

[0033] FIG. 14 is a cross-sectional view of an exemplary actuator, and also depicts an exemplary gate.

[0034] FIGS. 15-27, describe steps in operation of an exemplary microfluidic cartridge as further described herein.

[0035] Like reference symbols in the various drawings indicate like elements.

#### DETAILED DESCRIPTION

[0036] Analysis of biological samples often includes determining whether one or more polynucleotides (e.g., a DNA, RNA, tRNA, mRNA, or rRNA) is present in the sample. For example, one may analyze a sample to determine whether a polynucleotide indicative of the presence of a particular pathogen is present. As used herein, the terms polynucleotide and nucleic acid compound may be used interchangeably and are taken to mean polymeric organic molecules formed from recurring or non-recurring sequences of one or more of the naturally occurring nucleic acids, adenine, guanine, cytosine, thymine, and uracil.

[0037] Typically, biological samples are complex mixtures. For use herein, a sample may be provided as any matrix including but not limited to: a blood sample, a tissue sample (e.g., a swab of, for example, nasal, buccal, anal, or vaginal tissue), a biopsy aspirate, a lysate, as fungi, as bacteria, or as food samples such as are used in testing foodstuffs. Where found in food samples, the foodstuffs can include dairy products such as cheese or milk, and staples such as grain, corn, rice, or maize. Polynucleotides to be determined may be contained within particles (e.g., cells, such as white blood cells and/or red blood cells), tissue fragments, bacteria (e.g., gram positive bacteria and/or gram negative bacteria, fungi, spores). One or more liquids (e.g., water, a buffer, blood, blood plasma, saliva, urine, spinal fluid, or organic solvent) is typically part of the sample and/or is added to the sample during a processing step.

[0038] Methods for analyzing biological samples include steps of obtaining a biological sample in a form that can be handled in a laboratory (e.g., in the form of a swab), releasing polynucleotides from particles (e.g., bacteria or other cells) in the sample, amplifying one or more of the released polynucleotides (e.g., by PCR), and determining the presence (or absence) of the amplified polynucleotide(s) (e.g., by fluorescence detection).

[0039] Biological samples also typically include inhibitors (e.g., mucosal compounds, hemoglobin, faecal compounds, and DNA binding proteins). Such compounds inhibit attempts to determine the presence of polynucleotides in the sample. For example, such inhibitors can reduce the amplification efficiency of polynucleotides by PCR and other enzymatic techniques for determining the presence of polynucleotides. If the concentration of inhibitors is not reduced relative to the polynucleotides to be determined, the analysis can produce false negative results. Accordingly, preferred methods and related systems for preparing biological samples (e.g., samples having one or more polynucleotides to be determined) reduce the concentration of inhibitors relative to the concentration of polynucleotides to be determined.

System

[0040] FIG. 1 depicts an exemplary microfluidic system 10 for converting a sample containing one or more poly-

nucleotides into a form suitable for analyzing the one or more polynucleotides, for example according to methods described herein. FIGS. 2A-2F show exploded views of various aspects of exemplary system 10.

[0041] Four cartridge receiving elements 12 are depicted in FIG. 1, though it would be understood that other suitable embodiments of device 10 may have more, or fewer, receiving elements, such as but not limited to 1, 2, 3, 6, 8, 10, 12, 16, or 20 receiving elements. System 10 optionally has a closeable door 22, that covers the region of system 10 in which the cartridge receiving elements are situated. Door 22 may be transparent, for example made of Perspex or some similar material, so that a user may monitor visually the system's activity. Cartridge receiving elements 12 independently accept an insertable and removable cartridge such as a microfluidic cartridge as further described herein, and also such as a multi-sample cartridge, as further described herein, wherein a mechanical key (not shown) may facilitate accurate insertion of the cartridge. FIG. 1 shows that the optional door 22 is preferably closed during preparation of a sample. Door 22 is shown hinged at its top edge with one or more hinges 24, but may also be hinged at its lower, or its left, or right edges, consistent with the overall operation of system 10. Door 22 is further depicted with an optional handle 26 for ease of opening and closing. Door 22 is still further depicted in FIG. 1 with an optionally hingeable middle section, as accomplished by one or more hinges 28. Such an optionally hingeable middle section facilitates partial opening of the door, as well as to create a more manageable folded configuration of the door when open.

[0042] System 10 also preferably comprises an area 35 for storing reagents. Such an area may be located within housing 33 of system 10 but may also be on the outer surface of housing 33, as depicted in FIG. 1. Depicted in FIG. 1 are three reagent bottles 36 mounted externally to housing 33 via one or more mounting brackets 34. Reagent bottles 36 contain, respectively, release buffer, wash buffer, and neutralization buffer, and are configured to deliver the respective reagents to the samples during sample preparation. The external mounting of reagent bottles 36 advantageously permits a user to readily see when any one or more bottle requires re-filling. The incorporation of reagent bottles into system 10 is advantageous because it permits system 10 to be easily transportable from one location to another within a laboratory, without need for disconnecting and reconnecting delivery tubes from external reagent storage to the system. In other embodiments, however, where it is desired to operate system 10 for long periods of time without frequent user intervention to refill reagent bottles, the reagents may be supplied from larger containers, not attached to or contained inside system 10, but situated elsewhere and configured to deliver reagents to system 10 via one or more tubes, supply lines, or pipes.

[0043] System 10 also may comprise one or more stabilizing feet 30 that cause the housing 33 to be elevated above a surface on which system 10 is disposed, thereby permitting ventilation underneath system 10, and also providing a user with an improved ability to lift system 10. There may be 2, 3, 4, 5, or 6, or more feet 30, depending upon the size of system 10. Feet 30 are preferably made of rubber, or plastic, or metal, and elevate housing 33 of system 10 by from about 2 to about 10 mm above a surface on which it is situated.