

B_2 -F-3' and a second primer comprising U_2 and R domains in the order 5'- U_2 -R-3', where each pair of F and R oligonucleotides is capable of annealing specifically to a different target DNA sequence in the second set of multiple target sequences under stringent annealing conditions; and where amplicons for each of the multiple target DNA sequences of the second set are produced; and detecting the amplicons for each of the multiple target DNA sequences using a probe that anneals to sequence of the amplicon having the sequence of the B_2 domain or its complement. In one embodiment, U_1 , B_1 , F_1 , U_2 and R_1 domains are between 6 and 30 nucleotides in length. In one embodiment the probe is a molecular beacon. In one embodiment, the probe is a Taqman™-type probe.

[0008] In another aspect, the invention provides a microfluidic device, having (a) a first region comprising (i) a flow channel formed within an elastomeric material and having a first end and a second end in fluid communication with each other through said channel, where said channel may be branched or unbranched; (ii) an inlet for introducing a sample fluid in communication with said channel, said inlet; (iii) an outlet in communication with said flow channel; (iv) a plurality of control channels overlaying the flow channel(s), where an elastomeric membrane separates the control channels from the flow channels at each intersection, the elastomeric membrane disposed to be deflected into or withdrawn from the flow channel in response to an actuation force, and where, when the control channels are actuated the flow channel is partitioned into at least 1000 reaction chambers not in fluidic communication with each other; (b) a second region comprising a channel or chamber interposed between and in communication with said outlet in (a) and a flow channel in the third region; (c) a third region comprising a plurality of flow channels (e.g., blind flow channels), in fluidic communication with the channel or chamber of the second region, with a region of each flow channel defining a reaction site; (d) a control channel or channels that when actuated separates the first and second regions; (e) a control channel or channels that when actuated separates the second and third regions; and (f) a control channel or channels that when actuated separates the reaction sites of said flow channels from the other portions of control channels.

BRIEF DESCRIPTION OF THE FIGURES

[0009] FIGS. 1A and 1B show an exemplary design of a massively partitioning device (MPD) in valve off (FIG. 1A) and valve actuated (FIG. 1B) states.

[0010] FIG. 2 shows an exemplary design of a MPD with two banks: a first bank in which nucleic acids are partitioned and amplified in individual chambers, and a second bank in which subsequent analysis of the amplicon pool occurs.

[0011] FIGS. 3A-C are flow charts illustrating partition and analysis of nucleic acids using methods of the invention. FIG. 3A illustrates partition and analysis of nucleic acids in which multiple target sequences are amplified. FIG. 3B illustrates partition and analysis acids in which target sequences in only a single chamber are amplified. FIG. 3C illustrates analysis of nucleic acids of a single cell.

[0012] FIG. 4 is a flow chart illustrating partition of cells and analysis of their properties.

[0013] FIG. 5 is an illustration of primers used in the universal amplification method.

DETAILED DESCRIPTION

Definitions

[0014] The term "elastomer" has the general meaning used in the art. Thus, for example, Allcock et al. (Contemporary

Polymer Chemistry, 2nd Ed.) describes elastomers in general as polymers existing at a temperature between their glass transition temperature and liquefaction temperature. Elastomeric materials exhibit elastic properties because the polymer chains readily undergo torsional motion to permit uncoiling of the backbone chains in response to a force, with the backbone chains recoiling to assume the prior shape in the absence of the force. In general, elastomers deform when force is applied, but then return to their original shape when the force is removed. The elasticity exhibited by elastomeric materials can be characterized by a Young's modulus. The elastomeric materials utilized in the microfluidic devices disclosed herein typically have a Young's modulus of between about 1 Pa-1 TPa, in other instances between about 10 Pa-100 GPa, in still other instances between about 20 Pa-1 GPa, in yet other instances between about 50 Pa-10 MPa, and in certain instances between about 100 Pa-1 MPa. Elastomeric materials having a Young's modulus outside of these ranges can also be utilized depending upon the needs of a particular application. Microfluidic devices can be fabricated from an elastomeric polymer such as GE RTV 615 (formulation), a vinylsilane crosslinked (type) silicone elastomer (family). However, elastomeric microfluidic systems are not limited to this one formulation, type or even this family of polymer; rather, nearly any elastomeric polymer is suitable. Given the tremendous diversity of polymer chemistries, precursors, synthetic methods, reaction conditions, and potential additives, there are a large number of possible elastomer systems that can be used to make monolithic elastomeric microvalves and pumps (including, for example, perfluoropolyethers, polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), polyurethanes, and silicones, for example, or poly(bis(fluoroalkoxy)phosphazene) (PNF, Eypel-F), poly(carborane-siloxanes) (Dexsil), polyacrylonitrile-butadiene) (nitrile rubber), poly(1-butene), poly(chlorotrifluoroethylene-vinylidene fluoride) copolymers (Kel-F), poly(ethyl vinyl ether), poly(vinylidene fluoride), poly(vinylidene fluoride-hexafluoropropylene) copolymer (Viton), elastomeric compositions of polyvinylchloride (PVC), polysulfone, polycarbonate, polymethylmethacrylate (PMMA), polytetrafluoroethylene (Teflon), polydimethylsiloxane, polydimethylsiloxane copolymer, and aliphatic urethane diacrylate). The choice of materials typically depends upon the particular material properties (e.g., solvent resistance, stiffness, gas permeability, and/or temperature stability) required for the application being conducted. Additional details regarding the type of elastomeric materials that can be used in the manufacture of the components of the microfluidic devices disclosed herein are set forth in Unger et al. (2000) *Science* 288:113-116, PCT Publications WO 02/43615, WO 2005030822, WO 2005084191 and WO 01/01025; and U.S. patent publication No. 20050072946.

[0015] A "reagent" refers broadly to any agent used in a reaction, other than the analyte (e.g., cell or nucleic acid being analyzed). Exemplary reagents for a nucleic acid amplification reaction include, but are not limited to, buffer, metal ions, polymerase, reverse transcriptase, primers, template nucleic acid, nucleotides, labels, dyes, nucleases and the like. Reagents for enzyme reactions include, for example, substrates, cofactors, buffer, metal ions, inhibitors and activators. Reagents for cell-based reactions include, but are not limited to, cells, cell specific dyes and ligands (e.g., agonists and antagonists) that bind to cellular receptors.