

defining a reaction sites for subsequent analysis of the amplicon pool aliquots (e.g., the “third” region of the device described above in Section (viii)).

**[0117]** In one aspect, the invention provides a system for analysis of nucleic acids, proteins or cells comprising a massively partitioning device as described in Section (viii) above, where the MPD has 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.

**[0118]** Additional external components of the system may include sensors, actuators (e.g., pumps; see U.S. Pat. No. 6,408,878), control systems for actuating valves, data storage systems, reagent storage units (reservoirs), monitoring devices and signal detectors. Signal detectors may detect visible, fluorescent, and UV light (intensity, scattering, absorption) luminescence, differential reflectivity, electrical resistance, resistivity, impedance, or voltage, in chambers or reaction sites. In one embodiment the external component is a temperature control component such as a thermocycler (e.g., Peltier device, resistive heaters, and heat exchangers; see e.g., U.S. Pat. No. 6,960,437 B2).

**[0119]** 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, optionally including a plurality of flow channels defining a reaction sites for subsequent analysis of the amplicon pool aliquots, and optionally including an additional reagent positioned in the chambers of the MPD and/or the reaction sites for subsequent analysis of the amplicon pool aliquots. Exemplary additional reagents include enzymes (e.g., nuclease, polymerase, or ligase); primers and probes (PCR primers, molecular beacons, padlock probes, proximity ligation probes, Universal Amplification primers), amplification reagents and the like.

**[0120]** For illustration and not limitation, particular systems may comprise an MPD and a heat source (e.g., thermocycler) positioned to regulate the temperature of the contents of reaction chambers. In one embodiment, heat is transmitted from the heat source to the MPD by conduction (e.g., the heat source being adjacent and in contact with the MPD). In one embodiment the MPD is fixed (e.g., clamped) to the heat source.

**[0121]** For illustration and not limitation, particular systems may comprise an MPD and a signal detector positioned to detect signal emanating from reaction chambers in the MPD system. In one embodiment, a fluorescent signal is detected. In one embodiment the system includes an appropriately programmed computer coupled to the signal detector capable of storing information such as the position, intensity and/or duration of a signal emanating from reaction chambers in the MPD system.

**[0122]** For example, when the MPD comprises reaction chambers for analysis of amplicon pool aliquots, the signal detector, heat source, or other component of the system may be associated with those reaction chambers, reaction chambers produced in the partitioning step, or both. Other particu-

lar systems may comprise a MPD comprising prepositioned reagents in one or more reaction chambers or mixing chambers.

**[0123]** x) Illustrative Examples

**[0124]** The following prophetic examples are intended to illustrate aspects of the invention. However, they are for illustration only and are not intended to limit the invention in any fashion.

**[0125]** 1. SNP Analysis

**[0126]** In this illustration, 200 different genes of an individual are screened for the presence of mutations.

**[0127]** A sample containing genomic DNA from the subject is obtained. A small number of genome equivalents is sufficient for analysis. Thus the sample may be from a small number of cells (for example, fewer than 10 cells, and as few as one cell) which may be treated to release and fragment genomic DNA. Alternatively isolated or purified DNA may be used. Usually the DNA is fragmented by shearing, enzymatic or chemical cleavage, or other methods known in the art. In one embodiment the DNA is sheared by transport through a channel with varying cross-dimensions.

**[0128]** The reagents for amplification of target sequences are added to the sample. The amplification reagents include the following:

**[0129]** a) Primer pairs for each of the 200 gene segments to be analyzed. The primer pairs can be selected, for example, to (i) amplify a target polymorphic site sequence only if site has a particular sequence (i.e., a specified SNP allele is present) or (ii) amplify the target polymorphic site sequence using primers that flank the SNP site, so that the segment is amplified without regard to what SNP is present. Each primer pair includes both a target specific sequence and one of two 5' universal sequences shared by all of the forward or all of the reverse primers, allowing all of the amplicons produced using the target specific primers to be amplified with the same universal primers.

**[0130]** b) A pair of universal primers capable of amplifying all of the amplicons produced using the target specific primers.

**[0131]** Primers are selected so that all of the first round amplifications (using target specific primers) can occur under the same reaction conditions.

**[0132]** c) Amplification reagents (polymerase, cofactors, nucleotides, metal ions, buffer, etc.). The reagents may be added to the sample before partition, may be pre-positioned in the reaction chambers, or some reagents may be added and others pre-positioned.

**[0133]** The sample is injected into the partitioning channel system of a MPD having 40,000 chambers. After injection of the sample, valves are closed creating 40,000 isolated reaction chambers. Each reaction chamber contains all of the probes described above, and some of the chambers contain a nucleic acid molecule with a target sequence of interest as a consequence of the partitioning. The MPD is placed on a thermocycler and cycled using an appropriate protocol (e.g., 2 min at 51° C., 1 sec at 96° C. and 59 sec at 95° C., followed by 40 cycles of 1 min at 58° C., 1 sec at 96° C. and 59 sec at 95° C.). Following amplification, the valves are opened and sub-samples allowed to mix (e.g., by diffusion or active mixing) producing the amplicon pool.

**[0134]** A portion of the resulting amplicon pool is withdrawn from the MPD and distributed into two hundred (200) aliquots, and each aliquot is subjected to a different SNP assay. Alternatively, different sets of SNP assays can be con-