Electronic Supplementary Information

Bead-Enriched Catalyzed Hairpin Assembly for the Flow Cytometric Detection of microRNA *via* FRET signal readout

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1. Experimental details

Standard procedures of the homogeneous CHA for miRNA analysis. 100 pM H1 and 1 nM free H2 were mixed with different concentrations of let-7a in 100 μL of 4 × SSPE buffer solution. The mixture was incubated at 37 °C for 3 hours to conduct CHA reaction. Finally, the FRET signal is detected by using fluorescence spectroscopy.

Detection of let-7a level in total RNA extracted from cells. The HepG2 and Hela cell lines were purchased from the cell bank of Chinese Academy of Sciences. And they were cultured in Dulbecco's modified Eagle's medium (DMEM, Life). All the media were supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C under a humidified atmosphere containing 5% CO_2. The total RNA sample was extracted from the cells by using of RNAiso for Small RNA Kit (Takara, China) according to the manufacturer's instructions. The amount of extracted total RNA was quantified with a Nanodrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific,

USA). The total RNA extracted from cells was subject to the standard procedures of the bead-enriched CHA for miRNA analysis procedures stated in the main text to quantitatively evaluate the level of let-7a.

Quantification of let-7a in the total RNA by RT-PCR method. The stem-loop reverse-transcription PCR (RT-PCR) protocol is referred to the methods in the literatures with some modifications. **Reverse transcription reaction:** The reverse transcription reaction was carried out in the mixture with 1 µL target miRNA (or total small RNA sample), 1.2 µL of RNase-free water, 1 µL of 5 × RT buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$), 1 µL of 2.5 mM dNTPs, 0.2 µL of 200 U/µL ProtoScrip II reverse transcriptase, 0.5 µL of 1 µM stem-loop RT-Probe (5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACTA-3'), and 0.1 µL of 40 U/µL RNase inhibitor. The 5 µL mixture was treated with following conditions: 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C and then held at 4 °C. **Quantitative real-time PCR analysis:** 5 µL transcription product was added into the PCR reaction mixture with a final volume of 10 µL. The PCR reaction mixture consists of 200 nM forward primer (5'-GCCGCTGAGGTAGTAGGTTGTA-3') and 200 nM reverse primer (5'-GTGCAGGGTCCGAGGT-3'), 250 µM dNTPs, 0.4 × SYBR Green I, 0.5 U JumpStartTM Taq DNA Polymerase, and 1 × PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001(w/v) gelatin, pH 8.3). The 10 µL PCR reaction mixture was incubated in a StepOne Real-Time PCR System (Applied Biosystems, USA) according to the following thermal cycling conditions: hot start at 95 °C for 2 min, followed by 50 cycles of 95 °C for 15 s, and 60 °C for 1 min.

2. Nucleic acid sequences used in this work.

Table S1. DNA and RNA sequences used in the work

Name	Sequence from 5' to 3'
H1	TAGGTTGT(FAM)ATAGTTCCATGTAGAAACTATACAACCTACTAC CTCATTTTTTTTTT-Biotin
Free H2	CCATGTGTAGATAGGTTGTATAGTTCTACACATGGAACTATA-Cy3
Let-7a	UGAGGUAGUAGGUUGUAUAGU
Let-7e	UGAGGUAGGAGGUUGUAUAGU
Let-7f	UGAGGUAGUAGAUUGUAUAGU
Let-7g	UGAGGUAGUAGUUUGUACAGU
Let-7i	UGAGGUAGUAGUUUGUGCUGU
microRNA-21	UAGCUUAUCAGACUGAUGUUGA
microRNA-195	UAGCAGCACAGAAAUAUUGGC

FAM: 6-carboxyfluorescein; Cy3: Cyanine 3.
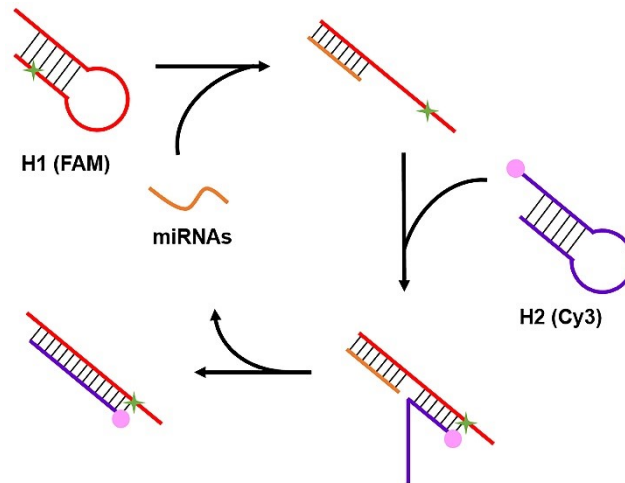
3. The detailed principle of miRNA-initiated CHA

Figure S1. Schematic illustration of the detailed principle of miRNA-initiated CHA based on the toehold-mediated strand displacement.

4. Detection results of let-7a with the proposed bead-enriched CHA assay by using 1.2×10^5 beads.

To evaluate the analytical performance of the proposed bead-enriched CHA assay, approximate 1.2×10^5 beads are employed to analyze the concentration of let-7a. As shown in Figure S2, the FRET signal continuously rises with the concentration of let-7a increasing from 50 pM to 300 pM. Notably, as low as 50 pM let-7a-produced FRET signal can be well discriminated from the blank signal.
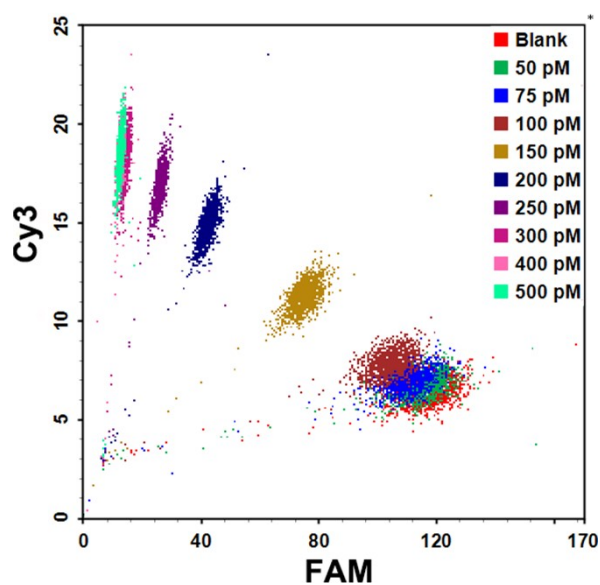

Figure S2. Fluorescence intensity of Cy3 *vs.* fluorescence intensity of FAM scattering plots of the beads in the presence of different concentration of let-7a by using 1.2×10^5 beads.

5. Fluorescence intensity of Cy3 *vs.* fluorescence intensity of FAM scattering plots of the beads in the presence of small concentration of let-7a by using 2.4 × 10³ beads.
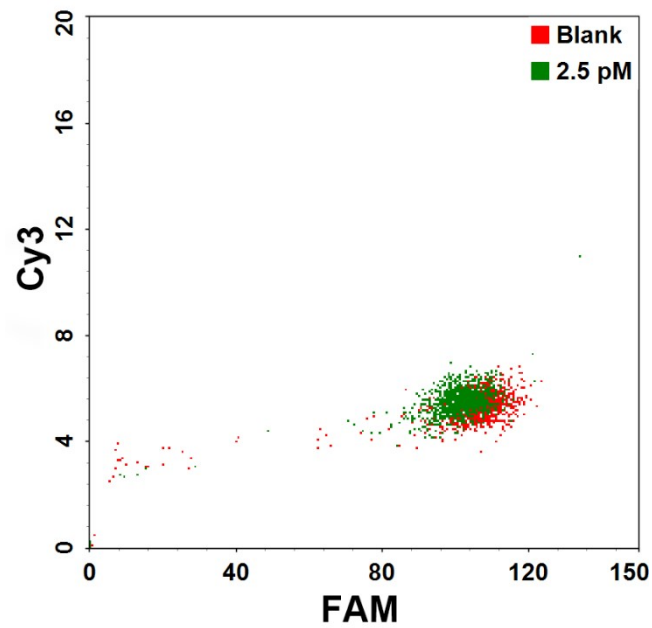

Figure S3. Fluorescence intensity of Cy3 vs. fluorescence intensity of FAM scattering plots of the beads in the presence of 0 and 2.5 pM of let-7a.

6. Calculation of the detection limit

In the Table S2, we provide the average FRET signal and standard deviation of nine parallel test results of the blank control. According to the 3σ criterion and the slope of the calibration curve, the detection limit of let-7a is calculated to be 0.7 pM (Equation 1). Moreover, from another angle, if we interrogate the detection limit value by using 2.5 pM target (whose FRET signal can be clearly discriminated from that of blank control) as a point on the calibration curve in the linear range, according to the Equation 2, the detection limit is calculated to be 0.65 pM. These results are consistent.

$$\text{Detection limit} = \frac{3\sigma}{S} = \frac{3 * 0.00243}{0.0104} = 0.7 \text{ pM (Equation 1)}$$

$$\frac{3\sigma}{lg(I_{Cy3}/I_{FAM})_{2.5\,pM} - lg(I_{Cy3}/I_{FAM})_{blank}} = \frac{Detection\ limit}{2.5\,pM} \quad \text{(Equation 2)}$$

Table S2. The relevant values for the datapoints produced by 0 (blank) and 2.5 pM let-7a.

Let-7a concentration	I_{Cy3}/I_{FAM}	$lg(I_{Cy3}/I_{FAM})$	Average of $lg(I_{Cy3}/I_{FAM})$	Standard deviation
0 (blank, n=9)	0.05066	-1.29534	-1.29232	0.00243
	0.05083	-1.29390		
	0.05072	-1.29479		
	0.05153	-1.28796		
	0.05082	-1.29395		
	0.05108	-1.29174		
	0.05100	-1.29240		
	0.05122	-1.29056		
	0.05126	-1.29024		
2.5 pM	0.05457	-1.26305	-1.26450	0.00126
	0.05430	-1.26522		
	0.05430	-1.26523		

7. Comparison of different nucleic acid detection methods based on CHA

Table S3. Comparison of different nucleic acid detection methods based on CHA

Sensing strategy	Analytical technique for signal readout	Step of the experimental operation	Detection limit/The lowest detectable concentration
The application of a three-arm nanostructure for nonenzymatic signal amplification	Fluorescence[S1]	one	29.3 pM
A method using a palindromic hybridization chain reaction combined with CHA	Fluorescence[S2]	two	10 pM
Based on cross-CHA on gold nanoparticles to generate cyclic amplification	Fluorescence[S3]	one	74 pM
A localized CHA-based DNA nanomachine	Fluorescence[S4]	one	58.1 pM
Coupling CHA with magnetic bead-confined 3D DNA walking	Fluorescence[S5]	two	47.4 pM
Combining CHA with the Luminex xMAP system	Fluorescence[S6]	two	2 pM
Coupling CHA with enzymatic repairing amplification	Fluorescence[S7]	two	50 fM
An electrochemical biosensor using localized DNA tetrahedron-assisted CHA	electrochemical[S8]	four	21 aM
Combining SERS techniques and CHA	SERS[S9]	three	2.53 aM
Coupling CHA with portable SERS reader	SERS[S10]	two	84 fM
Bead-enriched catalyzed hairpin assembly for the flow cytometric detection of microRNA	Fluorescence	one	0.7 pM (this work)

References

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