Electronic Supplementary Information

An Enzyme-Free Flow Cytometric Bead Assay for the Sensitive Detection of MicroRNAs Based on Click Nucleic Acid Ligation-Mediated Signal Amplification

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1. Optimization of the amount of probe A′, probe B′, and the number of thermal cycles

In this study, 0.2 μL of the original M-270 MBs were used for each reaction, which can theoretically capture ~0.4 pmols (40 nM in a 10 μL solution) biotinylated probe A according to the product instruction of M-270 MBs. So 40 nM (100% of the theoretical coverage rate of the used MBs) for both probe A, and probe B were chosen for miRNA analysis in this study. Under this condition, higher concentrations of probe A′ and B′ in homogeneous phase will lead to higher nonspecific amplification of CLCR. Meanwhile, the probability of random collisions between probes would also rise with the increase of thermal cycle number, leading to the increased background of CLCR. Therefore, we have simultaneously optimized the concentrations of the probe A′ and probe B′ along with different thermal cycle numbers towards the detection of let-7a miRNA target.

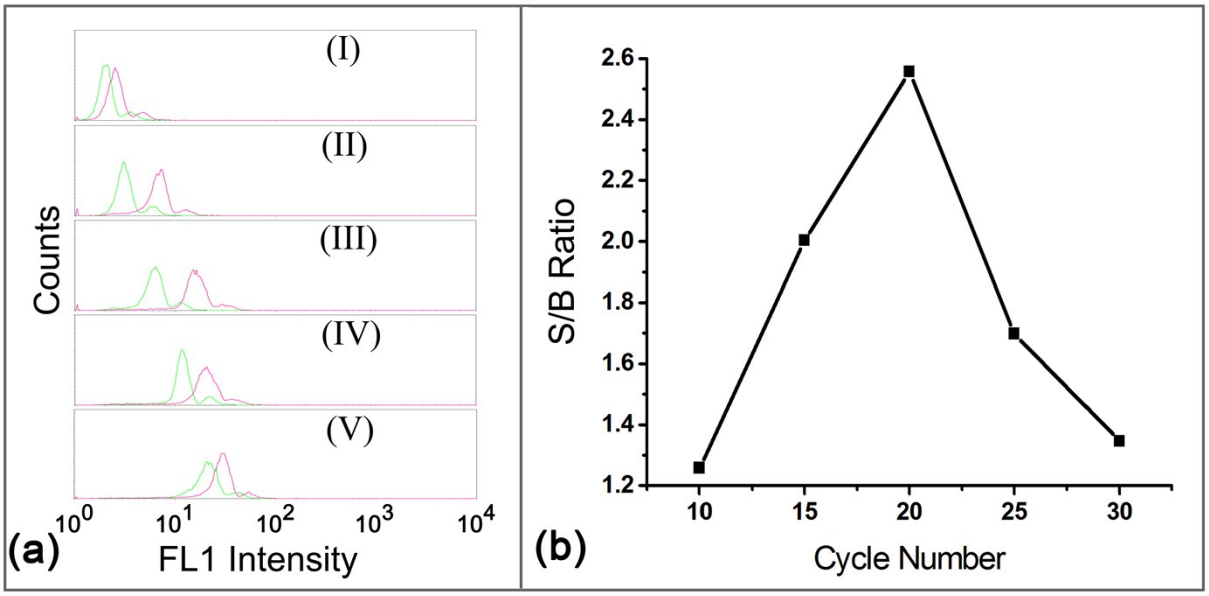

Fig. S1. (a) Effect of the thermal cycle number on the proposed FCBA strategy for let-7a analysis when the concentrations of probe A′ and probe B′ were both 40 nM. 200 pM of let-7a was used for this optimization (pink lines) in comparison with blank control without adding let-7a (green lines). The number of thermal cycle: (I) 10 cycle; (II) 15 cycle; (III) 20 cycle; (IV) 25 cycle; (V) 30 cycle; (b) The corresponding S/B ratios under different cycle numbers. S/B ratio refers to the ratio of the let-7a-produced MFI value to that of blank control. Other conditions: probe A, 40 nM; probe B, 40 nM; NaCl, 100 mM.

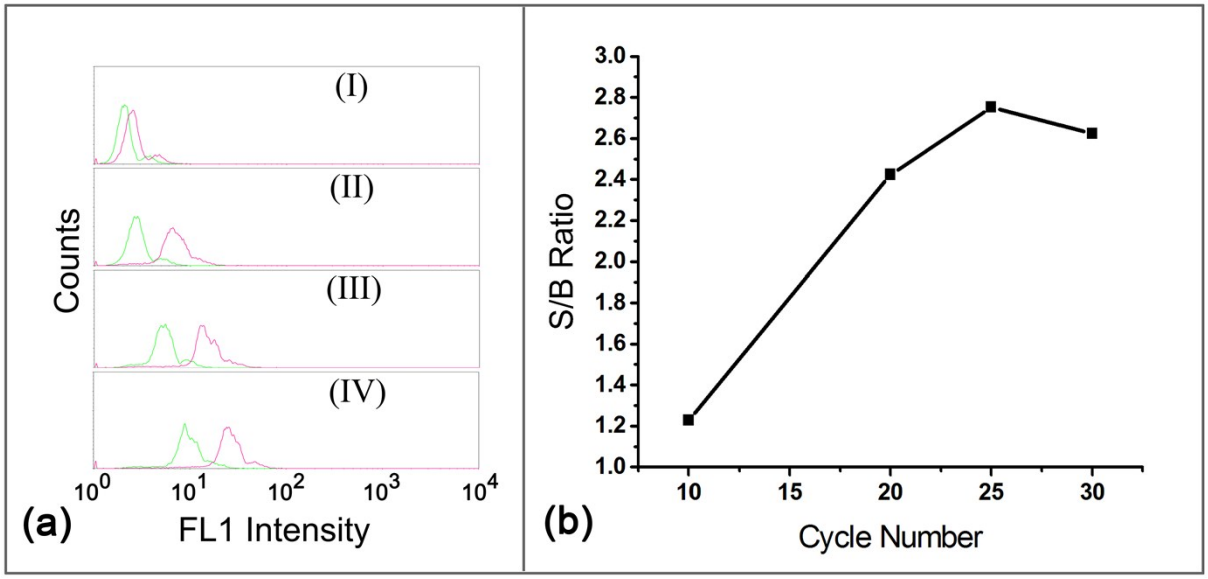

Fig. S2. (a) Effect of the thermal cycle number on the proposed FCBA strategy for let-7a analysis when the concentrations of probe A′ and probe B′ were both 20 nM. 200 pM of let-7a was used for this optimization (pink lines) in comparison with blank control without adding let-7a (green lines). The number of thermal cycle: (I) 10 cycle; (II) 20 cycle; (III) 25 cycle; (IV) 30 cycle; (b) The corresponding S/B ratios under different cycle numbers. S/B ratio refers to the ratio of the let-7a-produced MFI value to that of blank control. Other conditions: probe A, 40 nM; probe B, 40 nM; NaCl, 100 mM.

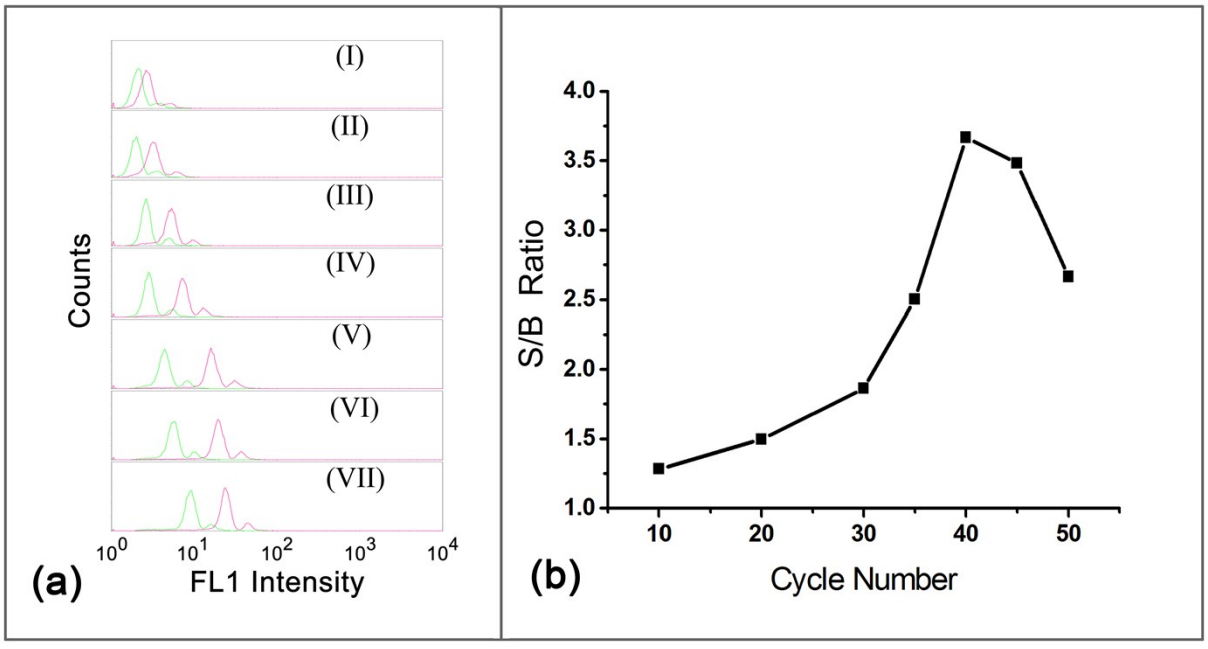

Fig. S3. (a) Effect of the thermal cycle number on the proposed FCBA strategy for let-7a analysis when the

concentrations of probe A′ and probe B′ were both 10 nM. 200 pM of let-7a was used for this optimization (pink lines) in comparison with blank control without adding let-7a (green lines). The number of thermal cycle: (I) 10 cycle; (II) 20 cycle; (III) 30 cycle; (IV) 35 cycle; (V) 40 cycle; (VI) 45 cycle; (VII) 50 cycle; (b) The corresponding S/B ratios under different cycle numbers. S/B ratio refers to the ratio of the let-7a-produced MFI value to that of blank control. Other conditions: probe A, 40 nM; probe B, 40 nM; NaCl, 100 mM.

We have optimized in detail the effect of thermal cycle numbers on the proposed miRNA assay at different concentrations of probe A′/probe B′. As can be seen from Fig. S1a, at a high concentration of probe A′/probe B′ (each 40 nM, identical to the dosage of probe A and probe B), the background signal of blank control increases more obviously than the 200 pM let-7a-produced signal as the cycle number increases from 10 to 40. This is probably because higher concentration of probe A′ and probe B′ will give rise to higher nonspecific amplification of CLCR along with the increased thermal cycle number. As shown in Fig. S1b, the ratio of let-7a-produced MFI value to that of blank (denoted as S/B ratio) reaches its maximum (2.56) at 20 cycles when the concentrations of probe A′ and probe B′ are both 40 nM. As can be seen from Fig. S2, when the concentrations of probe A′ and probe B′ are both fixed at 20 nM, the maximum S/B ratio reaches 2.75 at 25 cycles.

Fascinatingly, as can be seen from Fig. S3a, when the concentrations of probe A′ and probe B′ are both reduced to 10 nM, the fluorescence response produced by let-7a increases more obviously than the blank signal with the cycle number increased from 10 to 40. As the cycle number is further elevated from 40 to 50, the let-7a-aroused fluorescence signal increases slowly nevertheless the blank signal still keep increasing rapidly. As displayed in Fig. S3b, the highest value of S/B ratio (3.67) is obtained at 40 cycles when the concentration of probe A′ and probe B′ are 10 nM.

All of the above results suggest that the higher concentrations of probe A′/probe B′ along with the long cycles will lead to the high background signal and thus low detection sensitivity. One can see from Fig. S1 to S3 that the highest value of S/B ratio is obtained with 10 nM probe A′ and 10 nM probe B′ at 40 cycles. Therefore, taking into consideration of both the low background, low detection limit and the best discrimination between the target-produced signal and the blank control, 10 nM each of probe A′ and probe B′ and 40 thermal cycles are selected as the optimal conditions for miRNA detection in this work.

2. The effect of NaCl concentration on the CLCR reaction

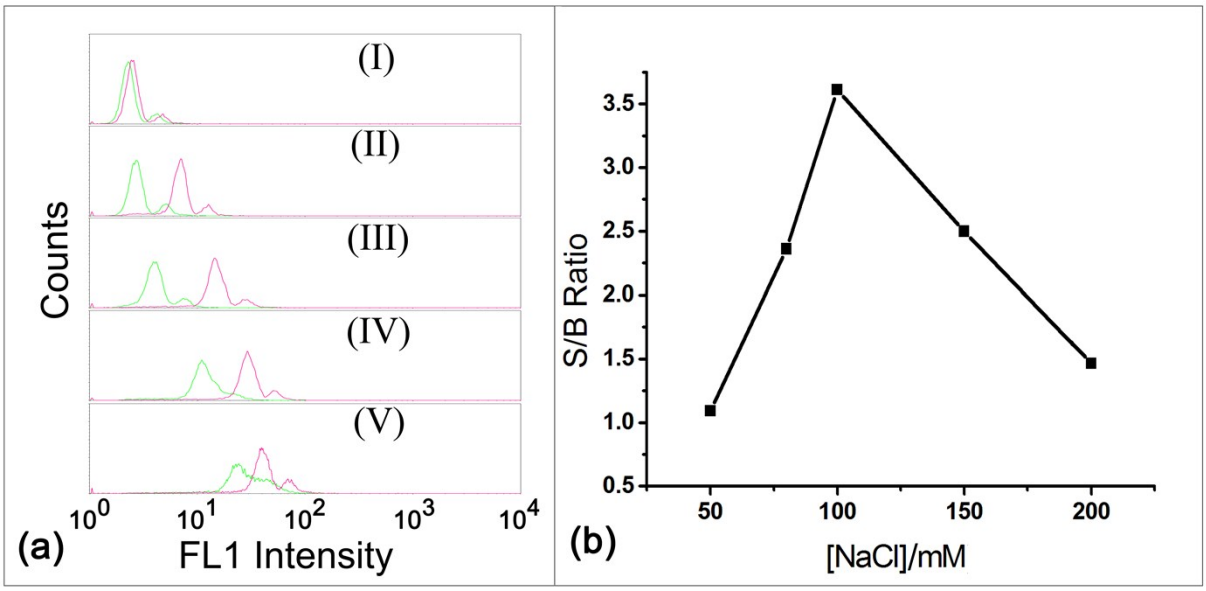

Fig. S4. (a) Effect of the NaCl concentration during the CLCR on the proposed FCBA strategy for the detection of let-7a. 200 pM of let-7a was used for this optimization (pink lines) in comparison with blank control (green lines). The concentration of NaCl: (I) 50 mM; (II) 80 mM; (III) 100 mM; (IV) 150 mM; (V) 200 mM; (b) The corresponding S/B ratios under different NaCl concentrations during the CLCR reaction. S/B ratio refers to the ratio of the let-7a-produced MFI value to that of blank control. Other conditions: the number of thermal cycle, 40; probe A, 40 nM; probe B, 40 nM; probe A′, 10 nM; probe B′, 10 nM.

In this study, the CLCR occurs based on the hybridization and ligation of several short ssDNAs. As is well known to us, the ionic strength will remarkably influence the hybridization reaction of short ssDNAs and thus the efficiency of CLCR. So we have also investigated the effect of ionic strength during the CLCR on the performance of the proposed miRNA assay by varying the concentration of NaCl from 50 mM to 200 mM, and the results are shown in Fig. S4. It can be observed that when the NaCl concentration increases from 50 mM to 100 mM, the fluorescence signals produced by let-7a increase sharply while the fluorescence signals produced by blank control change a little. The reason may be that during the miRNA-induced CLCR, all the involved probes including miRNA are quite short. So relatively higher ionic strength will enhance the hybridization efficiency between such probes and thus accelerate the target-mediated CLCR reaction. When the NaCl concentration continues to increase from 100 mM to 200 mM, the blank signal increases more remarkably than that aroused by let-7a. The reason may be that when the ionic strength is high enough, the electrostatic repulsion effect between different free ssDNAs will be shielded so that the nonspecific collision and ligation between such free ssDNAs will become serious. In this study, the highest ratio of the MFI

response produced by let-7a to that of the blank control is obtained at 100 mM NaCl (Fig. S4b). Thus, 100 mM NaCl is considered to be the optimal in this work to control the ionic strength during the CLCR reaction.

3. Investigation of the effect of the thermal cycling on the activity of STV (on the MBs) for binding biotinylated ssDNA probes

Some verification experiments were performed to prove that the thermal cycling in the proposed CLCR would not affect the activity of STV on the MBs for binding biotinylated DNA probes. On one hand, one batch of STV-functionalized M-270 MBs were firstly treated with the same thermal cycling conditions as the CLCR reaction (70 ºC for 30 s, and 25 ºC for 150 s, 40 cycles). Then certain amount of a 5′-biotinylated and 3′-FAM-modified ssDNA probe (biotin-AAAAA-FAM) was added and incubated with such MBs at room temperature for 30 min. On the other hand, as a comparison, the same amount of STV-functionalized M-270 MBs were directly incubated with the equivalent dosage of biotin-AAAAA-FAM ssDNA probe for 30 min without treatment of thermal cycling. Finally, the two batches of M-270 MBs were respectively detected by the flow cytometer. The effect of thermal cycling on the binding capacity of STV towards biotin can be evaluated by comparing the loaded FAM molecules on the two batches of MBs.

As can be seen from the FCM results in Fig. S5, when incubated with the same amount of biotin-AAAAA-FAM ssDNA probes, the fluorescence signals of the two batches of MBs keep almost the same irrespective of whether they are treated with thermal cycling or not. These results clearly prove that the thermal cycling conditions in the proposed CLCR will not affect the activity of STV anchored on the MBs for binding biotinylated DNA probes.

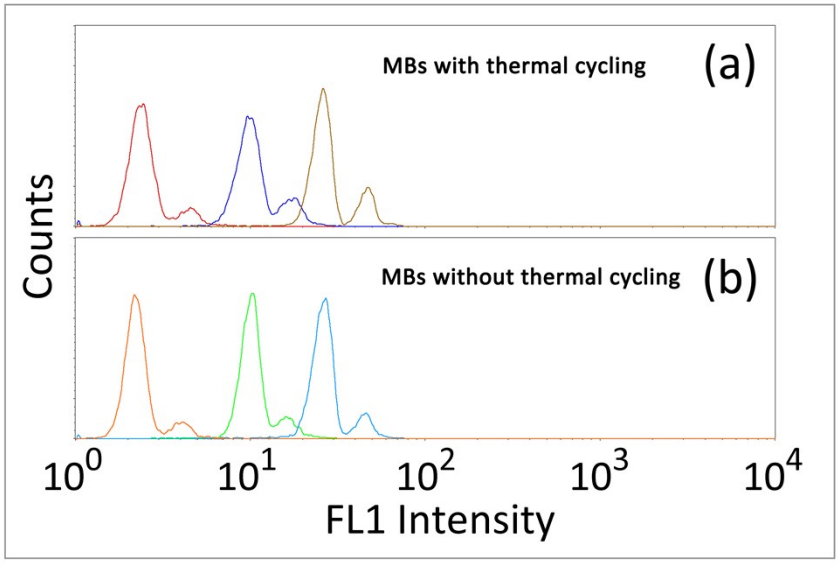

Fig. S5. Evaluation of the effect of the thermal cycling on the activity of STV (on the MBs) for binding biotinylated

ssDNA probes. (a) The MBs were firstly treated with thermal cycling and then respectively incubated with different concentrations of biotin-AAAAA-FAM ssDNA probe. The concentration of ssDNA probe from left to right is 0, 5 nM and 25 nM, respectively; (b) The M-270 MBs were directly incubated with the biotin-AAAAA-FAM ssDNA probe without treatment of thermal cycling. The concentration of ssDNA probe from left to right is 0, 5 nM and 25 nM, respectively. It should be noted that the conditions for FCM measurements are all the same.