Supplementary Material (ESI) for Lab on a Chip

Nanoliter Droplet Array for Real Time Reverse Transcription Polymerase Chain Reaction

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Cell cuture and RNA isolation.

The sequence of mature mir-122 was selected from the Sanger Center miRBase at http://microrna.sanger.ac.uk/sequences. Synthetic mature microRNA oligonucleotides were obtained from Shanghai GenePharma (Shanghai, China). Five cancer cell line including MCF-7, HepG-2, HL-60 and Hela kindly presented by Ms. Fengping Shen at Zhejiang University and Huh-7 purchased from CCTCC (Wuhan, China) were cultured in RPM1640 (for MCF-7, HepG-2 and HL-60) and DMEM (for Huh-7 and HeLa) (Hangzhou Jinuo Bio-pharmaceutical Tech. Co., Ltd., China), respectively. Total RNA was extracted using TRNzol (Tiangen, Beijing, China) according to the manufacturer's protocol and the concentration of total RNA was quantified by the value of A260 on a SP-752TM UV-Vis spectrophotometer (Spectrum Shanghai, China)

Data processing methodology.

For data analysis, the fluorescence intensity of each droplet in the image captured by CCD camera was read out using a program written in Labview and translated into amplification plot using Excel. The threshold for quantification was defined as 10-fold of the standard deviation (SD) value of the PCR plot baseline (usually of the first 10 cycles) in this work, and the cycle number at which the increment of fluorescent signal (Relative fluorescence intensity/ΔRn) became greater than the threshold was defined as Ct. The amplification plot showed the correlation of RNA input per droplet to the Ct value.

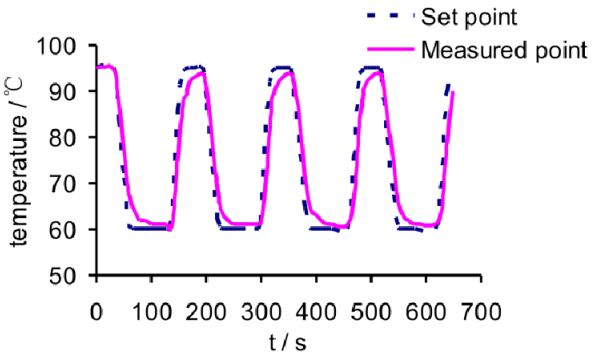

Fig. S1. The temperature set by the commercial thermal cycler and measured on the surface of the silicon chip, respectively.

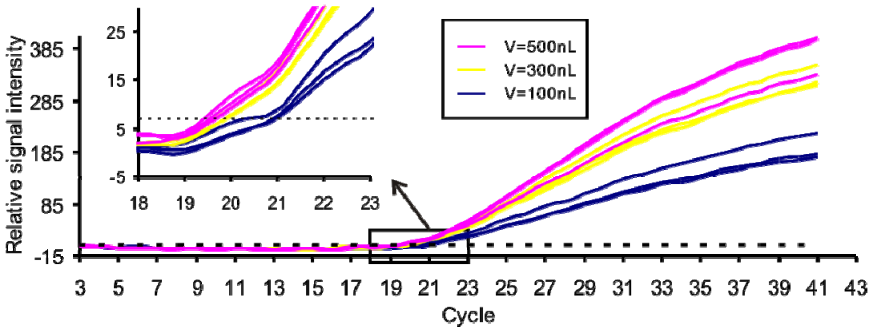

Fig. S2. The effect of droplet size on RT-PCR. The PCR solution was mixed in tube, and dispensed as droplets with 500 nL, 300 nL and 100 nL, respectively. With the same concentration of mir-122, the mean Ct value was 19.43, 19.75 and 20.67 respectively.

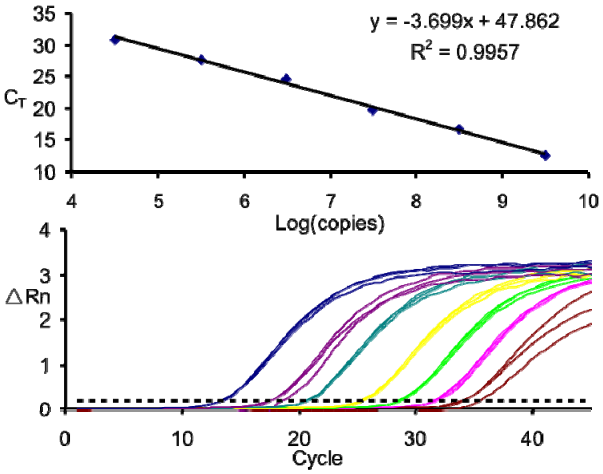

Fig. S3. Real-time RT-PCR assay of synthetic mir-122 with ABI 7900 HT. (a) the standard curve of miRNA. The input of mir-122 is 3.2×10^{9}, 3.2×10^{8}, 3.2×10^{7}, 3.2×10^{6}, 3.2×10^{5}, 3.2×10^{4}, and 0 copies/tube from left to right in the amplification plot (b).