

prises 20% PEG 8000, 2.5M NaCl (2× concentration). As shown in FIG. 28 (Which shows RNA purification using 0.125 uL SpeedBeads), bench experiments with SpeedBeads and DeAngelis buffer showed that at least 50 ug of total RNA could be purified with very high efficiency with a 0.25 ul packed bead bed. As shown in FIG. 29 (which shows RNA purification using 0.125/4 uL), equivalent results were obtained with ¼ the amount of SpeedBeads (13 ug×4=52 ug). And surprisingly, as shown in FIG. 30 (which shows RNA purification using 0.125/40 uL), even with 10× fewer SpeedBeads (0.125/40 ul) there was no sign of saturation up to 13 ug RNA (equivalent to 13 ug×40=520 ug), although recovery was reduced. Interestingly, in the experiment of FIG. 30, significant amounts of RNA were not recovered in the supernatant, indicating that bead loss, rather than bead saturation, was probably responsible for reduced RNA recoveries. These results indicate that 0.125 ul packed bead beds in chips should be capable of purifying at least 100 ug RNA with high efficiency.

#### D. Microfluidic RNA Recovery

**[0152]** The accuracy of mixing of RNA and 2XBB (actually dilution of 2XBB with water) was first characterized. This experiment relied on our observation that SpeedBead concentration can be sensitively monitored by absorbance at 400 nm (FIG. 31, left). FIG. 31 (right) shows that the % mixing error for four experiments was approximately +/-15%. FIG. 31 shows Bead Mixing Accuracy FIG. 31 Left shows a Standard curve relating bead concentration to A400. FIG. 31 Middle shows Final bead concentration after 1:1 dilution of 1.25% beads in 2XBB by Mix\_Out2 code chunk on a chip of this invention 1. FIG. 31 Right shows % mixing error. Most of this is likely attributable to pump filling inaccuracies caused by the relatively high viscosity of 2XBB. The sensitivity of RNA purification efficiency to this mixing ratio is presently uncharacterized.

**[0153]** FIG. 32 shows the results of three purification experiments with approximately 1.5 ug total RNA in a chip running the script. FIG. 32 shows Purification Yield and Purity. FIG. 32 Left shows Experiment 1 using 1.6 ug RNA. FIG. 32 Middle shows Experiment 2 using 1.7 ug RNA. FIG. 32 Right shows Experiment 3 using 1.7 ug RNA and increased # Binding Rxn Loader to 41. These results are also summarized in the FIG. 32 table. Average purification efficiencies were 61.3% to 69.8%, which is approximately 10-20% lower than the programmed RNA losses described above (expected yield as low as 79%). In addition to the programmed losses, additional losses may be incurred due to poor RNA-bead association, RNA or beads sticking to walls, etc. In this respect, one significant loss that we have consistently observed is the accumulation of beads in the dead volume formed by the adhesive layer attaching the chip to the fluidic manifold during transfer of bead binding mix to BPumps (step 4 above). We suspect that it is possible that up to 10% of the beads may become immobilized in this dead volume. Taking this additional loss into account, expected purification efficiencies should run around 70%.

**[0154]** With respect to purification efficiency, it is probably worth noting that Exp 3, in which # Binding Rxn Loader was increased from 39 to 41 had the highest mean and lowest CV among the three experiments. This indicates that the problem of bubble injection into BPumps may have been over-estimated.

**[0155]** The above described experiments were conducted with relatively small amounts of RNA (<5 ug) and small purification volumes (20 ul). In experiments with Message Amp III aRNA (15 ug) and liquid volume (120 ul) levels, additional effects on bead capture efficiencies were observed. The result of these effects was decreased bead capture and RNA purification efficiencies (about 50%, as discussed below). At present we believe that there are five major factors affecting bead capture and RNA purification efficiencies under Message Amp III conditions.

**[0156]** 1. Membrane Deformation. Efficient bead capture in BPumps relies on deformation of the PDMS membrane to the bottom of the 500 um milled-out pneumatic layer. The major factors affecting deformation are membrane modulus (flexibility), membrane thickness, and vacuum level. Experiments with different PDMS thicknesses and chemistries have shown that while increased membrane flexibility can improve deformation, bead collection efficiency, and RNA purification efficiency, it also decreases valve pressure operating margins. As illustrated in FIG. 33, this is because, when valves are closed, increased flexibility allows the membrane to deform up into valve cavities, cutting-off flow in "Bus" channels. Although this undesirable behavior can be reduced by decreasing valve closing (positive) pressures, this tends to increase valve leakage phenomena, generally degrading chip performance. FIG. 33 shows Bus Channel Cut-Off. PDMS membrane (red) deformation in three valve states. FIG. 33 A shows an Open Valve. The membrane is pulled down into the pneumatic layer. FIG. 33 B shows a Closed Valve. In normal operation, the membrane seals against valve seat, closing the valve. Flow through the Bus Channel is unimpeded. FIG. 33 C shows a Bus Channel Cutoff. With increased flexibility, membrane can deform up into valve cavities, cutting-off flow in the Bus Channel. Alternatively, chips can be designed without Bus channels by ensuring that valve cavities and input/output channels never overlap. Although this is a straightforward change, it decreases design flexibility. Fortunately, increased vacuum levels can improve membrane deformation into the pneumatic cavity without affecting valve closing phenomena. The relatively low vacuum pressure (18-21 in Hg) produced by the Hargraves pumps used throughout the project can be improved with stronger pumps, such as the KNF UN86. Vacuum levels exceeding 28 in Hg can be achieved, resulting in improved bead capture and RNA purification efficiencies.

**[0157]** 2. Magnetic Field. Magnetic field strength and bead capture efficiencies can be increased with larger magnets. However, unless careful field shaping and magnetic shielding is implemented, stray fields throughout the chip may tend to capture beads in undesired locations, decreasing chip operating efficiency.

**[0158]** 3. Buffer Viscosity. We have routinely observed that bead collection efficiencies are highest in water, and lowest in Bead Binding Buffer. The reason for this difference may be the high viscosity of the buffer, which is due to the presence of 10% PEG8000.

**[0159]** 4. Pumped Volume. We have also observed that bead capture efficiency is affected by the pumped volume. This is probably because, for a constant quantity of beads, increased pumped volumes result in greater net hydrodynamic drag on the beads, and therefore, greater bead losses from BPumps.

**[0160]** 5. RNA Quantity. We have recently observed an interesting and unexpected phenomenon associated with purification of relatively large amounts of RNA in chips of