

ducted using multiplex methods. The individual SNP assays can be carried out using, for example, a Taqman™-type probe, Molecular Beacon, Scorpion, or other detection methods and detected using a fluorescence detector.

**[0135]** 2. Analysis of Many Sequences Using Nucleic Acids from a Single Cell or Very Few Cells

**[0136]** In one embodiment, a nucleic acid analysis is conducted on a single cell. Such an analysis is useful for diagnostic or prognostic methods when tissue is limited such as, for example, genetic testing of a single blastocyst of a pre-implantation embryo produced using in vitro fertilization techniques. Such testing is also useful in the study or cloning of non-human animals. For example, blastocyst cells obtained from a non-human animal can be assayed for the presence, expression or characteristics of a transgene or endogenous gene. It can be verified that the genotype or expression profile of the embryo is consistent with the goals of the researcher prior to implantation into a surrogate mother, resulting in savings of time and resources. Such testing is also useful in forensic analysis in which very few cells may be available or in which cells must be analyzed individually because a sample is contaminated with cells from multiple sources.

**[0137]** Analysis of nucleic acids of a single cell is illustrated by the flow chart in FIG. 3C. It will be appreciated that the figure is provided to assist the reader in understanding the invention, and is not intended to limit the invention in any fashion.

**[0138]** In this method, the single cell (or small number of cells) is provided in a solution or combined with a solution. The cell is treated to release DNA. Any number of methods for cell lysis (e.g., using sonication, denaturants, etc.) are suitable. If genomic DNA is being analyzed it is fragmented. The desired fragment size will be based on the method of detection of the target sequence and the number of reaction chambers on the chip. The goal is to end up with large enough fragments so that target sequences can be amplified (typically >300 bp) and enough fragments so that different amplicons (i.e., amplicons corresponding to different sequences) will be generated in separate reaction chambers. Thus, the intended average size will depend on the number of target sequences to be detected and the number of partitions available. Fragments can be created by restriction digestion or other DNA fragmentation methods. In one embodiment, DNA is sheared by driving it through a narrow opening. Thus, a MPD can be designed with a via or flow channel of sufficiently small diameter narrowness to shear DNA to the desired fragment size. In an embodiment the diameter of the via or flow channel varies across its length (e.g., narrow-wide-narrow-wide) to drive the fragmentation. The sample is introduced into a MPD and the MPD valves are actuated to partition the DNA fragments, or RNA molecules, into separate reaction chambers.

**[0139]** Reagents sufficient to amplify each of the target sequences of interest are provided in each reaction chamber. For purposes of this example, assume 50 different loci containing SNPs are of interest, and at each of the 50 polymorphic loci there are two different possible sequences at the SNP site, with the 100 total different target sequences designated SNP 1A, 1B, 2A, 2B, . . . 50A, and 50B. As discussed above, the reagents can be added to the solution containing the intact cell, can be prelocated in the reaction chambers, or some combination of the two. In this example, the amplification reagents include primers sufficient to amplify gene segments spanning each of the 50 loci to produce the SNP site and 40

basepairs of flanking sequence on each side. UA amplification primers may be used. Amplification is then carried out (e.g., by thermocycling if the amplification method is PCR or reverse transcription-PCR). Amplicons are produced in those reaction chambers that contain a nucleic acid molecule (i.e., DNA fragment or RNA molecule) comprising one of the target sequences.

**[0140]** As described above, following amplification the valves are opened and amplicons allowed to mix to produce an amplicon pool. The pool is then divided into 100 different aliquots (optionally using a dual bank MPD as described in Section C (viii), above). In each aliquot a single assay is carried out for an individual SNP using, for example, a Taqman™-type probe, Molecular Beacon, Scorpion, or other detection methods known in the art.

**[0141]** 3. Detection of Pathogens Using the UA System

**[0142]** In this illustration, a sample is assayed for the presence of 150 different pathogens. Exemplary samples for the method include (i) a sample is obtained from a patient, (ii) an environmental sample (e.g., from a pond or reservoir) and (iii) a sample from a poultry processing facility.

**[0143]** The reagents for amplification of target sequences are added to the sample. The amplification reagents include the following:

**[0144]** a) Fifty UA primer pairs for gene segments found in fifty different bacterial pathogens. The forward primer of these UA primer pairs includes a recognition site for a molecular beacon labeled with the blue fluorescing dye Cy 5.5.

**[0145]** b) Fifty UA primer pairs for gene segments found in fifty different fungal pathogens. The forward primer of these UA primer pairs includes a recognition site for a molecular beacon labeled with the green fluorescing dye 6-FAM (Fluorescein).

**[0146]** c) Fifty UA primer pairs for gene segments found in fifty different viral pathogens. The forward primer of these UA primer pairs includes a recognition site for a molecular beacon labeled with the red fluorescing dye Cy 3.

**[0147]** d) Type 3 and Type 4 primers corresponding to the UA primer pairs Primers 5'-U<sub>A</sub>-3' and 5'-U<sub>B</sub>-3'.

**[0148]** Primer sequences are selected so that all of the UA primer pairs produce amplicons that can be amplified using the same Type 3 and Type 4 primers; and all of the first round amplifications can occur under the same reaction conditions.

**[0149]** e) Molecular beacons that recognize the recognition sites of the three UA forward primers and are labeled as indicated above.

**[0150]** f) Amplification reagents (polymerase, cofactors, nucleotides, metal ions, buffer, etc.). The reagents may be added to the sample before partition, may be prepositioned in the reaction chambers, or some reagents may be added and others prepositioned.

**[0151]** The sample is injected into the partitioning channel system of a MPD having 40,000 chambers. After injection of the sample each valve is 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. 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 device is imaged using a commercially available, modified, or custom made fluorescence reader.