SINGLE CELL MICRORNA QUANTIFICATION WITH TWO STEP RT-QPCR BASED ON FLEXIBLE NANOLITER-SCALE DROPLET ARRAY SYSTEM

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ABSTRACT

MicroRNAs have attracted increasing attention in recently years due to their roles in regulation gene expression and associations with many human diseases, such as cancers and heart disease. In this study, we presented a droplet-based microfluidic system for quantifying microRNA in single cell level with two-step real-time quantitative RT-PCR (RT-qPCR). All the steps of single cell processing, including droplet generation and single cell encapsulation, cell lysis, reverse transcription, and real-time PCR, were fully integrated on the nanoliter-scale droplet array system. As a demonstration, we applied the system to quantify the expression of microRNA-122 in single Huh-7 cells.

KEYWORDS

Single cell analysis, RT-qPCR, Droplet microarray

INTRODUCTION

Recent studies indicated that microRNAs participate in many important cell processes, including early development, cell proliferation, as well as cell apoptosis. The expression level in cells can reveal many human diseases. The ability to measure the microRNA expression in single-cell level could help to diagnose human disease at its early stage. Various microfluidic systems have been successfully developed for single cell RNA quantification, such as valve-controlled PDMS chips [1] and droplet microfluidics [2]. Droplet-based microfluidics holds many advantages for single cell analysis, including ultra-small reaction volume, parallel assays, high throughput, and minimal cross-contamination. However, in most of droplet systems, it's difficult to perform multi-step and multiplex droplet manipulation. We have developed a flexible droplet manipulation system namely DropLab [3]. In this work, we further developed the DropLab system into two-dimensional droplet array form and applied it to single-cell microRNA quantification with RT-qPCR technique [4].

EXPERIMENT

The procedure for single cell RT-qPCR was illustrated in Figure 1. A 16×16 hydrophilic spots array patterned on a silicon chip was used to immobilize the droplets during the whole process. The microchip was fixed on an x-y-z translational stage to precisely control the position of droplet dispensing. The droplets containing single cells or reagents were generated with a tapered capillary combined with a high-precision syringe pump. Droplet containing 1 nL cell suspension, 19 nL RT mixture and 30 nL PCR mixture were sequentially dispensed before heat lysis, reverse transcription and PCR, respectively. For real-time detection, the fluorescence image during each cycle was recorded by a CCD camera, and the fluorescence intensity of each droplet was read out using a program written with Labview.

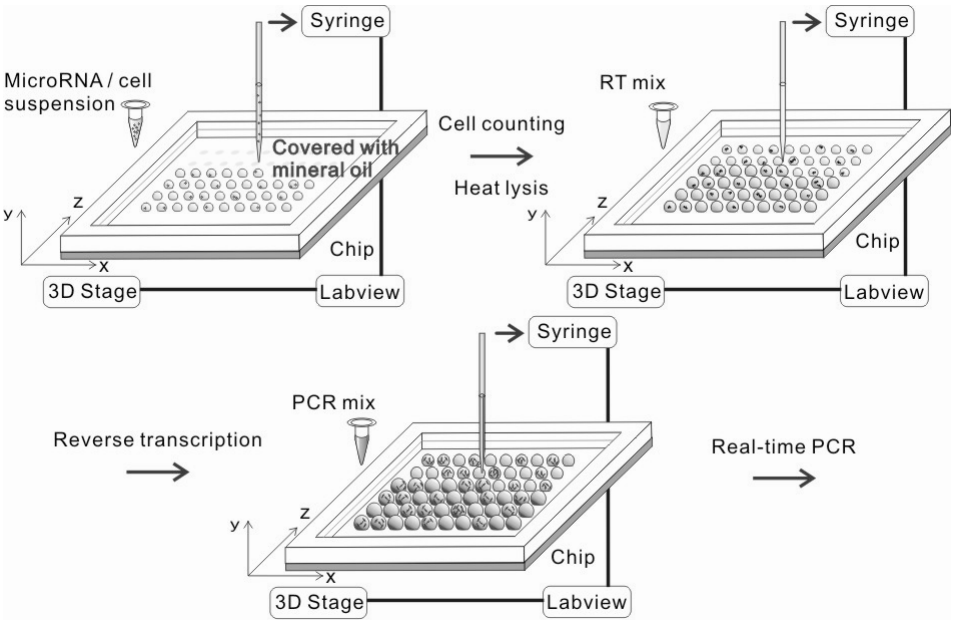

Figure 1. Schematic illustration of RT-qPCR process for single-cell microRNA quantification.

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RESULTS AND DISCUSSION

We applied this system to quantify microRNA in single cell level. The synthetic microRNA-122 was serially diluted over six orders (from 10^3 to 10^9) of magnitude to test the performance of real-time RT-qPCR. Excellent linear relationship between the log copy number of mir-122 input and threshold cycle (Ct) value was obtained (Figure 2). The low detect limit and large dynamic range proved the ability of the present system for microRNA quantification at single cell level.

The preliminary result of single cell assays was shown in Figure 3 and Figure 4. The mean CT value was 27.26 (SD=1.61), corresponding to the average of copy number per cell was 10122 (SD=5104). Clear difference of microRNA expression was observed among individual cells. The copy number of microRNA-122 in single Huh-7 cells was in the range of 3000-24000.

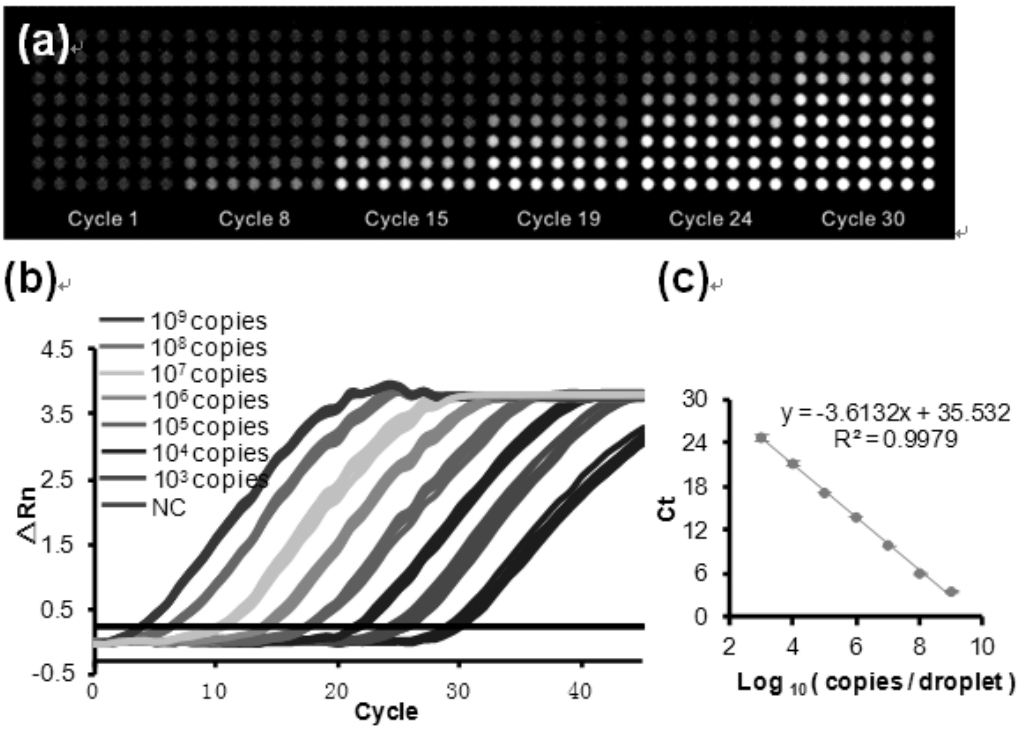

Figure 2. RT-qPCR assay of synthetic microRNA-122. (a) Fluorescence images of droplet array at different thermal cycles. (b) Amplification plot of mir-122 over six orders of magnitude. (c) Standard curve of mir-122.

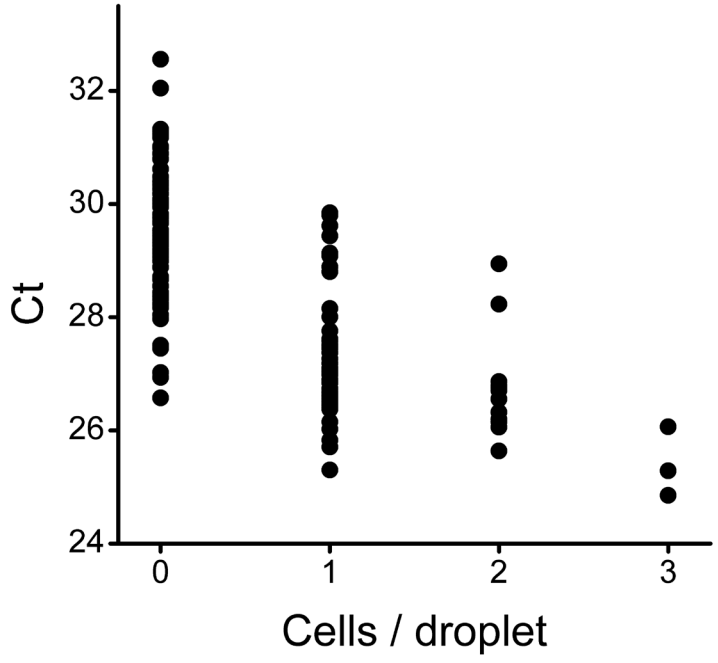

Figure 3. Scatter plot showing CT values from RT-qPCR measurement of mir-122 in Huh-7 cells.

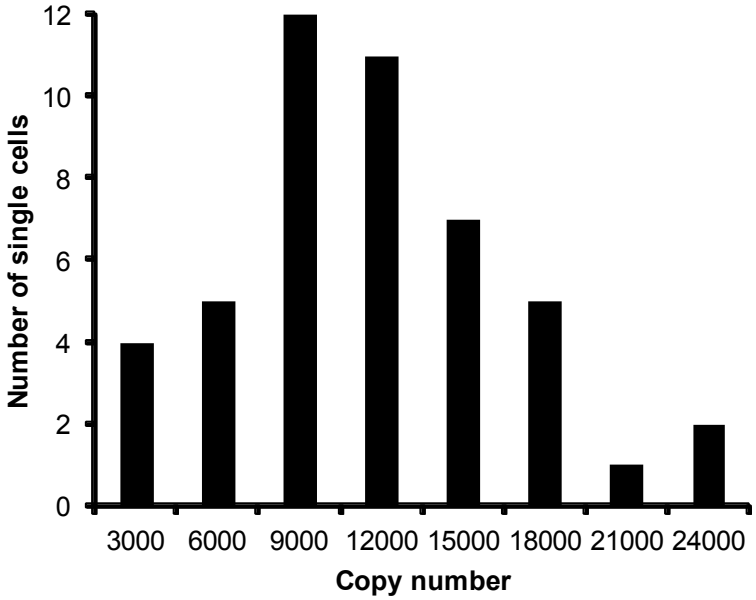

Figure 4. The copy number of mir-122 in single Huh-7 cells.

CONCLUTION

Here, we presented a nanoliter-scale droplet array system for single cell miRNA quantification with two-step real-time quantitative RT-PCR (RT-qPCR). With the flexible droplet manipulation ability, all steps of single cell processing, including cell encapsulation, cell lysis, reverse transcription and real-time PCR, were fully integrated. Single-cell measurement of microRNA-122 expression in Huh-7 cells was successfully performed on chip in 50nL droplet, and 256 parallel assays were achieved per run. The preliminary result showed great potential of this system in single cell gene analysis.

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