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(54) **Title**: FLUID PROCESSING AND TRANSFER USING INTER-CONNECTED MULTI-CHAMBER DEVICE

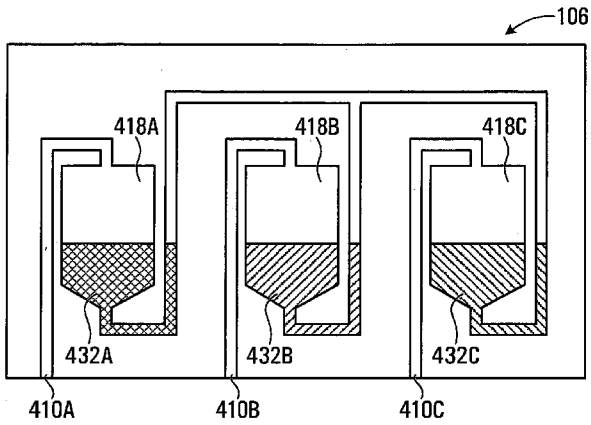


FIG. 13

(57) **Abstract**: A micro-fluidic device comprises a body. The body defines pneumatic ports, chambers for receiving liquids, and a connecting conduit. Each port is sealed with a seal and is shaped to couple to a pneumatic conduit through the seal. At least some of the chambers each have a top opening and a bottom opening. The top openings are in fluid communication with corresponding ports. The bottom openings are in fluid communication with one another through the connecting conduit, which is above the bottom openings. Selective application of pneumatic pressures to the chambers through the pneumatic conduits can transfer a liquid from one chamber to another through the connecting conduit, for example, for processing bio-samples within the device.

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FLUID PROCESSING AND TRANSFER USING INTER-CONNECTED MULTI-CHAMBER DEVICE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefits of U.S. provisional application No. 61/064,871, filed March 31, 2008, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to fluid processing, more particularly to fluid processing and transfer using inter-connected multi-chamber devices.

BACKGROUND OF THE INVENTION

[0003] Preparation of biological samples, such as DNA, RNA, mRNA and protein from clinical samples in the forms of solids and fluids, may involve a series of processing steps, such as tissue dissociation, cell separation, cell lysis, gene extraction, and/or washing. This sometimes requires complex fluidic delivery and processing protocols. In some conventional processing methods, samples and reagents are contained and manually transferred using test tubes and micropipettes. This is time-consuming, labor-intensive, and prone to human error. There is also a significant risk of cross-contamination of nucleic acids between different samples. Some of the manual steps and operations may be automated using robotic systems, but the robotic systems are difficult to use for handling small amounts of samples. Moreover, automation typically requires added costs and expensive equipments.

[0004] A chip-based or cartridge-based micro-system can process a small amount of sample fluid within a closed fluidic system, thereby reducing risks of cross-contamination. Typically, the fluid flow within the chip- or cartridge-based systems is driven and regulated using pumps and valves. These systems have complex structures and low reliability, and are expensive and inconvenient to use.

SUMMARY OF THE INVENTION

[0005] According to an aspect of the present invention, there is provided a micro-fluidic device comprising a body. The body defines first, second and third pneumatic ports; first, second and third chambers; and a connecting conduit. Each of the ports is sealed with a seal and shaped to couple to a pneumatic conduit through said seal. Each of the chambers is for receiving a liquid, and has a top opening and a bottom opening. The top opening of each chamber is in fluid communication with a respective port. The connecting conduit is above each one of the bottom openings. The bottom openings are in fluid communication with one another through the connecting conduit. Selective application of pneumatic pressures to the chambers through said pneumatic conduits can transfer a liquid from one of said chambers to another one of said chambers through said connecting conduit. Each one of said chambers may have a bottom surface sloped downwardly towards said bottom opening of said each chamber. At least a section of said connecting conduit may be at a level above a liquid level in said chambers. The body may include a top portion, a bottom portion, and a middle portion. The chambers and connecting conduit may be defined by said middle and top portions. At least a section of said connecting conduit may be adjacent said top portion. The top openings of said chambers may be adjacent said top portion. The ports may be defined by said bottom portion. The middle portion may define conduits each extending between one of the ports and its corresponding top opening. The middle portion may define conduits each extending between one of the bottom openings and the connecting conduit. The top, middle and bottom portions may be separate portions, and the middle portion may be sandwiched between said top and bottom portions. At least one of said top and bottom portions may be formed of a flat sheet. At least one of said top portion and said bottom portion may be made of a plastic material. The body may define more than three inter-connected chambers, such as six to eleven inter-connected chambers. The liquid in a chamber may comprise a reagent, a buffer, a sample, or a gene binding conditioner. At least two chambers may contain different liquids. At least a portion of said body may be made of a polymer. The polymer may comprise polycarbonate or poly(methyl methacrylate). The seal may be made of a plastic material. The body may define a gene extractor chamber. The gene extractor chamber may contain a gene

extractor and may be in fluid communication with said connecting conduit. The body may define a product chamber and a waste chamber, each in fluid communication with said gene extractor chamber. The device may be a cartridge. The pneumatic conduit may comprise a needle.

[0006] In accordance with another aspect of the present invention, there is provided an apparatus comprising a device as described in the preceding paragraph, and a station connectable to said device for selectively applying pneumatic pressures to said chambers through said ports of said device. The station may comprise a base configured for coupling with said device, a plurality of pneumatic conduits mounted on said base, shaped to couple to said ports through said seals when said device is coupled to said base, and a plurality of valves each connected to one of said pneumatic conduits for selectively regulating a fluid flow through said pneumatic conduits. A first set of the valves may be connected to a first pressure device for selectively applying to said ports a first pneumatic pressure, and a second set of the valves may be connected to a second pressure device selectively applying to said ports a second pneumatic pressure lower than said first pneumatic pressure. The first pneumatic pressure may be higher than one atmosphere, and the second pneumatic pressure may be lower than one atmosphere. The first pressure device may comprise a pressure pump, and said second pressure device may comprise a vacuum pump. The station may comprise a controller for controlling operation of said valves and said pressure devices. The station may comprise a computer in communication with said controller for controlling operation of said controller.

[0007] In accordance with a further aspect of the present invention, there is provided a method of operating the device described in the two preceding paragraphs, wherein at least one of the chambers contains a liquid. The method comprises coupling said pneumatic conduits to said ports; selectively applying different pneumatic pressures to said chambers through said ports, to cause said liquid to flow from one of said chambers to another one of said chambers. The pneumatic pressures may be selectively applied to transfer said liquid sequentially through more than two of said chambers.

[0008] Other aspects and features of the present invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] In the figures, which illustrate, by way of example only, embodiments of the present invention,

[0010] **FIG. 1** is a block diagram of a fluidic apparatus, exemplary of an embodiment of the present invention;

[0011] **FIG. 2** is a top plan view of a receptacle base for use in the apparatus of **FIG. 1**;

[0012] **FIG. 3** is a cross-sectional elevation view of the base of **FIG. 2** along the line **A-A**;

[0013] **FIG. 4** is a bottom, see-through perspective view of a cartridge for use in the apparatus of **FIG. 1**, exemplary of an embodiment of the present invention;

[0014] **FIG. 5** is a top exploded perspective view of the cartridge of **FIG. 4**;

[0015] **FIG. 6** is a bottom plan view of the cartridge of **FIG. 4**;

[0016] **FIG. 7** is a top perspective view of the top portion of the cartridge of **FIG. 4**;

[0017] **FIG. 8** is a bottom perspective view of the bottom portion of the cartridge of **FIG. 4**;

[0018] **FIG. 9** is a top plan view of the middle portion of the cartridge of **FIG. 4**;

[0019] **FIG. 10** is a bottom plan view of the middle portion of the cartridge of **FIG. 4**;

[0020] **FIG. 11** is a cross-sectional elevation view of the base of **FIG. 3** coupled to the cartridge of **FIG. 4** in operation;

[0021] **FIG. 12** is a schematic diagram of a part of the cartridge of **FIG. 4** illustrating its operation, exemplary of an embodiment of the present invention;

[0022] **FIGS. 13**, **14** and **15** are schematic diagrams of a part of the cartridge of **FIG. 4** illustrating its operation, exemplary of an embodiment of the present invention;

[0023] **FIG. 16** is an exploded perspective view of an alternative cartridge, exemplary of an embodiment of the present invention;

[0024] **FIG. 17** is a bottom perspective view of the middle portion of the cartridge of **FIG. 16**;

[0025] **FIG. 18** is a schematic diagram of the alternative cartridge illustrating its operation, exemplary of an embodiment of the present invention; and

[0026] **FIG. 19** is a perspective view of an alternative base suitable for coupling with the alternative cartridge.

DETAILED DESCRIPTION

[0027] Exemplary embodiments of the present invention utilize pneumatic pressures to selectively transfer liquids between inter-connected fluid chambers in a micro-fluidic device. The chambers are connected to pneumatic ports through which pneumatic pressures may be selectively applied in individual chambers above the respective liquid level. The chambers may be interconnected through a connecting conduit that is at least partially positioned above the liquid levels in the chambers for preventing unintended liquid transfer between the chambers.

[0028] **FIG. 1** is a schematic block diagram showing a system **100** for processing fluidic biological samples, exemplary of an embodiment of the present invention.

[0029] System **100** includes station **102** which can be a desktop station. Station **102** is connected to a computer **104** and can receive a cartridge **106**, as will be further detailed below.

[0030] Station **102** also includes an embedded controller **108**, which communicates with computer **104**, such as through a USB connection. Computer **104** may be a specially designed computer or a general-purpose computer loaded with specially designed program for control the operation of system **100**.

[0031] Station **102** also includes a plurality of pumps **110A** and **110B** (also collectively and individually referred to as pump **110**), miniature valve arrays **112A** and **112B** (also collectively and individually referred to as valve array or valve **112**) and a valve array control board **114**. One of the pumps, such as pump **110A**, is a vacuum pump; another pump, such as pump **110B**, is a pressure pump. The pumps may be miniature pumps, such as syringe pumps or peristaltic pumps. In some embodiments, the flow rate provided by the pumps may be from about 0.01 to about 50 ml/min. In other embodiments, the flow rate may be different. The valves may be miniature electromagnetic valves. The pumps may be air pumps. However, in different applications, different gas or fluid pumps may be used. In different embodiments, a pump **110** may be replaced with another pneumatic

source, which may be an external vacuum or pressure source. In some cases, the atmosphere may provide a suitable pneumatic source.

[0032] Controller **108** is adapted and configured to control the operation of pumps **110** and valve arrays **112** through valve array control board **114**. Controller **108** may include a microcontroller unit such as a PIC ("Programmable Intelligent Computer) microcontroller, or a programmable logic controller such as an Omron programmable logic controller, or the like.

[0033] Valves **112** are connected to a pneumatic interface **116**. Valves **112** may include miniature valves available from SMC™, or pinch valves available from Cole-Parmer™, or other commercially available or specially designed valves.

[0034] Interface **116** may include a receptacle/coupling unit or base **300** illustrated in **FIGS. 2** and **3**. Base **300** includes a base portion **302** and a wall portion **304** shaped for coupling with cartridge **106**. A number of pneumatic conduits, in this case needles **306**, extend upwardly from and through base portion **302**. Each needle **306** has an internal conduit and is connected to a different valve in the valve array **112** for transferring a fluid through the valve **112** and the needle **306**.

[0035] An exemplary cartridge **106** is shown in **FIGS. 4** to **10**, exemplary of an embodiment of the present invention.

[0036] As shown in **FIGS. 4**, **5** and **6**, the cartridge body **400** is formed of three portions, a top portion **402**, a middle portion **404**, and a bottom portion **406**. The three portions may be separate portions and the middle portion **404** is sandwiched between top and bottom portions **402** and **406**. The three portions **402**, **404**, **406** may be affixed together in any suitable manner. For example, the portions may be bolted together using bolts and nuts (not shown) or glued together using an adhesive. In some embodiments, it may be convenient to removably mount the top and bottom portions to the middle portion. For example, as illustrated in **FIGS. 4** to **10**, bolt holes **408** may be provided for securing the portions together.

[0037] As shown in **FIG. 5**, top portion **402** may be shaped as a flat plate with bolt holes **408**. Alternatively, top portion **402** may be formed of a thin film or sheet

such as a plastic tape which has an adhesive side. For example, the adhesive side of the tape may have a glue layer on its surface.

[0038] As shown in **FIGS. 6** and **8**, bottom portion **406** may be generally plate-shaped but provided with bolt holes **408**, pneumatic ports **410** sealed with breakable seals **411** (not shown but see **FIG. 11**), a well **412**, and fluid conduits or channels **414** and **416**. Seals **411** may be formed of an adhesive tape. Pneumatic ports **410** may be sealed using the adhesive tape. Alternatively, bottom portion **406** may also be formed of a flat sheet such as a plastic sheet with an adhesive side that faces middle portion **404**. Each port **410** is shaped to couple to a pneumatic conduit, such as needle **306**, through seal **411**.

[0039] **FIGS. 9** and **10** are top and bottom views of the middle portion **404** respectively. As shown, middle portion **404** defines a number of chambers **418A**, **418B**, **418C**, **418D**, **418E**, **418F**, **418G**, **418H**, **418I**, and **418J** (also collectively and individually referred to as chamber **418A**). Each chamber **418** has a top opening **420** and a bottom opening **422** (for easy viewing, only **422A**, **422B**, **422G** and **420J**, and **422J** are labeled in **FIG. 9**).

[0040] Each top opening **420** is connected with a pneumatic conduit or channel **424**, which extends initially laterally and then downwardly to a respective pneumatic port **410** in bottom portion **406**, so that the corresponding chamber **418** is in communication with the respective port **410** (for easy viewing, only channels **424A** and **424J** are labeled in **FIG 9** and **FIG. 10**).

[0041] Each bottom opening **422A** to **422H** is connected with a liquid conduit or channel **426** (labeled as **426A** to **426H** respectively), which extends initially laterally and then upwardly towards a top connecting conduit **428** that extends along the top surface of middle portion **404**. Bottom openings **422I** and **422J** are connected to well **412** through conduits or channels **414** and **416** in the bottom portion **406**. Well **412** is connected with a conduit or channel **430**, which extends upwardly to connecting conduit **428**.

[0042] Thus, chambers **418** are each in fluid communication with a respective port **410** through its top opening **420**, and are in fluid communication with one another through their bottom openings **422** and connecting conduit **428**.

[0043] When the three portions are affixed together such as with bolts and nuts or with an adhesive tape or thermo-diffusion bonding, the channels are tightly sealed and form closed fluid conduits for allowing fluid communication between the chambers and between the corresponding pairs of pneumatic ports and chambers. The contacting surfaces between portions **402** and **404**, and between portions **404** and **406**, may contain, or coated with, a sealing material. Well **412** when covered by bottom surface of middle portion **404** forms another fluid chamber, which may serve, for example, as a gene extraction chamber, as will be discussed further below. The chamber formed by well **412** is not connected directly to a pneumatic port, as it is not necessary to do so, as can be understood by persons skilled in the art.

[0044] As can be appreciated, cartridge body **400** initially defines a closed fluid network therein. Top and middle portions **402**, **404** when affixed together define chambers **418** and top connecting conduit **428** therebetween. Top openings of chambers **418** are in fluid communication with corresponding ports **410**. Bottom openings **420** of chambers **418** are in fluid communication with one another through connecting conduit **428**.

[0045] Top openings **420** and connecting conduit **428** may be positioned at the same level, adjacent top portion **402**, as illustrated in the figures. In different embodiments, connecting conduit **428** may be positioned higher or lower than top openings **420** but it should be at a level above the highest bottom opening **422** of the chambers **418** that are interconnected through connecting conduit **428** to prevent unintended transfer or mixing of the liquids contained in the chambers **418** through the connecting conduit **428**. As can be appreciated, the allowable highest liquid level in the chambers **428** is dependent on the level of the highest section of the connecting conduit **428**. Thus, a higher connecting conduit **428** (up to the top of the chambers) may be advantageous as it can allow more effective utilization of the chamber volume.

[0046] A chamber **418** may have a generally elongated cylindrical shape and a sloped bottom, and may extend vertically between top and bottom portions **402** and **406** (as better illustrated in **FIG. 11**). However, in different embodiments, chambers **418** may have a different cross-sectional shape and may be longer or shorter.

[0047] Cartridge body **400** or its portions may be formed using any suitable material. For example, each portion may be formed from a polymeric material, such as polycarbonate, poly(methyl methacrylate) (PMMA), or the like. Top portion **402** may be formed of a thin sealing material such as a plastic sheet with or without an adhesive surface. Seals **411** may be formed of any suitable sealing material such as a plastic film that can be conveniently broken with the needles **306**. The plastic film may or may not have an adhesive layer on its surface.

[0048] The body **400** may be formed using traditional machining techniques, such as microinjection molding and computerized numerically controlled (CNC) machining, or using plastic injection molding, as can be understood by persons skilled in the art.

[0049] The internal surfaces of the chambers and channels may be cleaned or sterilized when desired or needed. In some cases, the internal surfaces of the chambers and channels may be coated with another polymer material such as Teflon to modify the surface properties.

[0050] The sizes or dimensions of the cartridge **400** and the internal chambers **418**, fluid conduits/channels, or openings may vary depending on the application. For biological applications, the sample amount is typically small, thus the fluidic chambers and channels may have dimensions on the order of micro-meters. When the dimensions are too large, it may be difficult to use pneumatic pressures to transfer small amounts of fluids. On the other hand, the dimensions should be large enough to allow sufficient transfer rate of the fluids under pneumatic pressures. In typical applications, the fluid channels and openings may have dimensions in the range of about 0.2 mm to about 1 mm. The chambers should have sufficient volumes for performing the particular desired process or treatment. Each chamber **418** or other fluid chambers may have a volume on the order of 1 micro liter to 100 milliliter.

[0051] The particular shapes of the openings, channels, conduits, and ports may very in different embodiments and depending on the application.

[0052] In use, one or more chambers **418** may be filled, such as being pre-loaded, with a liquid. The liquid may be a sample, a buffer, or a reagent liquid, or any other desired liquid. A chamber may also be used to store a product such as purified target genes, or to store wastes. Different chambers **418** may be initially loaded with different fluids.

[0053] The fluids may be loaded before covering the top surface of middle portion **404** with top portion **402**. After loading the fluids, top portion **402** is attached and affixed to middle portion **404** to close and seal the exposed openings at the top of middle portion **404** and to prevent leakage or contamination during transportation or operation. In some embodiments, a fluid may be initially loaded into a chamber through a port **410** before the port **410** is sealed. However, in many applications, loading from the top with top portion **402** removed may be more convenient.

[0054] As illustrated in **FIG. 11**, when cartridge body **400** is coupled to base **300**, seals **411** at ports **410** may be selectively broken by needles **306** which will pierce through the seals **411**, thus establishing fluid communication between corresponding valves in valve array **112** and chambers **418**, through ports **410**, conduits **424** and top openings **420**.

[0055] The valves in valve arrays **112A** and **112B** are each connected to a corresponding needle **306** and configured to selectively regulate a fluid flow through that needle **306**. The pumps **110** are connected to the valve arrays **112A** and **112B** for selectively applying a positive or negative pressure to the ports **310** through values **112**.

[0056] Computer **104** controls the operation of controller **108**, which in turn controls the operation of pumps **110** and valves **112** to selectively apply pressured air (positive pressure) or vacuum (negative pressure) to ports **410**, through needles **306**. Depending on whether a positive or a negative pressure is applied at a port **410**, a fluid may be selectively transferred to or from the corresponding chamber

418. If no change is needed for a particular chamber, the port for that chamber may remain closed, such as by closing the corresponding valve.

[0057] For example, to perform gene extraction for PCR (polymerase chain reaction), cartridge **400** may be loaded with fluids as illustrated in **FIG. 12**, which schematically shows a similar cartridge with some modifications from cartridge **400**. One modification is that some chambers are omitted in **FIG. 12** as they are not used for this particular operation; further the bottom. Another modification is that bottom openings **424I** and **424J** are connected to connecting chamber **428** through gene extraction chamber **412**, not directly. However, the operation procedure for transferring liquids between chambers **418B** to **418F** can be the same despite these changes. Assuming chambers **418B**, **418C**, **418D**, **418E**, **418F** are loaded with sample, lysis buffer, conditioner, wash buffer, and elute buffer respectively. A gene extractor is deposited in well **412**. The gene extractor may include a magnetic-based extractor, silica membrane, silica beads or another material that can attach to genes in a fluid with a high iron concentration. The genes may include DNA (Deoxyribonucleic acid), RNA (Ribonucleic acid), or mRNA (Messenger RNA). The gene extractor may be selected based on the particular genes to be extracted. Chambers **418I** and **418J** can be used for storing the product, extracted genes, and the waste produced by the reactions respectively. DNA or RNA degradation reagent may also be preloaded in one or more chambers.

[0058] In some cases, adjustment of the gene binding condition may be required. The buffers loaded in the chambers may include buffers for gene binding (such as pure or 70% ethanol) based on the target gene binding conditions.

[0059] In one embodiment, to perform sample lysing, the valves **112** corresponding to conduits **424D**, **424E**, **424F** are closed. The valve **112B** connected to conduit **424B** is open and a pressurized gas such as air is supplied thereto, thus creating a positive pressure inside chamber **418B**. The valve **112A** connected to conduit **424C** is open and a vacuum pressure is applied thereto, thus creating a negative pressure above the liquid in chamber **418**C. The pressure differential thus drives sample liquid from chamber **418B** to chamber **418C**. Gas bubbles may also be created inside chamber **418C**, facilitate mixing of the sample with the lysis buffer, after the sample liquid is completely transferred to chamber

418C. The sample may thus be mixed with the lysis buffer thoroughly to lyse the sample and to protect the genes from degradation.

[0060] A similar procedure may be used to transfer the lysed sample to chamber **418D** to mix with the binding conditioner, thus adjusting the sample's gene condition.

[0061] The conditioned sample may be then transferred to well **412** directly or through one or more of chambers **418E** and **418F**, depending on the particular application as will be understood by those skilled in the art.

[0062] When magnetic beads are used as the gene extractor for gene extraction, the lysed sample may be mixed with the magnetic beads in well **412** using a similar procedure, without first going through the other chambers.

[0063] Gene extraction may also be performed in well **412** with the pre-treated sample fluid transferred from another chamber such as chamber **424D**. The target genes in the sample will be attached to the gene extractor. The remaining liquid may be transferred to chamber **418J** as waste.

[0064] Gene purification may be performed by transferring the wash buffer from chamber **424E** to well **412**. One wash buffer or different types of wash buffers may be used to wash the genes in multiple steps. If multiple wash buffers are used, two or more chambers may be allocated to store wash buffers. In one embodiment, wash buffer may be transferred from chamber **418E** to chamber **418J**, through well **412** and conduit **414**. The waste may be later discharged from chamber **418J** if the cartridge is to be recycled, or disposed with the cartridge.

[0065] Gene elution may be performed by transferring the elution buffer from chamber **418F** to chamber **418I** through well **412** and conduit **416**. The elution buffer will release the genes attached to the gene extractor and carries the genes to chamber **418F**. The target genes collected in chamber **418I** may be withdrawn using pipette or syringe for downstream applications, such as gene amplification and detection. Extracted genes or other reaction products may be transferred to a detection chamber (not shown) for detection of certain signals or target. The

detection chamber may be provided within cartridge **116** or may be provided in an external device (not shown) which can receive the material from cartridge **116**.

[0066] The operation of selectively transfer a fluid between the chambers is further illustrated with a simplified schematic diagram of the cartridge shown in **FIGS. 13, 14** and **15**. For simplicity, only three chambers are shown in these figures but it should be understood that more chambers may be operated in a similar manner.

[0067] As shown in **FIG. 13**, in an exemplary application, each of chambers **418A**, **418B** and **418C** is filled with a liquid reagent **432A**, **432B** or **432C** respectively. Ports **410A**, **410B** and **410C** are initially sealed.

[0068] As shown in **FIG. 14**, in order to mix reagents **432A** and **432C** in chamber **418C**, a positive gas (using air or another gas, such as an inert gas including N_2) pressure may be applied at port **410A** and a vacuum pressure may be applied at port **410C**, while port **410B** may remain closed such as by closing the corresponding valve **112**. The pneumatic pressure will drive reagent **432A** through bottom opening **422A**, connecting conduit **428** and bottom opening **420C** into chamber **418C**. Thus, reagents **432A** and **432C** can be brought into contact or mixed in chamber **418C**. Due to the positive pressure within chamber **418B**, reagent **432A** is prevented from flowing into chamber **418B**. The reagent in chamber 418A can be completely transferred to chamber **418C**.

[0069] As shown in **FIG. 15**, reagent **432A** may be completely transferred to chamber **418C**, while reagent **432B** remains in chamber **418B**.

[0070] Because the connecting conduit **428** is above the liquid level in the chambers, and the pneumatic pressure is applied through the top opening, air can bubble through the liquids without driving the liquid out of the cartridge through the pneumatic ports. Conveniently, the air bubbles can be used to mix the reagents in the chambers such as in chamber **418C** as shown in **FIG. 15**.

[0071] Conveniently, fluid transfer, mixing and processing may be effected without using any internal valves, pumps or other fluid regulating devices in the cartridge. The fluid transfer may be effected entirely by selectively applying

pneumatic pressures to different ports. This process may be automated using computer **104** and controller **108**. In some embodiments, the chambers may be interconnected such that a fluid may be transferred from any selected chamber to another selected chamber, or may be transferred sequentially from one to another through a series of chambers.

[0072] As can be understood, the exemplary cartridges described herein is simple in construction and can be made at relatively inexpensively. Since there is no moving parts in the cartridge it is very reliable. Since the sample and reagents can remain in a closed system during transportation and even during operation, the risk of contamination is significantly reduced.

[0073] The cartridge can also be conveniently configured and adapted for use in different applications, with different materials. Embodiments of the present invention can also be conveniently integrated with other systems such as a gene amplification unit or a gene detection unit.

[0074] In another embodiment, cartridge **116** may have an alternative construction, such as having a cartridge body **400'** illustrated in **FIGS. 16**, **17** and **18**. In cartridge body **400'**, processing chambers and channels are provided within middle portion **404'**. Top and bottom portions **402'** and **406'** may be formed of flat sheets with no fluid channel or cavities. The flat sheets may have an adhesive surface, and may be formed of polymer tapes. The conduits and channel features may be entirely provided within the middle portion. The pneumatic ports **410'** are also provided within middle portion **404'** and are sealed by bottom portion **406'**. Thus, bottom portion **406'** serves as a seal in this embodiment.

[0075] Cartridge body **404'** may define three or more chambers, which are used to initially receive a sample, a wash buffers and an elute buffer, respectively; a gene extractor chamber **412'**; and two chambers for storing extracted and purified genes and wastes respectively during operation, as illustrated in **FIGS. 16**, **17** and **18**. The chamber may be formed of through holes in middle portion **404'**.

[0076] As better shown in **FIGS. 16** and **18**, the chambers in the alternative cartridge **400'** may include a gene extractor chamber **412'** and a waste chamber

418'. The remaining chambers shown may be used as reagent chambers for receiving samples and other reagent fluids or the extracted genes. A difference between the cartridges **400** and **400'** is that the chambers for storing the waste and the product genes in cartridge **400'** do not have a bottom opening but their top openings are connected to both the pneumatic ports and the extraction chamber **412'**. As can be seen, a plurality of connecting conduits **428'** are directly connected to the top opening of gene extractor chamber **412'** in a radial arrangement, which are respectively connected to the other corresponding chambers. The other chambers, which have top and bottom openings similar to those described above with regard to body **400**, are arranged around chamber **412'**.

[0077] As illustrated, extractor chamber **412'** is located at the center of middle portion **404'** in the sense that other fluid chambers, except the waste chamber, are located around chamber **412'**. A gene extractor **434**, which may have a cup-shape, may be placed at the bottom of extractor chamber **412'**.

[0078] During use, pre-loaded sample and washing buffers may be transferred, under pneumatic pressure, to gene extractor chamber **412'** through channels **428'**. A pre-loaded elute buffer may also be transferred to gene extractor chamber **412'** to elute genes via channel **428'**.

[0079] For coupling to cartridge body **400'**, the receptacle base of the pneumatic interface **116** may be modified as shown in **FIG. 19**, so that modified base **300'** can be properly coupled to the alternative cartridge **400'** and the needles **306'** are arranged to match the locations of the pneumatic ports **410'** on the cartridge **400'**. Needles **306'** can thus pass through bottom portion **406'** (acting as a seal) and communicate with pneumatic ports **410'** when cartridge **400'** is coupled to base **300'**.

[0080] In use, the alternative cartridge **400'** may be operated in a similar manner as described above with regard to cartridge body **400**.

[0081] As can be understood, it is not necessary that the cartridge in an embodiment of the present invention to be provided with an internal integrated

detector, or a detection window. Instead, the cartridge may be coupled to another unit that includes detectors and other detection devices. For this purpose, the body of the cartridge may be made of a material that is suitable for use with the particular detection method to be used. For example, the body may be made of a transparent material when an optical detection method is to be used, or a non-magnetic material when a magnetic probe is to be used.

[0082] As described above and shown in the figures, it is not necessary that every chamber in the cartridge is connected to a pneumatic port. The top and bottom openings of a chamber may be respectively connected to different connection channels or conduits.

[0083] Also as shown in **FIG. 11**, **12** to **15**, and **18**, the bottom of a chamber may have a bottom surface that is sloped downwardly towards the bottom opening of the chamber. The bottom surface may be generally cone-shaped. Such a sloped bottom surface may be advantageous as the fluid in the chamber can be conveniently withdrawn through the bottom opening due to the gravitational force. In other words, the dead volume in the chamber is reduced or minimized with such a sloped bottom. While the bottom opening is shown to be located at the centre of the chamber in the figures, this is not necessary. The bottom opening may be relocated off centre. As long as the bottom opening is at the lowest level in the chamber and the bottom surface is sloped downwardly towards it, the same benefit may be obtained.

[0084] Of course, modification to the system and cartridge described herein is possible. For example, the cartridge body may have different constructions depending on the particular application. The shapes and number of chambers may vary. Each chamber may have more than one top or bottom openings. The top or bottom openings may be moved to the side wall of the chamber as long as they remain separated by a sufficient vertical distance to allow a sufficient amount of liquid to be received without losing liquid, or allow all liquid in the chamber be withdrawn through the bottom opening by applying pneumatic pressure.

[0085] As now can be understood, it is not necessary that the body of cartridge **106** is formed of three separate portions or layers. The cartridge may be formed

from a single block of materials. However, layered construction may be convenient for manufacture and pre-loading of the fluids.

[0086] It is not necessary that the pneumatic ports **410** are located at the bottom portion of the cartridge body **400**. The ports may be positioned elsewhere such as at the top or on the size of the body **400**.

[0087] The top portion **402**, the seals **411**, or the bottom portion **406**, may be removable and replaceable so that the cartridge **106** may be conveniently re-used.

[0088] Multiple connecting conduits **428** may be provided between different chambers. To isolate the liquids in different chambers, it my be sufficient that at least a portion of each connecting conduit **428** is at a level above the highest liquid level in the chambers **418** that are interconnected through that connecting conduit **428**.

[0089] It is not necessary that the pump is provided within station **102**. For example, when available, any compressed air supply or source may be used to apply positive air pressure to the ports. Any available vacuum source may also be used.

[0090] In the embodiments shown in the drawings, the cartridge **106** may include six to eleven fluid chambers. In different embodiments, the number of chambers may be different depending on the particular application. Further, it is not necessary that all the chambers are at the same level or that all chambers are inter-connected. For example, a chamber may be positioned above or higher than another chamber. It should be understood that the term "above" as used herein do not require that one chamber is directly above another chamber. It is sufficient if the bottom level of one chamber is at a higher level than the bottom level of the other chamber. Further, it should be understood that the terms "higher" or "lower" is used with the assumption that the cartridge body is placed in an upright position with the top portion on top.

[0091] While needles **306** and seals **411** may be conveniently used in some embodiments, particularly when the size of the ports are small, the pneumatic conduits and the coupling between a pneumatic port **410** and a pneumatic conduit

for selectively applying a pneumatic pressure may be provided in different manners. For example, other types of coupling arrangements may be used in different embodiments.

[0092] In different embodiments, a different base station that is connectable to the cartridge **106** may be used for selectively applying pneumatic pressures to the chambers **418** through ports **410**. The base station may include a base configured for coupling with the cartridge body **400**, pneumatic conduits mounted on the base for coupling with ports **410** to selectively apply the pneumatic pressures through the seals **411**. The base station may also include valves each connected to a pneumatic conduit for regulating the fluid flow therethrough. A controller may be provided for control the operation of the valves. Each pneumatic conduit may be connected to two valves, which are in turn connected to two different pressure devices, one for applying a first pneumatic pressure and the other for applying a second pneumatic pressure lower than the first pneumatic pressure, so that the difference in the pneumatic pressures is sufficient to cause fluid transfer between the chambers in the cartridge. The pressure devices may include pumps, a pressured gas (such as pressured air) line, or a vacuum line. The first pressure may be higher than one atmosphere and the second pressure may be lower than one atmosphere. In some embodiments, both pressures may be higher than one atmosphere. The pumps may be separately provided or integrated with the base station. The applied pressures and the valves may be automatically or manually controlled.

[0093] As now can be understood, the methods and apparatus described herein can be used to transfer and process raw biological materials that contain genes protein and reagents. After pre-treatment, the target genes or protein can be extracted using the same apparatus. Different raw biological materials can be processed using embodiments described herein, which include blood, cultured cells, serum, other types of body fluids, and the like. The target genes can be DNA, RNA, mRNA, protein, or the like.

[0094] Exemplary embodiments described herein can be used in various applications, including as part of a clinical or point-of-care disease diagnostics system, for sample preparation in research or clinical use, in forensic studies or

sample processing, in the field of disease or medical research, in the field of chemistry or biological research such as molecular chemistry research, or in other similar fields.

[0095] Other features, benefits and advantages of the embodiments described herein not expressly mentioned above can be understood from this description and the drawings by those skilled in the art.

[0096] Of course, the above described embodiments are intended to be illustrative only and in no way limiting. The described embodiments are susceptible to many modifications of form, arrangement of parts, details and order of operation. The invention, rather, is intended to encompass all such modification within its scope, as defined by the claims.

WHAT IS CLAIMED IS:

1. A micro-fluidic device, comprising:

a body defining

first, second and third pneumatic ports, each sealed with a seal and shaped to couple to a pneumatic conduit through said seal;

a first chamber for receiving a first liquid, having a first top opening and a first bottom opening, said first top opening in fluid communication with said first port;

a second chamber for receiving a second liquid, having a second top opening and a second bottom opening, said second top opening in fluid communication with said second port;

a third chamber for receiving a third liquid, having a third top opening and a third bottom opening, said third top opening in fluid communication with said third port; and

a connecting conduit above each one of said first, second and third bottom openings, said bottom openings in fluid communication with one another through said connecting conduit,

wherein selective application of pneumatic pressures to said chambers through said pneumatic conduits transfers a liquid from one of said chambers to another one of said chambers through said connecting conduit.

2. The micro-fluidic device of claim 1, wherein each one of said chambers has a bottom surface sloped downwardly towards said bottom opening of said each chamber.

3. The micro-fluid device of claim 1 or claim 2, wherein at least a section of said connecting conduit is at a level above a liquid level in said chambers.

4. The micro-fluidic device of any one of claims 1 to 3, wherein said body comprising a top portion, a bottom portion, and a middle portion, said chambers and connecting conduit defined by said middle and top portions.

5. The micro-fluidic device of claim 4, wherein at least a section of said connecting conduit is adjacent said top portion.

6. The micro-fluidic device of claim 4 or claim 5, wherein said top openings of said chambers are adjacent said top portion.

7. The micro-fluidic device of any one of claims 4 to 6, wherein said ports are defined by said bottom portion.

8. The micro-fluidic device of any one of claims 4 to 7, wherein said middle portion defines a first conduit extending between said first port and said first top opening, a second conduit extending between said second port and said second top opening, and a third conduit extending between said third port and said third top opening.

9. The micro-fluidic device of claim 8, wherein said middle portion defines a fourth conduit extending between said first bottom opening and said connecting conduit, a fifth conduit extending between said second bottom opening and said connecting conduit, and a sixth conduit extending between said third bottom opening and said connecting conduit.

10. The micro-fluidic device of any one of claims 4 to 9, wherein said top, middle and bottom portions are separate portions, said middle portion being sandwiched between said top and bottom portions.

11. The micro-fluidic device of claim 10, wherein at least one of said top and bottom portions is formed of a flat sheet.

12. The micro-fluidic device of any one of claims 4 to 11, wherein at least one of said top portion and said bottom portion is made of a plastic material.

13. The micro-fluidic device of any one of claims 1 to 12, wherein said body defines more than three inter-connected chambers.

14. The micro-fluidic device of claim 13, wherein said body defines six to eleven inter-connected chambers.

15. The micro-fluidic device of any one of claims 1 to 14, wherein said liquid comprises a reagent, a buffer, a sample, or a gene binding conditioner.

16. The micro-fluidic device of any one of claims 1 to 15, wherein at least two of said chambers contain different liquids.

17. The micro-fluidic device of any one of claims 1 to 16, wherein at least a portion of said body is made of a polymer.

18. The micro-fluidic device of claim 16, wherein said polymer comprises polycarbonate or poly(methyl methacrylate).

19. The micro-fluidic device of any one of claims 1 to 18, wherein said seal is made of a plastic material.

20. The micro-fluidic device of any one of claims 1 to 19, wherein said body defines a gene extractor chamber, said gene extractor chamber containing a gene extractor and being in fluid communication with said connecting conduit.

21. The micro-fluidic device of claim 20, wherein said body defines a product chamber and a waste chamber, each in fluid communication with said gene extractor chamber.

22. The micro-fluidic device of any one of claims 1 to 21, wherein said device is a cartridge.

23. The micro-fluidic device of any one of claims 1 to 22, wherein said pneumatic conduit comprises a needle.

24. An apparatus comprising a device as defined in any one of claims 1 to 23, and a station connectable to said device for selectively applying pneumatic pressures to said chambers through said ports of said device.

25. The apparatus of claim 24, wherein said station comprises:

a base configured for coupling with said device,

a plurality of pneumatic conduits mounted on said base, shaped to couple to said ports through said seals when said device is coupled to said base, and

a plurality of valves each connected to one of said pneumatic conduits for selectively regulating a fluid flow through said pneumatic conduits.

26. The apparatus of claim 25, wherein a first set of said plurality of valves are connected to a first pressure device for selectively applying to said ports a first pneumatic pressure, and a second set of said plurality of valves are connected to a second pressure device selectively applying to said ports a second pneumatic pressure lower than said first pneumatic pressure.

27. The apparatus of claim 26, wherein said first pneumatic pressure is higher than one atmosphere, and said second pneumatic pressure is lower than one atmosphere.

28. The apparatus of claim 27, wherein said first pressure device comprises a pressure pump, and said second pressure device comprises a vacuum pump.

29. The apparatus of any one of claims 26 to 28, wherein said station comprises a controller for controlling operation of said valves and said pressure devices.

30. The apparatus of claim 29, wherein said station comprises a computer in communication with said controller for controlling operation of said controller.

31. A method of operating the device of any one of claims 1 to 24, wherein at least one of said chambers contains said liquid, comprising:

coupling said pneumatic conduits to said ports;

selectively applying different pneumatic pressures to said chambers through said ports, to cause said liquid to flow from one of said chambers to another one of said chambers.

32. The method of claim 31, comprising selectively applying said pneumatic pressures to transfer said liquid sequentially through more than two of said chambers.

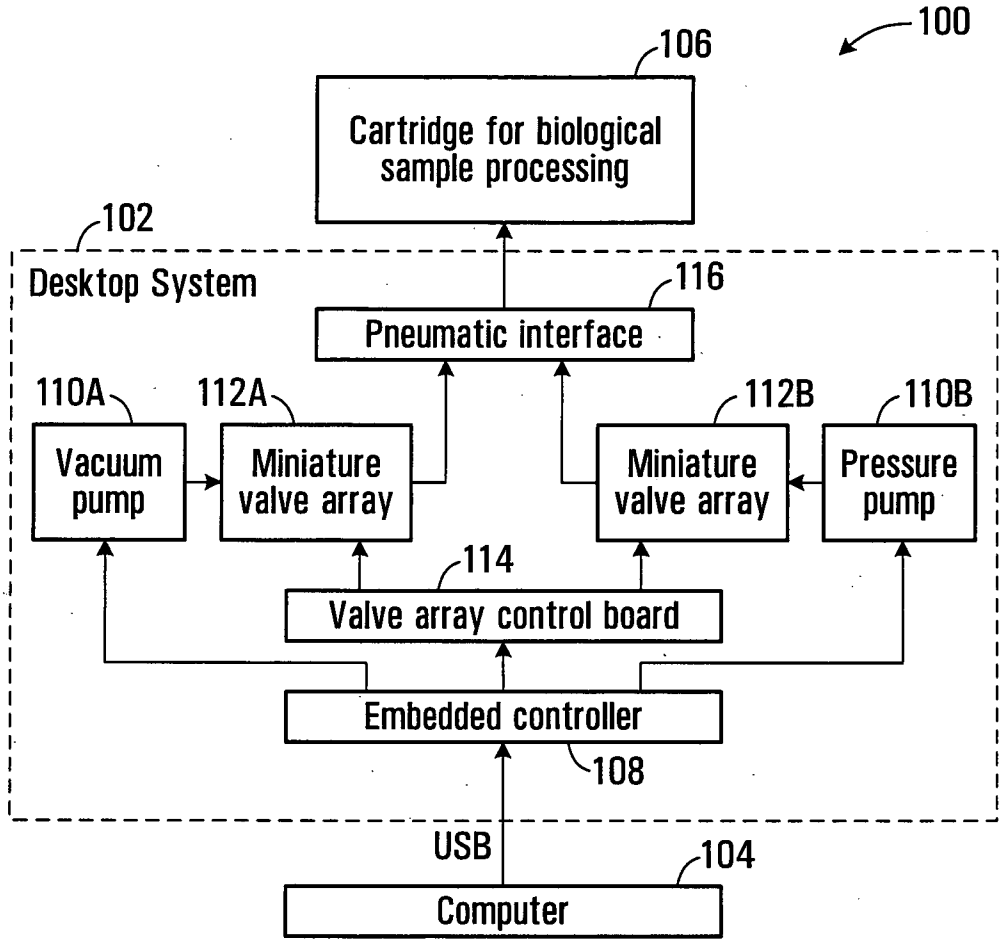

100
106
Cartridge for biological sample processing
102
Desktop System
116
Pneumatic interface
110A
112A
112B
110B
Vacuum pump
Miniature valve array
Miniature valve array
Pressure pump
114
Valve array control board
Embedded controller
108
USB
104
Computer

FIG. 1

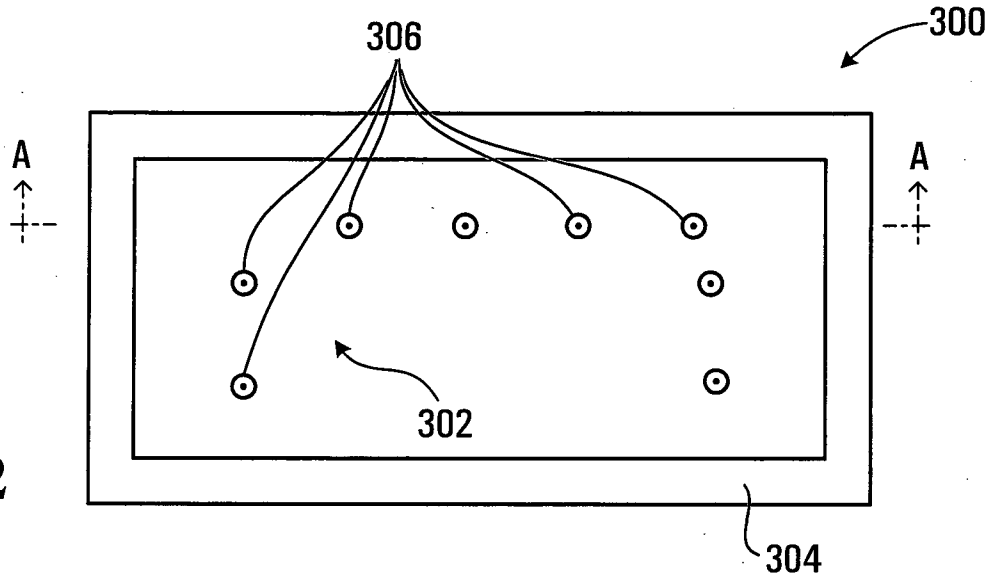


FIG. 2

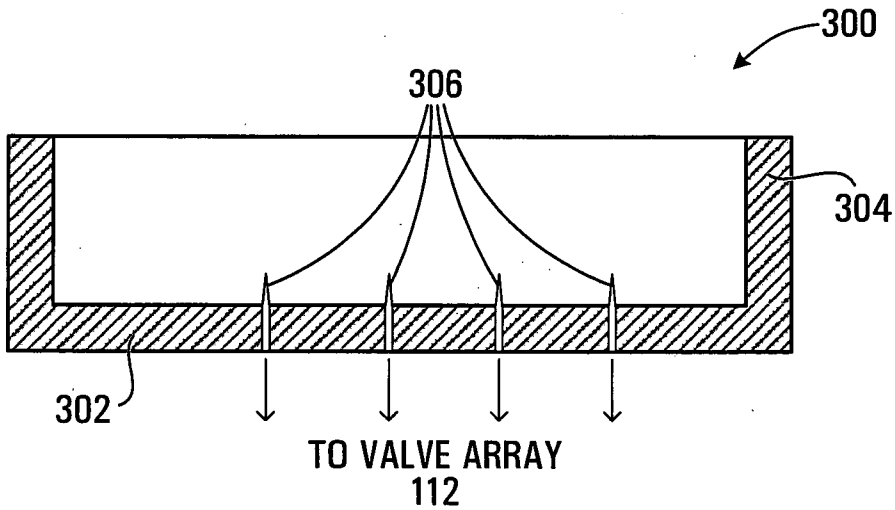


FIG. 3

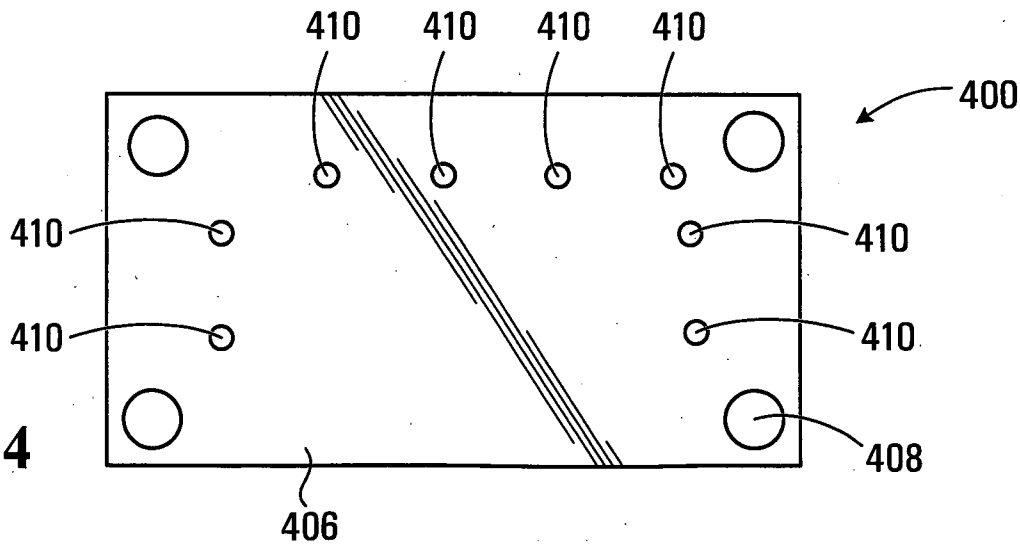


FIG. 4

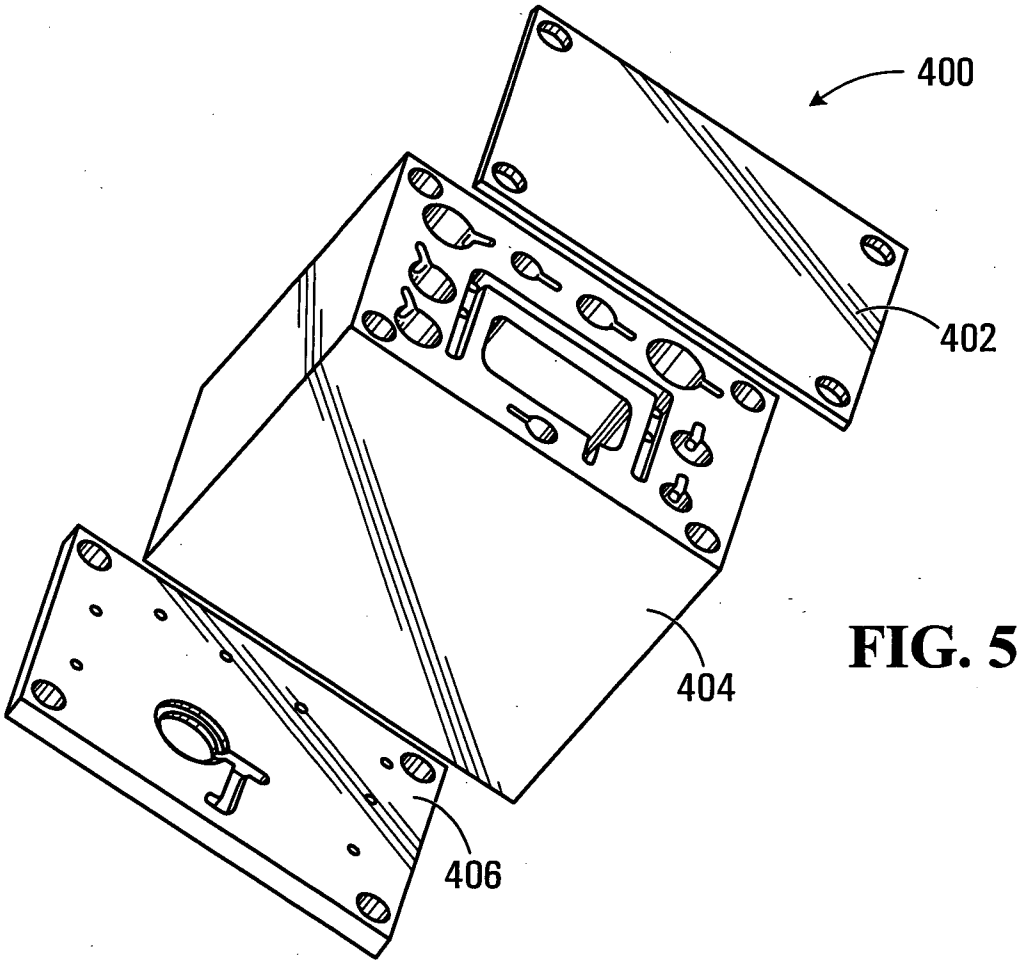


FIG. 5

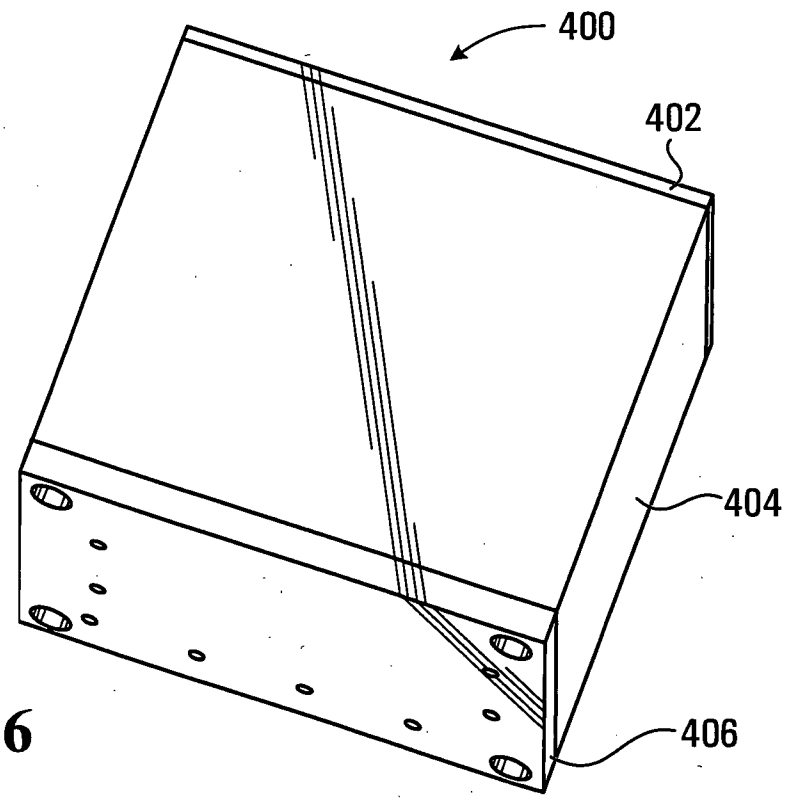


FIG. 6

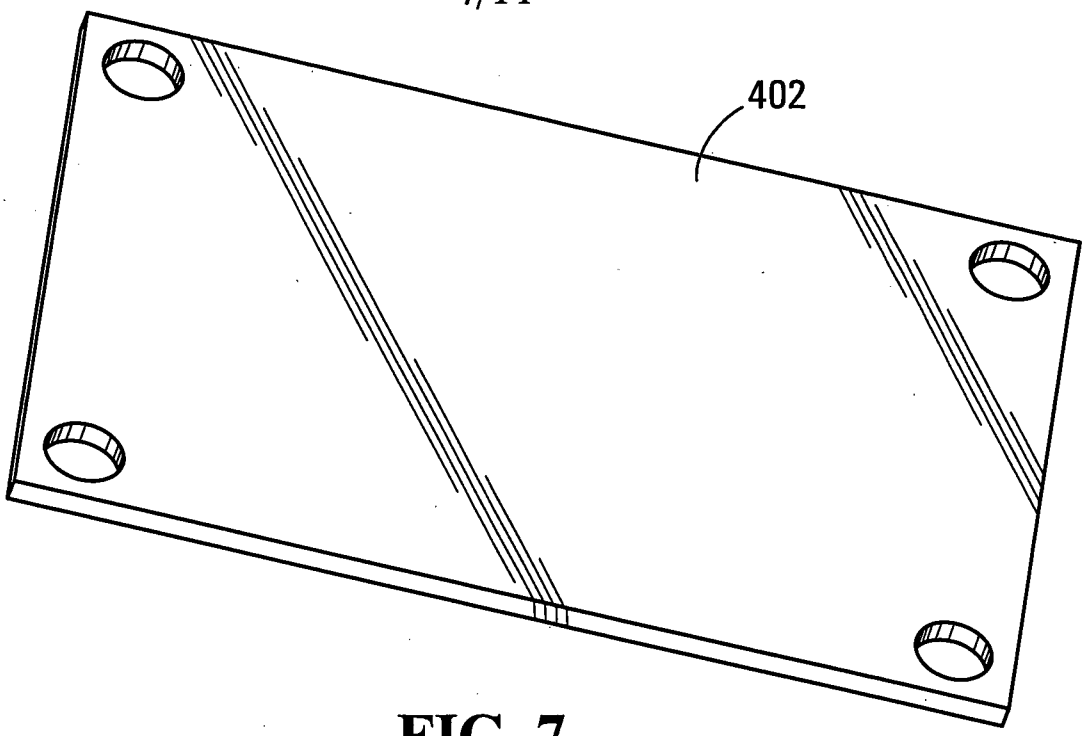


FIG. 7

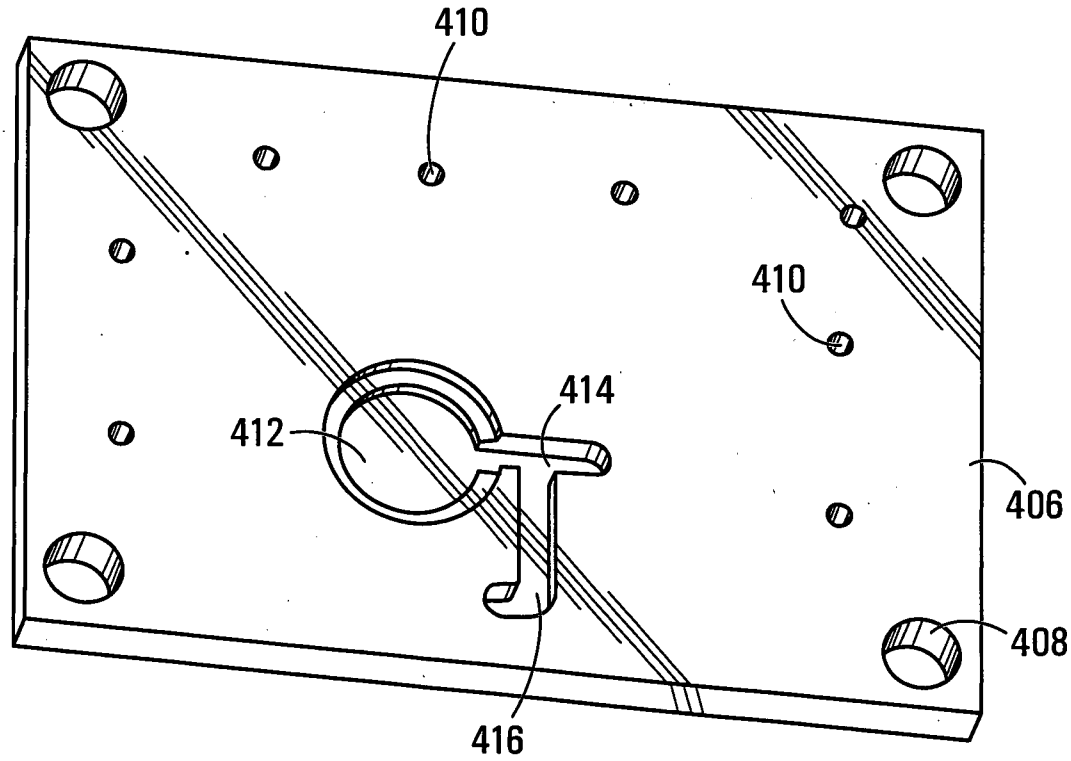


FIG. 8

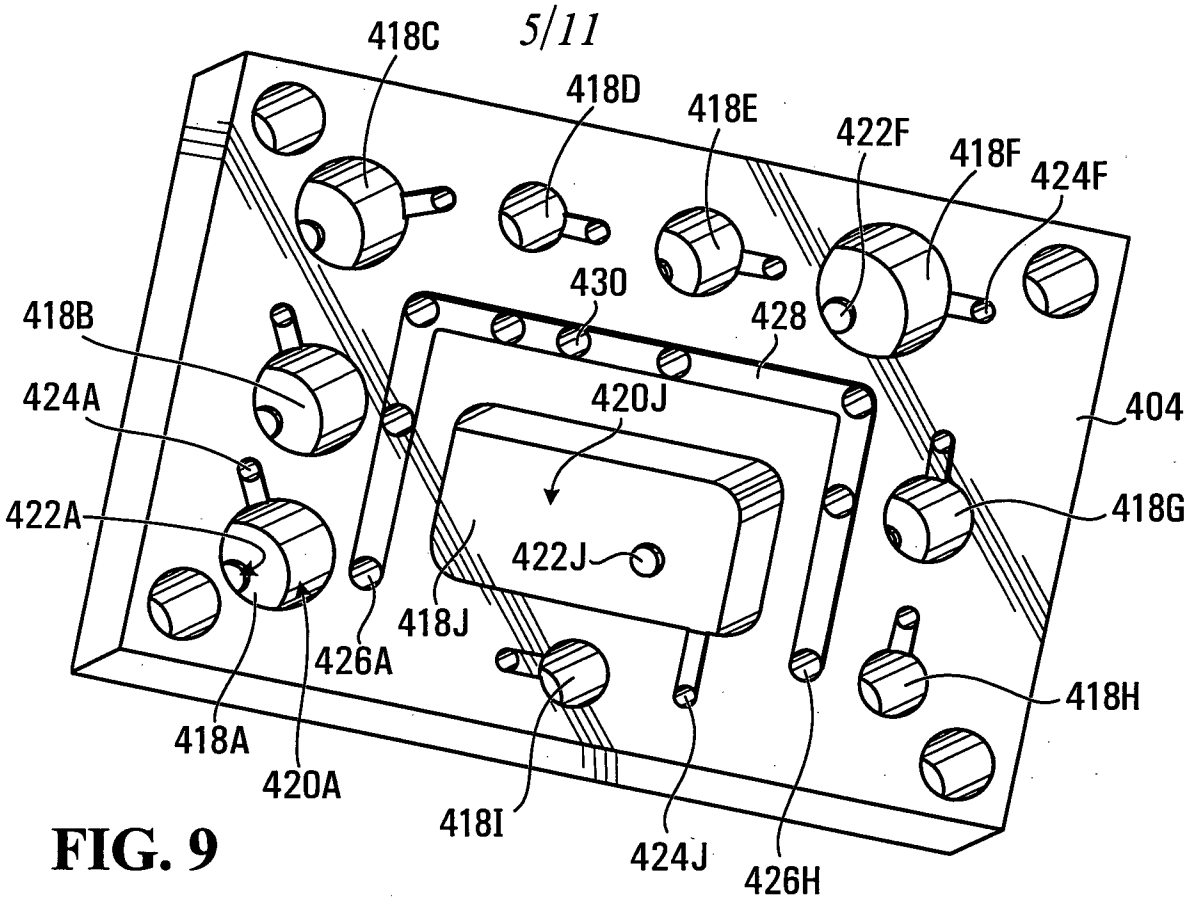

418C
418D
418E
422F
418F
424F
430
428
418B
424A
420J
404
422A
418G
422J
418J
426A
418H
418A
420A
418I
424J
426H

FIG. 9

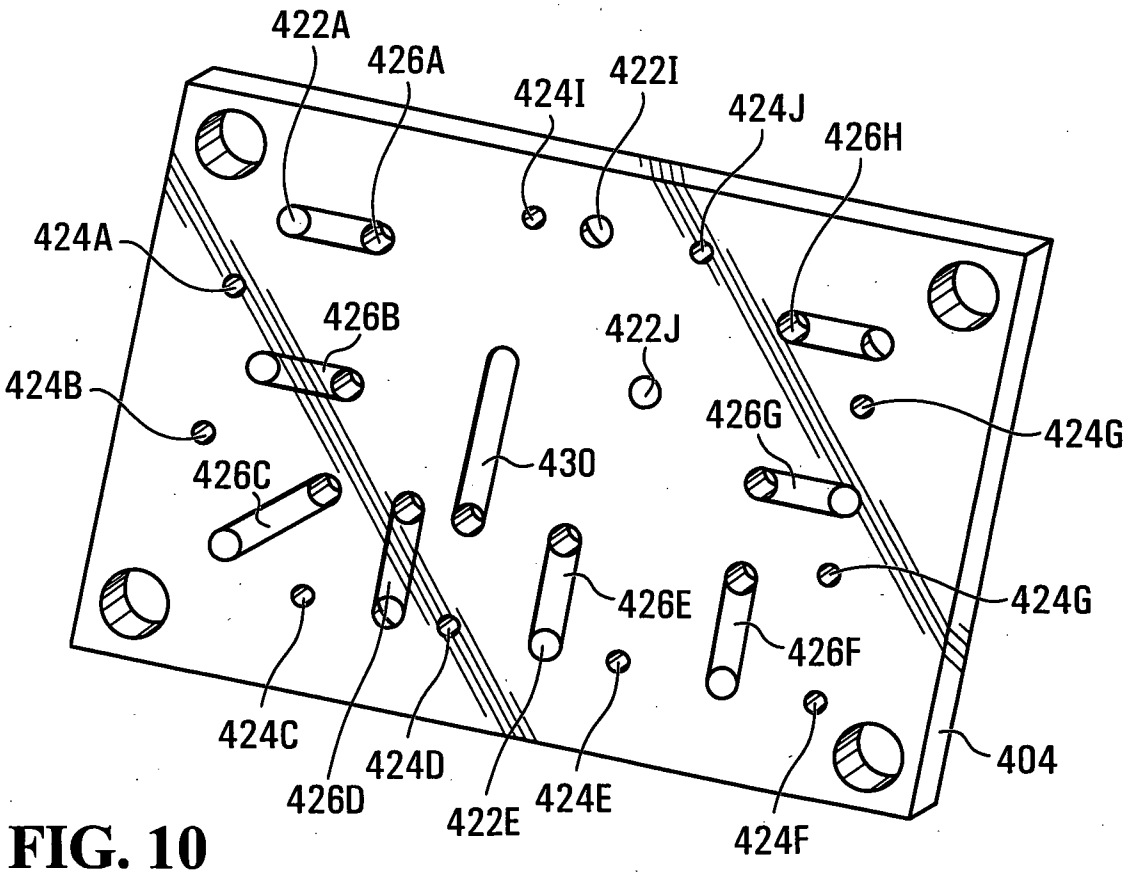

422A
426A
424I
422I
424J
426H
424A
426B
422J
424B
426G
424G
426C
430
426E
424G
426F
424C
424D
404
426D
422E
424E
424F

FIG. 10

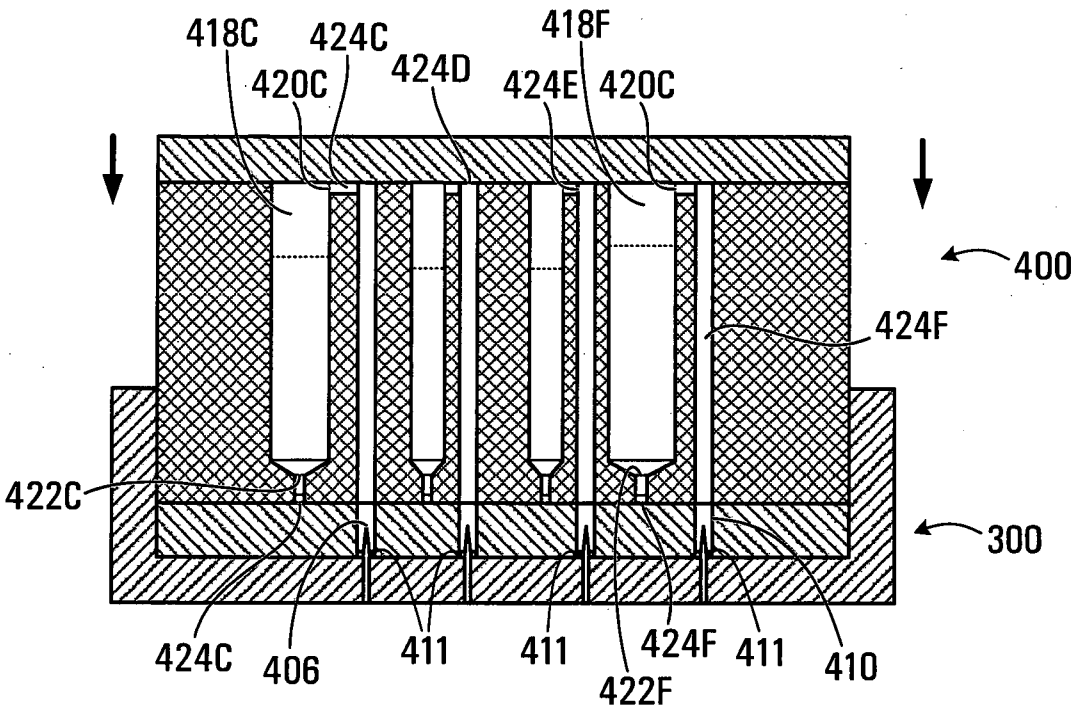

418C
424C
420C
424D
418F
424E
420C
400
424F
422C
300
424C
406
411
411
424F
422F
411
410

FIG. 11

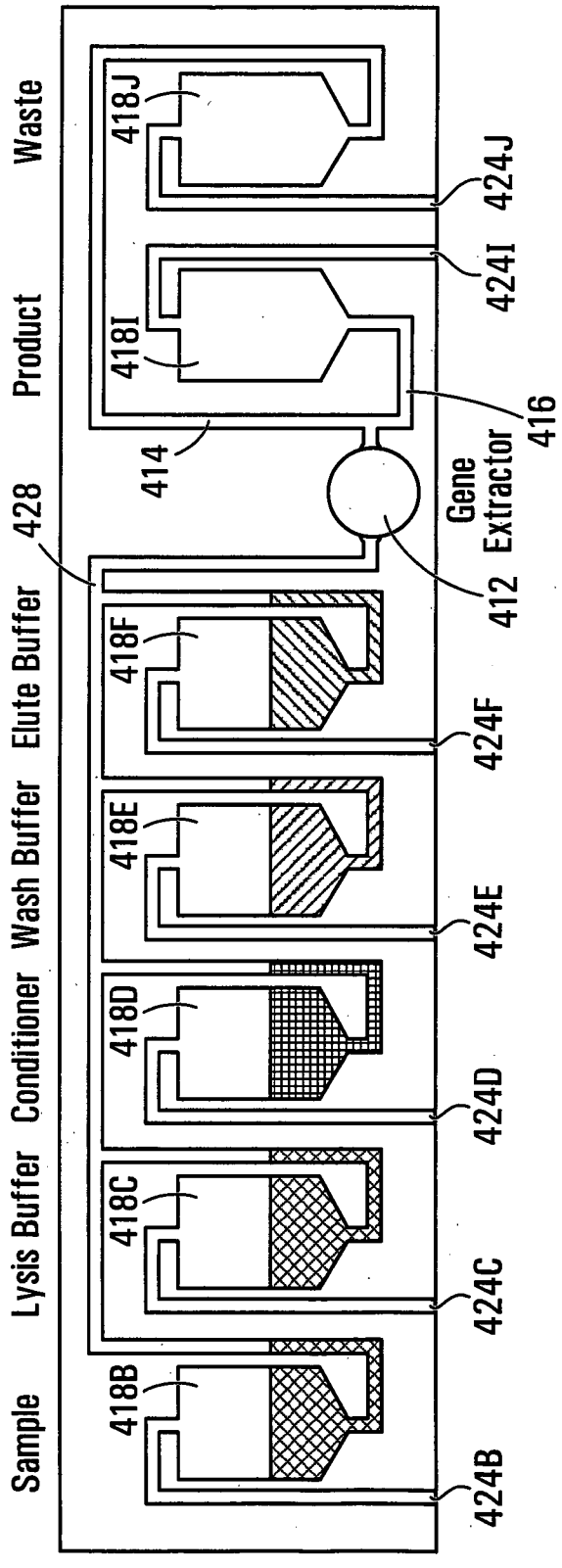

Sample
Lysis Buffer
Conditioner
Wash Buffer
Elute Buffer
Product
Waste
418B
418C
418D
418E
418F
418I
418J
424B
424C
424D
424E
424F
424I
424J
414
416
412
428
Gene Extractor

FIG. 12

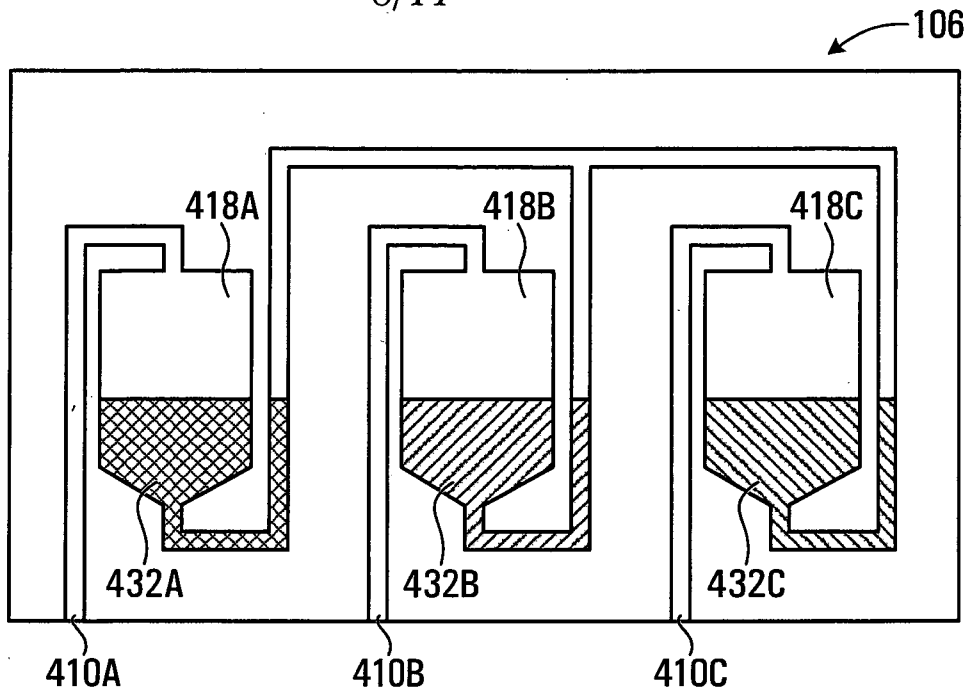

106
418A
418B
418C
432A
432B
432C
410A
410B
410C

FIG. 13

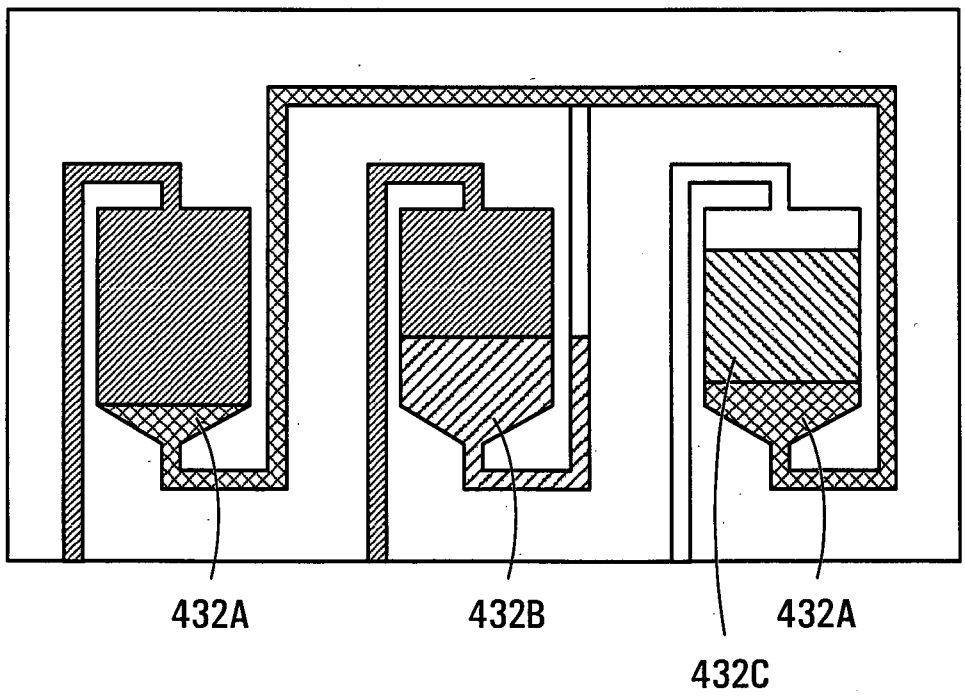

432A
432B
432C
432A

FIG. 14

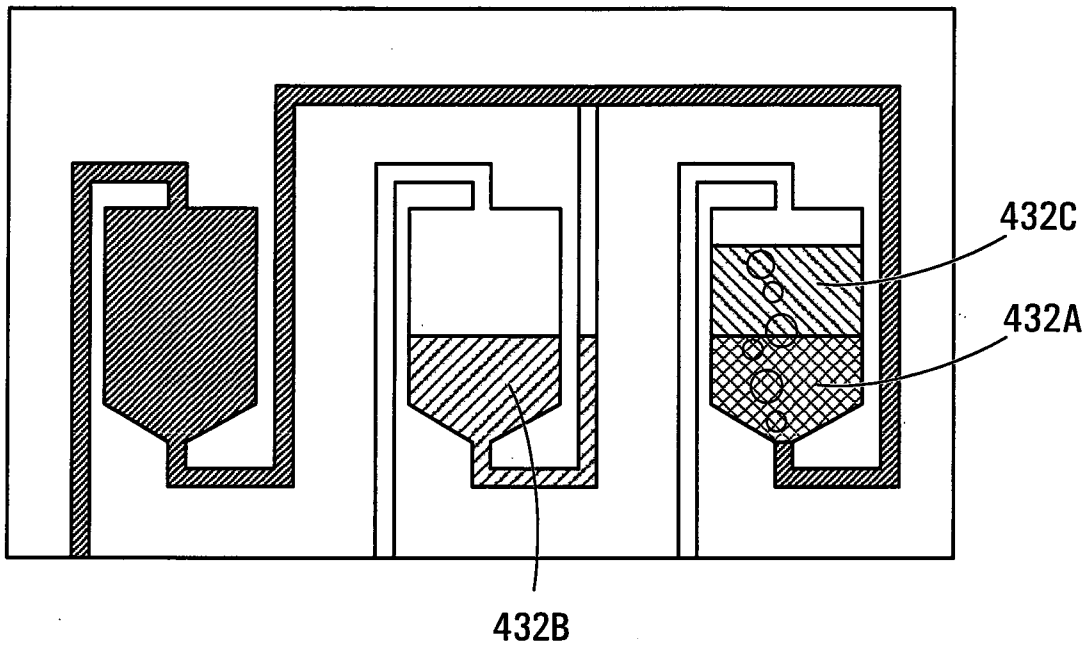


FIG. 15

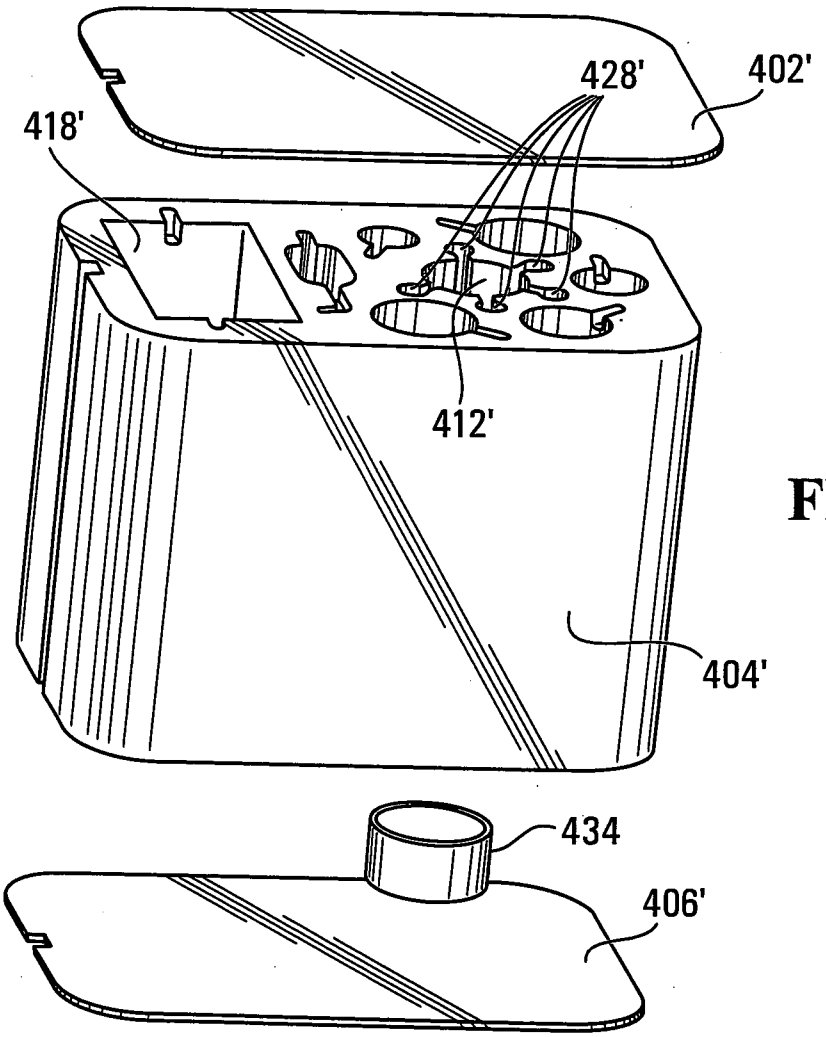


FIG. 16

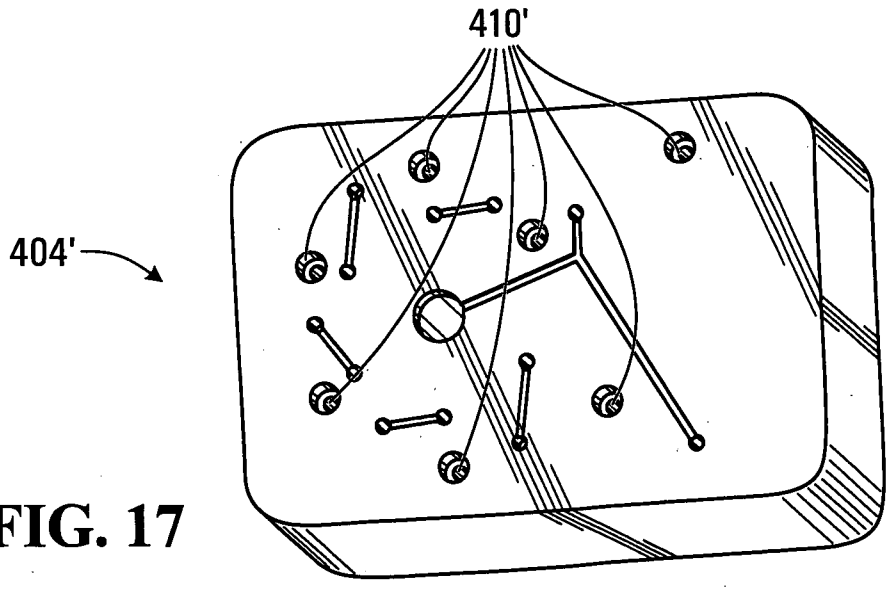


FIG. 17

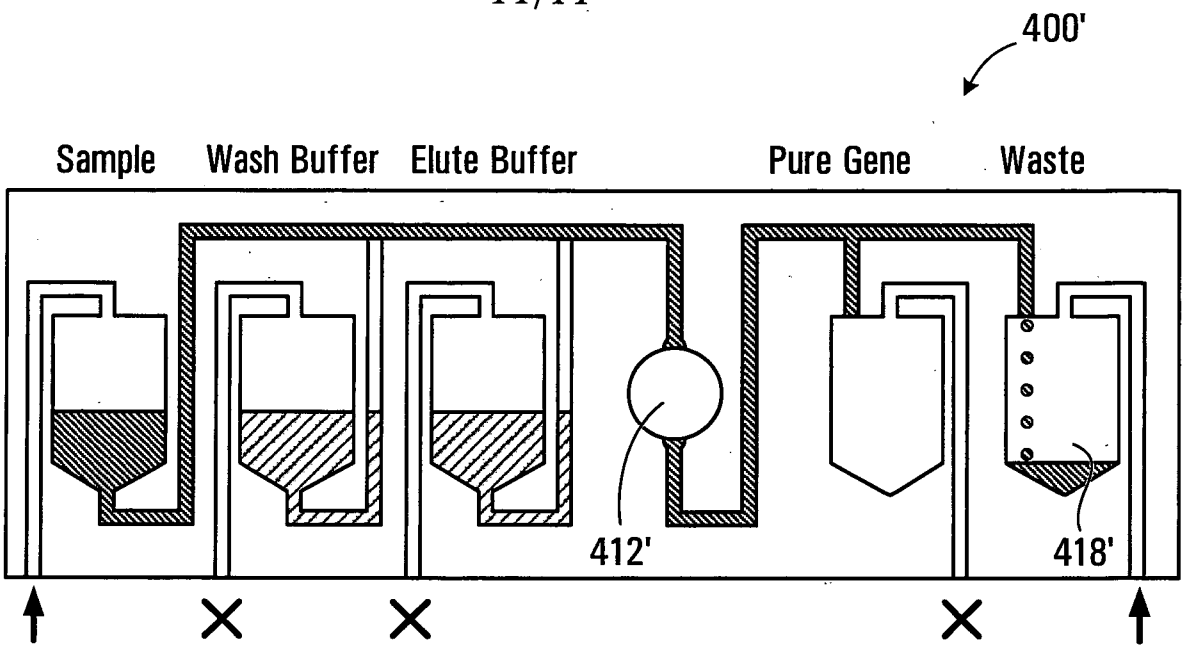

400'
Sample
Wash Buffer
Elute Buffer
Pure Gene
Waste
412'
418'

FIG. 18

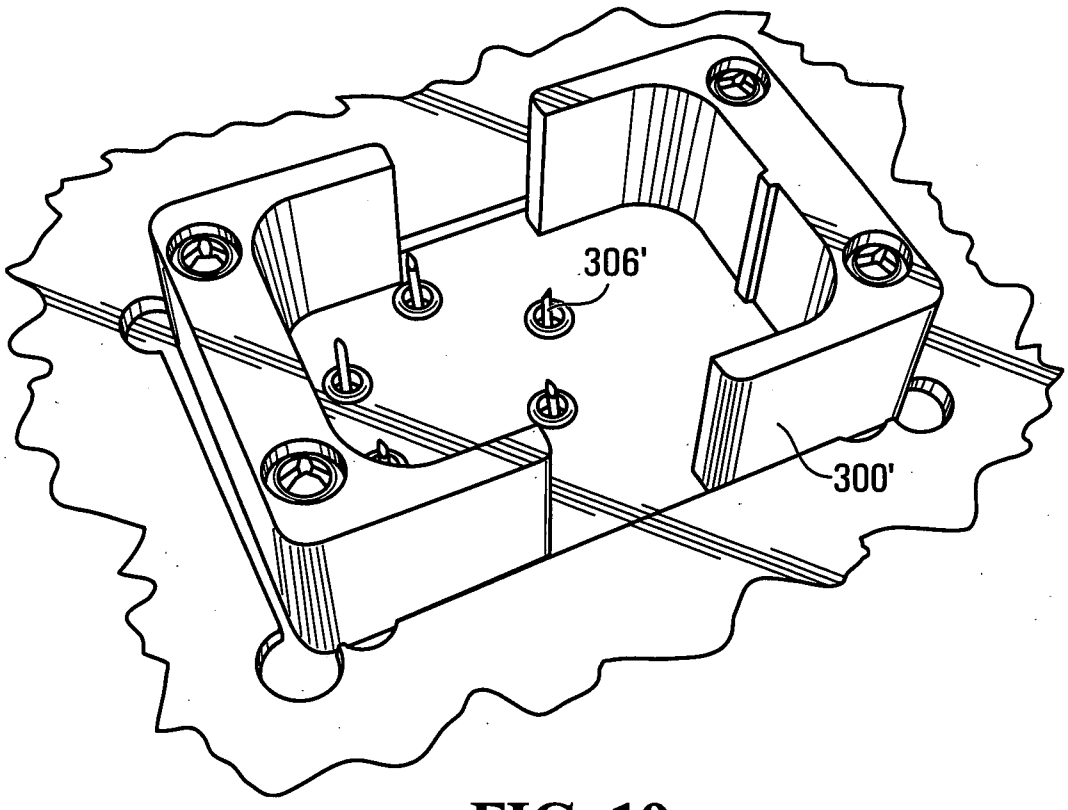

306'
300'

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2008/000222

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

G01N 35/10 (2006.01) ***B81B 1/00*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI and EPODOC:
/ic B81B or B65D or G01N AND
cartridge? or modul+ or cassette or (plug 3d in) or disposable or (single 3d use) AND
chambers or vessels or volumes or receptacles or wells or reservoir?) AND
fluid+ (3d) processing) or (fluid+ (3d) handling) or (fluid+ (3d) react+ or +fluid+ AND
dock+ or plug+ or mate+ or mating or removabl+ AND
ports or actuators or inlets or intakes or entries or entrances or duct+ or contact+ or seal? or
piston? or diaphragm? or membrane? AND
sample? (4d) (ingredient? or reactant? or +agent? or analyte? or marker? or indicator? or specimen? or gene_binding or buffer+ or dye?) AND
mix+ or react+ or lys+ or condition+ or elut+ or wash+ or rins+ or irrigate+ or extract+

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/0148091 A1 (KITAGUCHI et al.) 7 July 2005 Abstract, Figures 8 & 9, paragraph 301.	
A	US 2004/0053403 A1 (JEDRZEJEWSKI et al.) 18 March 2004	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 September 2008	19 SEP 2008

Name and mailing address of the ISA/AU

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2008/000222

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1203959 A1 (ASAHI KASEI KABUSHIKI KAISHI) 8 May 2002	
A	WO 2001/011374 A3 (THERMO BIOSTAR, INC.) 15 February 2001	
A	US 6167910 B1 (CHOW) 2 January 2001	
A	US 6068751 A (NEUKERMANS) 30 May 2000	
A	WO 1999/067646 A (CHEN & CHEN, LLC) 29 December 1999	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SG2008/000222

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	2005148091	AU	63217/00	CN	1370278	EP	1203959
		WO	0113127				
US	2004053403	AU	39865/01	AU	43269/01	CA	2400159
		CA	2401118	CN	1444646	CN	1920560
		EP	1261689	EP	1272768	US	6454924
		US	6720157	US	6730516	US	2001036669
		US	2001036674	US	2003013203	US	2004142491
		WO	0162887	WO	0163241	AU	63217/00
		CN	1370278	EP	1203959	US	2005148091
		WO	0113127				
WO	0111374	AU	65241/00	CN	1556922	EP	1250602
		JP	2006138866	US	6656428		
US	6167910	US	6321791	US	6494230	US	6648015
		US	6857449	US	2002023684		
US	6068751	NONE					
WO	9967646	AU	49576/99	AU	2002246765	CA	2301153
		CA	2433347	CA	2460192	CA	2632856
		CN	1272919	CN	1406154	CN	1585674
		EP	1005656	EP	1347833	EP	1427531
		US	6318191	US	6748332	US	6780617
		US	6964862	US	7337072	US	2002049557
		US	2002086417	US	2003049833	US	2004223878
		US	2005019875	US	2006154341	US	2008038813
		US	2008145275	WO	02057798	WO	03022435

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX