

enzymes (e.g., mixtures of endo- and exo-proteases such as pronase) that cleave peptide bonds. Exemplary protease resistant ligands include, for example, poly-D-lysine and other ligands that are enantiomers of ligands susceptible to enzymatic attack.

[0078] Particles for retaining polynucleotides are typically formed of a material to which the ligands can be associated. Exemplary materials from which particles can be formed include polymeric materials that can be modified to attach a ligand. Typical polymeric materials provide or can be modified to provide carboxylic groups and/or amino groups available to attach ligands. Exemplary polymeric materials include, for example, polystyrene, latex polymers (e.g., polycarboxylate coated latex), polyacrylamide, polyethylene oxide, and derivatives thereof. Polymeric materials that can be used to form particles **218** are described in U.S. Pat. No. 6,235,313 to Mathiowitz et al., which is incorporated herein by reference. Other materials include glass, silica, agarose, and amino-propyl-tri-ethoxy-silane (APES) modified materials.

[0079] Exemplary particles that can be modified with suitable ligands include carboxylate particles (e.g., carboxylate modified magnetic beads, such as Sera-Mag Magnetic Carboxylate modified beads, Part #3008050250, Seradyn, and Polybead carboxylate modified microspheres, available from Polyscience, catalog no. 09850). In some embodiments, the ligands include poly-D-lysine and the beads comprise a polymer (e.g., polycarboxylate coated latex).

[0080] In general, the ratio of mass of particles to the mass of polynucleotides retained by the particles is no more than about 25 or more (e.g., no more than about 20, no more than about 10). For example, in some embodiments, about 1 gram of particles retains about 100 milligrams of polynucleotides.

[0081] The particles typically have an average diameter of about 20 microns or less (e.g., about 15 microns or less, about 10 microns or less). In some embodiments, particles **218** have an average diameter of at least about 4 microns (e.g., at least about 6 microns, at least about 8 microns).

[0082] The density of particles **218** in the lysis pellets is typically at least about  $10^8$  particles per milliliter (e.g., about  $10^9$  particles per milliliter).

[0083] In some embodiments, at least some (e.g., all) of the particles are magnetic. In alternative embodiments, few (e.g., none) of the particles are magnetic.

[0084] In some embodiments, at least some (e.g., all) of the particles are solid. In some embodiments, at least some (e.g., all) of the particles are porous (e.g., the particles may have channels extending at least partially within them).

[0085] In an embodiment in which heat is applied to the sample in bulk lysis chamber **264**, the volume of sample in chamber **264** is such that the upper level of the liquid is in contact with the inside surface **283** of an area **266** of chamber **264**. Area **266** is preferably flat and is configured to receive heat from a heat source, whereby the heat effectuates lysis of the cells in the liquid sample. Preferably the heating is by contact heating and preferably it causes the sample to reach a temperature of between 55 and 85° C., and still more preferably between 65 and 75° C. It is noted that the material from which the cartridge is made is typically a good insulator and therefore the outside of the cartridge may

have to reach a temperature of 20-40° C., e.g., 30° C., in excess of the desired temperature of the sample.

[0086] After the sample has been lysed in lysis chamber **264**, the lysed sample flows through outlet **282** into microfluidic network **201**.

[0087] Cartridge **200** still further comprises a reagent inlet **280** in communication with microfluidic network **201**. Typically reagent inlet **280** is of the form of a pierceable inlet, such as a septum. Reagent inlet **280** may also be configured to make a tight seal with a nozzle of a reagent delivery head, as further described herein in connection with system **10**.

[0088] Cartridge **200** further comprises an outlet **236** by which a prepared sample can be removed (e.g., expelled or extracted). Outlet **236** is preferably configured to direct prepared sample into a PCR tube (not shown in FIG. 4) such as are used in the art. Preferably such a PCR tube **237** is detachable from cartridge **200** and is typically one of those used throughout the biotechnology industry, and is thus typically made of a plastic material such as polypropylene, and configured to fit other laboratory equipment such as a thermal cycler for performing PCR, or other equipment for performing analyses such as TMA, SDA, and NASBA. A PCR tube such as is used herein typically has an effective volume of 0.2 ml, though may also have an effective volume of 0.6 ml. Representative PCR tubes for use with the methods and apparatus described herein are available from suppliers that include USP, Inc., San Leandro, Calif. (see <http://www.uspinc.com/PCRTubes.htm>). Preferably, PCR tubes for use with the present invention are connected to one another in strips of 8 and are used with a multi-sample cartridge as further described herein.

[0089] Cartridge **200** also has a waste chamber **269** that receives waste from microfluidic network **201** via inlet hole **270**. When liquid from microfluidic component **201** flows into waste chamber **269** via hole **270** and is followed by air expelled through hole **270**, the liquid has a tendency to foam, and overflow from vent **262**. To reduce this phenomenon, waste chamber **269** may contain one or more tablets of an anti-foaming agent such as, but not limited to, Simeicone. When used, the tablets are typically 1-4 mm in diameter.

[0090] FIG. 5 shows a perspective view of an underside of multi-sample cartridge **200** showing microfluidic component **201** having representative microfluidic channels **285**. A nozzle **284** is situated about an outlet **236**, and is configured to mate with a top of a PCR tube, to thereby minimize waste during expulsion of polynucleotide containing sample from the microfluidic network into the PCR tube.

[0091] FIG. 6 shows a close-up of an exemplary nozzle **284**, showing outlet hole **236** in a raised conical area **286** situated concentrically with respect to the outer rim of nozzle **284**. One of ordinary skill in the art would understand that this configuration may be tailored to suit many different shapes and geometries of PCR tube, as used in the art, and is therefore not limited to the configuration depicted in FIG. 6.

[0092] In operation, microfluidic component **201** is situated in close proximity to an array of heaters so that the various elements of the microfluidic component can be controllably and selectively heated. FIG. 7 shows, in overview, a schematic of an array of heaters **501**, disposed in a contact heating layer, is disposed in relationship to various