

BRIEF DESCRIPTION OF THE FIGURES

[0019] FIG. 1A-E shows a microfluidic chip of the disclosure, a method of design and fabrication. (A) Shows a layout of the microfluidic chip design, which, in this embodiment, contains four parallel channels (each including seven wells for residing of different aqueous droplets). Exemplary dimensions are listed in the figure. (B) Shows a picture of Master-3# (see FIG. 1C(3)), used for one-step PDMS replication. (C) Shows a schematic flow of a microfluidic chip fabrication: (1) Master-1#, made, in this embodiment, from NOA81 and containing only lower channel structures; (2) Master-2#, punched PDMS substrate that was replicated from Master-1#; (3) Master-3#, molded from Master-2# by one-time curing process; (4) microfluidic chip, with PDMS substrate replicated from Master-3# incorporating pre-formed holes. (D-E) Shows a schematic depiction of a device/system of the disclosure.

[0020] FIG. 2A-B shows parallel handling of multiple superparamagnetic particles (SMP) droplets on a microfluidic chip platform. (A) Picture of syringe pump-based handling stage (1) and lateral view to show relative positions (2). (B) Pictures showing the process of SMP droplets manipulation. Pictures 11)~14) show the process of SMP-1 droplets splitting, collection and retaining. Pictures 21)~24) represent the process of SMP-2 droplets moving from wash wells to elution wells.

[0021] FIG. 3A-D shows a scheme of microfluidic chip-based miRNA purification. Prior to use, the microfluidic chip was primed with mineral oil and buffers/reagents at designated wells with multichannel pipette. (A) SMP-1 droplets containing the adsorbed large DNA/RNA molecules was split from the sample droplet and finally collected and trapped in the collection well; (B) A suspension of SMP-2 in ethanol was added into the sample droplet to give a solution of 70% ethanol, facilitating adsorption of small RNAs to the SMP-2; (C) SMP-2 droplets containing the adsorbed small RNAs passed through three W5 buffer droplets, then the adsorbed small RNA molecules were eluted to Sigma water droplets; (D) Reaction pre-mix was added to the eluted sample to perform on-line reaction (such as RT reaction).

[0022] FIG. 4A-C shows microfluidic chip-based small RNA selective binding and extraction. In these experiments, mir-191 (10^8 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 (A) and (B), respectively. (C) is the real-time RT-qPCR results from eluted and collected miRNAs. CE conditions were the same as in FIG. 7 except that separation field for RNA ladder was 364 V/cm.

[0023] FIG. 5A-D shows the performance of a microfluidic chip for miRNA purification. In these experiments, hsa-mir-191 was spiked into 2.5 μ l of *E. coli* cell lysate matrix with copy numbers of 10^5 , 10^6 , 10^7 , 10^8 , 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. (A) Representative amplification profiles. (B) Calibration curve using the data from (A). Amplification efficiency (101.2%, $R^2 > 0.998$) was calculated from the copy numbers in PCR and the Ct values. (C) Recovery (20%-30%) of different miRNA input. (D) Reproducibility of interchip ($Ct = 17.62 \pm 0.31$, $n = 4$) and intra-

chip ($Ct = 17.48 \pm 0.25$, $n = 4$) miRNA SPE was measured by using 10^8 copies of mir-191 input.

[0024] FIG. 6A-B shows microfluidic chip-based miRNA analysis from cell lysate. For microfluidic chip-based method, 2.5 μ l of cell lysate (80 cell/ μ l) 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 microfluidic chip for RT reaction. (A) shows very close Ct values between chip-based and kit-based methods from lysates of two cell lines, Jurkat and 293T; each data was repeated at least three times. (B) Amplification profiles and the calibration curve ($R^2 > 0.98$) for sensitivity test. Sensitivity of cell numbers was measured by purifying a serial dilutions of 293T cell lysate (200, 40, 8, 1.6 cells input) via microfluidic chip and performing on-line RT reaction and off-line real-time qPCR detection.

[0025] FIG. 7A-B shows CE-LIF analysis of 63 nt DNA oligos. CE conditions: separation matrix 5% PVP with 4 M urea and 2xSYBR Gold dye in 1xTBE buffer; capillary 75 μ m id with 55/40 cm 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 to 1 nM.

[0026] FIG. 8A-B shows a calibration curve for real-time PCR quantitation. In these experiments, hsa-mir-191 was spiked into 2.5 μ l of *E. coli* cell lysate matrix with a copy number of 10^9 and the eluent were subjected to RT reaction. Serial 10-fold dilutions of the RT product were used as standards to perform real-time qPCR. Pipette collected eluent (2.5 μ l) was added to 5 μ l of RT pre-mix to perform RT reaction; then 0.67 μ l of diluted RT product was mixed with 9.33 μ l of PCR pre-mix to run real-time qPCR. (A) Representative amplification profiles. (B) Calibration curve using the data from (A). Amplification efficiency (102.2%, $R^2 > 0.998$) was calculated from the copy numbers in PCR and the Ct values.

[0027] FIG. 9 shows expression levels of hsa-mir-191 in two cell lines. Data was from FIG. 6A. The results demonstrate a higher expression level of hsa-mir-191 in 293T cell line than that in Jurkat cell line.

[0028] FIG. 10A-B shows results upon integrating PCR on the microfluidic chip platform. (A) Microfluidic chip-based PCR. In these experiments, 1.5 μ l of serial 10-fold dilutions of RT product with copy numbers of 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , was mixed with 6 μ l of PCR pre-mix to run thermal cycling (28 cycles) on regular PCR machine. PCR product detection was performed by on-line imaging. Each concentration was repeated once and error bars represent standard deviation. (B) Microfluidic chip-based SPE+ RT-PCR. In these experiments, four samples (two blanks and two cell lysate samples) run on-line SPE+ RT-PCR, and the control wells run on-chip PCR with 3 standard concentrations (copy numbers of 10^4 , 10^5 , 10^6) used to estimate the concentration range of the target in sample.

DETAILED DESCRIPTION

[0029] As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents