

accepts the first sample, and wherein the second microfluidic network accepts the second sample.

[0074] FIG. 6A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 for use with a heater unit described herein. FIG. 6B shows a close-up view of a portion of the cartridge 200 of FIG. 6A illustrating various representative components. The cartridge 200 may be referred to as a multi-lane PCR cartridge with dedicated sample inlets 202. For example sample inlet 202 is configured to accept a liquid transfer member (not shown) such as a syringe, a pipette, or a PCR tube containing a PCR ready sample. More than one inlet 202 is shown in FIGS. 6A, 6B, wherein one inlet operates in conjunction with a single sample lane. Various components of microfluidic circuitry in each lane are also visible. For example, microvalves 204, and 206, and vents 208, are parts of microfluidic circuitry in a given lane. Also shown is an ultrafast PCR reactor 210, which, as further described herein, is a microfluidic channel in a given sample lane that is long enough to permit PCR to amplify polynucleotides present in a sample. Above each PCR reactor 210 is a window 212 that permits detection of fluorescence from a fluorescent substance in PCR reactor 210 when a detector is situated above window 212. It is to be understood that other configurations of windows are possible including, but not limited to, a single window that straddles each PCR reactor across the width of cartridge 200.

[0075] In preferred embodiments, the multi-sample cartridge has a size substantially the same as that of a 96-well plate as is customarily used in the art. Advantageously, then, the cartridge may be used with plate handlers used elsewhere in the art.

[0076] The sample inlets of adjacent lanes are reasonably spaced apart from one another to prevent any contamination of one sample inlet from another sample when a user introduces a sample into any one cartridge. In an embodiment, the sample inlets are configured so as to prevent subsequent inadvertent introduction of sample into a given lane after a sample has already been introduced into that lane.

[0077] In certain embodiments, the multi-sample cartridge is designed so that a spacing between the centroids of sample inlets is 9 mm, which is an industry-recognized standard. This means that, in certain embodiments the center-to-center distance between inlet holes in the cartridge that accept samples from PCR tubes, as further described herein, is 9 mm. The inlet holes are manufactured conical in shape with an appropriate conical angle so that industry-standard pipette tips (2 μ l, 20 μ l, 200 μ l, volumes, etc.) fit snugly. The apparatus herein may be adapted to suit other, later-arising, industry standards not otherwise described herein.

[0078] FIG. 7 shows a plan view of an exemplary microfluidic cartridge 700 having 12 lanes. The inlet ports 702 have a 6 mm spacing, so that, when used in conjunction with an automated sample loader having 4 heads, spaced equidistantly at 18 mm apart, the inlets can be loaded in three batches of 4 inlets: e.g., inlets 1, 4, 7, and 10 together, followed by 2, 5, 8, and 11, then finally 3, 6, 9, and 12, wherein the 12 inlets are numbered consecutively from one side of the cartridge to the other.

[0079] FIG. 8 shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS. 6A, 6B and 7. Other configurations of microfluidic network would be consistent with the function of the cartridges and apparatus described herein. In sequence, sample is introduced through liquid inlet 202, flows into a

bubble removal vent channel 208 (which permits adventitious air bubbles introduced into the sample during entry, to escape), and continues along a channel 216. Throughout the operation of cartridge 200 the fluid is manipulated as a microdroplet (not shown in FIG. 5), and the various microfluidic components are actuated or controlled by application of heat from the heater unit further described herein. Valves 204 and 206 are initially open, so that a microdroplet of sample-containing fluid can be pumped into PCR reactor channel 210 from inlet hole 202 under influence of force from the sample injection operation. Upon initiating of processing, the detector present on top of the PCR reactor checks for the presence of liquid in the PCR channel, and then closes valves 204 and 206 to isolate the PCR reaction mix from the outside.

[0080] The reactor 210 is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein. Both valves 204 and 206 are closed prior to thermocycling to prevent any evaporation of liquid, bubble generation, or movement of fluid from the PCR reactor. End vent 214 prevents a user from introducing any excess amount of liquid into the microfluidic cartridge, as well as playing a role of containing any sample from spilling over to unintended parts of the cartridge. A user may input sample volumes as small as an amount to fill from the bubble removal vent to the middle of the microreactor, or up to valve 204 or beyond valve 204. The use of microvalves prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully complete a PCR thermocycling reaction. The application of pressure to contact the cartridge to the heater unit assists in achieving better thermal contact between the heater and the heat-receivable parts of the cartridge, and also prevents the bottom laminate structure from expanding, as would happen if the PCR channel was partially filled with liquid and the entrapped air would be thermally expanded during thermocycling.

[0081] Further aspects of a microfluidic cartridge that adapt it to carrying out PCR efficiently are described in U.S. patent application Ser. No. _____, entitled "Microfluidic Cartridge and Method of Making Same" and filed on even date herewith.

[0082] FIGS. 9A-C show various views of an exemplary microfluidic cartridge as further described herein. FIG. 9A shows an exploded view; FIG. 9B shows a perspective view; and FIG. 9C shows a cross-sectional view. Referring to FIGS. 9A-C, an exemplary microfluidic cartridge 200 includes first 220, second 222, third 224, fourth 226, and fifth layers 228, 230 (as shown) that enclose a microfluidic network having various components configured to process multiple samples in parallel that include one or more polynucleotides to be determined.

[0083] Microfluidic cartridge 200 can be fabricated as desired, for example, according to methods described in U.S. patent application Ser. No. _____, entitled "Microfluidic Cartridge and Method of Making Same" and filed on even date herewith. Typically, the microfluidic cartridge layer includes a layer 228, 230 of polypropylene or other plastic label with pressure sensitive adhesive (typically between about 50 and 150 microns thick) configured to seal the wax loading holes of the valves, trap air used for valve actuation, and serve as a location for operator markings. In FIG. 29A, this layer is shown in two separate pieces, 228, 230, though it would be understood by one of ordinary skill in the art that a single piece layer would be appropriate.