

nents: Eberwine enzyme reactions and Solid Phase Reversible Immobilization (SPRI) aRNA clean-up. These processes are discussed in detail herein.

**[0108]** Any process that alters relative mRNA abundance levels may potentially interfere with accurate gene expression profiling. An important aspect of the Eberwine amplification procedure is that it can employ a linear amplification reaction that can be less prone to bias mRNA populations than exponential amplification methods such as PCR.

**[0109]** The original Eberwine protocol has been streamlined and simplified by commercial vendors such as Ambion. As shown in FIG. 25, the Ambion procedure comprises three binary (two component) additions followed by an RNA purification process. Each binary addition can be followed by incubation(s) at specific temperatures, as indicated in FIG. 25. The initial reverse transcription (RT) reaction can have three inputs (primer, total RNA, and reverse transcriptase [RT] Mix); however, total RNA and primer can conveniently be premixed. Typical volumes for this first reaction can be 5 ul RNA+Primer 5 ul RT Mix. Only mRNA hybridizes to the oligo dT primer and is transcribed into DNA. The second-strand reaction can be initiated by addition of 20 ul of a Second-Strand Mix, and the final T7 amplification reaction can be initiated by addition of 30 ul of a T7 Mix. Synthesized RNA can be labeled at this stage by incorporation of biotin-labeled ribonucleotides. Mixes contain buffers (Tris), monovalent and divalent salts (KCl, NaCl, MgCl<sub>2</sub>), nucleotides, and DTT, along with enzymes as indicated. Typically, enzymes can be premixed with concentrated mixes just prior to use. The process can be implemented using three sequential enzyme reactions, including reverse transcription, DNA polymerization, and RNA polymerization. The three steps can be implemented without intermediate clean-up steps. A heat-kill step can be included after the DNA polymerization or second-strand synthesis (step 2).

**[0110]** After synthesis, aRNA can be purified to remove enzymes, buffers, salts, unincorporated nucleotides, pyrophosphate, etc. Purification can rely on commercial kits exploiting the association of aRNA with silica membranes or beads in the presence of chaotropic salts such as guanidinium hydrochloride (GuHCl) or thiocyanate (GuSCN). After binding, the silica is washed with 70% ethanol (EtOH), dried, and aRNA is eluted with water.

**[0111]** As described above, the Eberwine mRNA amplification procedure can be a cascade of three binary additions. To execute the Eberwine sequence, assume that Ras1R contains RT Mix, Ras2R contains second-strand synthesis (2S) Mix, and Ras3R contains T7 Mix, as shown in FIG. 19. As indicated in FIG. 25 for Message Amp III, a 2x volume of 2S Mix will be added to the RT reaction, and a 1x volume of T7 Mix will be added to the 2S reaction. This requires a 2:1 pumping ratio (AB:CD) for the 2S Mix addition, and a 1:1 ratio for the T7 Mix addition.

**[0112]** Assume that 4-Cycle pumping assembled the first (RT) reaction with a 1:1 mixture of total RNA from Sample and 2x RT Mix from Ras1R in the Out1 reservoir. After an appropriate incubation period, the second-strand reaction may be assembled in the Out2 reservoir by drawing from Out1 (rather than from Sample), and drawing from Ras2R (rather than from Ras1R). In other words, in cycle A, Vr2 is opened rather than Vr1; in cycle B, V3 is opened rather than V2; in cycle C, V2 is opened rather than V1; and in cycle D,

V3 is opened instead of V2. Note that to obtain the required 2:1 mixing ratio, for every cycle drawing from Out1, two cycles will draw from Ras2R.

**[0113]** After another appropriate incubation period, the third (T7) reaction may be assembled in the reservoir connected to Out1 with a similar process (drawing from Ras3R and Out2, 1:1 ratio). Thus the final T7 reaction will reside in the Out1 reservoir. After an appropriate incubation period, aRNA will be ready for purification.

**[0114]** Each of these steps can be carried out on the devices described herein. For example, reagents and sample can be supplied through ports in the cartridge and then delivered to the microfluidic chip. The on-chip valves can be used to pump the reagents and samples to chambers and reservoirs in the cartridge and the microfluidic chip through channels. Temperature control can be accomplished using internal or external heating and cooling devices. The reaction products can be moved to product outlet ports of the cartridge for further handling. Alternatively, the reaction products can be purified or separated using the devices of the invention.

#### B. Separation and Cleanup

**[0115]** A variety of separations can be performed using the devices described herein. These separations include chromatographic, affinity, electrostatic, hydrophobic, ion-exchange, magnetic, drag-based, and density-based separations. In some embodiments of the invention, affinity or ion-exchange interactions are utilized to bind materials to solid-phase materials, such as beads. The beads can be separated from fluid solutions using any method known to those skilled in the art.

**[0116]** In some embodiments, separation and cleanup can include solid phase reversible immobilization (SPRI). SPRI can utilize a variety of chemistries, including guanidinium-based purification chemistries and magnetic bead-based chemistry. Guanidinium buffers can be toxic, near-saturated solutions prone to crystal particulate formation. Guanidinium buffers can promote binding to silica (glass) surfaces. Other chemistries that can be utilized include PEG/salt-driven association of nucleic acids with magnetic beads that can be covered with carboxylated polymers (deAngelis et al., Nucl. Acids Res. 23, 4742). Typically, beads in 2x buffer (20% PEG8000, 2.5M NaCl) are combined with RNA in a 1:1 ratio. After a brief incubation period, RNA-bead complexes are captured with a magnet, the beads are washed with 70% EtOH, briefly dried, and RNA is eluted in a small volume of water. Carboxylated polymer double shell magnetic beads (SpeedBeads) are available from Seradyne (<http://www.seradyne.com/micro/particle-overview.aspx>).

**[0117]** Magnetic separation can be used to capture and concentrate materials in a single step using a mechanistically simplified format that employs paramagnetic beads and a magnetic field. The beads can be used to capture, concentrate, and then purify specific target antigens, proteins, carbohydrates, toxins, nucleic acids, cells, viruses, and spores. The beads can have a specific affinity reagent, typically an antibody, aptamer, or DNA that binds to a target. Alternatively electrostatic or ion-pairing or salt-bridge interactions can bind to a target. The beads can be paramagnetic beads that are only magnetic in the presence of an external magnetic field. Alternatively, the beads can contain permanent magnets. The beads can be added to complex samples such as aerosols, liquids, bodily fluids, extracts, or food. After (or before) binding of a target material, such as DNA, the bead can be cap-