

the sample and certain other sample components (e.g., inhibitors). Typically, the affinity beads retain at least about 50% of polynucleotides (at least about 75%, at least about 85%, at least about 90%) of the polynucleotides present in the sample that entered processing region 220.

[0153] After completion of lysis and capture of DNA onto reagent beads, the lysed sample flows through hole H1 and into the microfluidic component, as shown in FIG. 15. Hole H1 is always open to permit sample to flow through but passage of sample is effectively controlled by gate G1 and thus sample does not exit through H1 until G1 is opened. The sample flows past valve V1, junction 259 and along channel 239 towards capture filter C. Motion towards gate G5 is impeded.

[0154] Gate G1 is opened, for example by heating, and continual expansion of air from chamber 264 forces the sample to flow along channel 239 to capture filter C. Pressure within chamber 264 drives the lysed sample material (containing lysate, polynucleotides bound to particles, and other sample components) along the pathway. During this flow, as depicted in FIGS. 16A and 16B, the DNA capture beads get trapped at the inline filter (C.). Preferably filter C. is a 8 micron filter. Valve V2 has remained open during this process.

[0155] Next, after a period of time (e.g., between about 2 and about 5 minutes), as depicted in FIG. 17, the excess pressure in the bulk lysis chamber is vented to atmosphere through hole H4 to the waste chamber by opening gate G1.

[0156] Valve V1 is now closed, as shown in FIG. 18, to prevent any liquid leaking back into the bulk lysis chamber during further liquid processing, and thereby sealing off the lysis chamber.

[0157] In a next step, wash reagent is input, preferably automatically by a system such as system 10, through the pierceable inlet 280 and via hole H1, forced through channels 208, 211, 213, 239, 210, 236, and 234, along the shaded flow path in FIG. 19, to wash the filter, C. Gates G1 and G3 are opened to open this flow path, whereas G2 and V1 remain closed. Typically, the wash liquid is a solution having one or more additional components (e.g., a buffer, chelator, surfactant, a detergent, a base, an acid, or a combination thereof). A typical volume of wash buffer used in this step is 50 μ l. Exemplary solutions include those, for example, made from a solution of 10-50 mM Tris at pH 8.0, 0.5-2 mM EDTA, and 0.5%-2% SDS, a solution of 10-50 mM Tris at pH 8.0, 0.5 to 2 mM EDTA, and 0.5%-2% Triton X-100.

[0158] Thereafter, FIG. 20, the bead column is purged with air by introducing air, for example between 10 and 100 μ l of air, through the reagent inlet. The result is a purging of wash buffer through hole H4 into the waste chamber.

[0159] Next, in FIG. 21, release buffer is input from the reagent inlet 280 to replace the wash solution, and the end terminus of the release buffer liquid volume passes through column C. An exemplary release liquid is a hydroxide solution (e.g., a NaOH solution) having a concentration of, for example, between about 2 mM hydroxide (e.g., about 2 mM NaOH) and about 500 mM hydroxide (e.g., about 500 mM NaOH). In some embodiments, liquid in reservoir 281 is an hydroxide solution having a concentration of about 25 mM or less (e.g., an hydroxide concentration of about 15 mM). A typical volume of release buffer is 50 μ l.

[0160] Valves V2 and V3 are now closed, to seal off the column C, as shown in FIG. 22. The bead column C is heated to 70-90° C. for 3-4 minutes to release the DNA from the affinity beads in the presence of release buffer, FIG. 23.

[0161] Neutralization buffer (about 5 μ l) is next input through the reagent inlet, and sent to the vent V by opening gate G4, as shown in FIG. 24. Valve V4 is now closed, FIG. 25.

[0162] A further 0-45 μ l of neutralization buffer is pumped into the microfluidic component through inlet 280, and mixed with released DNA by opening gates G4, G5, and G6, as shown in FIG. 26.

[0163] Upon input from the user, air is again pumped through the reagent inlet, and gates G5 and G6 are opened to combine neutralization buffer with the released DNA. This step is not generally automated because it is preferred to start the reaction in a controlled manner. The mixture is pumped through a specified channel volume, using for example pressurized air transmitted through the reagent inlet, to intermix and neutralize the DNA, before ejecting the mixed sample into a PCR tube, as shown in FIG. 27.

[0164] The neutralized DNA (or RNA) is forced into the PCR tube at the end of the sample processing unit. The liquid in which the polynucleotides are released into a PCR tube typically includes at least about 50% (e.g., at least about 75%, at least about 85%, or at least about 90%) of the polynucleotides present in the sample that was introduced into the bulk lysis chamber. The concentration of polynucleotides present in the release liquid may be higher than in the original sample because the volume of release liquid is typically less than the volume of the original liquid sample. For example the concentration of polynucleotides in the release liquid may be at least about 10 times greater (e.g., at least about 25 times greater, at least about 100 times greater) than the concentration of polynucleotides in the sample introduced to device 200. The concentration of inhibitors present in the liquid into which the polynucleotides are released is generally less than concentration of inhibitors in the original fluidic sample by an amount sufficient to increase the amplification efficiency for the polynucleotides.

[0165] The time interval between introducing the polynucleotide containing sample to the bulk lysis chamber, and releasing the polynucleotides into the PCR tube is typically about 15 minutes or less (e.g., about 10 minutes or less, about 5 minutes or less). The PCR tube containing PCR-ready DNA is ready for further processing in a bench scale PCR detection machine, and can thus be removed.

[0166] A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the disclosure. Accordingly, other embodiments are within the scope of the following claims.

What is claimed is:

1. A microfluidic system for converting a sample containing one or more polynucleotides into a form suitable for analyzing the one or more polynucleotides, the system comprising:

a cartridge receiving element in communication with an insertable and removable cartridge;