

tured by application of a magnetic field. Unbound or loosely bound material is removed by washing with compatible buffers, which purifies the target from other, unwanted materials in the original sample. Beads can be small (nm to μm) and can bind high amounts of target. When the beads are concentrated by magnetic force they can form bead beds of just nL- μL volumes, thus concentrating the target at the same time it is purified. The purified and concentrated targets can be conveniently transported, denatured, lysed or analyzed while on-bead, or eluted off the bead for further sample preparation, or analysis.

[0118] Separations are widely used for many applications including the detection of microorganisms in food, bodily fluids, and other matrices. Paramagnetic beads can be mixed and manipulated easily, and are adaptable to microscale and microfluidic applications. This technology provides an excellent solution to the macroscale-to-microscale interface: beads can purify samples at the macroscale and then concentrate to the nanoscale (100's of nL) for introduction into microfluidic or nanofluidic platforms. Magnetic separations can be used as an upstream purification step before real-time PCR, electrochemiluminescence, magnetic force discrimination, magnetophoretic, capillary electrophoresis, field-flow separations, or other separation methods well known to one skilled in the art.

[0119] The devices of the invention can accommodate the use of magnetic beads. For example, beads or bead slurry can be supplied to a port of a cartridge. The beads can be mixed or suspended in solution within the cartridge using pumping, magnetic fields, or external mixers. The beads can then be pumped to desired chambers or reservoirs within the microfluidic device or cartridge. Beads can be captured within a chamber using a magnetic field. Beads in a solution can be captured as the solution travels through the magnetic field, or beads can be captured in a stagnant solution.

[0120] RNA purification can involve operation of the Bead Rail rather than the Reagent Rail. Thus, during this phase of chip operation, valve Vr will remain closed and Vb will open. As described above, 4-Cycle pumping can be used to mix 2x Bead Slurry from Ras1B (FIG. 19) with aRNA from the Out 1 reservoir, into the Out2 reservoir. The next step, after a brief incubation period, is collection of RNA—bead complexes in BPump. To do this, assume first that the BPump membrane remains pulled down into the 500 μm deep pneumatic cavity. Then, 2-Cycle pumping (analogous to cycles AB or CD in FIG. 20) can be used to pump the bead binding mixture from the Out2 reservoir, through BPump, and out to E11. RNA-bead complexes are captured in the BPump, as they are pulled down out of the main flow path by the magnet positioned immediately beneath the chip (in the pneumatic manifold). After capture, beads are washed with 100% EtOH, and dried by (2-Cycle) air pumping from Ras4B (which is empty).

[0121] RNA elution can rely on “disruptive mixing” of beads (initially captured in the BPump) and water from Ras3B. This can be accomplished through the use of the BPump membrane to (2-Cycle) pump water from Ras3B to the Out1 reservoir. The packed bead bed, deposited on the BPump membrane, can be rapidly disrupted and mixed with water as the BPump membrane reciprocates. Finally, beads and released aRNA can be pumped back through BPump to E12. Beads are recaptured in BPump, and aRNA (in water) ends up in E12.

III. Examples

A. Script for RNA Purification

[0122] Scripts can be written to operate and/or automate the systems, devices, and methods described herein. The following is an example of a script for performing RNA purification.

[0123] As shown in FIG. 26 (left), the script is organized into 11 code chunks. Each chunk has associated run-time parameters which are shown on the right. Four points where RNA purification losses may occur are indicated in red. Chunks are discussed below. Unless otherwise noted, pump cycles are executed by chip pumps (Pump). Chip pumps move 0.5 ul/stroke and BPumps move 1 ul/stroke.

[0124] 1. BPump_Initialization. BPump chambers are cleaned as the BPump membrane pumps water and then EtOH (# BPump Cleaner=10). BPumps are left filled with EtOH, bubble-free, and ready to accept Bead-RNA mix later in the script.

[0125] 2. Prime_For_Mixing. RNA (Out1) and 2XBB (Ras1B) are primed (# Out1 RNA Prime=12 and # Ras1B 2XBB Prime=4, respectively). Priming removes any air in manifold dead volumes, and assures that subsequent mixing will be accurate.

[0126] 3. Mix_Out2. Twenty cycles of eight-step pumping mix RNA (10 ul) and 2XBB (10 ul) in Out2 (total volume 20 ul). Note that the #Binding Rxn Mixer=23 cycles. This is because three cycles are used to re-prime 2XBB from Ras1B at 10 cycle intervals (at cycles 0, 10, and 20) as specified by BBufLoadMod=10. A 100 sec binding reaction incubation is programmed (Binding Reaction Inc=100000), after mixing is completed.

[0127] 4. Load_BPump. To minimize introduction of air bubbles into BPumps during transfer of the RNA-bead binding reaction to BPumps, Out2 is first primed to remove any accumulated air (# Out2 Mix Prime=2). This is a (first) programmed loss of RNA, as up to 1 ul out of 20 ul (5%) is deliberately lost to priming. After Out2 priming, the binding reaction is pumped through BPumps to waste ports W. As the mixture traverses BPumps, RNA-bead complex is captured by magnets positioned underneath BPumps. To maximize bead capture, an additional dwell time is introduced into each pump cycle (BeadDwell=2500). Note that # Binding Rxn Loader=39 intentionally leaves 0.5 ul (second programmed loss, 2.5%) behind in Out2, again to avoid introduction of air bubbles into BPumps. Finally, during transfer, additional (third programmed) losses of $3 \times 2.5\%$ are incurred by periodic Out2 re-priming at cycles 0, 15, and 30 (MixLoadMod=15). Total programmed maximum losses are therefore $5 + 2.5 + 7.5 = 15\%$ at this point.

[0128] 5. Wash_BPump. After Wash priming (Ras2B EtOH Prime=32 5), the accumulated RNA-bead bed is washed with 100% EtOH (Ras2B Wash=50). Note that only about 12.5 ul 100% EtOH is loaded into the Ras2B pipette tip, as the rest of the cycles are reserved for pumping of air to dry the washed bead bed.

[0129] 6. PreElute_Empty_Out2. Since Out2 will next be used to hold elution material, it must be cleaned prior to use. The first step in this process is removal of any remaining RNA-bead binding mix from Out2. Ten pump cycles are hardwired into the script at this point.

[0130] 7. PreElute_Prime_Elution. Elution (water) is primed (Ras3B Water Prime=2) to eliminate any air bubbles and to wash processor channels.