

Automatic Extraction and Processing of Small RNAs on a Multi-well/Multi-channel (M&M) Chip

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Video 1. Magnetic droplets splitting, collection and retaining

Video 2. Magnetic droplets moving from wash well to elution well

Supplemental Experimental Methods

Chemicals and materials

The Sylgard® 184 silicone elastomer kit was obtained from Dow Corning (Midland, MI). The NOA81 optical adhesive was from Norland Products (Cranbury, NJ). RNA isolation buffers were included in the PureLink™ miRNA Isolation Kit (Life Technologies, Carlsbad, CA). The silica magnetic particles with diameter of 1 µm (40 mg/ mL in water) were purchased from Bioclone Inc. (San Diego, CA). The TaqMan™ microRNA RT (reverse transcription) kit and primers specific for amplification of hsa-mir-191 were acquired from Applied Biosystems (Life Technologies). Taq™ 5×Master Mix for PCR was from New England BioLabs (Ipswich, MA). Mineral oil (M5904) and the nuclease-free water were from Sigma (St. Louis, MO). Other chemicals and materials, like dimethyl dichlorosilane (DMDCS), ethanol, polyvinylpyrrolidone (PVP, average Mw 1,300,000), buffers for capillary electrophoresis (CE), and glass slides, were from Fisher Scientific (Pittsburgh, PA). The target miRNA, hsa-mir-191 (5'-CAACGGAAUCCCAAAGCAGCUG -3'), and oligos with lengths of 15 nt and 63 nt

were synthesized by Integrated DNA Technologies (Coralville, IA). The SYBR Gold dye (10,000× concentration) and the 0.1–2 kb RNA ladder were purchased from Invitrogen (Life Technologies).

Manipulation of magnetic particles

A syringe pump (NE-300, New Era Pump Systems Inc., Farmingdale, NY) was used to enable automatic manipulation of the magnetic droplets on our M&M chip (Figure S1, Supporting Information). Prior to use, the M&M chip was treated by DMDCS and then filled with mineral oil. Binding and washing buffers were deposited at designated wells with a multichannel pipette, forming droplets in the wells. A permanent neodymium magnet (1" cube) was fixed between the two guiding rods of the syringe pump (Fig. S1A-1). One end of the M&M chip was attached to the pusher block of the syringe pump by adhesive tape, and the other end was supported by a home-made plastic block assembled on the guiding rods (Fig. S1A-2). In this way, the chip was right above the magnet, and could be moved horizontally across the magnet at various speeds (between 0.2~0.4 mm/ sec) controlled by the syringe pump. The magnetic particles were pipetted into the first row of wells that were lined up with the edge of the cubical magnet. As the chip was driven by the syringe pump to move across the magnet, the magnetic particles were retained by the large magnetic field gradient around the magnet edge, creating a relative movement between the chip and the particles (Fig. S1B). The particles could then travel from well to well (Fig. S1B-1); and the unwanted particles could be collected at the first well connecting all four channels (Fig. S1B-2).

MicroRNA purification and cell lysate preparation

We used the lysis/binding (L3) and washing (W5) buffers included in the PureLink™ miRNA Isolation Kit for cell lysis and miRNA purification. The E.coli cells were cultured in Luria-Bertani Broth, and lysed with buffers from the QIApre Spin Minipre Kit (Qiagen, Valencia, CA). The Jurkat cells were grown in RPMI-1640 medium (ATCC) and the 293T cells in DMEM medium (ATCC), both supplied with 10% fetal bovine serum (FBS) and 1% penicillin. Cell suspension (up to 1×10^6) was centrifuged at 2,000 rpm for 5 minutes to pellet cells. Cell pellets were re-suspended in 300 μ L L3 buffer (up to 3.3×10^3 cells/ μ L) and mixed well by vortexing.

Capillary Electrophoresis

The effect of size selection was evaluated by CE using a home-built system equipped with a 488 nm laser for laser-induced-fluorescence (LIF) detection, with operation conditions shown in Supporting Information.³² The sieving matrix of 5% PVP (average Mw 1,300,000) was prepared in $1 \times$ TBE buffer and contained 4 M urea and $2 \times$ SYBR Gold dye. Prior to use, the fused-silica capillary (75- μ m i.d., 365- μ m o.d., Polymicro Technologies, Phoenix, AZ) with a total length of 55 cm and an effective length of 40 cm was rinsed sequentially with 0.1 M HCl, deionized water, methanol, and the sieving matrix. Each sample was spiked with a 15 nt DNA oligo as the internal standard (IS), and injected at -10 kV for 15 s. The same voltage was also used for separation.

Figure S1. Parallel handling of SMPs in multiple channels on the M&M chip. (A) Picture of the syringe pump-based handling stage (1-top view and 2-lateral view). (B) Pictures showing the process of SMP manipulation. Series 1 shows the movement of SMP-1 from being split from the main droplet in the wells, to collected and retained. Series 2 represents the process of SMP-2 moving from the wash wells to the elution wells.

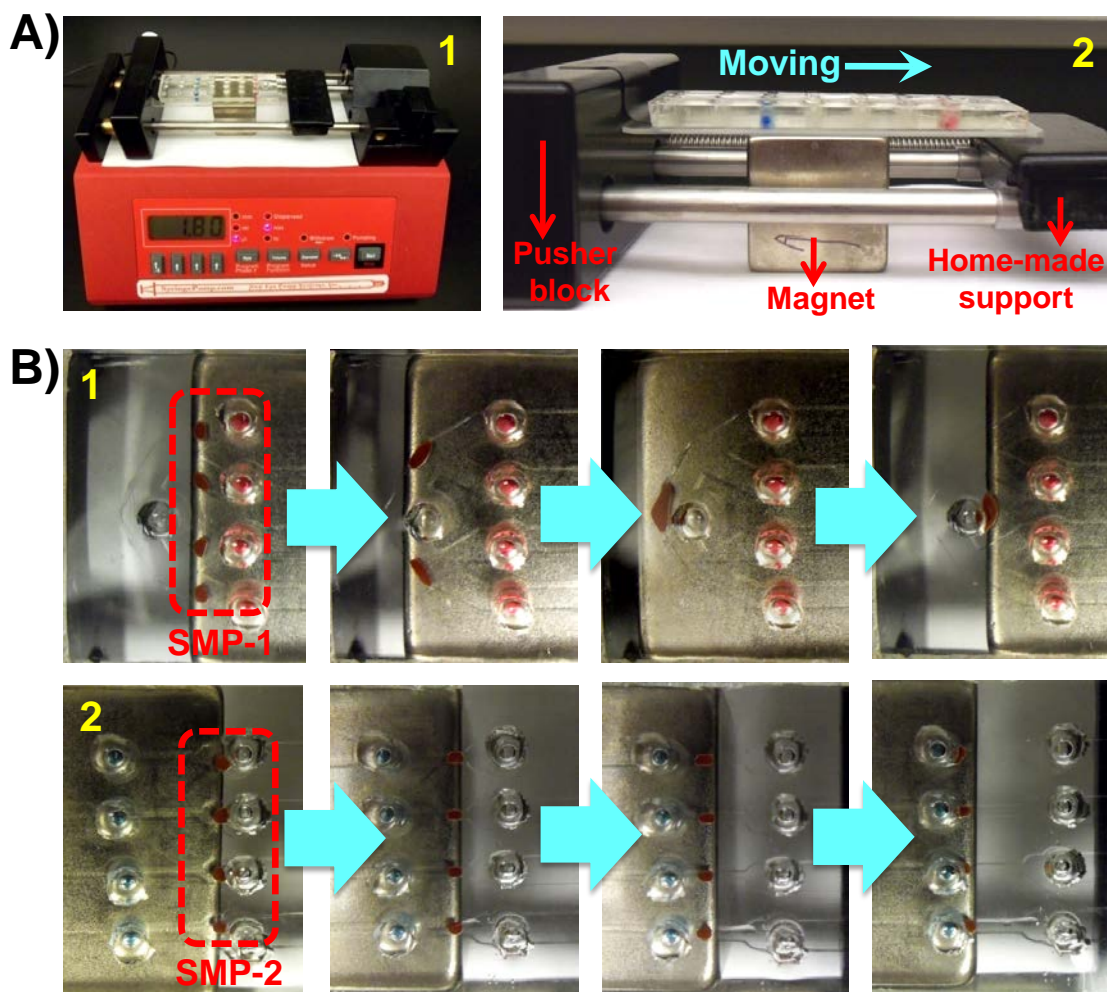


Figure S2. Assessment of inter- and intra-chip extraction reproducibility. In these experiments, 10^8 copies of hsa-mir-191 was spiked into 2.5 μL of *E.coli* cell lysate and extracted by the on-chip SPE. About 1% of the eluent was amplified by the TaqMan real-time RT-qPCR kit. Three repeats were performed on the same chip for inter-chip reproducibility evaluation or on the chips fabricated from the same master for intra-chip reproducibility assessment. The Ct values obtained from qPCR were shown in the plot.

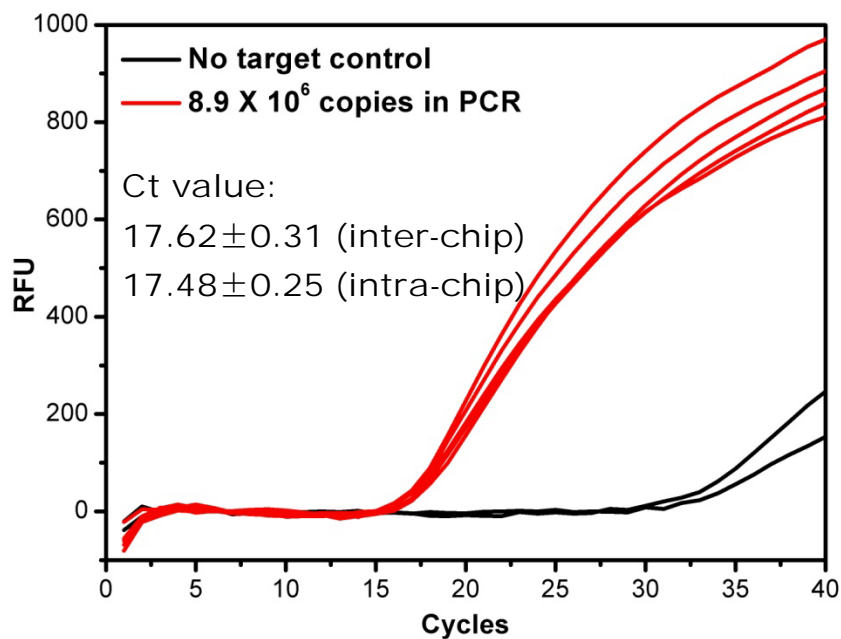


Figure S3. Detection limit of CE-LIF analysis for 63-nt DNA oligos. CE conditions: separation matrix 5% PVP with 4 M urea and 2×SYBR Gold dye in 1×TBE buffer; capillary 75 μm id with 55-cm total length and 40-cm effective length; injection -182 V/ cm for 15 sec, separation -182 V/ cm. Before CE runs, each sample was spiked with a 15-nt oligo as an internal standard (IS). (A) Separation of 63-nt and 15- nt oligos, indicating a detectable 63-nt oligo concentration of 0.25 nM. (B) Calibration curve of 63-nt oligo concentration versus normalized fluorescence intensity, showing good linearity in the concentration range of 0.25 nM-1 nM.

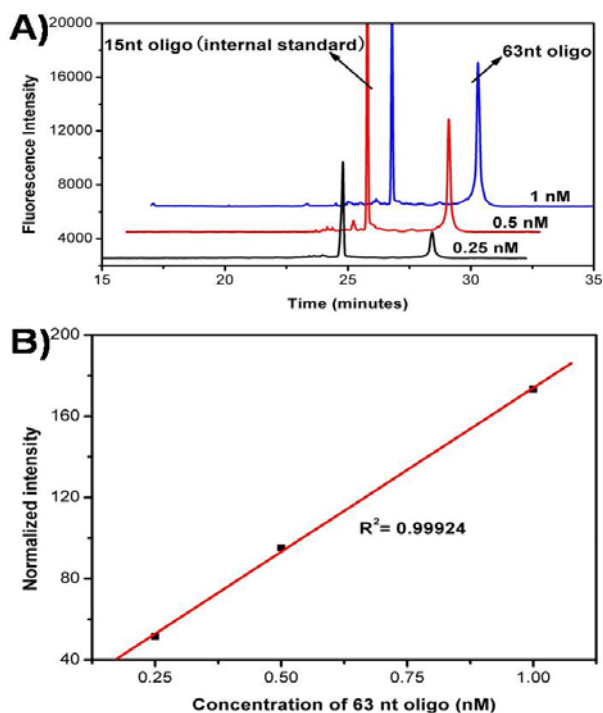


Figure S4. Comparison of on-chip and in-tube miRNA RT reactions. In these experiments, 10^6 copies of hsa-mir-191 were spiked into 2.5 μL of E.coli cell lysate matrix to perform on-chip SPE and the purified miRNAs were eluted into 0.915 μL of RNase-free water. After adding 0.585 μL of RT pre-mix to each eluent to run on-chip or in-tube RT reactions (1.5 μL volume), the cDNAs were amplified by adding 6 μL of TaqMan qPCR pre-mix. Each experiment was repeated at least twice and error bars represent standard deviations. On-chip RT reaction shows a lower Ct value than that of the in-tube RT reaction, indicating more efficient small-volume RT reactions on the M&M chip.

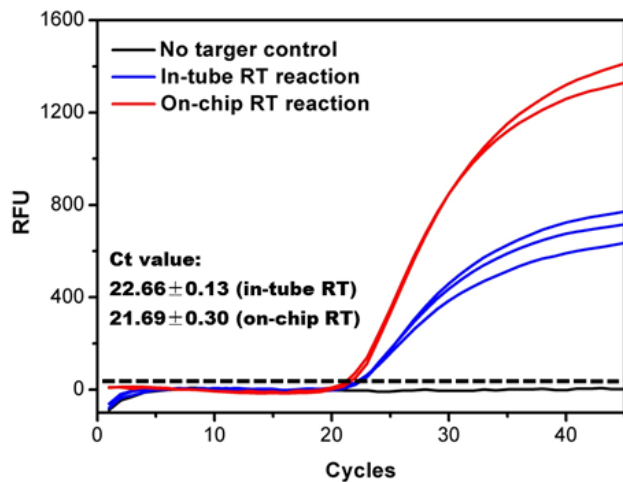


Figure S5. Real-time PCR profile for the calibration curve shown in Figure 5B in the main text.

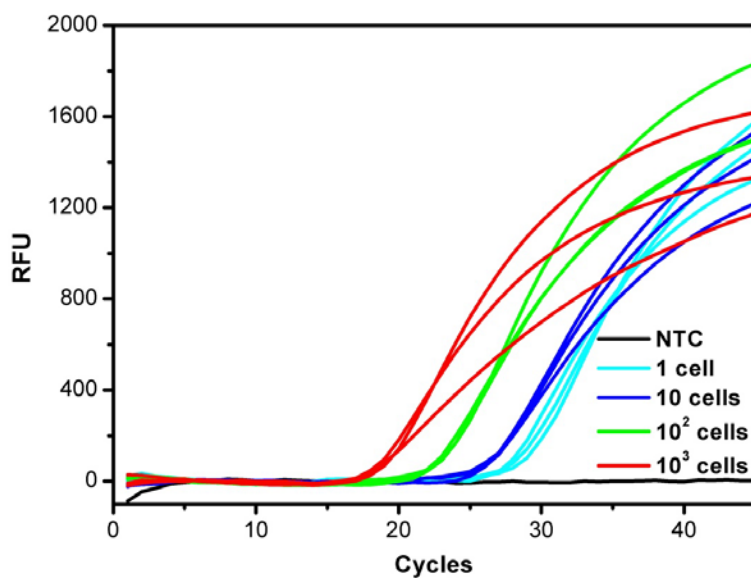


Figure S6. The M&M chip-based thermal cycling profile. The temperature control was performed on a PCR machine with a 0.4 mm-thick brass plate placed under the M&M chip. A calibrated thermocouple (0.076-mm diameter) was inserted into the center of a droplet within the reaction well to measure the real temperature.

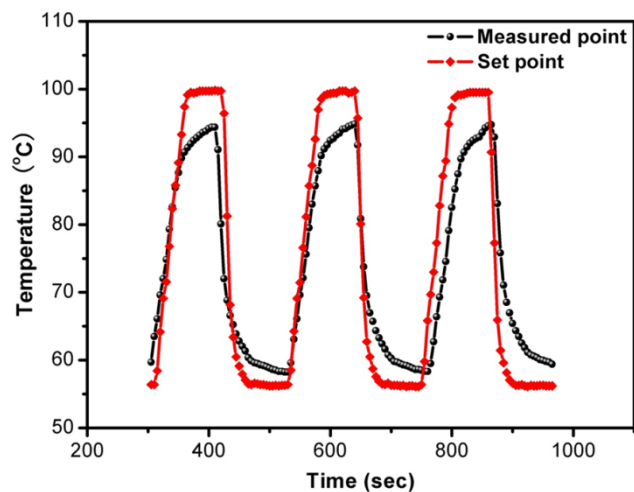


Figure S7 On-chip PCR for detection of standard miRNA solutions.

