

durations of the thermal cyclers to fit the actual temperature profiles in the droplets, a 0.076 mm-diameter T-type thermocouple (5SC-TT-T-40-36, OMEGA) was inserted into the center of the reaction well. M2 chip fluorescence images were taken by using a Typhoon Image Scanner (GE healthcare) with the following settings: 488 nm laser excitation, 530SP emission filter, 530V PMT gain and normal sensitivity. Image processing was done by using ImageQuant (GE healthcare) or ImageJ (NIH) software.

**[0066]** Capillary Electrophoresis (CE) analysis. A home-built CE system equipped with a 488 nm laser (Lei R., *Anal. Chem.* 2009, 81, 5510-5516) was used for the separation and detection of miRNA and size markers. An established CE condition (Ni L., *Anal. Chem.* 2009, 81, 4906-4913) was utilized with some modifications: separation matrix 5% Polyvinylpyrrolidone (PVP, average Mw 1,300,000; from Fisher Scientific) with 4 M urea and 2×SYBR Gold dye (Invitrogen) in 1×TBE buffer. Prior to use, the 55 cm fused-silica capillary (75 μm i.d., 365 μm o.d., Polymicro Technologies, Phoenix, Ariz.) with an effective length of 40 cm was rinsed sequentially with 0.1 M HCl, deionized water, methanol, and the sieving matrix using syringes and a hand pump. Before CE runs, each sample was spiked with a 15 nt DNA oligo as an internal standard (IS). Sample injection was carried out at -10 kV for 15 s, and the separation voltage was -10 kV.

**[0067]** The target miRNA, 15 nt and 63 nt oligos (consisted of only A, C and G bases, used as internal standard and size marker, respectively) were from Integrated DNA Technologies (Coralville, Iowa). The 0.1-2 kb RNA ladder was purchased from Invitrogen (Carlsbad, Calif.). The target strand, hsa-mir-191 was identified as a potential target for Hepatocellular Carcinoma (HCC) therapy and was found to be a regulator of a variety of cancer-related pathways. This miRNA was found to be highly consistent in its expression across 13 normal tissues and five pair of distinct tumor/normal adjacent tissues and was statistically superior to the most commonly used reference RNAs used in miRNA qRT-PCR experiments.

**[0068]** Rapid prototyping of M2 chip. The M2 chip was designed to process multiple samples simultaneously. In this work, the chip contained four parallel channels, each including seven wells for loading of different aqueous droplets (FIG. 1A), enabling purification and reaction of four miRNA samples in parallel. By using regular PDMS molding method to fabricate the PDMS/glass chip, 29 holes needed to be punched each time after PDMS replication, which was tedious and difficult to ensure the consistency of the hole positions and shapes. To simplify and speed up the fabrication process, a rapid prototyping technique was used to make the molding master for convenient PDMS replication with an inexpensive, UV-curable adhesive, NOA 81. This thiolene-based optical adhesive functions as a negative resist in conventional lithography with faster curing rate (down to seconds) and higher feature structures (up to millimeters, see FIG. 1B). After curing, it has very good adhesion to glass, but does not bind to cured PDMS (due to the oxygen dissolved in PDMS that inhibits NOA81 polymerization) [Lab Chip, 2008, 8, 492-494], which enables the open-faced methodology (FIG. 1C-1) [J. Micromech. Microeng. 14 (2004) 153-158] and is fully compatible with PDMS replication techniques. It was feasible to use NOA81 to mold PDMS substrate (FIG. 1C-2) or to be molded by PDMS master (FIG. 1C-3). The three-dimensional Master-3# (containing 360 μm-height channels and 4.6 mm-height pillars) was formed by one time

UV exposure (FIG. 1C-3) and was utilized to make consistent M2 chip by one-step PDMS replication (FIG. 1C-4); no further punching procedure was required. Fabrication of such high structures by conventional lithography would be both challenging and time consuming, requiring expensive masks or deep etching techniques. To control the height of the PDMS on Master-3#, a certain amount of PDMS prepolymer was used, or a negative pressure was employed to remove the extra PDMS. The Master-3# was mechanically stable and reusable, thus reducing the fabrication cost and turnaround.

**[0069]** Parallel handling of multiple SMP droplets. The M2 chip was designed to operate using only regular laboratory equipment (such as pipettes, magnet, syringe pump and PCR machine), without the need for any kind of microvalves/pumps or external connections. The presented M2 chip consisted of four parallel sample processing units and a SMP-trapping structure (FIG. 1A). Each processing unit included seven wells in a linear configuration connected by microchannels with different widths, and can be used to complete the whole process of miRNA purification and reaction. The seven wells in one unit, for residing different aqueous droplets, were arranged according to the pattern of the standardized 96-well microtiter plates, ensuring compatibility with existing high-throughput laboratory infrastructure (e.g. multichannel pipettes, liquid handling robots, PCR machine). Parallel units can be simultaneously processed by moving the M2 chip using a syringe pump with a magnet underneath the microchip holding the SMPs at the edge area of the magnet (FIG. 2A), enabling simultaneous purification of four miRNA samples. It was easy and convenient to assemble and operate the syringe pump-based handling stage. An automatic purification protocol could be obtained by programming the direction and speed of the pump.

**[0070]** Upon M2 chip priming with mineral oil and reagents, all the droplets were submerged inside the oil, and the whole process was carried out in an oil medium, making the SMP droplet movement easier due to the shorter contact line, and thus the decreased friction between the droplet and the surface. Moreover, a thick layer of mineral oil functioned as a good evaporation barrier during operation. By moving the M2 chip with the syringe pump, different droplets in each processing unit were confined within interconnected wells because of high surface tension and friction. The SMPs, on the other hand, were pulled by the magnetic force and retained at the area of large magnetic field gradient. As a result, the large droplet containing the SMPs elongated, forming regions with and without SMPs. In the end, a small droplet containing SMPs was split from the parent droplet (FIG. 2B) and moved towards the neighboring droplet, finally merged with it, triggering dilution of impurities adsorbed on the SMPs. The wide channels between the sample well and the wash wells facilitated the handling of SMPs, while the narrow channels connecting the wash well and elution well enabled a small critical volume (~0.8 μL), making it possible to incorporate miRNA two-step RT-PCR with SPE on M2 chip.

**[0071]** Small RNA selective binding and extraction. For miRNA purification, it is extremely important to enrich small RNA molecules, such as microRNAs (miRNAs) and short interfering RNAs (siRNAs), in a separate fraction and remove larger RNA and genomic DNA molecules to minimize background on downstream applications. Currently, commercial kits usually employ two-spin column process to purify small RNA species from biological samples and only allow the separation of small RNAs from >200 nt RNAs. The disclo-