Supporting Information

Silica Bead Immobilization Method

To simplify device fabrication and the on-chip operation, beads for RNA purification were immobilised within the RNA purification chamber (RPC). Beads can be positioned precisely into the RPC by loading a limited volume of 3 µL of a 68% (bead: deionised H_2O volume) solution of 10-µm plain silica beads (PSi-10.0, G. Kisker Gbr., Germany) into the input port (IP), with all other ports on the chip sealed except for the waste output (WO). This dense solution of beads is left to dry at room temperature for 3 hr. This packs the dry silica beads into the extraction chamber. After the beads dried in place, similar to conventional PDMS glass bonding, the silica beads are immobilised via bonding to the PDMS walls of the extraction chamber by using UV ozone treatment. The chip with the packed dried silica beads was treated with ozone for 10 min to activate the PDMS, then left to bond overnight at room temperature. Finally, a bead-washing step with deionised H_2O (dH_2O) is performed to remove unbound beads and leave a layer of silica beads bonded to the PDMS walls of the RPC. To demonstrate that the beads were immobilized on the surface of the PDMS within the RPC chamber a new PDMS-PDMS chip was fabricated instead of the reported PDMS-glass. The immobilization strategy followed was exactly the same except that 10 µm green-fluorescent silica beads were used. The microfluidic chip was then cut across the RPC chamber to generate a cross-sectional view of the bonded bead and imaged with a fluorescent microscope, as shown in Figure 1.

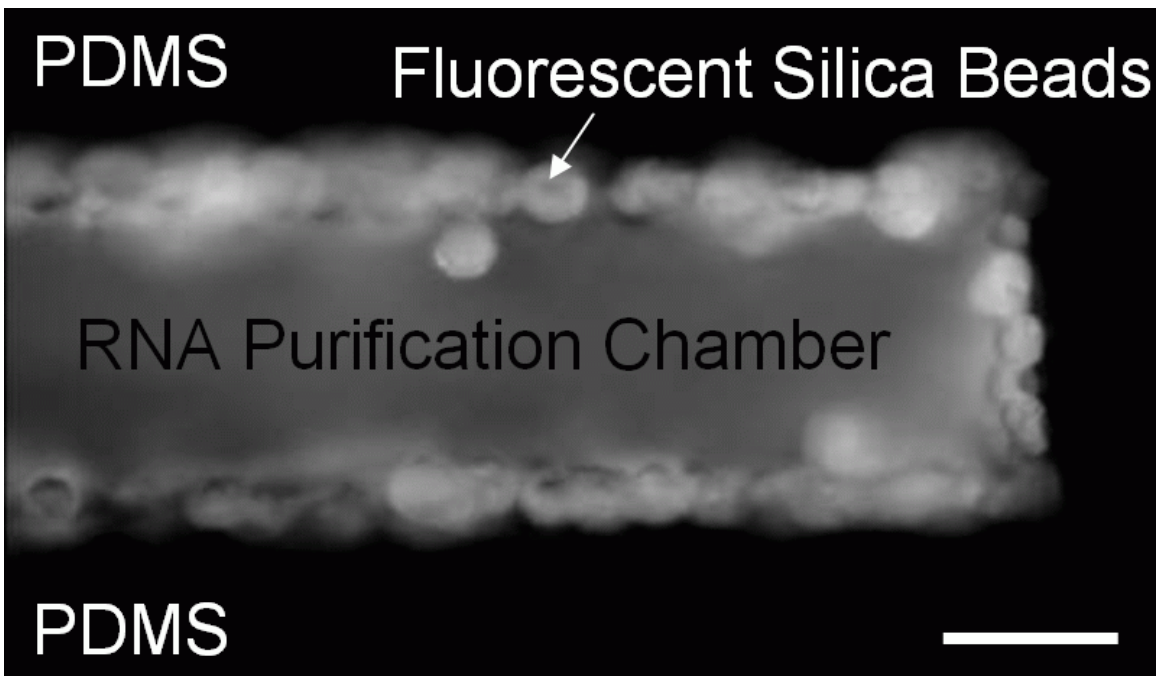

Fig. 1 Fluorescent image of a cross section of the RPC chamber showing a layer of immobilized green fluorescent silica beads on the PDMS walls. Please note that in this case the entire device is made of PDMS, for ease of cutting the bottom glass cover was replaced with a flat PDMS layer. Scale bar 30 *µ*m

Comparison: Microfluidic RNA purification and Real Time NASBA protocol versus conventional RNA purification and Real Time NASBA protocol

Compared to the conventional method the on-chip RNA purification and Real Time NASBA reaction is done in approx. 10 time less volume, result discrimination can be done approx. 10 times faster (within the first 3 min. compared to 25 min. in the conventional case) and the direct pipetting steps are

reduced from 11 to 2 and the sample transfer steps from 3 to 1, this reduces the sample contamination probability (Fig. 2).

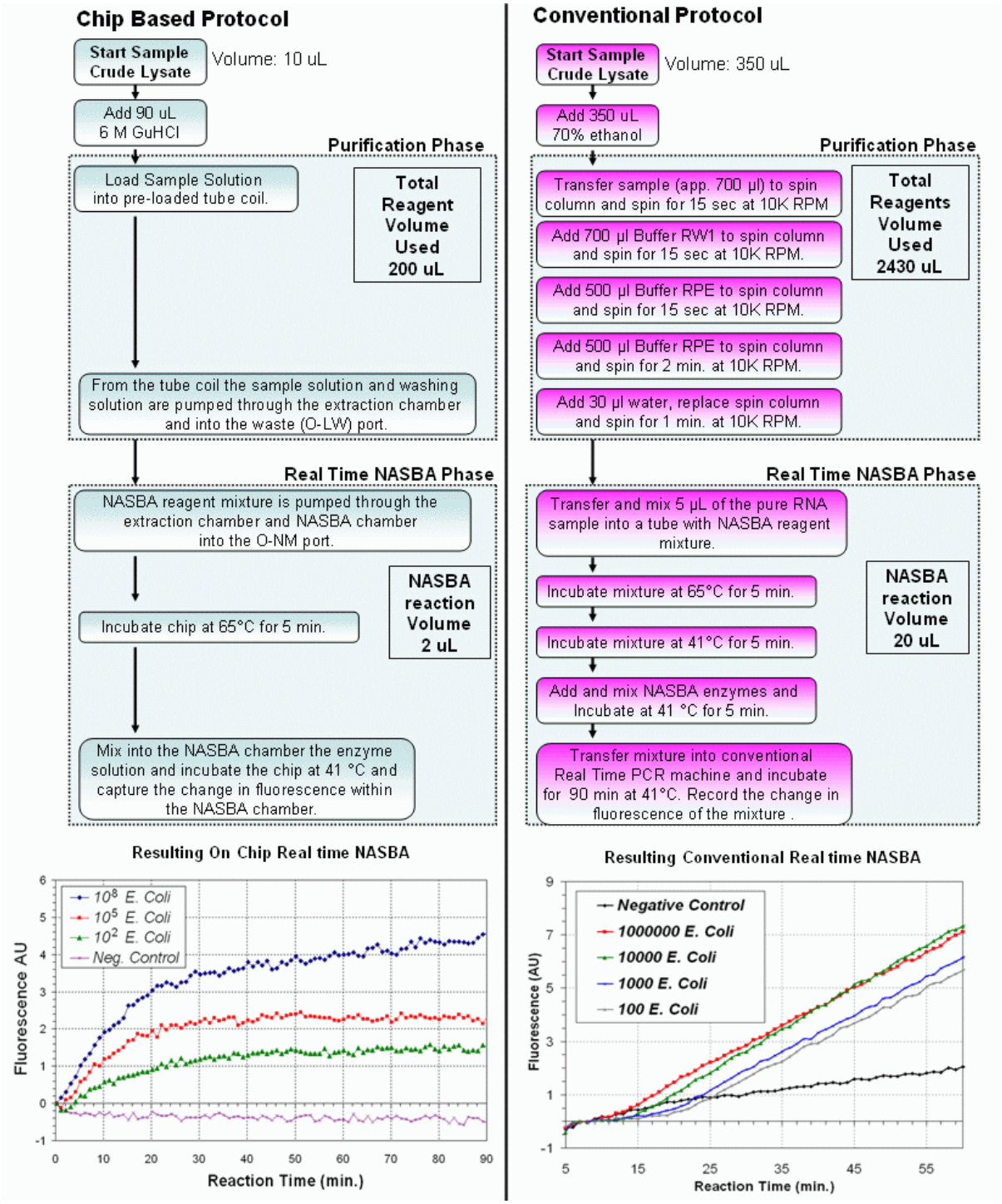

Fig. 2 Microfluidic RNA purification and Real Time NASBA protocol versus conventional RNA purification and Real Time NASBA protocol. In the microfluidic case approx. ten time less volume is used, results are obtained in approximately one tenth of the time and direct pipetting steps are reduced from 11 to 2.