

This section also describes devices (i.e., massively partitioning devices, MPDs) useful in carrying out analyses according to the method.

**[0049]** Analytical methods described in this section can be used for deleting the presence or absence of a target sequence, detection of polymorphisms; single polynucleotide polymorphism (SNP) analysis; haplotype analysis; amplification of a segment for sequence determination, gene expression analysis, quantification of nucleic acids, analysis of cells (see Section D, below), as well as other applications that will be apparent to one of skill guided by this disclosure. Although this section focuses on analysis of nucleic acids it will be appreciated by the reader that many aspects of the description in this section will be applicable, with appropriate modification, to analysis of other molecules and of cells. FIGS. 3A and 3B are flow charts illustrating partition and analysis of nucleic acids in which multiple targets are amplified in which amplicons may be pooled. FIG. 3B illustrates partition and analysis of nucleic acids in which, in one embodiment, target molecules in only a single chamber are amplified.

**[0050]** i) Samples Containing Nucleic Acids

**[0051]** In one step of the assay method, a sample containing a plurality of nucleic acid molecules is partitioned into a plurality of sub-samples, at least two of which each comprise at least one nucleic acid molecule.

**[0052]** Samples that may be analyzed according to the invention are any fluid sample that contains nucleic acids. A variety of types of samples can be used, so long as at least some nucleic acids can be partitioned from each other by the MPD. The nucleic acids can be free in solution or can be contained in particles or within cells suspended in a fluid. Samples may be processed so that any nucleic acids in the sample can be amplified. For example, in samples containing cells or viruses, the cells or viruses can be lysed or disrupted, using such routine methods as exposure to enzymes (such as lysozyme), detergents, denaturants (such as guanidine salts) and/or physical disruption) before the sample. Any method of liberating nucleic acids that results in nucleic acid molecules sufficiently intact and purified to amplify fragments is suitable. Examples of samples containing nucleic acids are cell lysates or cell fractions, water samples containing microorganisms, purified DNA resuspended in an aqueous buffer, sputum, blood, nucleated blood cells, tissue or fine needle biopsy samples, urine, peritoneal fluid, fecal samples, and floral fluid, or cells therefrom. Exemplary samples include cells and cell lysates (e.g., eukaryotic cells, human cells, animal cells, plant cells, fetal cells, embryonic cells, stem cells, blood cells, lymphocytes, bacterial cells, recombinant cells and cells infected with a pathogen, tissue samples), viruses, purified or partially purified DNA or RNA, environmental samples (e.g., water samples), food samples, forensic samples, plant samples and the like. It will be apparent that the sample can contain other compounds and macromolecules in addition to nucleic acids. If necessary for the functioning of a microfluidic device non-nucleic acid components and/or particulates can be removed by filtration, sedimentation or other methods.

**[0053]** In one embodiment of the invention, the nucleic acids are contained in cells, organelles, or viruses and the nucleic acids are not released (e.g., the cells are not lysed) until at least after a partitioning step. Particular aspects of this embodiment are discussed in Section D, below.

**[0054]** Analysis of nucleic acids in a sample generally involves determining whether the sample contains a nucleic

acid having a particular target sequence. A target sequence may be predefined (i.e., known prior to analysis) or may be a sequence in a segment of a nucleic acid defined by other parameters (e.g., defined as the segment of a gene that can be amplified by a particular primer pair).

**[0055]** A target sequence can be any nucleic acid sequence of interest, such as a sequence associated with a gene, a sequence that identifies a particular allele or polymorphism, a sequence that, alone in combination with other genotypic or phenotypic markers, identifies the presence in the sample of a particular organism or strain, and the like. A target sequence can also include sequences flanking a sequence of interest, such as the sequences flanking a SNP. In addition, in some cases as will be recognized from context, a "target sequence" is a sequence added during an amplification step. For example, the B and U sequences discussed below in the context of a Universal Amplification method can be referred to as "target sequences" recognized by a probe or amplification primer.

**[0056]** A target sequence can be found in DNA (including genomic, mitochondrial DNA, viral DNA, recombinant DNA and complementary cDNA made from RNA) or in RNA (including rRNA, mRNA and iRNA). If a target sequence is detected in a sample it is possible to deduce that the sample contains a nucleic acid molecule containing the detected sequence or its complement. For example, a sample from a human patient can be analyzed to determine whether a viral nucleotide sequence (the target sequence) is detectable in the sample, in order to diagnose (or rule out) viral infection. As another example, genomic DNA from a human patient can be analyzed to determine whether a particular polymorphism is or is not present in a subject's genome.

**[0057]** In some cases, it will be advantageous to fragment the nucleic acid molecules prior to the partitioning step. For example, to characterize two genes on different regions of a eukaryotic chromosome it may be useful to fragment the DNA to produce smaller nucleic acid molecules so that the genes can be separately partitioned (i.e., partitioned into different sub-samples). Fragmentation can be accomplished enzymatically (e.g., using restriction enzymes), mechanically or chemically. In one embodiment, shearing is accomplished by passing the DNA through a channel of a MPD with a diameter (bore size) that is sufficiently small, or which varies in diameter along the length of the channel, so as to shear large nucleic acids as they pass through. A sample containing a single DNA molecule (e.g., a single chromosome) contains a plurality of nucleic acids upon fragmentation of the single molecule.

**[0058]** ii) Partitioning of a Sample Containing Nucleic Acid Molecules

**[0059]** Methods for partitioning a sample using a MPD are provided in Section B, above. The terms "to partition," "partitioning," "partitioned," and grammatical equivalents refer to the process of separating a sample into a plurality of sub-samples using a MPD. A sample is partitioned by introducing the sample into the flow channels and reaction sites with valves open and then closing the valves to isolate each sub-sample. It will be recognized that each sub-sample is contained (for at least a period of time) in a separate reaction chamber such that the sample is isolated from (not in fluidic communication with) other sub-samples. It is sometimes convenient to refer to the "sample" even after it has been partitioned into subsamples. Thus, in some contexts "sample" can