

samples. It is to be understood that, unless specifically made clear to the contrary, where the term PCR is used herein, any variant of PCR including but not limited to real-time and quantitative, and any other form of polynucleotide amplification is intended to be encompassed. The microfluidic cartridge need not be self-contained and can be designed so that it receives thermal energy from one or more heating elements present in an external apparatus with which the cartridge is in thermal communication. An exemplary such apparatus is further described herein; additional embodiments of such a system are found in U.S. patent application Ser. No. _____, entitled "Microfluidic System for Amplifying and Testing Polynucleotides in Parallel", and filed on even date herewith, the specification of which is incorporated herein by reference.

[0107] By cartridge is meant a unit that may be disposable, or reusable in whole or in part, and that is configured to be used in conjunction with some other apparatus that has been suitably and complementarily configured to receive and operate on (such as deliver energy to) the cartridge.

[0108] By microfluidic, as used herein, is meant that volumes of sample, and/or reagent, and/or amplified polynucleotide are from about 0.1 μ l to about 999 μ l, such as from 1-100 μ l, or from 2-25 μ l. Similarly, as applied to a cartridge, the term microfluidic means that various components and channels of the cartridge, as further described herein, are configured to accept, and/or retain, and/or facilitate passage of microfluidic volumes of sample, reagent, or amplified polynucleotide. Certain embodiments herein can also function with nanoliter volumes (in the range of 10-500 nanoliters, such as 100 nanoliters).

[0109] One aspect of the present technology relates to a microfluidic cartridge having two or more sample lanes arranged so that analyses can be carried out in two or more of the lanes in parallel, for example simultaneously, and wherein each lane is independently associated with a given sample.

[0110] A sample lane is an independently controllable set of elements by which a sample can be analyzed, according to methods described herein as well as others known in the art. A sample lane comprises at least a sample inlet, and a microfluidic network having one or more microfluidic components, as further described herein.

[0111] In various embodiments, a sample lane can include a sample inlet port or valve, and a microfluidic network that comprises, in fluidic communication one or more components selected from the group consisting of: at least one thermally actuated valve, a bubble removal vent, at least one thermally actuated pump, a gate, mixing channel, positioning element, microreactor, a downstream thermally actuated valve, and a PCR reaction chamber. The sample inlet valve can be configured to accept a sample at a pressure differential compared to ambient pressure of between about 70 and 100 kilopascals.

[0112] The cartridge can therefore include a plurality of microfluidic networks, each network having various components, and each network configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined.

[0113] A multi-lane cartridge is configured to accept a number of samples in series or in parallel, simultaneously or consecutively, in particular embodiments 12 samples, wherein the samples include at least a first sample and a second sample, wherein the first sample and the second sample each contain one or more polynucleotides in a form suitable for amplification. The polynucleotides in question

may be the same as, or different from one another, in different samples and hence in different lanes of the cartridge. The cartridge typically processes each sample by increasing the concentration of a polynucleotide to be determined and/or by reducing the concentration of inhibitors relative to the concentration of polynucleotide to be determined.

[0114] The multi-lane cartridge comprises at least a first sample lane having a first microfluidic network and a second lane having a second microfluidic network, wherein each of the first microfluidic network and the second microfluidic network is as elsewhere described herein, and wherein the first microfluidic network is configured to amplify polynucleotides in the first sample, and wherein the second microfluidic network is configured to amplify polynucleotides in the second sample.

[0115] In various embodiments, the microfluidic network can be configured to couple heat from an external heat source to a sample mixture comprising PCR reagent and neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

[0116] At least the external heat source may operate under control of a computer processor, configured to execute computer readable instructions for operating one or more components of each sample lane, independently of one another, and for receiving signals from a detector that measures fluorescence from one or more of the PCR reaction chambers.

[0117] For example, FIG. 9 shows a plan view of a microfluidic cartridge 100 containing twelve independent sample lanes 101 capable of simultaneous or successive processing. The microfluidic network in each lane is typically configured to carry out amplification, such as by PCR, on a PCR-ready sample, such as one containing nucleic acid extracted from a sample using other methods as further described herein. A PCR-ready sample is thus typically a mixture comprising the PCR reagents and the neutralized polynucleotide sample, suitable for subjecting to thermal cycling conditions that create PCR amplicons from the neutralized polynucleotide sample. For example, a PCR-ready sample can include a PCR reagent mixture comprising a polymerase enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid and a plurality of nucleotides, and at least one probe that is selective for a polynucleotide sequence. Exemplary probes are further described herein. Typically, the microfluidic network is configured to couple heat from an external heat source with the mixture comprising the PCR reagent and the neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

[0118] In various embodiments, the PCR reagent mixture can include a positive control plasmid and a plasmid fluorogenic hybridization probe selective for at least a portion of the plasmid, and the microfluidic cartridge can be configured to allow independent optical detection of the fluorogenic hybridization probe and the plasmid fluorogenic hybridization probe.

[0119] In various embodiments, the microfluidic cartridge can accommodate a negative control polynucleotide, wherein the microfluidic network can be configured to independently carry out PCR on each of a neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized poly-