

sure demonstrates the separation and extraction of small RNAs from precursor miRNA (pre-miRNA, ~70 nt) and other ≥ 63 nt RNA molecules using the M2 chip-based protocol. The method is based on the selective binding of RNA molecules of different sizes to the SMPs when different ethanol concentrations are present in the binding solution: under low ethanol concentration (35%), large DNA/RNA molecules preferentially bind to the SMPs; at high ethanol concentration (70%), small RNAs can efficiently bind to the SMPs. Incorporating a SMP-trapping structure in the chip design (FIG. 1A), a magnetic droplet based, two-binding SPE procedure was used to perform on-chip miRNA purification (see Materials and Methods Section and FIG. 3). The integrated SMP-trapping structure was used to collect the waste SMP-1 and separate the SMP-1 and SMP-2 to prevent the interference with each other during operation. If desired, the large DNA/RNA species adsorbed on SMP-1 can also be eluted and analyzed.

[0072] A CE-LIF (laser induced fluorescence) method was applied to demonstrate the M2 chip-based selective binding and extraction of small RNAs. A 63 nt oligo (mixed base A, C, G), physically mimicking pre-miRNAs but with less susceptibility to RNase degradation, was used as a size marker. Experiments show that the CE-LIF method can detect as low as 0.25 nM of 63 nt oligo (FIG. 7, ESI†). With this sensitivity, CE-LIF method was used to analyze the isolated large DNAs/RNAs (eluted from SMP-1) and small RNAs (eluted from SMP-2). CE conditions were the same as in FIG. 7, except that separation field for RNA ladder was 364 V/cm. In these experiments, mir-191 (108 copies) was spiked into 0.7 μ L of 63 nt oligo (1 μ M) or 0.1-2 kb RNA ladder (80 ng/ μ L), and the CE results can be seen in FIG. 4A and FIG. 4B, respectively. FIG. 4C was the results of real-time RT-qPCR from eluted and collected miRNAs. These results show that after M2 chip-based purification process, large DNA/RNA molecules can only be detected in recovered fractions eluted from SMP-1, and are undetectable from recovered fractions eluted from SMP-2. Re-isolated mir-191 in different samples can be amplified by RT-qPCR and show similar cycle threshold (Ct) values. For SMP-1/sample mixing procedure, on-chip mixing and in-tube vortexing shows no significant difference. These experiments demonstrate the capability of the presented M2 chip-based protocol for miRNA selective binding and high-purity extraction from 63 nt RNA molecules, which may inhibit expression analysis of miRNA molecules.

[0073] Performance for miRNA purification. The performance of the M2 chip platform was demonstrated in FIG. 5. In these experiments, hsa-mir-191 was spiked into 2.5 μ L of *E. coli* cell lysate matrix with copy numbers of 105, 106, 107, 108, and the eluents were amplified by using TaqMan microRNA real-time RT-qPCR method. Pipette collected eluents (2.5 μ L) were added to 5 μ L of RT pre-mix to perform RT reaction; then 0.67 μ L of RT product was mixed with 9.33 μ L of PCR pre-mix to run real-time qPCR. Data represent three independent experiments and error bars represent standard deviations. FIG. 5A is the representative amplification profiles. Amplification efficiency (101.2%, $R^2 > 0.998$) was calculated from the copy numbers in PCR and the Ct values (FIG. 5B). Recovery (20%-30%) of different miRNA input (FIG. 5C) was determined according to a calibration curve. Reproducibility of intership-(Ct=17.62 \pm 0.31, n=4) and intrachip-(Ct=17.48 \pm 0.25, n=4) miRNA SPE was measured by using 10^8 copies of mir-191 input (FIG. 5D). The results demonstrate efficient and reproducible purification of high-

quality miRNAs from cell lysate matrix using the M2 chip platform. Reliable performance and linearity in downstream RT-qPCR of the purified miRNA can be obtained.

[0074] MicroRNA analysis from cell lysate. The performance of the M2 chip-based SPE and RT reaction of miRNA from cell lysate samples were tested and compared with a commercial kit. In these experiments, around 10^6 cells were resuspended in lysis/binding (L3) buffer, mixed well and then diluted to a concentration of 80 cell/ μ L using L3 buffer. For M2 chip-based method, 2.5 μ L of cell lysate was loaded and finally eluted into 0.915 μ L of RNase-free water for on-chip RT reaction. Using commercial spin columns, 100 μ L of cell lysate was required for sample load and finally eluted into 100 μ L of RNase-free water, from which 0.915 μ L of eluent was injected into the control well of the M2 chip for RT reaction. The control wells can be utilized to perform negative and/or positive controls, or to obtain a calibration curve by running the standard serial dilutions. After addition of 0.585 μ L of RT pre-mix to the eluted miRNAs, on-line RT reaction was run and the RT products were collected by pipette to perform real-time qPCR (7.5 μ L reaction). FIG. 6A shows very close Ct values between chip-based and kit-based method from lysates of two cell lines, Jurkat and 293T; each data was repeated at least three times. The results indicated the comparable performance of the M2 chip and the commercial kit for purification of miRNAs from cell lysate samples, but the M2 chip-based method required only a small fraction (1:40) of the sample volume used by spin column procedure. Furthermore, it was feasible to integrate SPE and RT reaction on the M2 chip, enabling on-line transcription of unstable miRNA to relatively stable cDNA to minimize experimental loss, bias, or variations for downstream applications. The results also demonstrate a higher expression level of hsa-mir-191 in 293T cell line than that in Jurkat cell line (FIG. 9).

[0075] Sensitivity of cell numbers was measured by purifying a serial dilutions of 293T cell lysate (200, 40, 8, 1.6 cells input) via M2 chip and performing on-line RT reaction and off-line real-time qPCR detection. Amplification profiles and the calibration curve ($R^2 > 0.98$) can be seen in FIG. 6B, which indicates a single cell level of mir-191 detection from 293T cell lysate.

[0076] To improve the sample processing throughput of the current M2 chip platform, there are several options available: using the same chip size with a more compact design, using a larger chip size with the same design scheme, or operating several chips simultaneously on a multichannel syringe pump. The last one may provide more flexibility in different applications. For further performance improvement of the M2 chip, the possibility of integrating PCR (FIG. 10(A)) and SPE+ RT-PCR (FIG. 10(B)) was tested on the M2 chip platform for miRNA analysis. Although the preliminary results seem not comparable with the real-time qPCR results, which may be attributed to the inefficient temperature control of M2 chip as well as the PCR product end-point detection method, further developments with micro temperature controller and real-time fluorescence detector are expected to enable the M2 chip platform as a fully integrated microdevice for parallel miRNA purification, amplification and detection/quantitation.

[0077] A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the description. Accordingly, other embodiments are within the scope of the following claims.