

withdrawn from the device, thereby pooling and mixing any amplicons present in the sample. Any method that results in a distribution of amplicons sufficient to carry out subsequent detection steps may be used.

**[0105]** It is not necessary that the various amplicons diffuse (or are mixed) to equilibrium in the post-amplification sample, but it is desirable that sufficient mixing occur so that an aliquot of post-amplification sample contains a number of molecules of each amplicon (e.g., at least 10, at least 100, at least 1000, or at least 5000 molecules) from each sub-sample in which amplicons were produced. For illustration, consider carrying out an amplification reaction that produces 100,000 copies of each of three distinct amplicons, i.e., amplicon A in chamber 1, amplicon B in chamber 2, and amplicon C in chamber 3. The sub-samples are pooled (by releasing valves) and one-fifth of the volume of the post-amplification sample removed from the device or from the partition region of the device (see Section C (viii), below). If the amplicons had diffused to equilibrium in the post-amplification sample, and assuming no loss of material, the one-fifth volume would contain about 20,000 molecules of each of the three amplicons. If diffusion was less than complete, the one-fifth volume could contain unequal amounts of each amplicon, for example 50,000 molecules of amplicon A, 20,000 molecules of amplicon B and 15,000 molecules of amplicon C.

**[0106]** Alternatively, all or substantially all, of the post-amplification sample (or substantially all of it) can be withdrawn from the device, thereby mixing any amplicons present in the sample.

**[0107]** vii) Subsequent Analysis of Amplicons

**[0108]** Following pooling (e.g., diffusion and/or mixing) all or a portion of the amplicon pool can be used for subsequent analyses. Typically the amplicon pool is divided into a plurality of aliquots and each aliquot separately analyzed to determine a property (e.g., a nucleotide sequence or the presence or absence of a predetermined nucleotide sequence) of an amplicon or amplicons in that aliquot. If desired, the volume of the amplicon pool can be increased by addition of a suitable solution such as aqueous buffer or a reaction mixture containing amplification and/or detection reagents. The amplicon pool can be divided into aliquots manually or can be divided using an appropriately designed MPD (see Section C (viii), below).

**[0109]** It will be apparent from the discussion above that each of the aliquots from the amplicon pool will contain essentially the same set of amplicons (i.e., the same amplicon species will be represented in each aliquot). Each of the aliquots can be used for a different analysis. For example, a first aliquot can be assayed for the presence of a first target sequence (e.g., a first SNP in an amplified gene sequence), a second aliquot can be assayed for the presence of a second target sequence (e.g., a second SNP in the same amplified gene sequence), a third aliquot can be assayed for the presence of a third target sequence (e.g., a first SNP of a different amplified gene) and so on. It will be appreciated that target sequences of interest are not limited to SNPs.

**[0110]** The subsequent analysis of amplicons can be carried out using any desired technique including, without limitation, hybridization to a target nucleic acid or array of targets, PCR amplification, FRET-assays, hybridization to probes, and the like.

**[0111]** viii) Devices

**[0112]** As noted above, massive partitioning is accomplished using an elastomeric MPD. Subsequent analyses can

also be accomplished using any suitable assay. In certain embodiments, an elastomeric microfluidic device is used for subsequent analyses. In some embodiments the initial partitioning and amplification, the subsequent mixing of applications, and the subsequent analysis of amplicons are carried out using different sections (i.e., different banks) of the same the same device. For example, an elastomeric device can be fabricated with three regions: a first region that is a MPD in which target sequences, cells or molecules are amplified in individual chambers to produce amplicons and then allowed to mix to produce an amplicon pool, a second region (which can be as small as a single flow channel) by which the amplicon pool is transferred to the third region, and a third region having a plurality of flow channels with a region of each flow channel defining a reaction site in which subsequent analysis of the amplicon pool occurs. In one embodiment, the flow channels in the third region are blind flow channels with reaction sites near the channel terminus. A schematic of an exemplary device is shown in FIG. 2. In this schematic five control channels (1, 2, 3, 4 and 5) are shown in black. A branched flow channel system is shown in gray. It will be appreciated, as discussed above, that the flow channel configuration need not be branched. In the device shown, a sample containing an amplification mixture and nucleic acids is injected into inlet A with the valves formed by control channels 1 and 2 open, and control channel 3 actuated (closed). Control channel 2 is then actuated to isolate the sample in multiple chambers (B). The samples are then subject to thermocycling and optionally detection of the amplification products (e.g., using a commercially available fluorescence reader). Control channel 1 is then closed and control channel 2 opened to allow mixing of amplicons in the various chambers to produce an amplicon pool, a portion of which is then pumped into blind channels (D) with control channels 3, 4 (if present) and 5 open. Alternatively, control channels 2 and 3 are both opened and a portion of the amplicon pool is pumped to a mixing chamber (C) with control channel 4 closed. Control channel 3 is then closed and channels 4 and 5 are opened and portion of the amplicon pool is pumped into blind channels (D). Control channel 5 is then closed isolating blind reaction chambers (E) and the subsequent round of analysis takes place.

**[0113]** In one embodiment the device is configured so that reagents can be added to the pooled amplicon sample; for example, the mixing chamber (C) shown in FIG. 2 may be fluidically linked to a reservoir containing reagents (e.g., nuclease, probes or primers) that can be added prior to distributing the amplicon pool into a reaction chambers.

**[0114]** ix) Systems

**[0115]** In one aspect, the invention provides a system for analysis of nucleic acids, proteins or cells comprising a massively partitioning device and an external collection reservoir for collection of an amplicon pool. The external reservoir can be any type of container or tube that is fluidically connected to the MPD, so that the contents of the MPD chambers can be transferred from the MPD to the reservoir. Transfer can be by displacement of the MPD contents using a displacement fluid, by active pumping, or by other means.

**[0116]** In one aspect, the invention provides a system for analysis of nucleic acids, proteins or cells comprising a massively partitioning device, optionally including an integral or external reservoir for collection of an amplicon pool, and an (additional) external component. In one embodiment the MPD includes a region with a plurality of flow channels