

nucleotide sample and PCR amplicons of the negative control polynucleotide. Each lane of a multi-lane cartridge as described herein can perform two reactions when used in conjunction with two fluorescence detection systems per lane. A variety of combinations of reactions can be performed in the cartridge, such as two sample reactions in one lane, a positive control and a negative control in two other lanes; or a sample reaction and an internal control in one lane and a negative control in a separate lane.

[0120] FIG. 10A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 according to the present technology. FIG. 10B shows a close-up view of a portion of the cartridge 200 of FIG. 10A 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. 10A, 10B, 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 hydrophobic vents 208 for removing air bubbles, 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.

[0121] 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, such a cartridge may be used with plate handlers used elsewhere in the art.

[0122] 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. 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 can be manufactured conical in shape with an appropriate conical angle so that industry-standard pipette tips (2  $\mu$ l, 20  $\mu$ l, 200  $\mu$ l, volumes, etc.) fit snugly therein. The cartridge herein may be adapted to suit other, later-arising, industry standards not otherwise described herein, as would be understood by one of ordinary skill in the art.

[0123] In one embodiment, an exemplary microfluidic cartridge has 12 sample lanes. The inlet ports in this embodiment 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 four 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 as shown.

[0124] A microfluidic cartridge as used herein may be constructed from a number of layers. Accordingly, one aspect of the present technology relates to a microfluidic cartridge that comprises a first, second, third, fourth, and fifth layers wherein one or more layers define a plurality of microfluidic networks, each network having various components configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined. In various embodiments, one or more such layers are optional.

[0125] FIGS. 11A-C show various views of a layer structure of an exemplary microfluidic cartridge comprising a number of layers, as further described herein. FIG. 11A shows an exploded view; FIG. 11B shows a perspective view; and FIG. 11C shows a cross-sectional view of a sample lane in the exemplary cartridge. Referring to FIGS. 11A-C, an exemplary microfluidic cartridge 400 includes first 420, second 422, third 424, fourth 426, and fifth layers in two non-contiguous parts 428, 430 (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.

[0126] Microfluidic cartridge 400 can be fabricated as desired. The cartridge can include a microfluidic substrate layer 424, typically injection molded out of a plastic, such as a zeonor plastic (cyclic olefin polymer), having a PCR channel and valve channels on a first side and vent channels and various inlet holes, including wax loading holes and liquid inlet holes, on a second side (disposed toward hydrophobic vent membrane 426). It is advantageous that all the microfluidic network defining structures, such as PCR reactors, valves, inlet holes, and air vents, are defined on the same single substrate 424. This attribute facilitates manufacture and assembly of the cartridge. Additionally, the material from which this substrate is formed is rigid or non-deformable, non-venting to air and other gases, and has a low autofluorescence to facilitate detection of polynucleotides during an amplification reaction performed in the microfluidic circuitry defined therein. Rigidity is advantageous because it facilitates effective and uniform contact with a heat unit as further described herein. Use of a non-venting material is also advantageous because it reduces the likelihood that the concentration of various species in liquid form will change during analysis. Use of a material having low auto-fluorescence is also important so that background fluorescence does not detract from measurement of fluorescence from the analyte of interest.

[0127] The cartridge can further include, disposed on top of the substrate 424, an oleophobic/hydrophobic vent membrane layer 426 of a porous material, such as 0.2 to 1.0 micron pore-size membrane of modified polytetrafluoroethylene, the membrane being typically between about 25 and about 100 microns thick, and configured to cover the vent channels of microfluidic substrate 424, and attached thereto using, for example, heat bonding.

[0128] Typically, the microfluidic cartridge further includes a layer 428, 430 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 in substrate 424, trap air used for valve actuation, and serve as a location for operator markings.