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Electronic Supplementary Information (ESI)

## Droplet-based PCR in a 3D-printed microfluidic chip for miRNA-21 detection

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**Fig. S1** The design details of 3D-printed chip. The length and width of the chip were 100 mm and 70 mm, respectively.



Fig. S2 The side view of the chip. (a) The design scheme. (b) The printed chip.



Fig. S3 The droplets were generating (a), collected (b) and counting (c) when the continuous phase flow rate was 20  $\mu$ L/min and the dispersed phase flow rate was 4  $\mu$ L/min. Scale bars were 500  $\mu$ m.



Fig. S4 The droplets were generating (a), collected (b) and counting (c) when the continuous phase flow rate was 21  $\mu$ L/min and the dispersed phase flow rate was 3  $\mu$ L/min. Scale bars were 500  $\mu$ m.



Fig. S5 The droplets were generating (a), collected (b) and counting (c) when the continuous phase flow rate was 22  $\mu$ L/min and the dispersed phase flow rate was 2  $\mu$ L/min. Scale bars were 500  $\mu$ m.



Fig. S6 The droplets were generating (a), collected (b) and counting (c) when the continuous phase flow rate was 23.5  $\mu$ L/min and the dispersed phase flow rate was 0.5  $\mu$ L/min. Scale bars were 500  $\mu$ m.



Fig. S7 The droplets were generating (a), collected (b) and counting (c) when the continuous phase flow rate was 23.75  $\mu$ L/min and the dispersed phase flow rate was 0.25  $\mu$ L/min. Scale bars were 500  $\mu$ m.



**Fig. S8** The variable coefficients of the actual temperatures with different setting temperatures.



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**g. S9** The optimization of the high temperature for miRNA-21 amplification in commercial thermal cycler when the low temperature was set as 60 °C.



**Fig. S10** The optimization of the low temperature for miRNA-21 amplification in commercial thermal cycler when the high temperature was set as 95 °C.