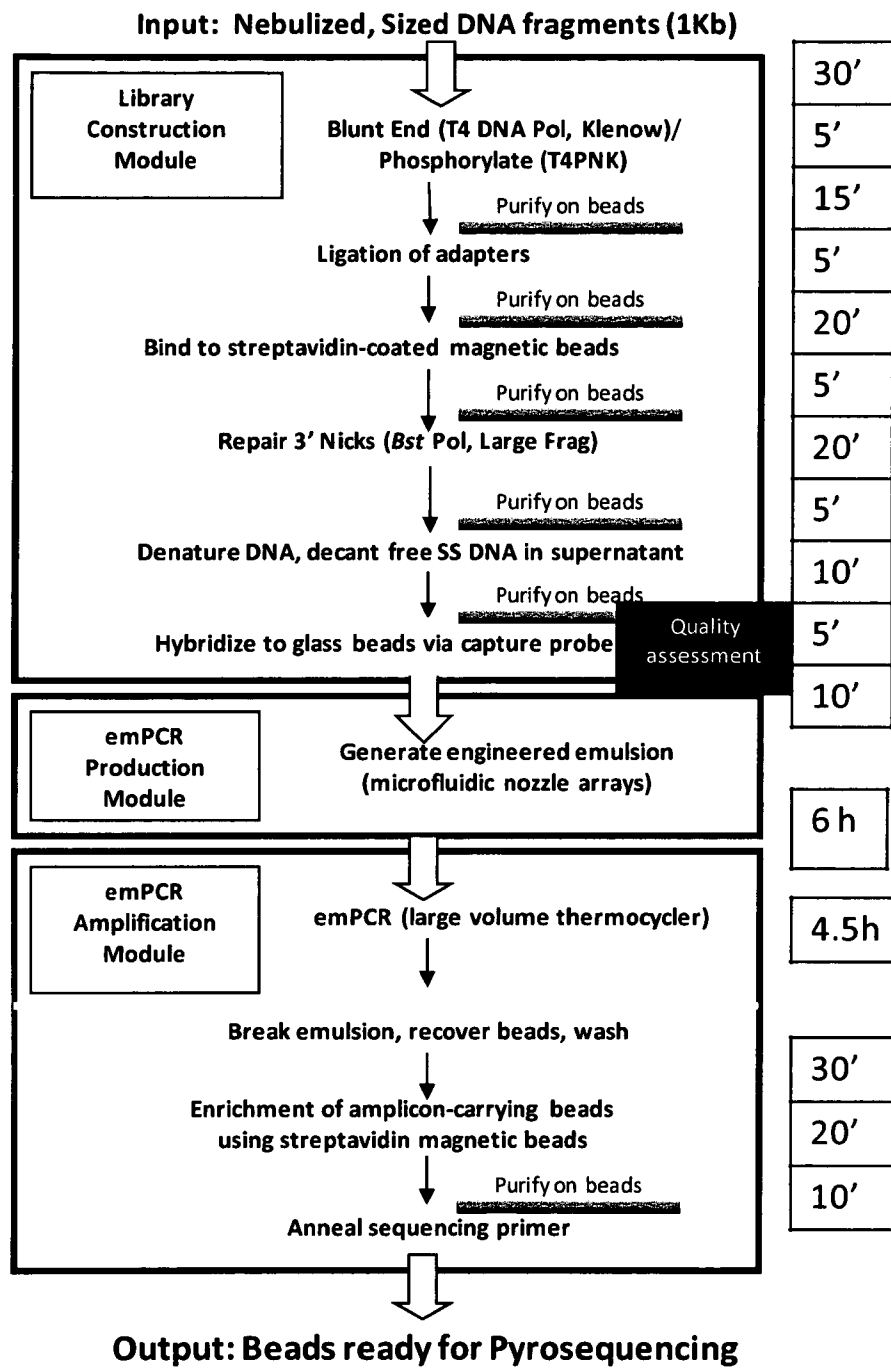
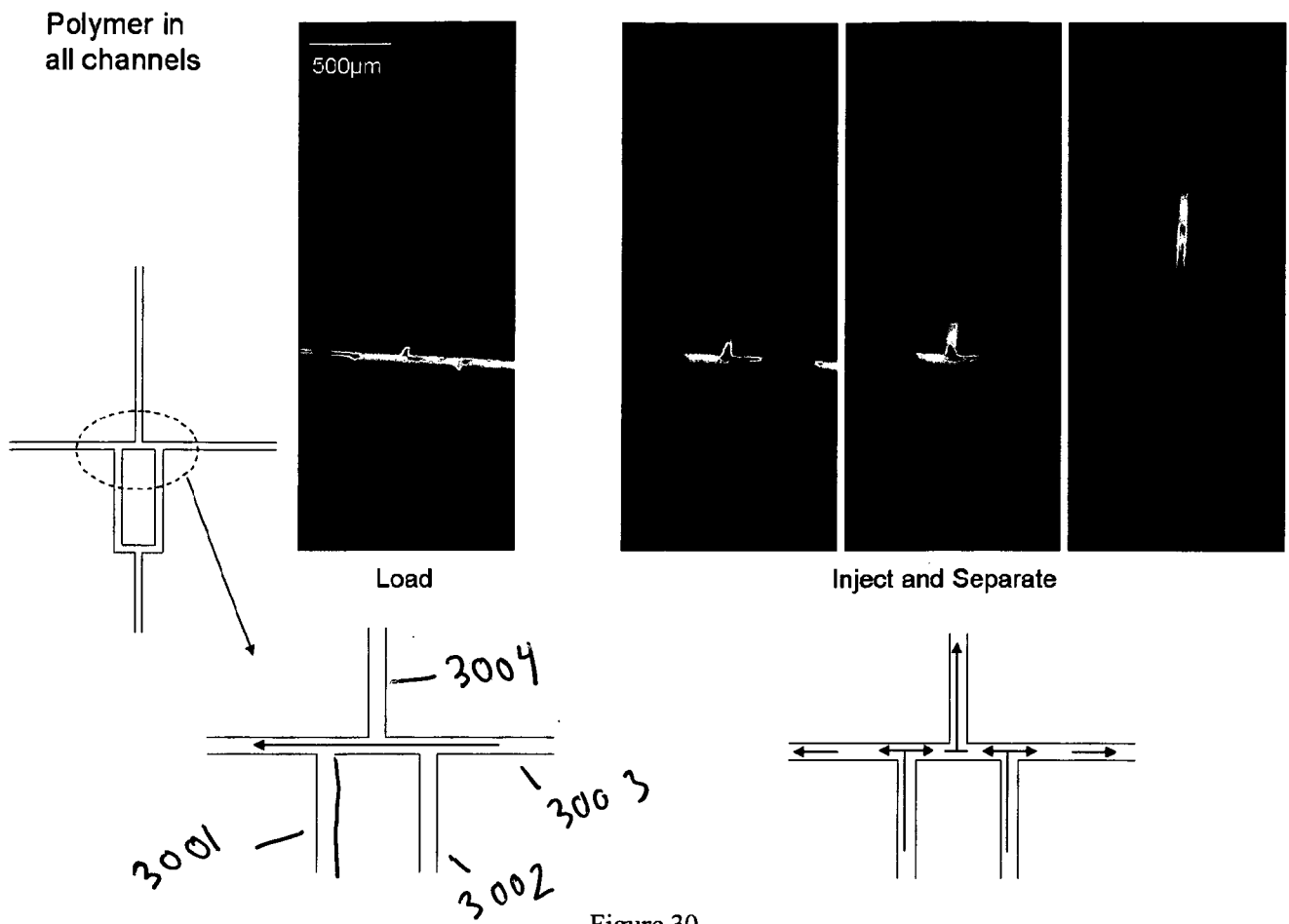


Figure 29



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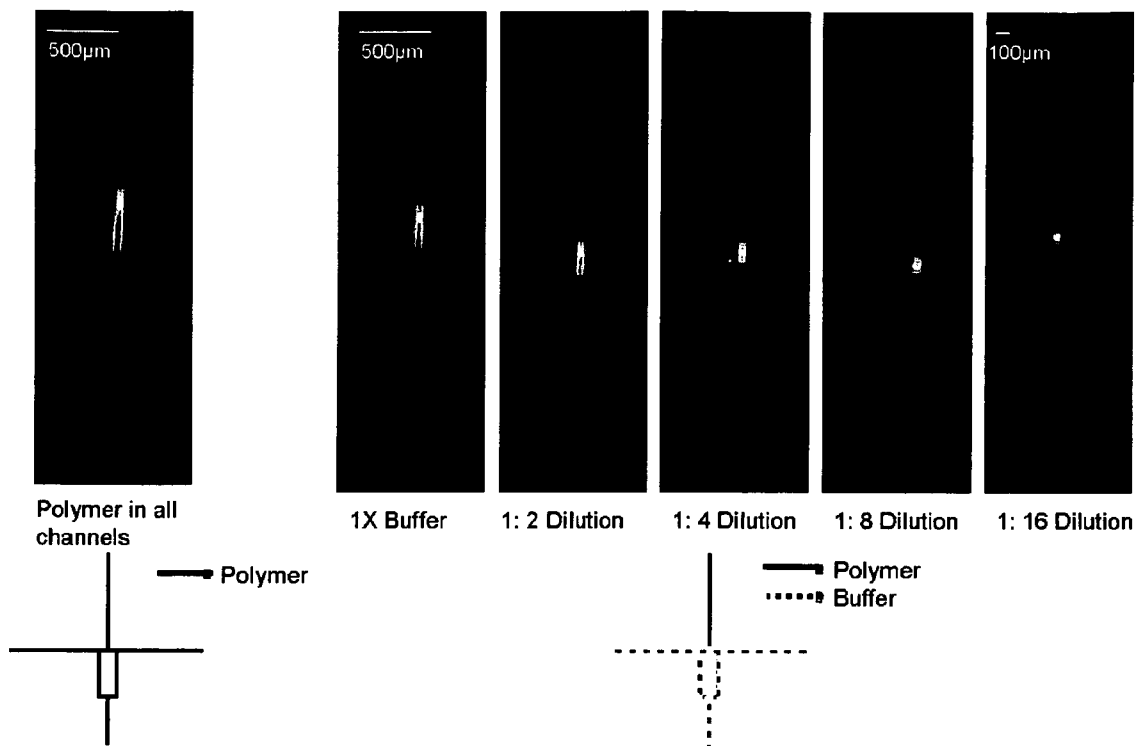


Figure 31

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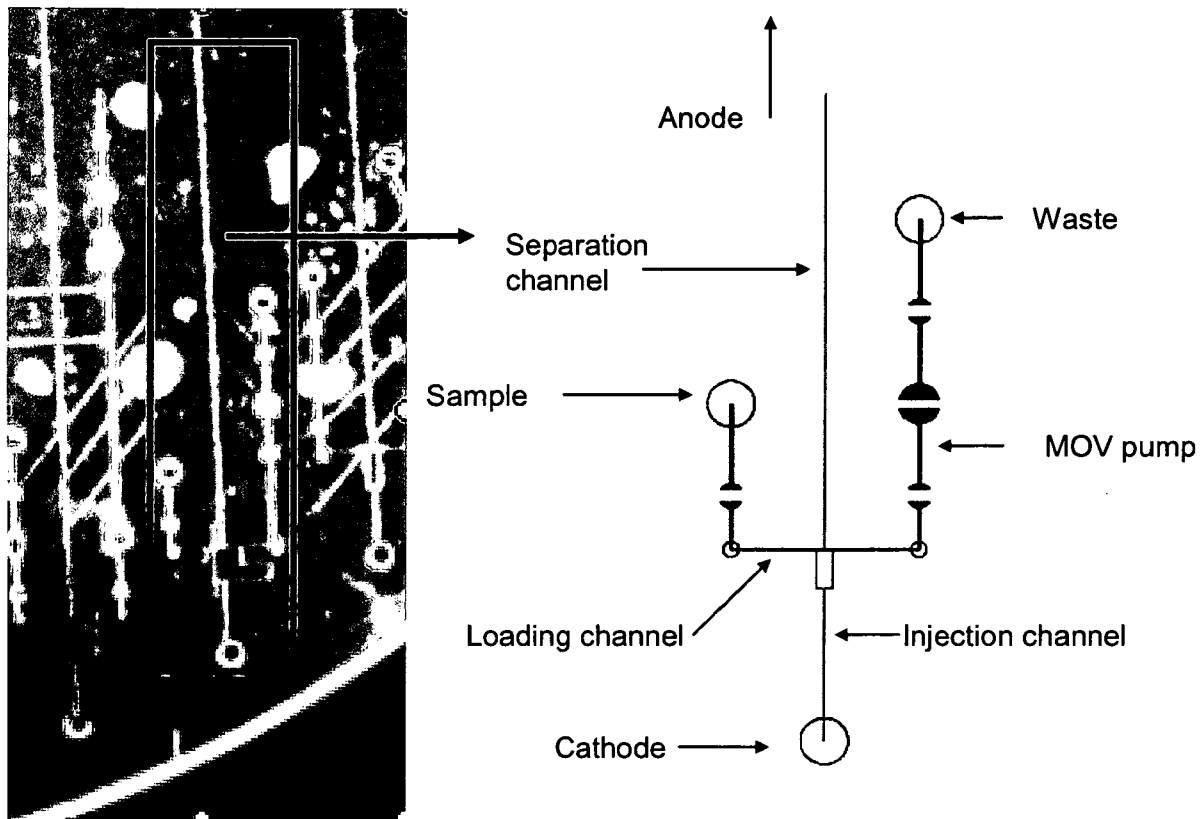
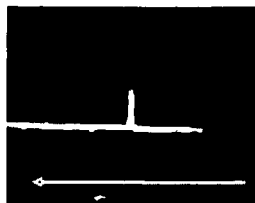


Figure 32

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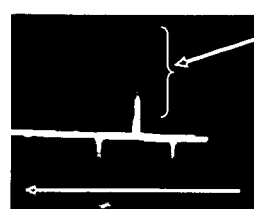
STR Sample Loading



Initiate sample
pumping across
loading channel



Pump
through-stroke

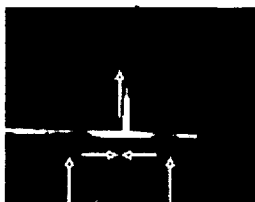


Complete pump
loading

Polymer
filled
Channel

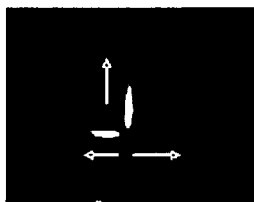
STR Sample Injection

Initiate Injection



Applying high
voltage across
the forked
injector

Pull back



Apply high voltage across
load channel to prevent
sample bleed into
separation channel

Electrophoresis



Apply high
voltage across
cathode and
anode

Figure 33

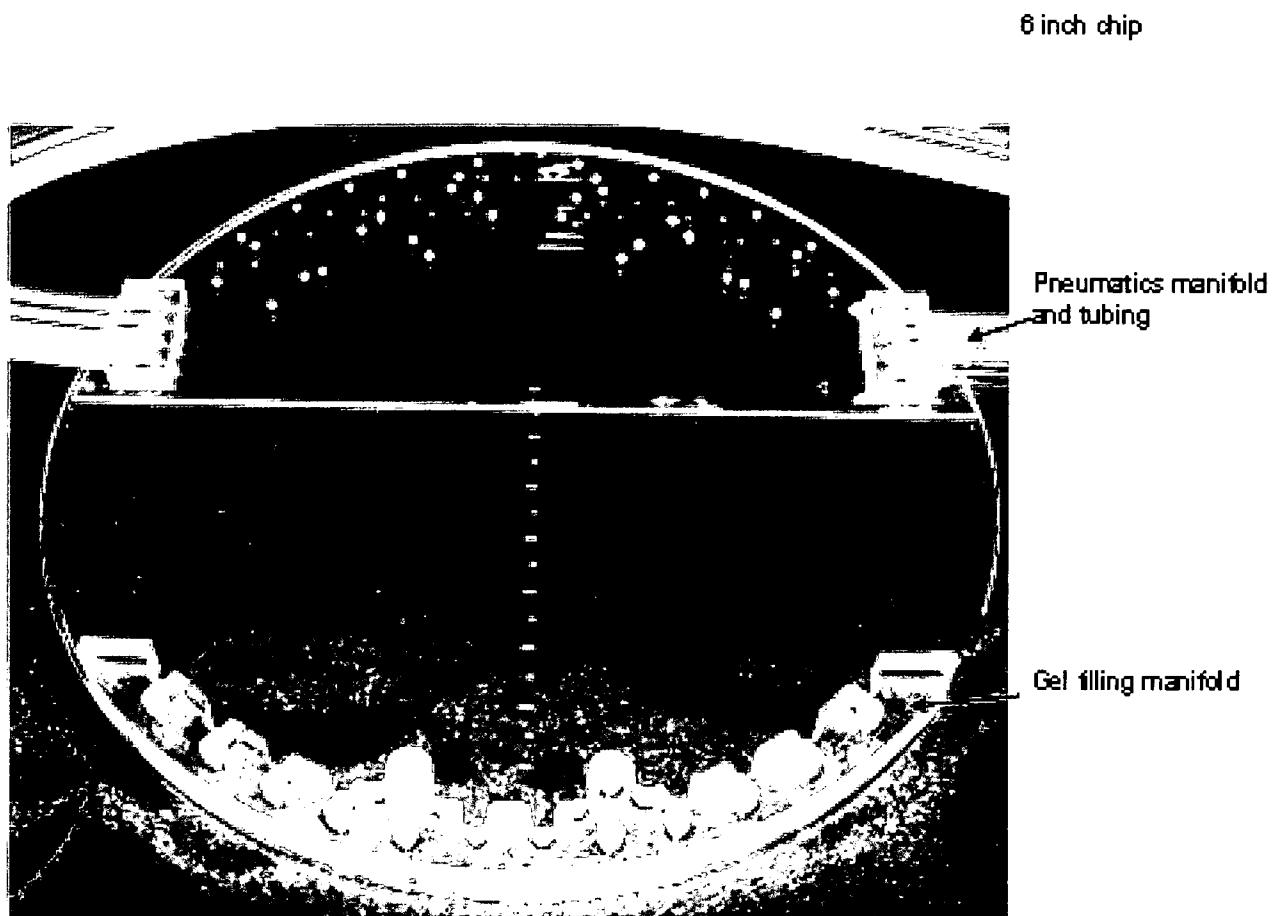


Figure 34

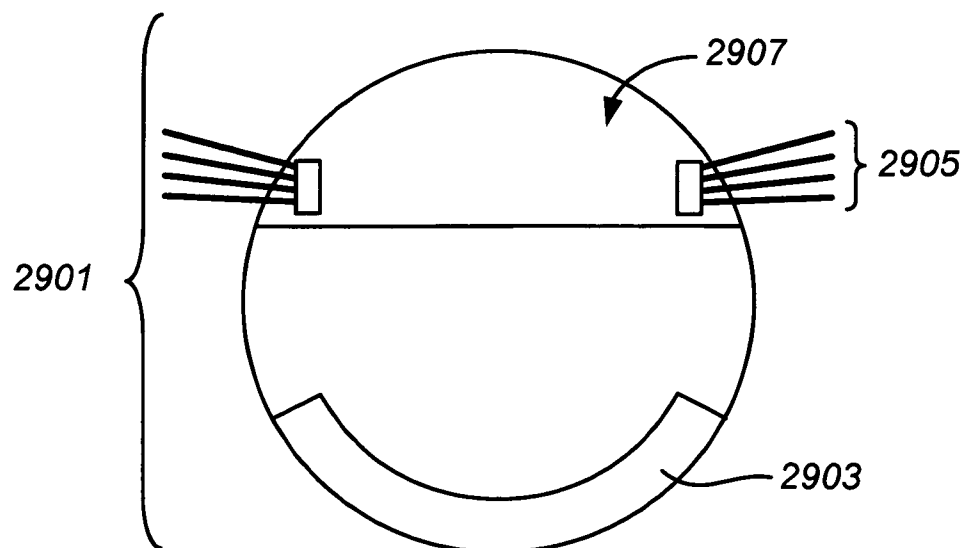


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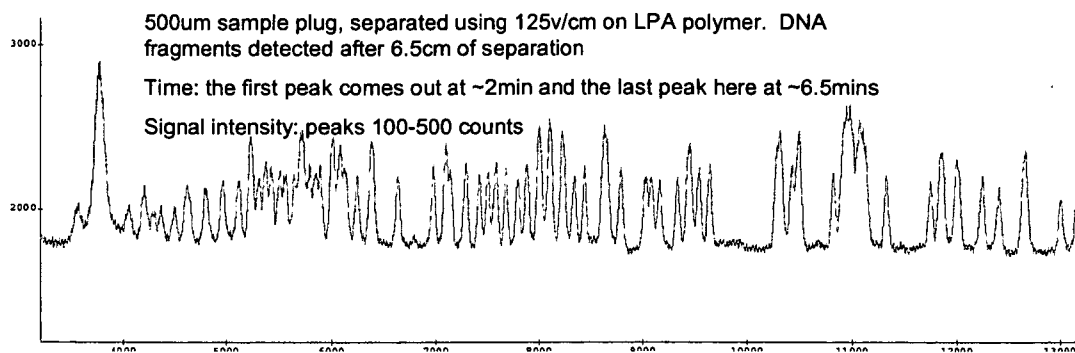


Figure 36

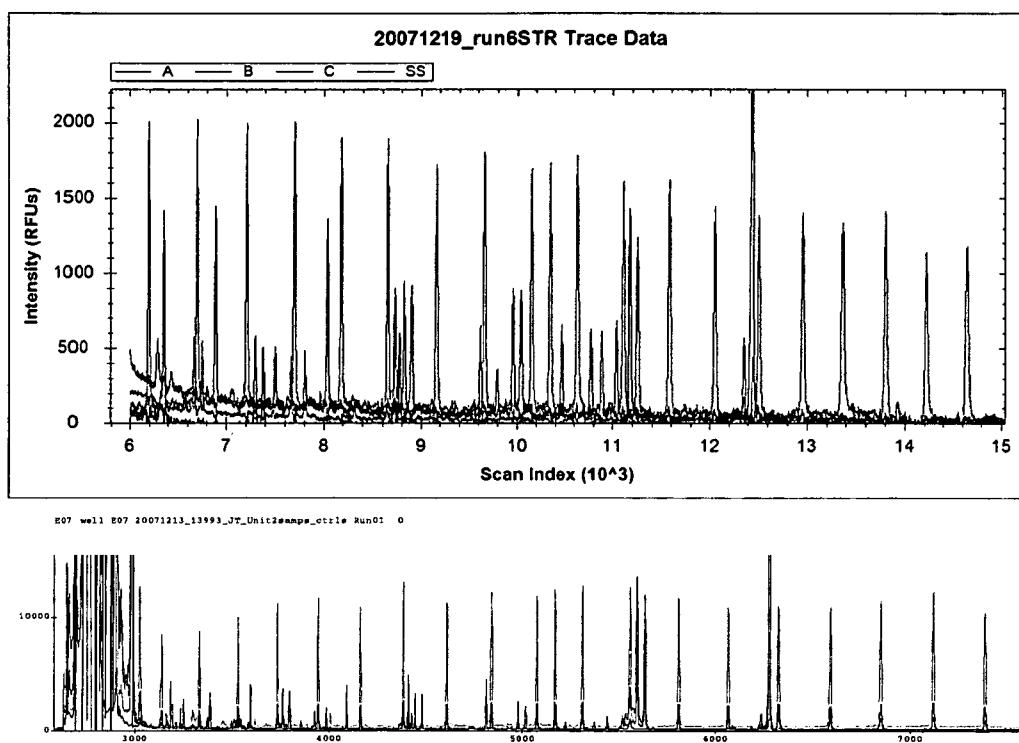


Figure 37

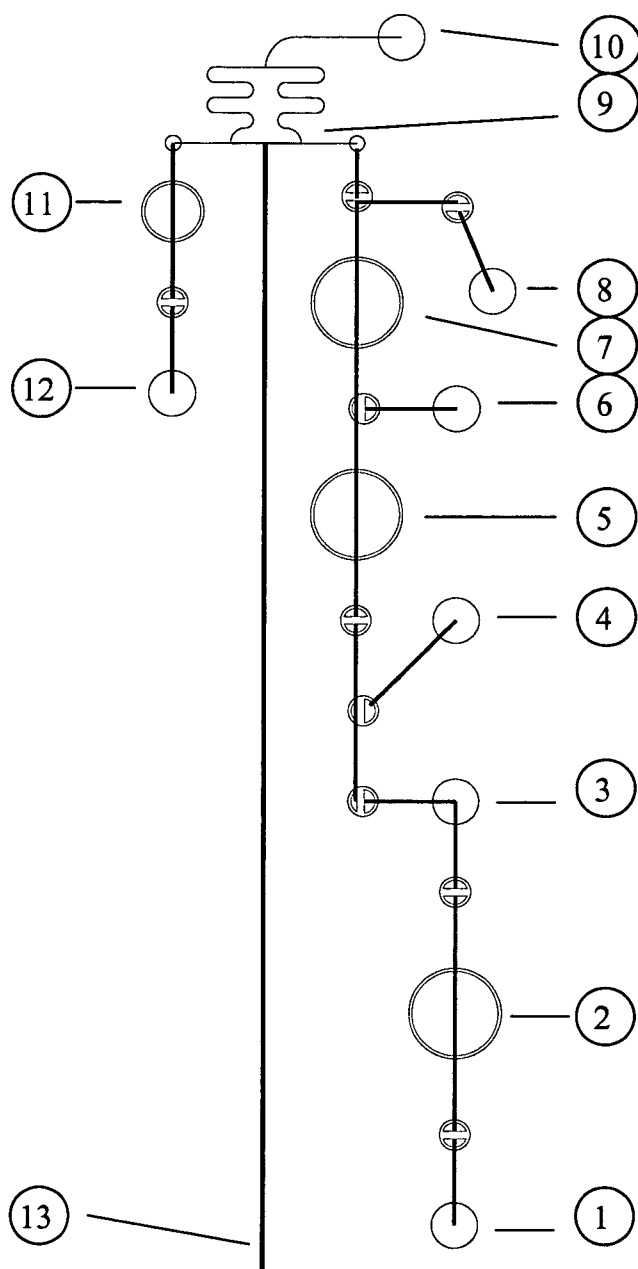


Figure 38

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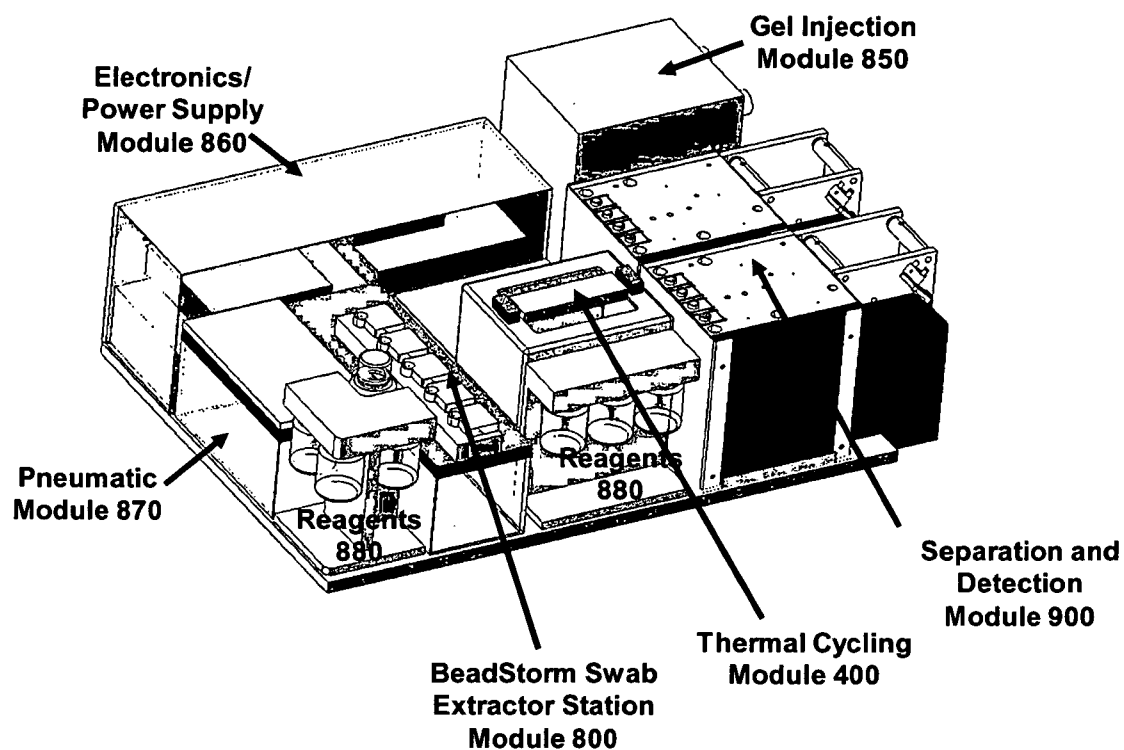


Figure 39

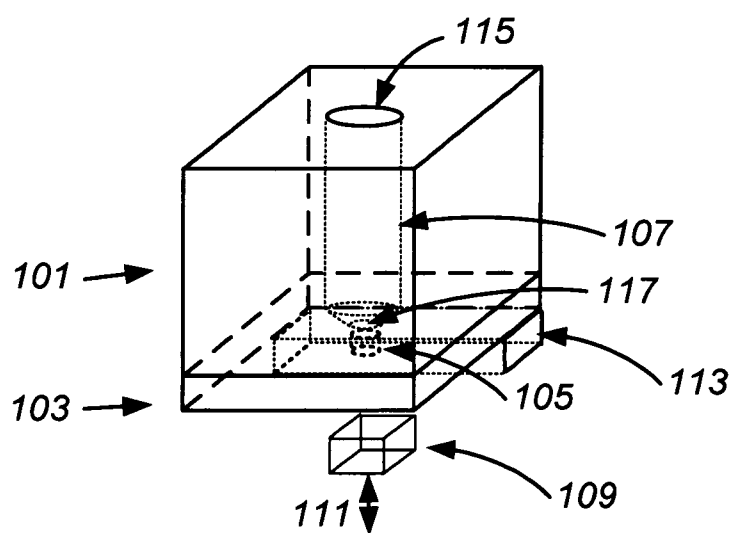


Figure 40

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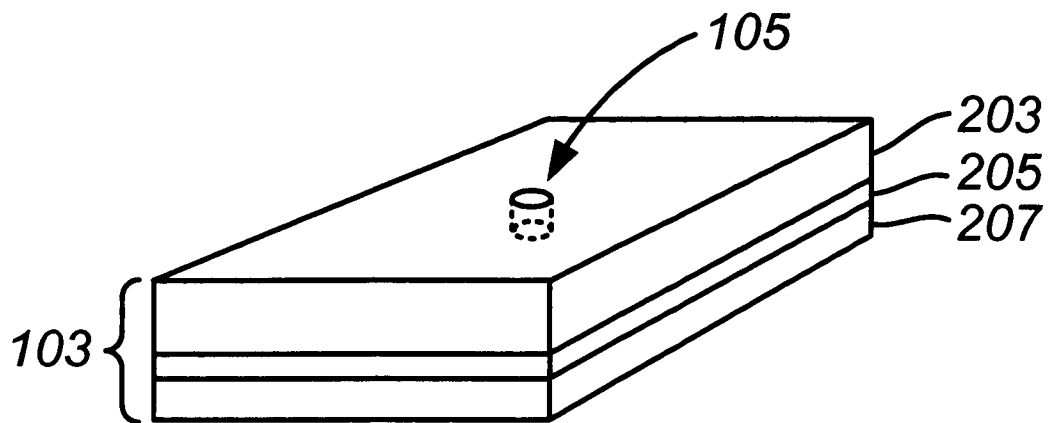


Figure 41

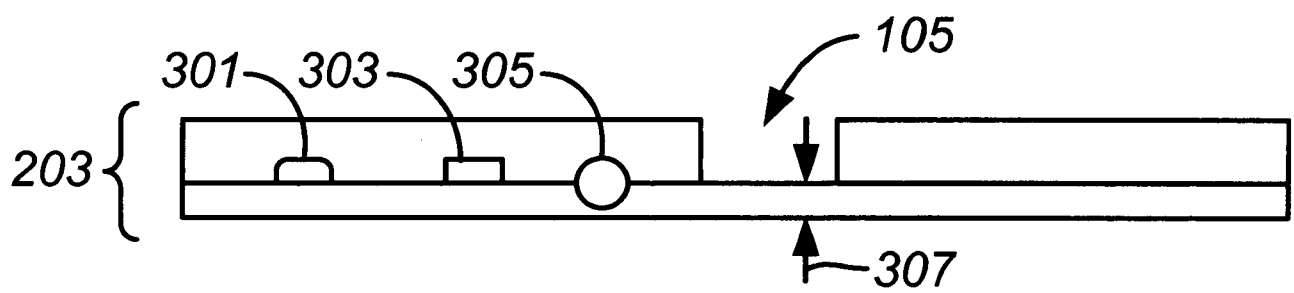


Figure 42

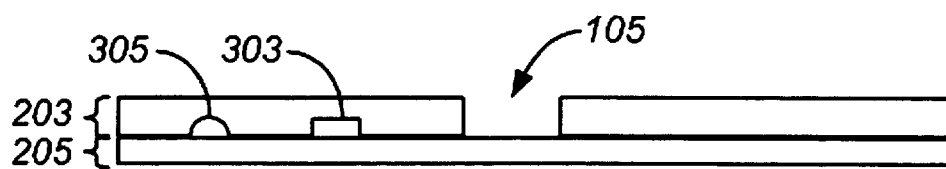


Figure 43

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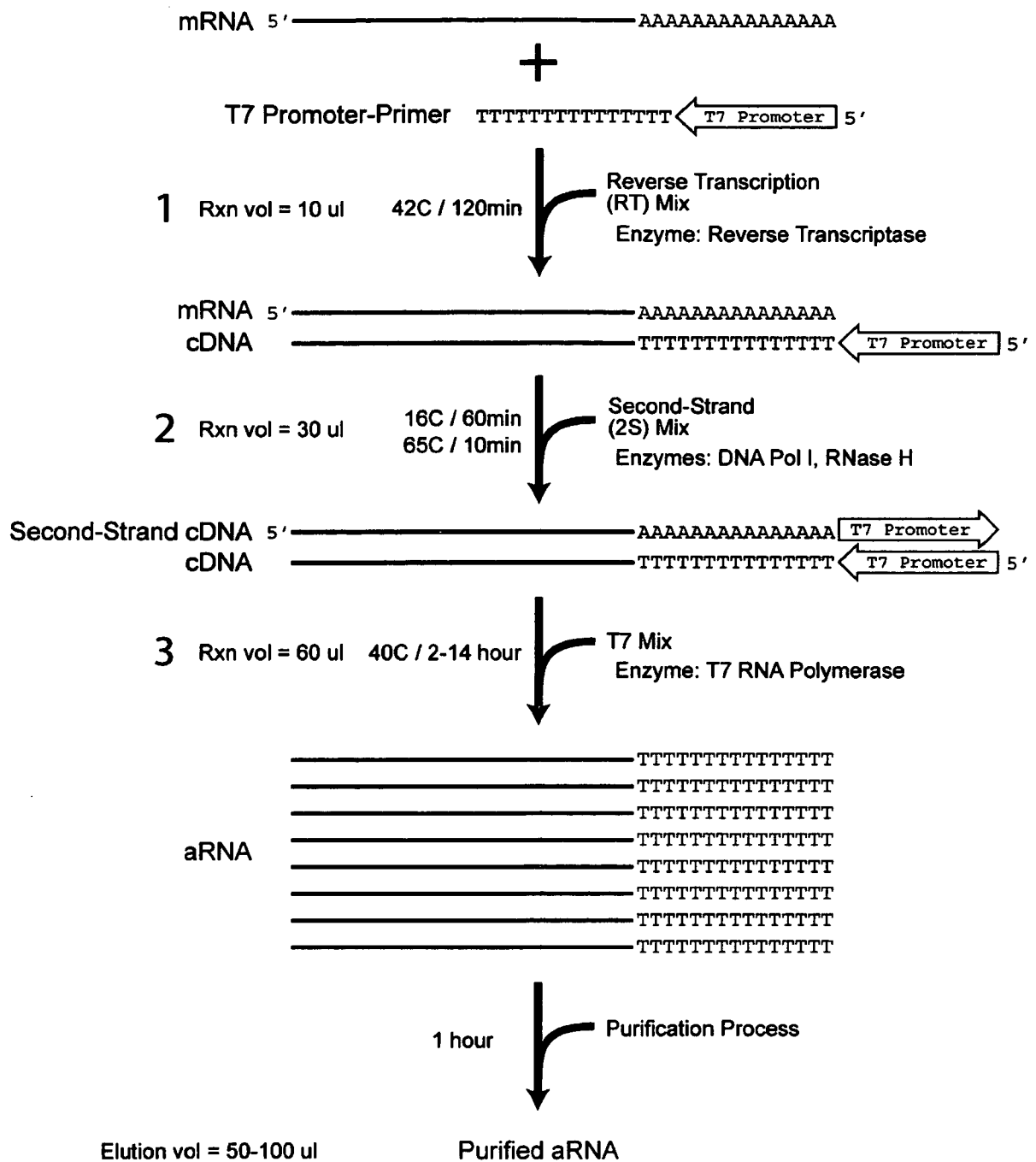


Figure 44

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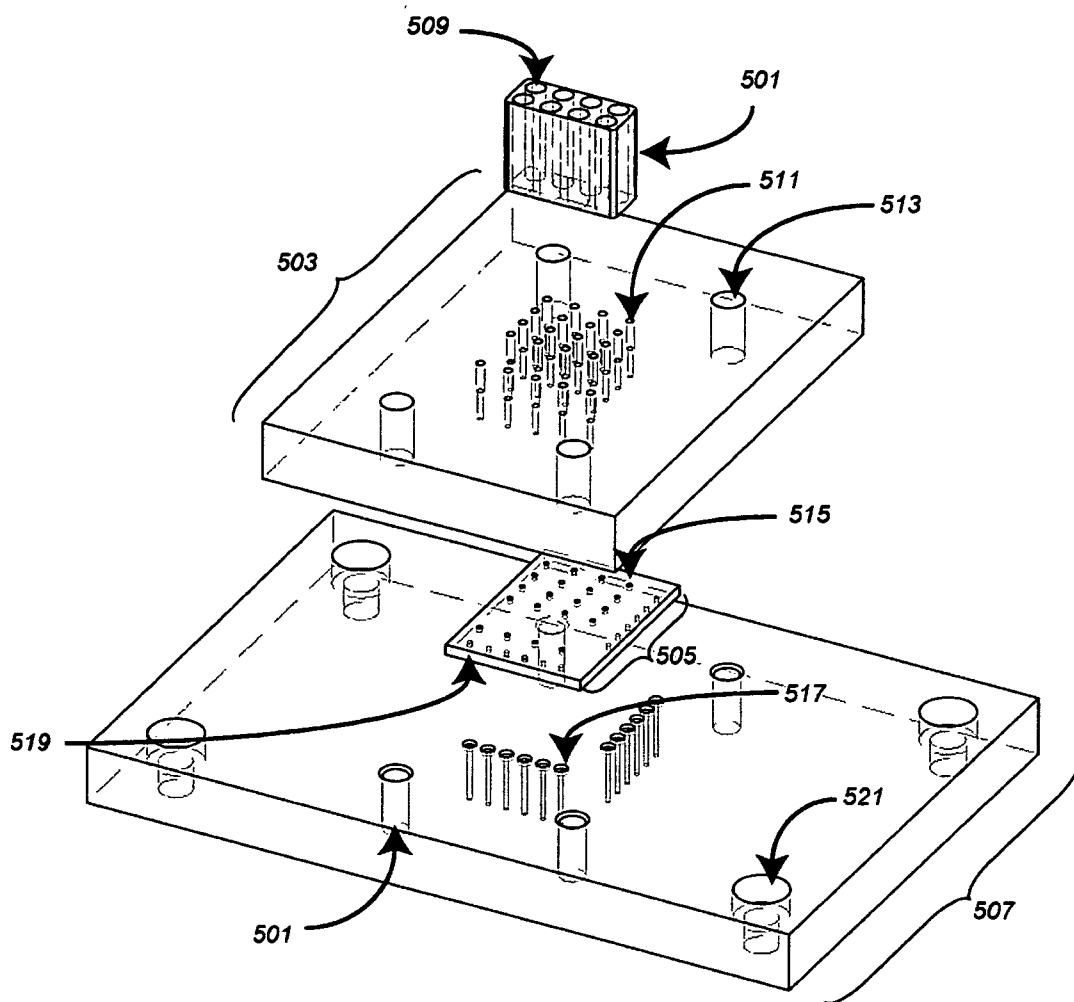


Figure 45

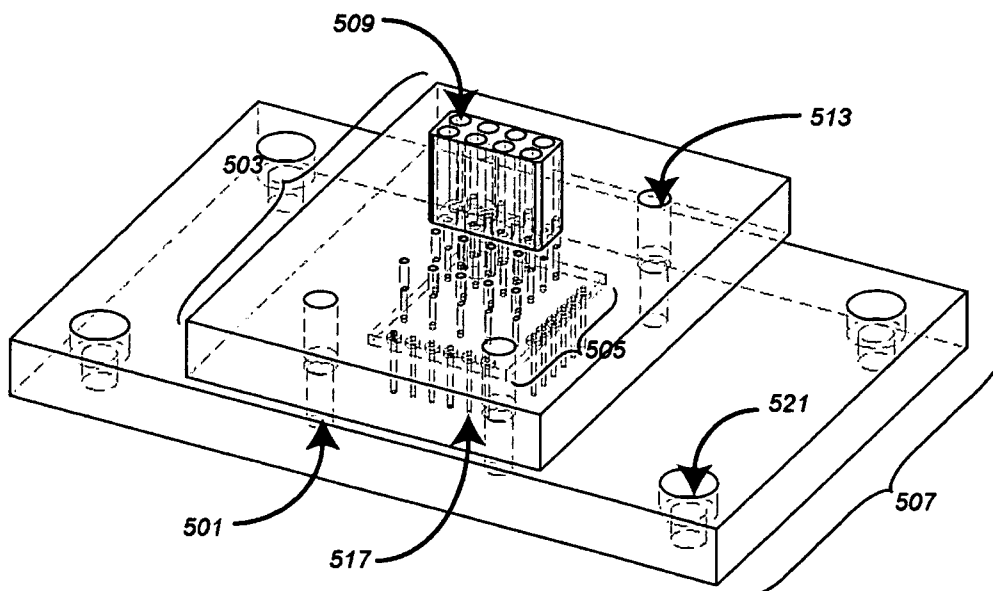


Figure 46

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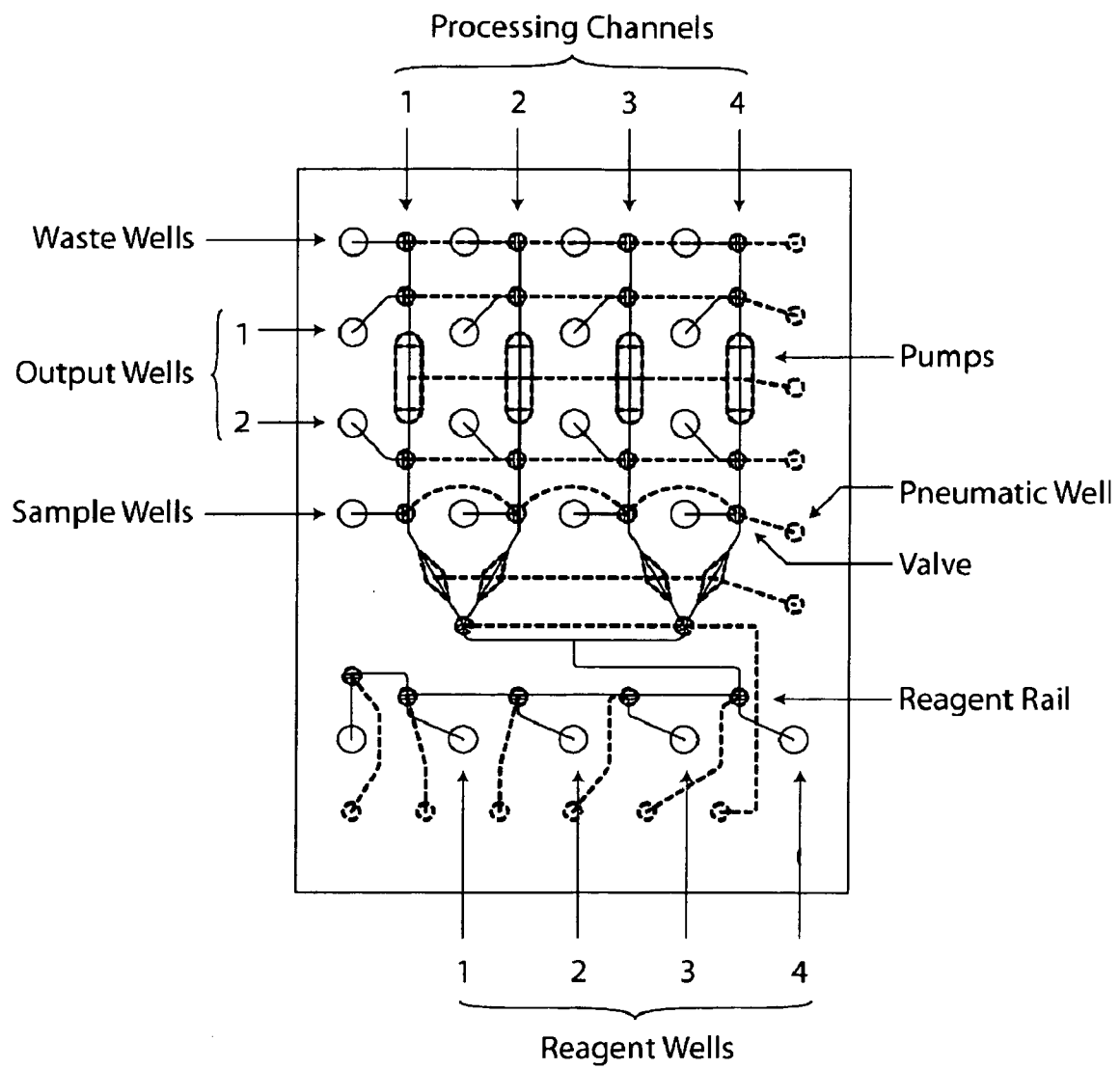


Figure 47

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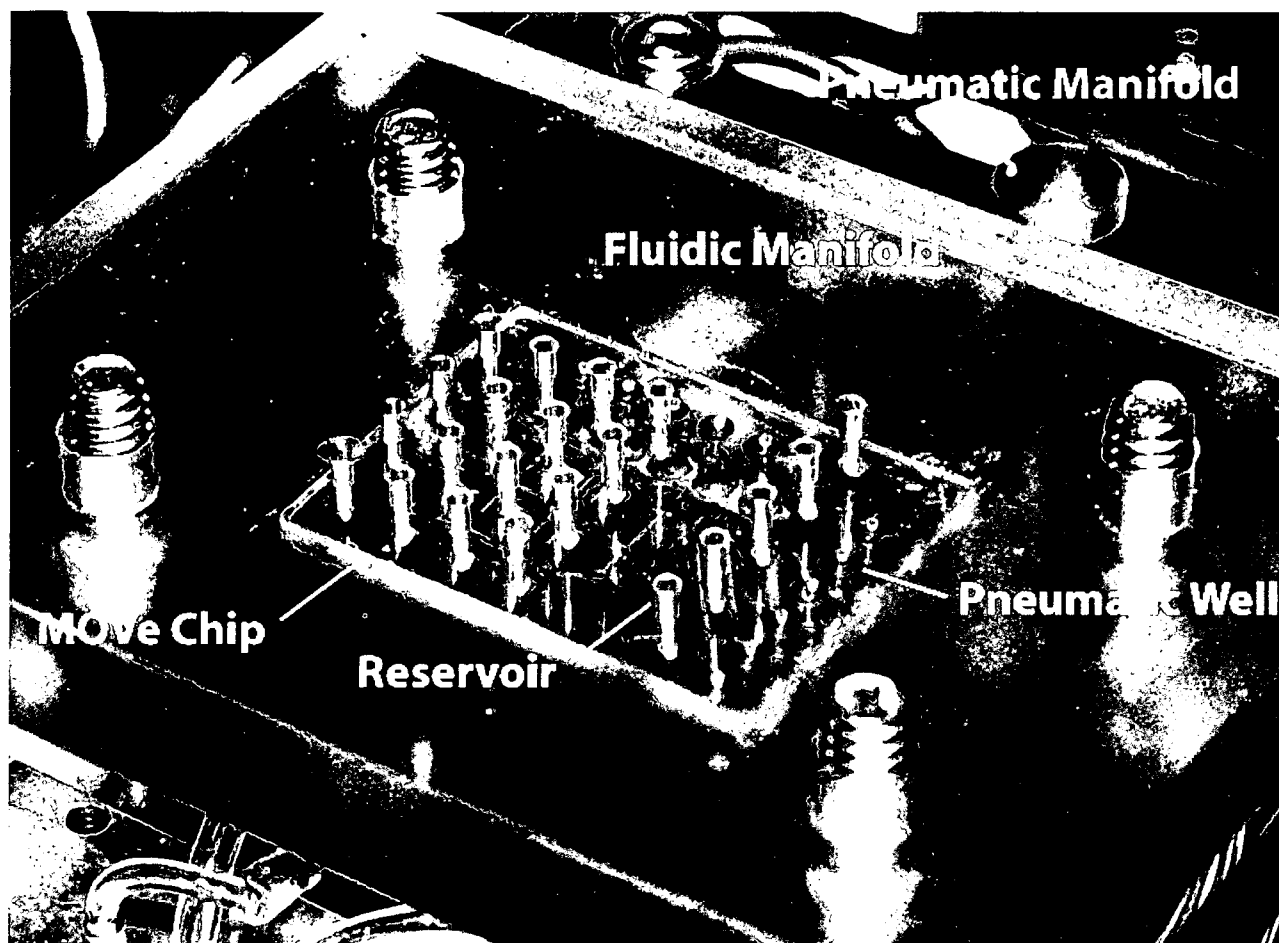


Figure 48

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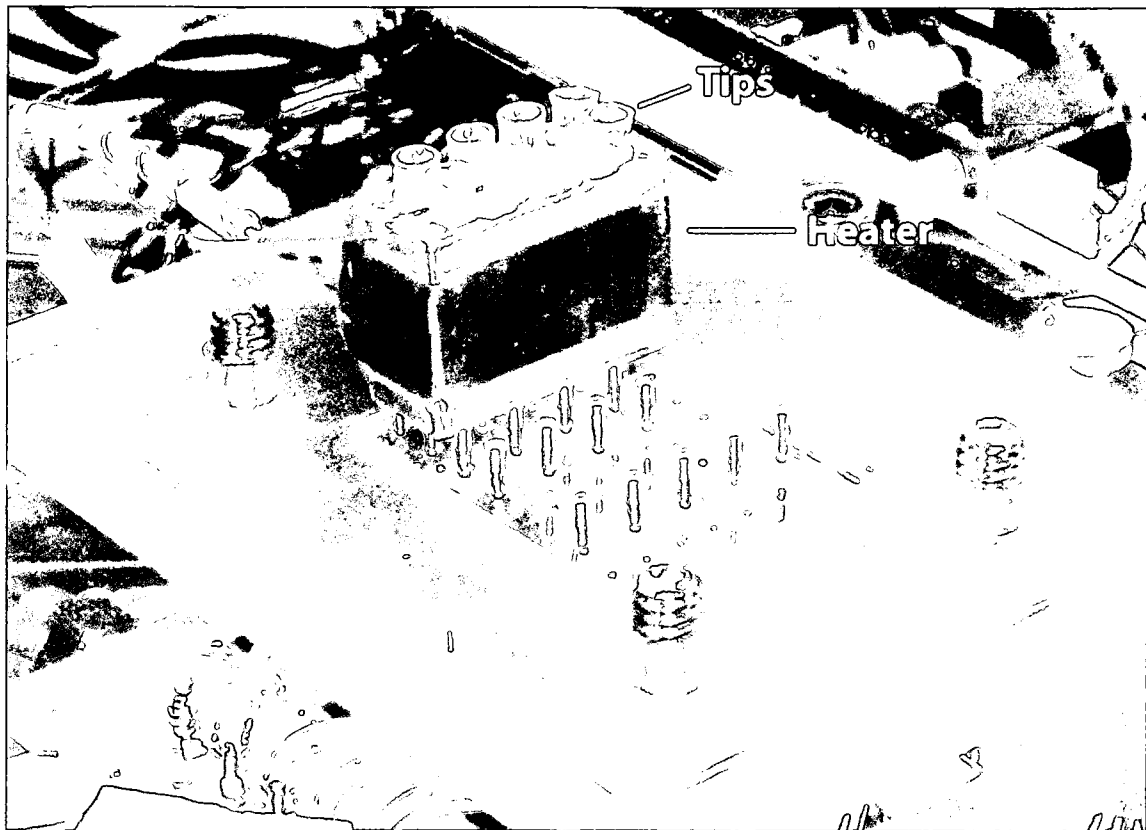


Figure 49

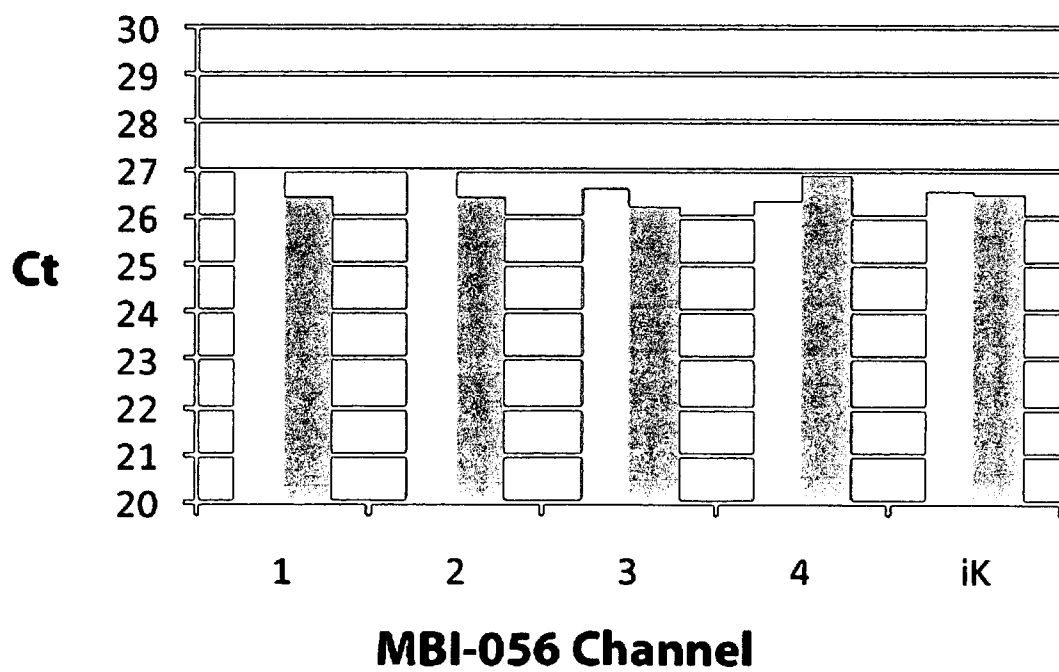


Figure 50

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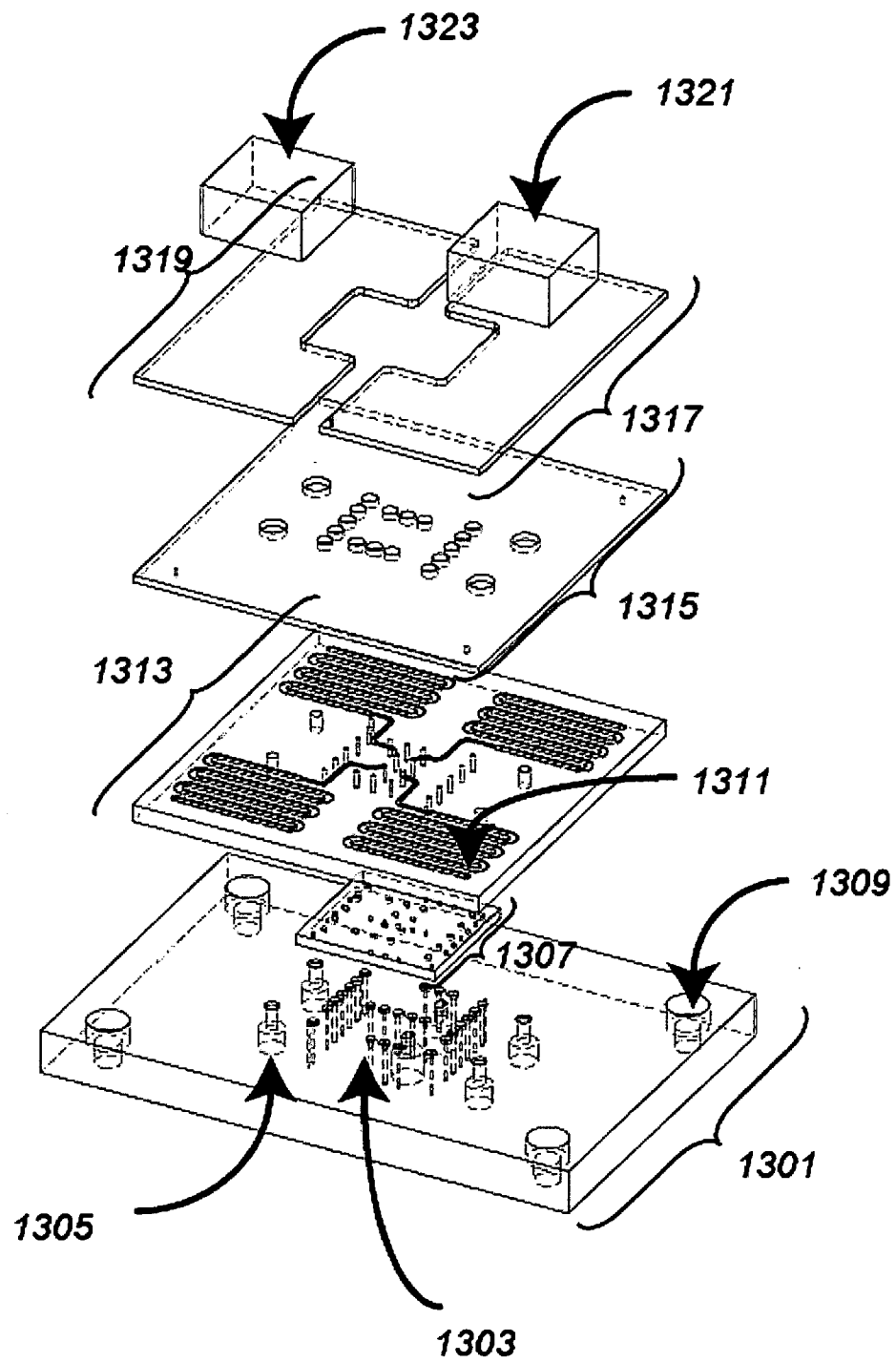


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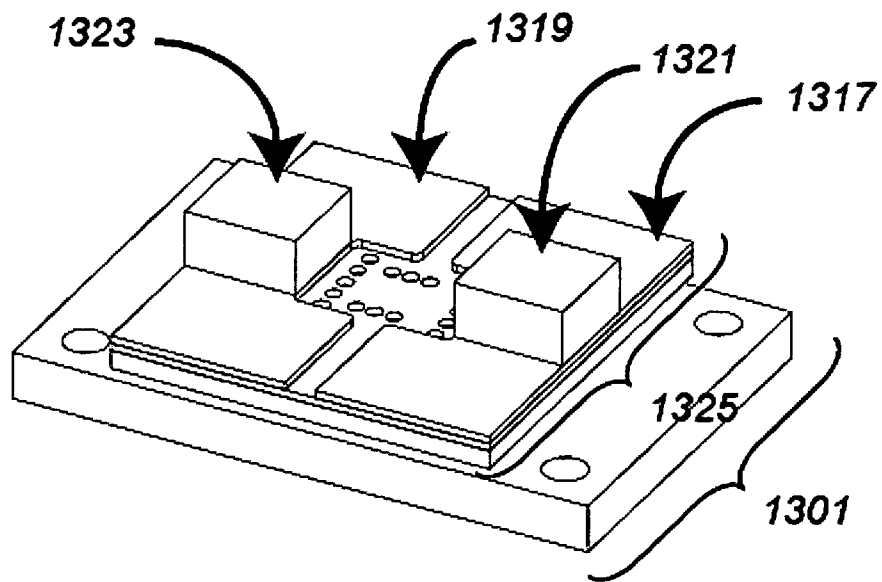


Figure 52

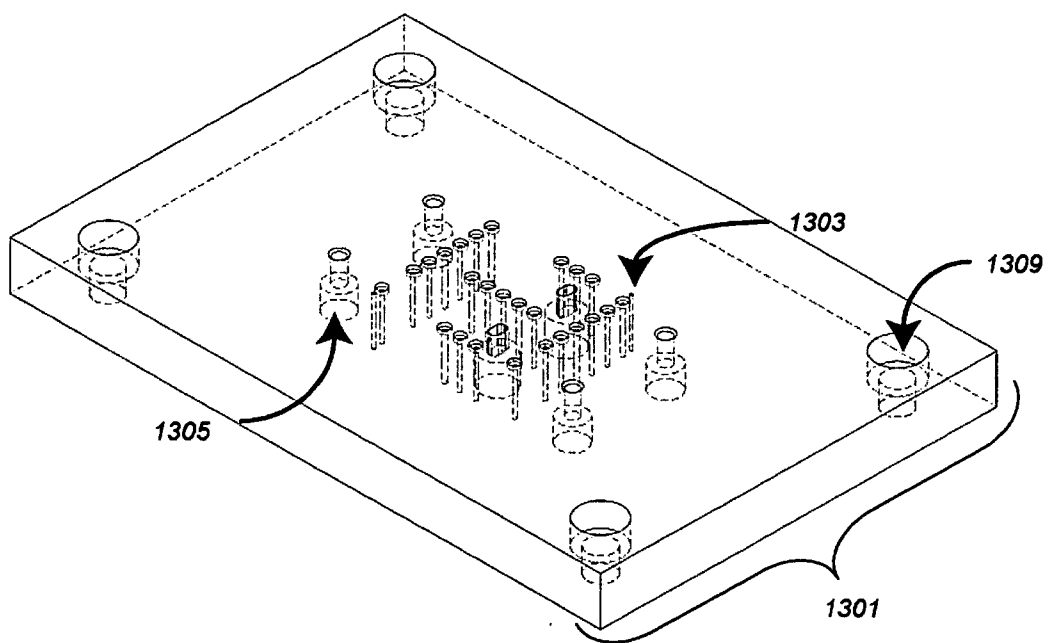


Figure 53

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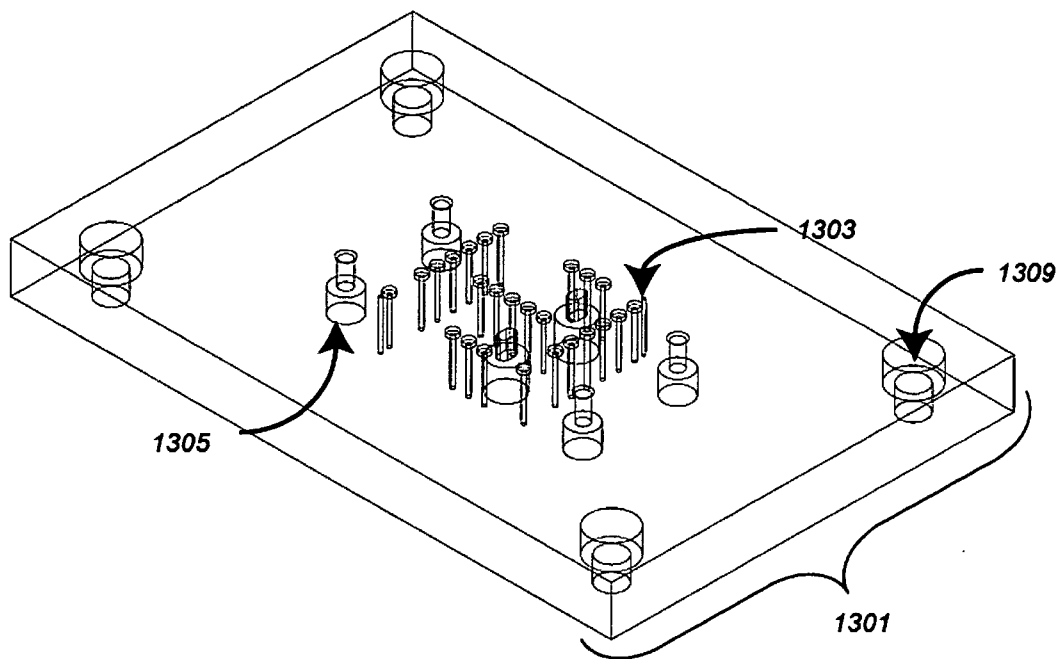


Figure 54

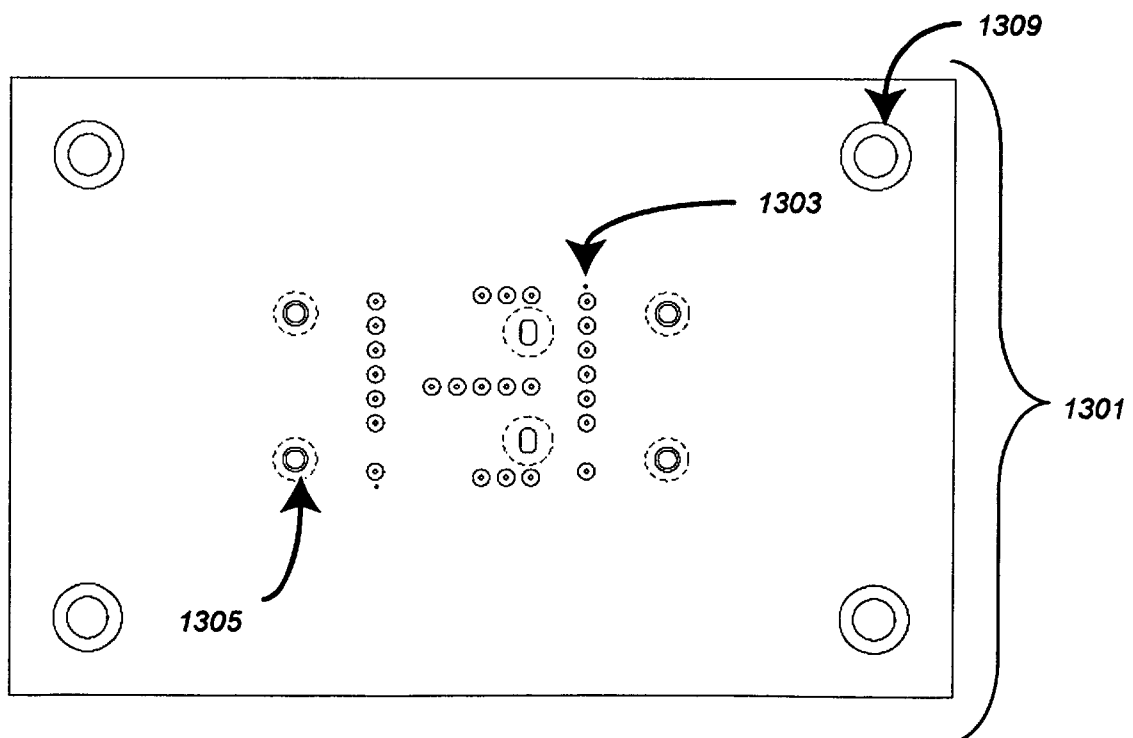


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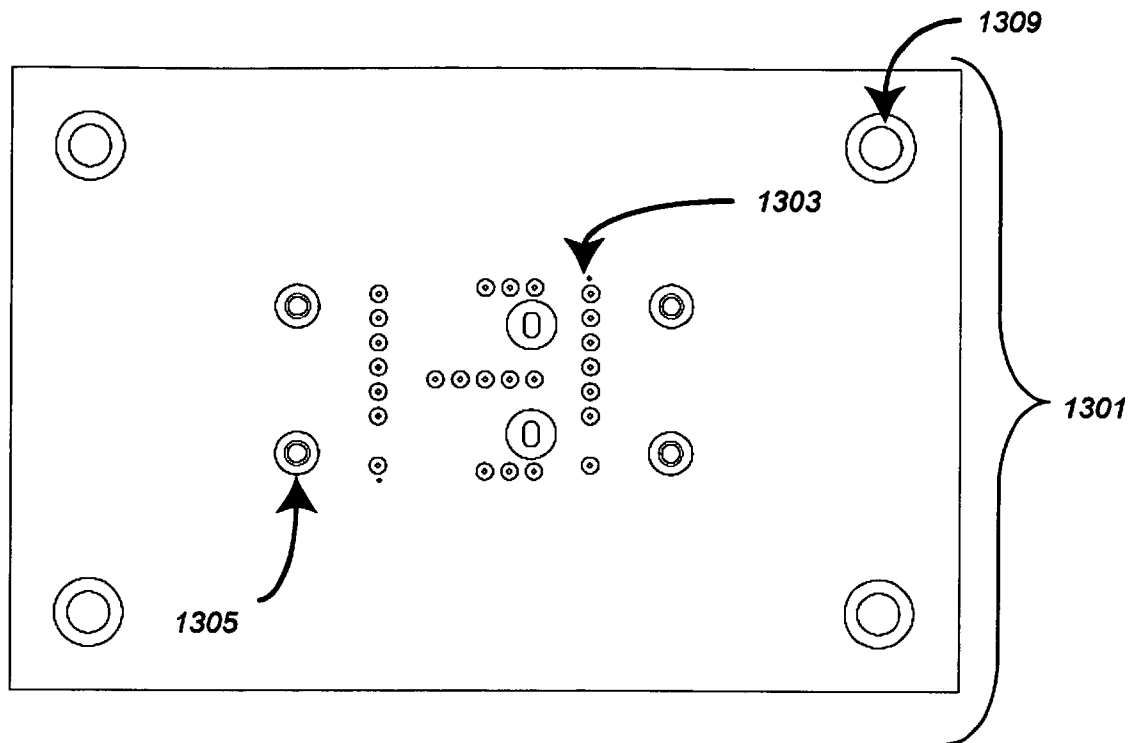


Figure 56

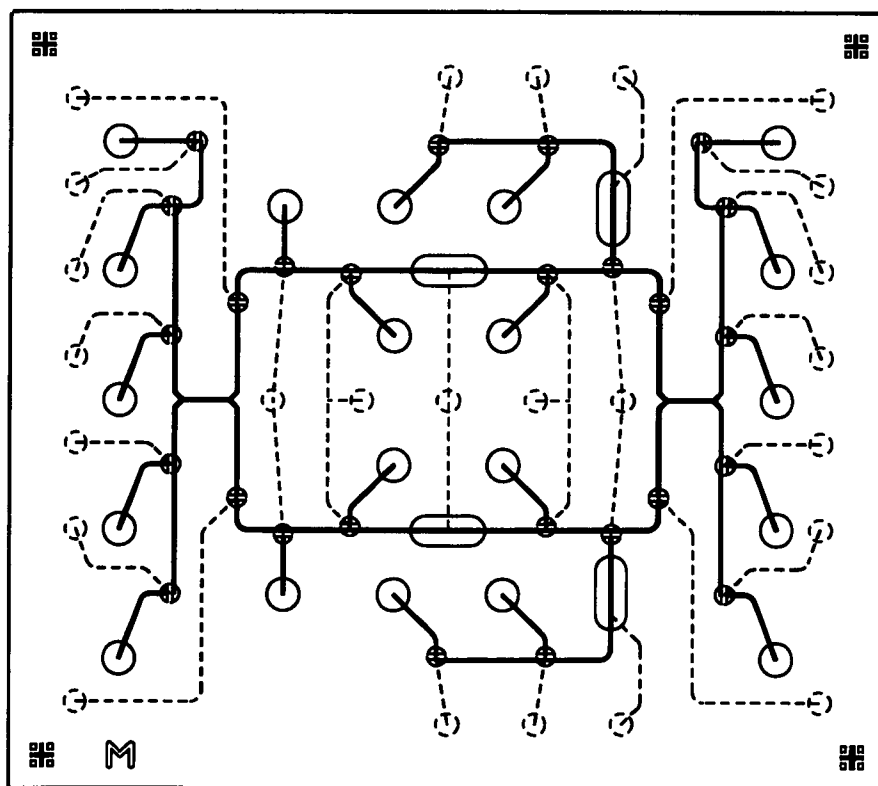


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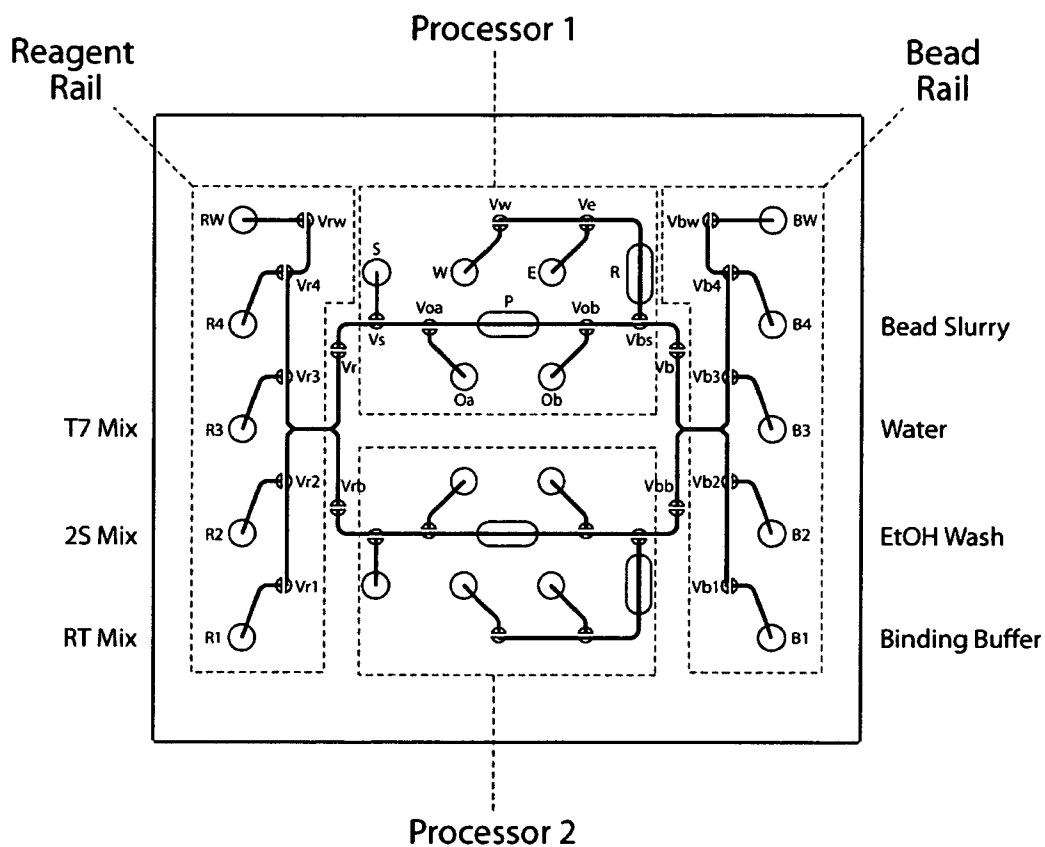


Figure 58

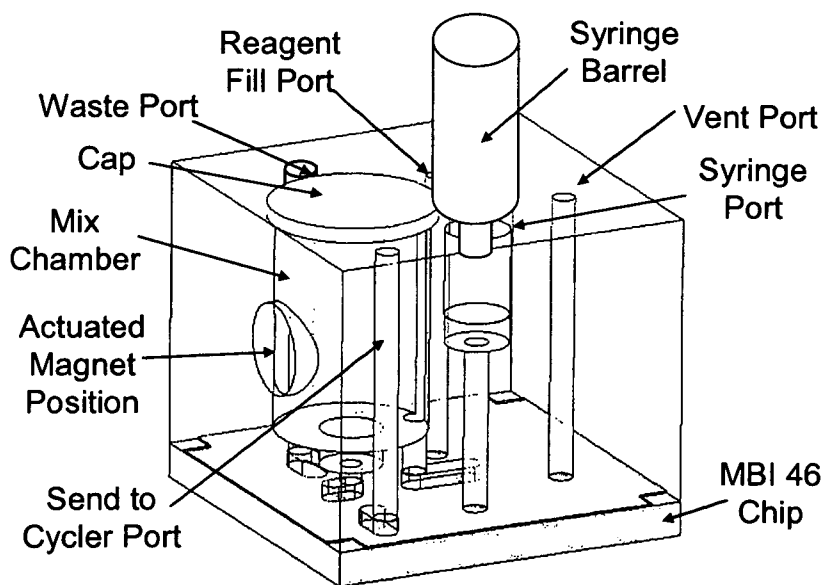


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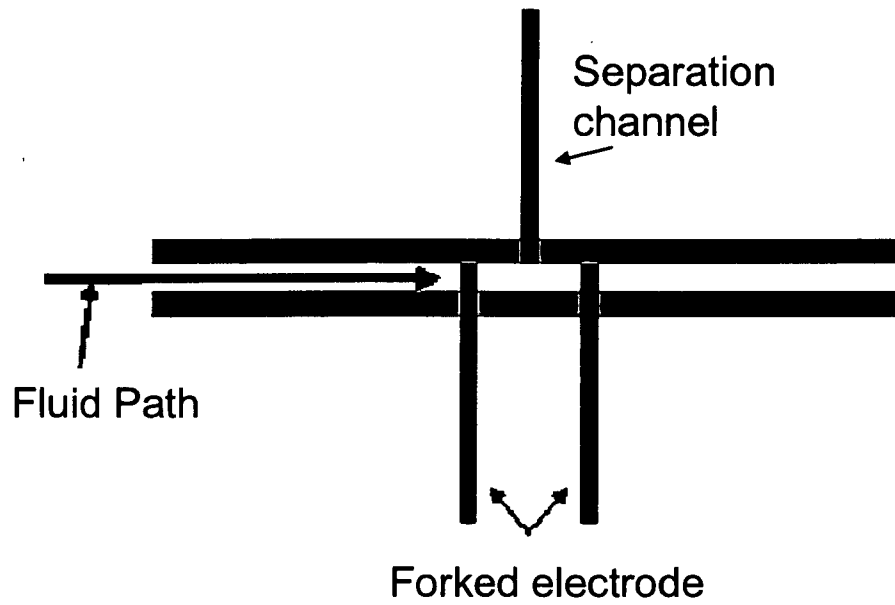


Figure 60

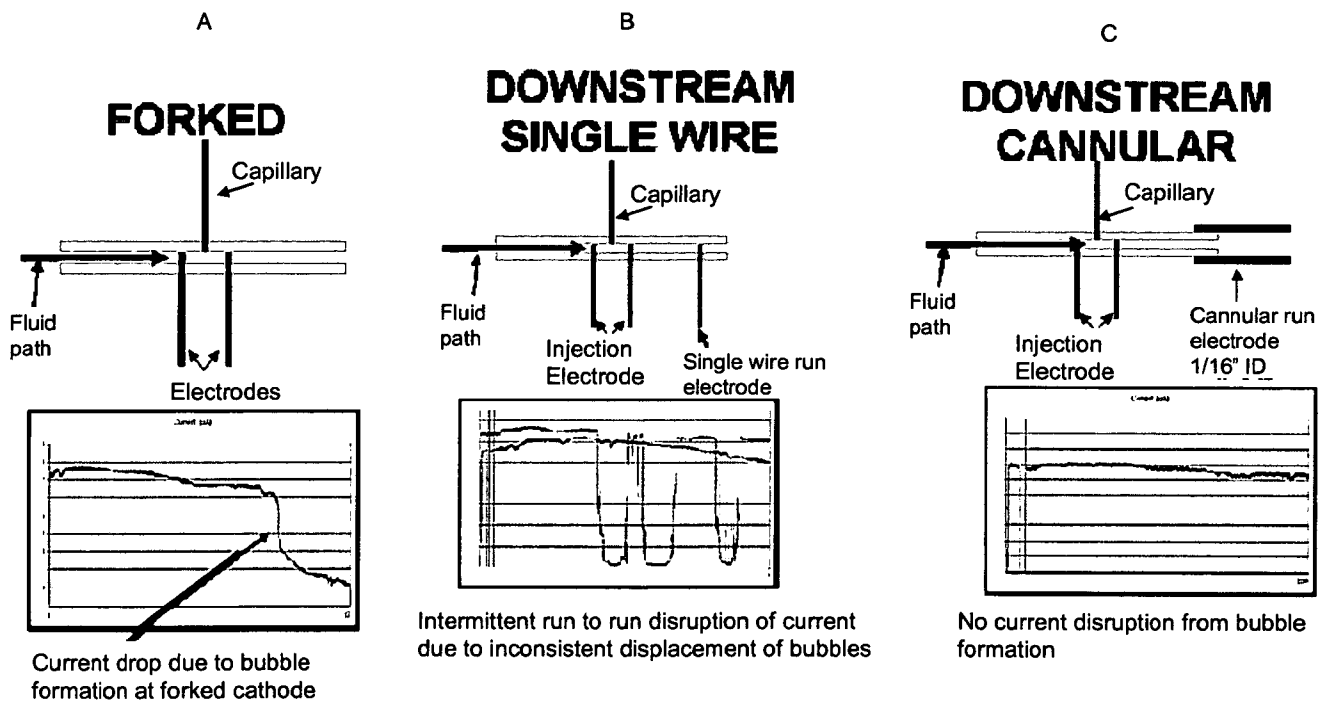


Figure 61

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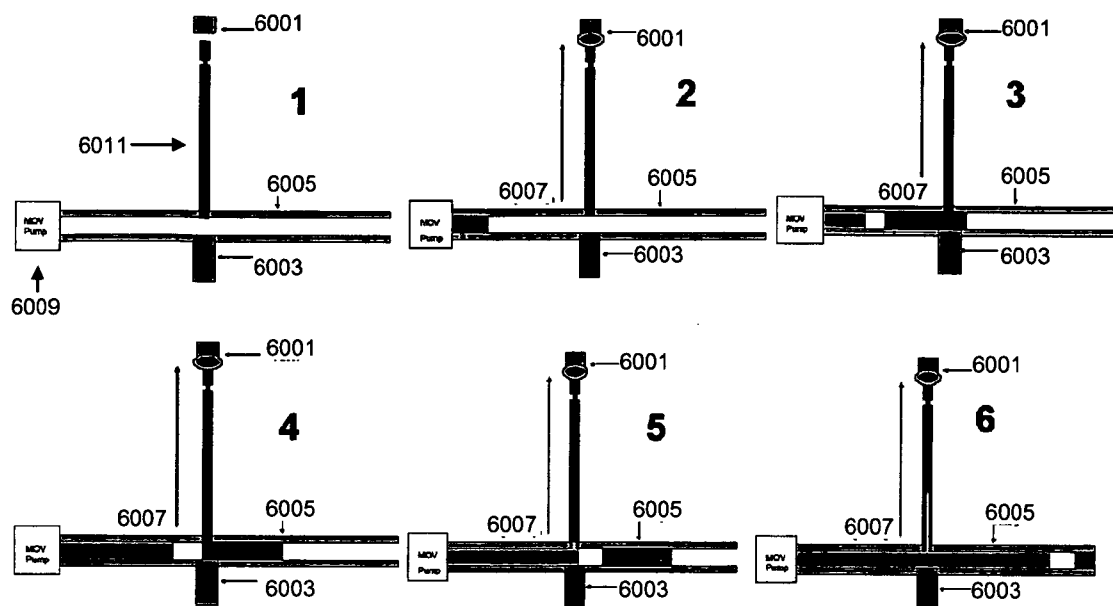


Figure 62

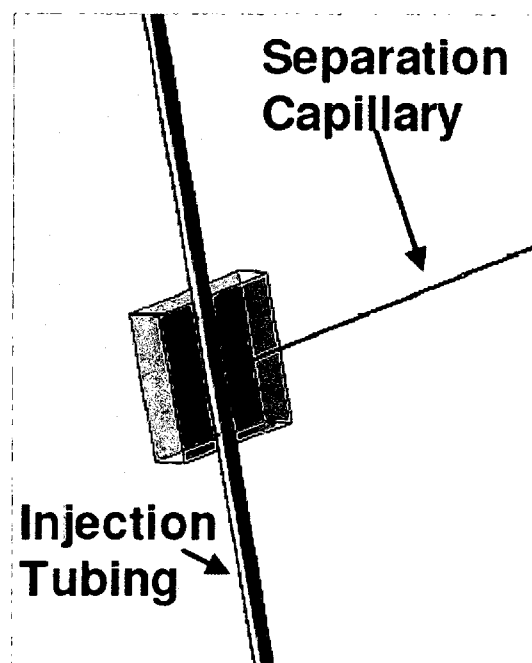


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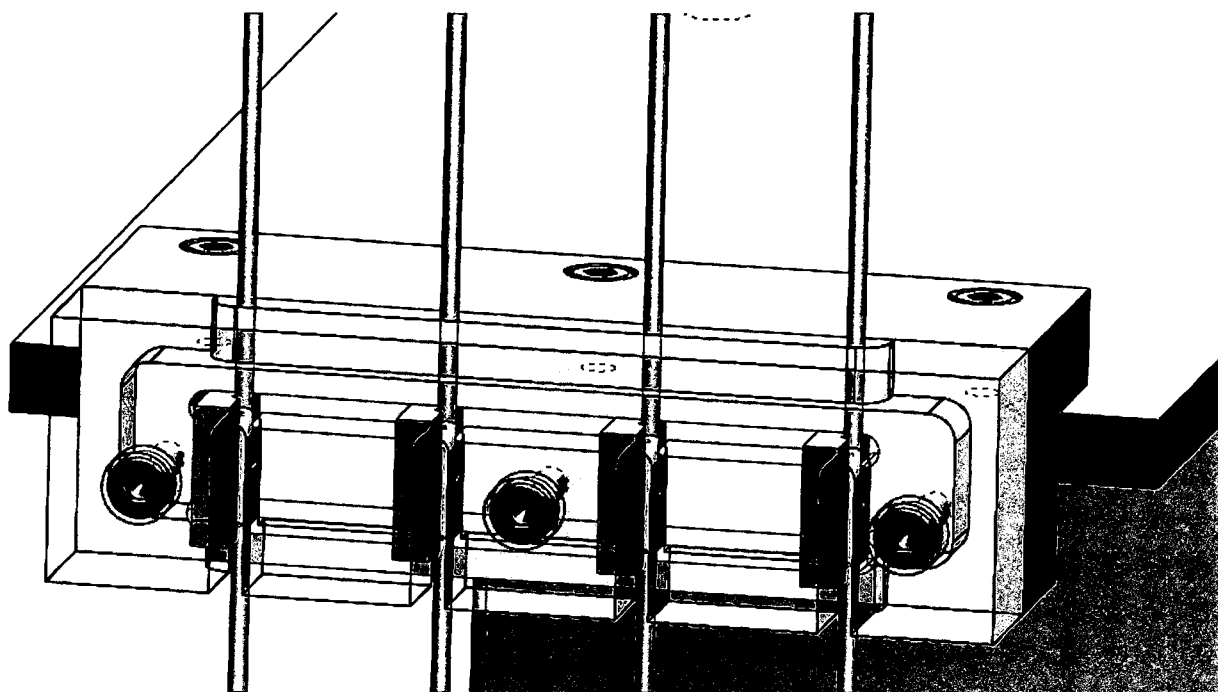


Figure 64

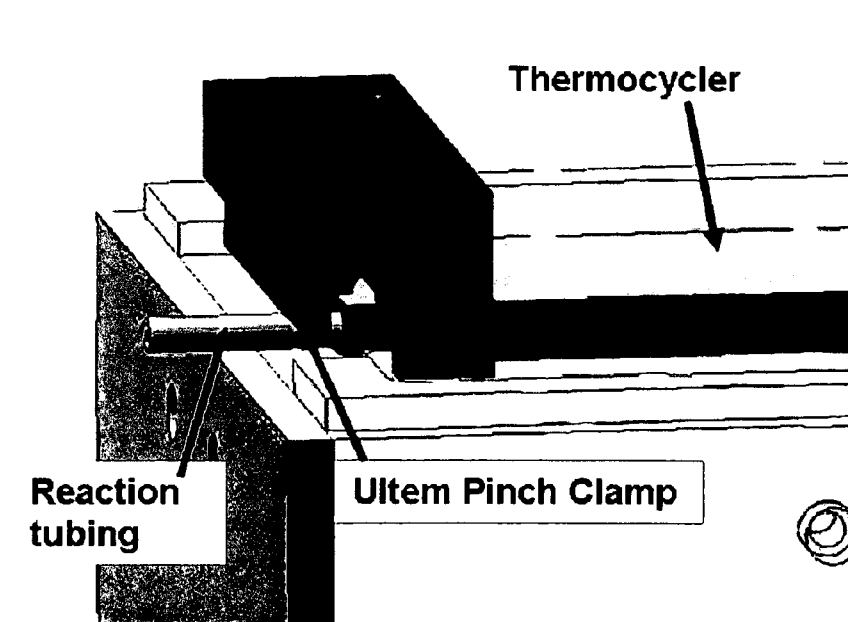


Figure 65

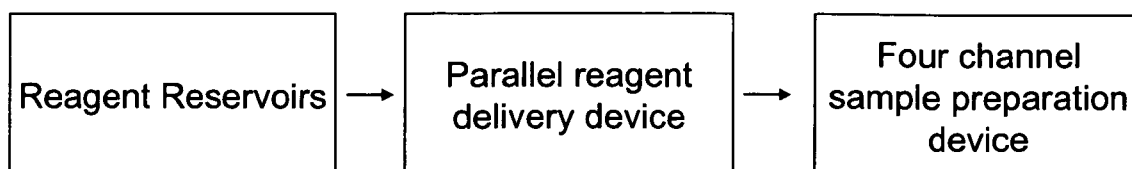


Figure 66.

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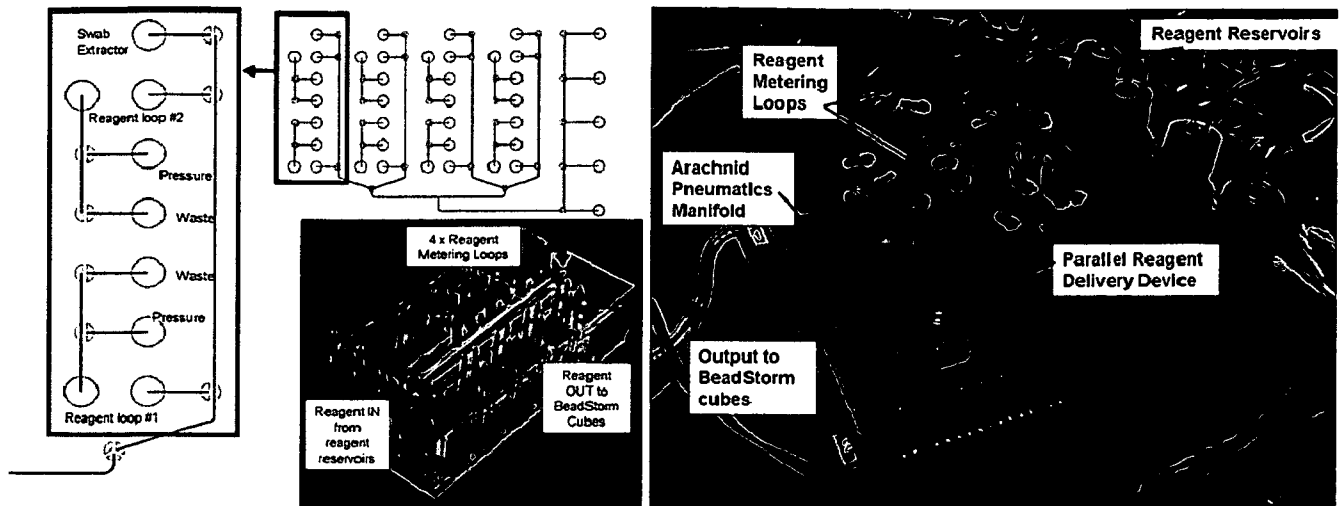


Figure 67.

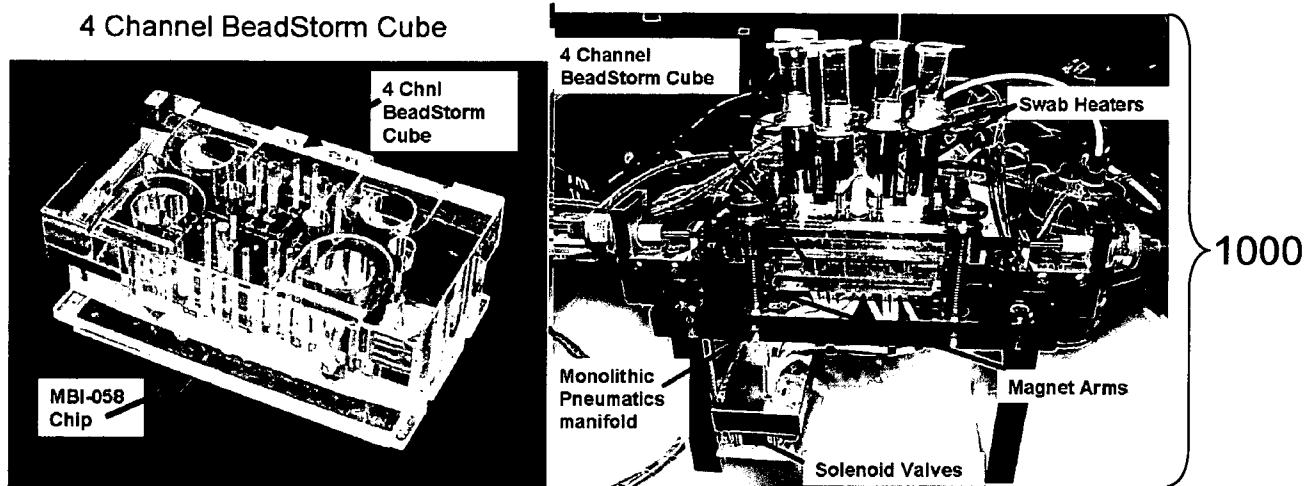


Figure 68.

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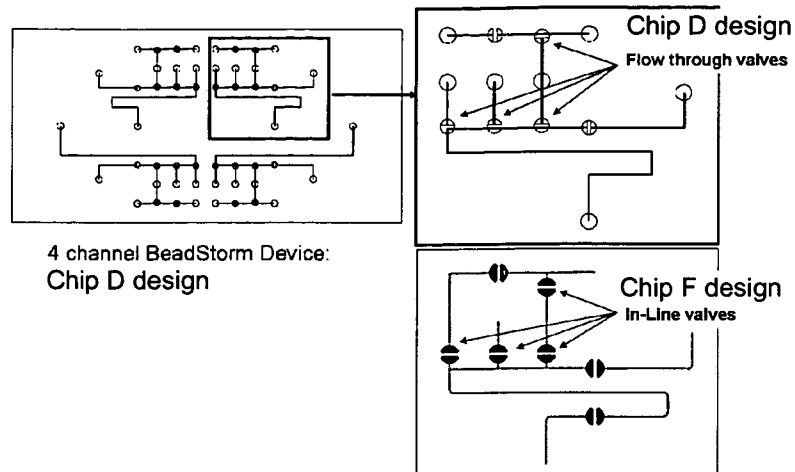


Figure 69.

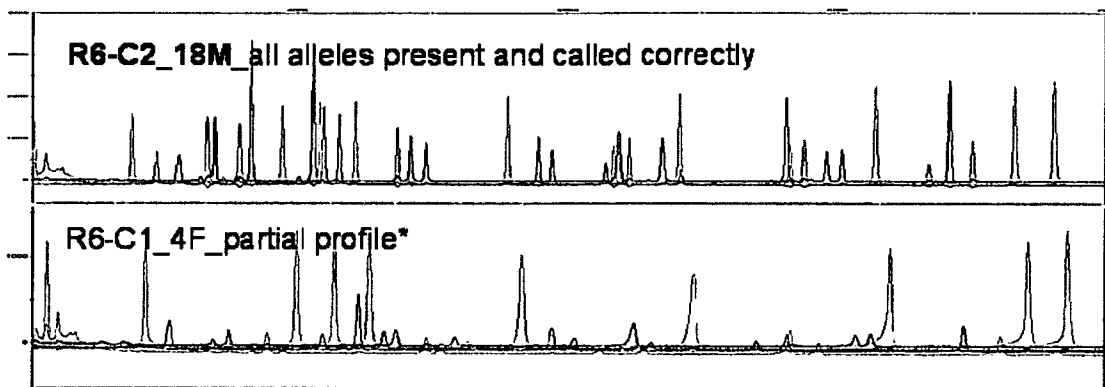


Figure 70.

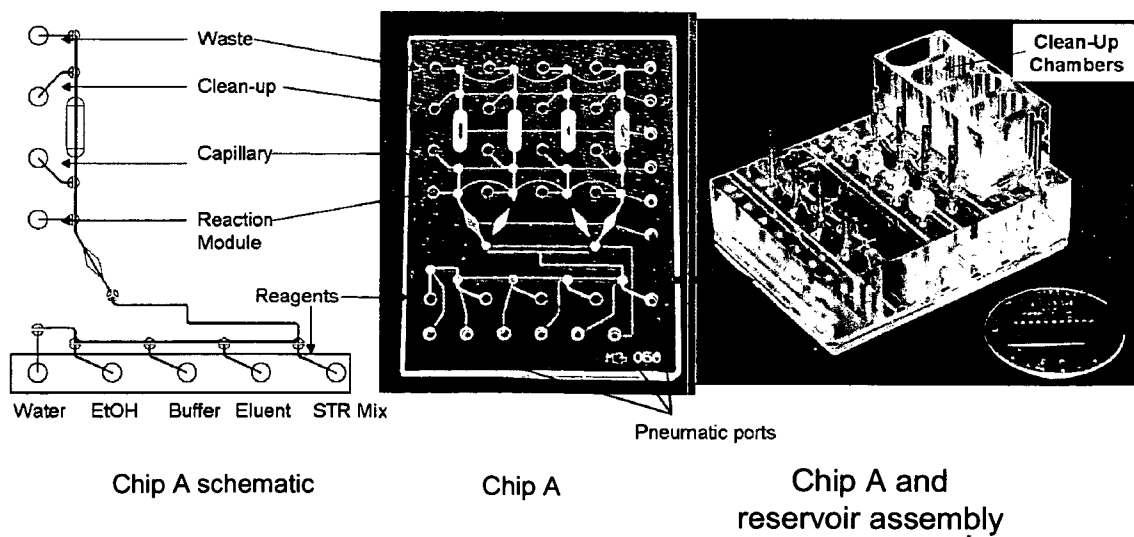


Figure 71.

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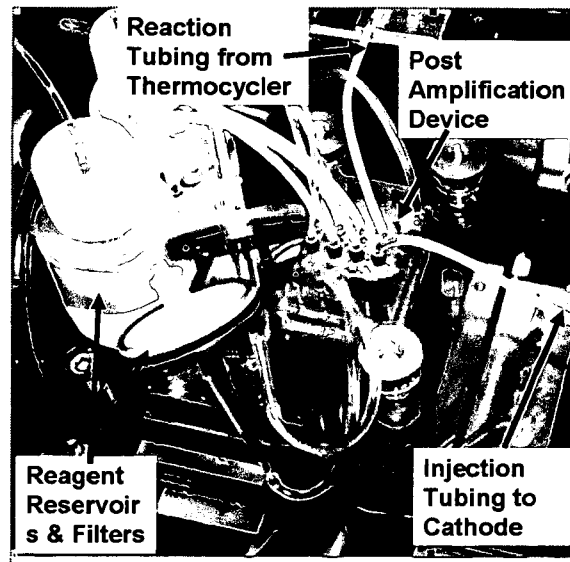


Figure 72

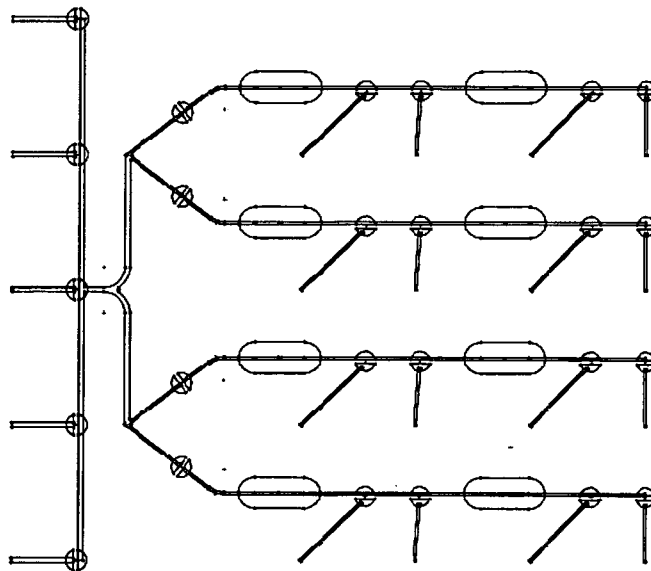


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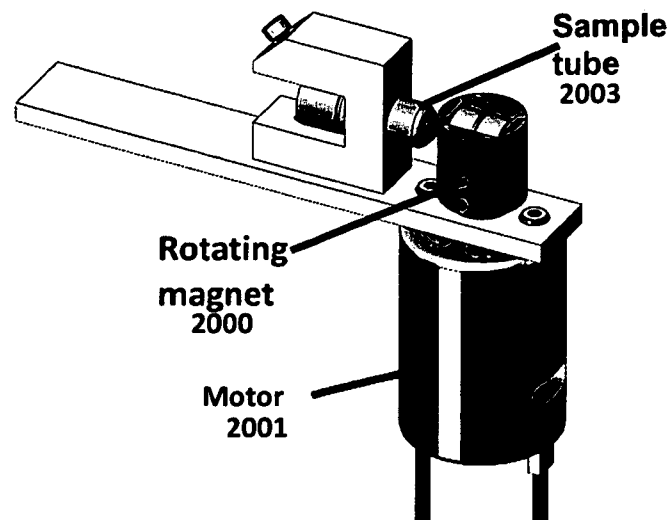


Figure 74

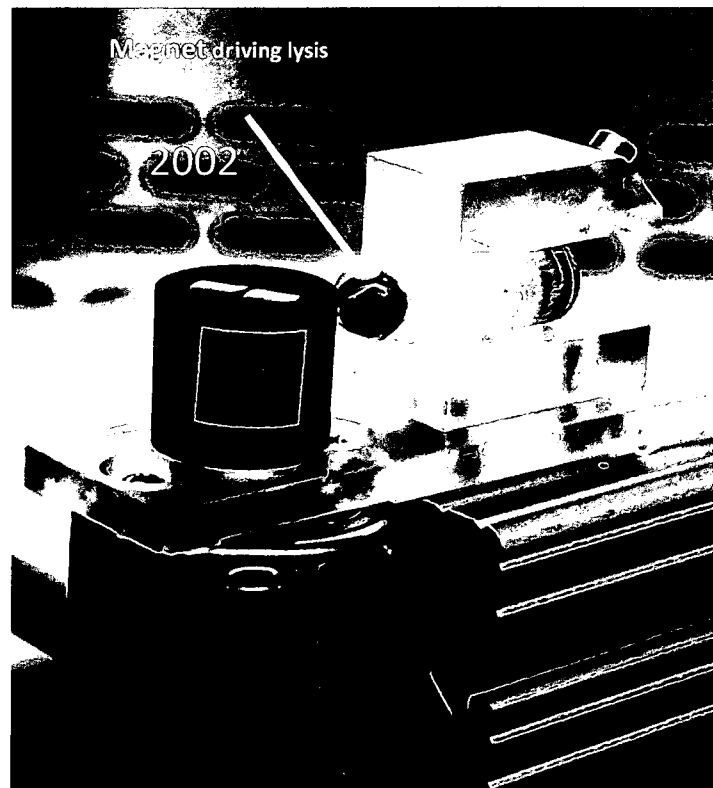


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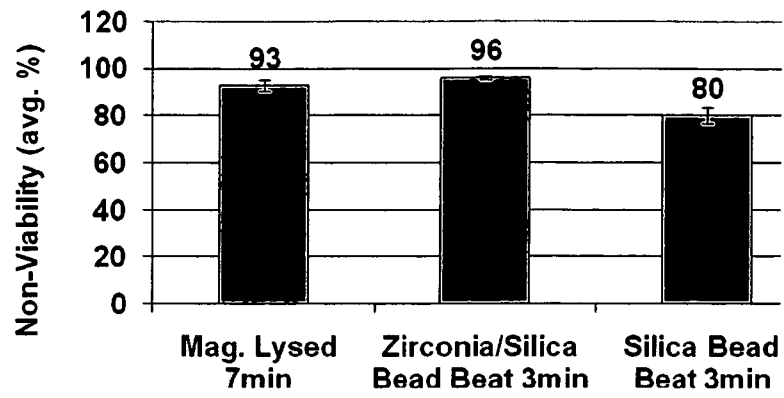


Figure 76