

strain. The cells are injected into a MPD and control channels actuated to create separate reaction chambers (e.g., a sample containing about 100 bacterial cells is partitioned into 2000 chambers) all or most of which contain zero or one cell. The device is placed on the thermocycler or amplification is otherwise initiated. Detection in a chamber of only a red signal indicates the presence in the sample of non-resistant *S. aureus*; detection in a chamber of both a red and green signal indicates the presence in the sample of drug resistant *S. aureus*; detection or no signal indicates no *S. aureus* bacteria are present in the sample.

[0179] 2. Quantitation of Cells in a Population Having Specific Properties

[0180] In one aspect, the method is used for quantization of cells in a heterogeneous population having specific properties. For illustration, a cell population (e.g., peripheral blood mononuclear cells (PBMC)) containing cytotoxic T lymphocytes (CTL) (effector cells) can be partitioned and the ability of the cells to be stimulated by an antigen tested. The antigen reagent can be prepositioned in chambers or combined with cells immediately before partition. The proportion or type of cells activated in the presence can be assayed using any of a variety of assays for effector cell activation. For example, by performing in vitro stimulation after limiting dilution of circulating CTLs with the gag antigens of human immunodeficiency virus (HIV), the precursor population of gag-specific CTL can be quantitated and/or characterized. See, e.g., Koup "Limiting dilution analysis of cytotoxic T lymphocytes to human immunodeficiency virus gag antigens in infected persons: in vitro quantitation of effector cell populations with p17 and p24 specificities" *J Exp Med.* 1991 Dec. 1; 174(6): 1593-600.

[0181] 3. Characterization of a Rare Cell in a Background of Other Cells

[0182] It is often advantageous to quantitate and/or characterize rare cells in a background of other cells. For example, in cancer, individual disseminated cancer cells may be found in blood. Further, biopsies may recover only a few malignant cells in a background of normal cells. The methods of the present invention allow the malignant cells to be isolated, identified based on a property (e.g., antigen, mutation or expression pattern) unique to the cancer cell, and then a different property of the cell determined.

[0183] In another example, nucleated fetal red blood cells are found at low levels in the blood of pregnant women and are a potential source of information about the fetal genome including any sequences associated with disease or propensity to disease. However, even enriched 10,000-fold fetal cells may be less than 0.1% of a sample making analysis by conventional methods difficult. Cells from a sample enriched for fetal NRBCs can be partitioned using the methods disclosed herein. Chambers containing fetal cells can be identified using a fetal-specific probe (e.g., a probe specific for the Y chromosome; abundance of RNA encoding fetal forms of hemoglobin) and assayed for several genetic characteristics

using a multiplex assay. Other examples of rare cells in a background of different cells include, for example, a virally infected cell in a background of uninfected cells, a cell expressing a gene in a background of cells not expressing the gene; and the like.

[0184] In one embodiment, the MPD is used to partition a mixed population of cells to detect a property characteristic of a rare cell type in the population, i.e., cells comprising less than about 1%, more often less than about 0.1%, and very often less than about 0.01% of the cells in the population. There are many cases in which it advantageous to determine the properties of a rare cell in a population of other cells. The methods of the present invention enable analysis of a rare cell without background or interference for other cells. In general, the method involves partitioning cells and assaying individual cells for at least two properties at least one of which identifies the rare cell.

[0185] 4. Expression Analysis of Individual Cells

[0186] In one embodiment the nucleic acid being analyzed is or includes RNA, and the expression level of specified genes in an individual cell is determined. Again using the example of a virus-infected cell, the expression profile for several host genes in a single cell can be correlated with the presence or absence of virus or with viral load. Gene expression profiles can also be correlated with cell identity (e.g., different expression profiles for different cells in a sample containing a heterogeneous mixture of cells) or cell response to a stimulus (e.g., the presence of a ligand that binds a cell receptor).

[0187] Because expression analysis typically involves a quantitative analysis, detection is typically achieved using one of the quantitative real time reverse transcriptase PCR methods described above. Thus, if a TaqMan approach is utilized, the reagents that are introduced (or previously spotted) in the reaction sites can include one or all of the following: primer, labeled probe, nucleotides and polymerase. Another approach is Ribo-SPIA (see above).

[0188] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes can be made and equivalents can be substituted without departing from the scope of the invention. In addition, many modifications can be made to adapt a particular situation, material, composition of matter, process, process step or steps, to achieve the benefits provided by the present invention without departing from the scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

[0189] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an indication that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same.

SEQUENCE LISTING

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