Duplex-Specific Nuclease (DSN) and Catalytic Hairpin Assembly (CHA)-mediated Dual Amplification Method for MiRNA-146b Detection

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Table S1. The sequences of DNA and RNA used in the study are as follows (5′-3′)

Name	Sequences (5′ – 3′)
BTR probe-a	ACATCATCATACATCATCATACATCAGCCTATGGAATTCAGTTCTCA-Biotion
BTR probe-b	Biotion-CAGCCTATGGAATTCAGTTCTCAACATCATCATACATCATCATACAT
HP-8a	ACATCATCATACATCATCATACATCAGCCTATGGAATTCAGTTCTCAATGTATGA
HP-12a	ACATCATCATACATCATCATACATCAGCCTATGGAATTCAGTTCTCAATGTATGATGAT
HP-12b	GTATGATGATGTCAGCCTATGGAATTCAGTTCTCAACATCATCATACATCATCATACAT
HP1	BHQ1-ATGTATGATGATGTATGATGATGTTCCAATCACAACACATCATCATACATCATC-FAM
HP2	GTATGATGATGTGTTGTGATTGGAATCATCATACATTCCAATCACAACACATCA
HP3	GTTGTGATTGGAATGTATGATGATACATCATCATACATCATCATACATTCCAAT
mi-RNA-146a	UGAGAACUGAAUUCCAUGGGUU
mi-RNA-146b	UGAGAACUGAAUUCCAUAGGCUG
mi-RNA-let-7a	UGAGGUAGUAGGUUGUAUAGUU
mi-RNA-200a	UAACACUGUCUGGUAACGAUGU
mi-RNA-499a	UUAAGACUUGCAGUGAUGUUU
mi-RNA-199	CCCAGUGUUUAGACUAUCUGUUC
mi-RNA-429	UAACACUGUCUGGUAACGAUGU
mi-RNA-141	CAUCUUACUGGGCAGCAUUGGA

* The bases differing from those in miR-146b are marked in red.

Table S2. Comparison of different strategies for detection of miRNAs using CHA reaction

Analytical methods	Target	Sample	Mechanism	LOD	Linear range	Ref.
Fluorescence	miRNA-141	Human serum	DNA-templated silver Nanoclusters	297.1 pM	0-200 nM	[1]
Fluorescence	miRNA-21	Cell	Catalytic Hairpin Assembly RNA circuit that is Genetically Encoded	2.5 nM	2.5 nM - 250 nM	[2]
Fluorescence	miRNA-203a	Cell	Based on cross-catalytic hairpin assembly on gold nanoparticles	74 pM	0.1 nM - 150 nM	[3]
Fluorescence	miRNA-146b	Human serum	Combination of DSN and fluorescence of CHA dual signal amplification	2 fmol (40 pM)	20 fmol-1000 fmol	This work

Table S3 Recovery of miR-146b spiked into human serum samples. (n=3)

Added (fmol)	Found (fmol)	Recovery (%)	RSD (%)	Mean Recovery (%)
1000	1103.77 ± 25.41	110.37	2.30	98.94
600	603.14 ± 4.85	100.52	0.81	
400	364.75 ± 20.58	91.18	5.64	
200	187.34 ± 19.17	93.67	10.23	

Optimization of concentrations of three hairpin primers

In this DSN-CHA system, the CHA reaction was used to detect front-end DSNSA. Therefore, this parameter was optimized firstly. As shown in Figure S1, the abscissa (x) represents the concentration of hairpin primers, and the ordinate (y) represents the background-subtracted sample fluorescence intensity. To investigate the influence of the amount of HP1, HP2, and HP3 on the miR-146b detection, we select various concentrations of HP1, HP2, and HP3 from 2 to 16 pmol. When the concentration of all three hairpin primers exceeds 8 pmol, the difference value of fluorescence was lower and lower. As shown in Figure S1a, the fluorescence intensity of the sample group is increased when the concentration of the hairpin primer is increasing. However, the fluorescence intensity of the blank group was also increased, and their different value was significantly reduced. Therefore, the concentration of all three hairpin primers was 8 pmol, the background-subtracted sample fluorescence intensity is the most suitable for miRNA detection.

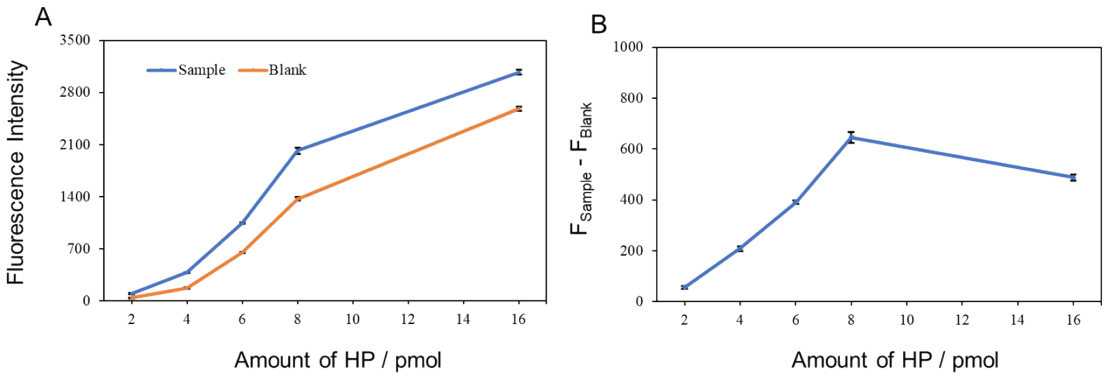

Figure S1. (A) Influence of three hairpin primers on the fluorescence intensity. (B) Influence of three hairpin primers on the difference value of fluorescence intensity. Experimental conditions: miR-146b (0.2 pmol) + DSN (0.2U) + SA-MBs (40 μg) + BTR-a probe (6.25 pmol) + incubation temperature (45°C) + incubation time (120 min). The detection procedure was carried out as described in the Experimental Section.

Optimization of concentration of BTR-a

As shown in Figure S2b, when the concentration of BTR-a exceeds 6.25 pmol, the background-subtracted sample fluorescence intensity was gradually decreased. Figure S2a shows that when the BTR-a concentration was 10 pmol, the fluorescence intensity of the sample group was similar to that of the 6.25 pmol, but the blank group was rising. The main reason for this phenomenon is that the concentration of BTR-a is significantly excess; the DSN enzyme could hydrolyze BTR-a without hybridizing miRNA, which leads to the enhancement of the fluorescence signal in the blank group. Therefore, according to the optimized data, we choose the concentration of the BTR-a to be 6.25 pmol optimizing the parameters remaining.

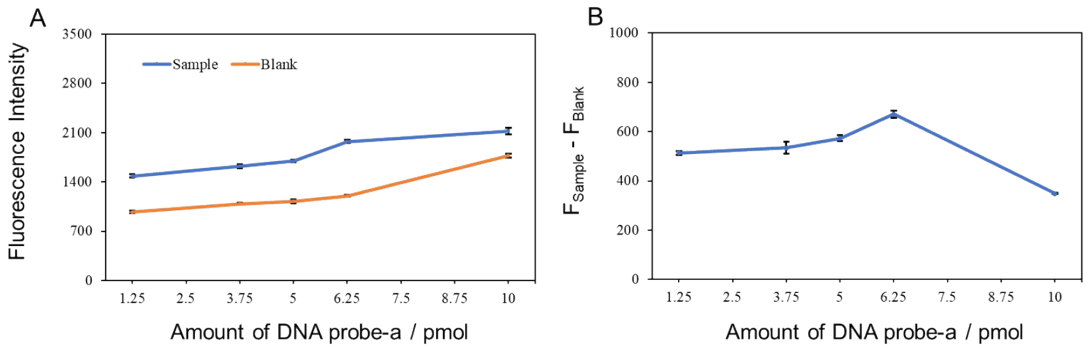

Figure S2. (A) Influence of BTR-a probe on the fluorescence intensity. (B) Influence of BTR-a probe on the difference value of fluorescence intensity. Experimental conditions: miR-146b (0.2 pmol) + DSN (0.2U) + SA-MBs (40 μg) + HP1 (8 pmol) + HP2 (8 pmol) + HP3 (8 pmol) + incubation temperature (45°C) + incubation time (120 min). The detection procedure was carried out as described in the Experimental Section.

Optimization of the amount of SA-MB

As shown in Figure S3b, when the SA-MB was 4 μL (40 μg), the result showed that the background-subtracted sample fluorescence intensity was the most pertinent data. However, Figure S3a showed that fluorescence intensity of blank group increased with the increase of the amount of SA-MBs, which was not expected. Because the more SA-MBs there are, the more BTR-a were removed, and the fluorescence intensity of the blank group reached an equilibrium with the increase in the amount of SA-MBs. Subsequently, we measured the fluorescence intensity from CHA reaction, it was similar to 4 μL of SA-MB of blank group. So, we think excess 4 μL SA-MB could reduce the ability to capture BTR probes. Therefore, we chose 4 μL (40 μg) as the optimal volume of SA-MBs for better detection intensity and selectivity.

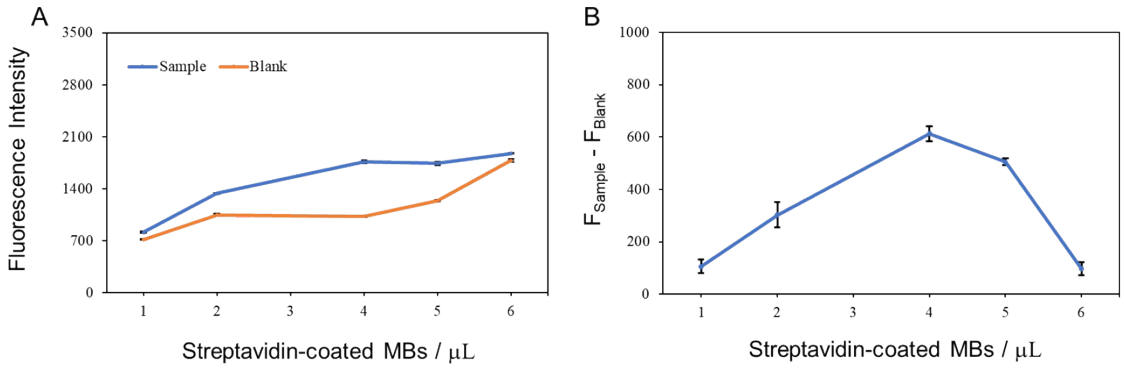

Figure S3. (A) Influence of Streptavidin magnetic beads on the fluorescence intensity. (B) Influence of Streptavidin magnetic beads on the difference value of fluorescence intensity. Experimental conditions: miR-146b (0.2 pmol) + DSN (0.2U) + BTR-a probe (6.25 pmol) + HP1 (8 pmol) + HP2 (8 pmol) + HP3 (8 pmol) + incubation temperature (45°C) + incubation time (120 min). The detection procedure was carried out as described in the Experimental Section.

Optimization of DSN dosage

As shown in Figure S4, when DSN enzymes were 0.2 U and 0.4 U, their effects on the experiment result were the same. In order to reduce the consumption of reagents, we choose 0.2 U DSN enzyme as the optimal condition parameter. However, as shown in Figure S4a, we found that the fluorescence intensity of the blank group also increased as the amount of DSN increased from 0.05 U to 0.5 U. This proves the previous conjecture that the DSN enzyme can hydrolyze ssDNA, but this ability was inhibited when competition existed.

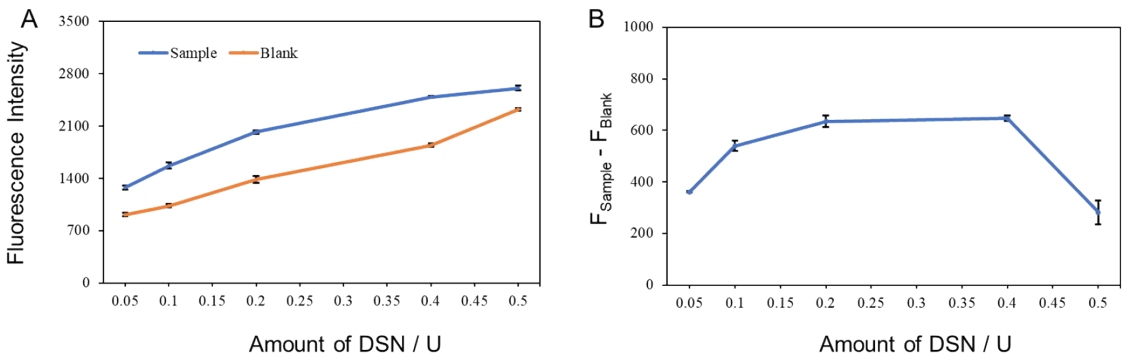

Figure S4. (A) Influence of Streptavidin magnetic beads on the fluorescence intensity. (B) Influence of Streptavidin magnetic beads on the difference value of fluorescence intensity. Experimental conditions: miR-146b (0.2 pmol) + DSN (0.2U) + SA-MBs (40 μg) + BTR-a probe (6.25 pmol) + HP1 (8 pmol) + HP2 (8 pmol) + HP3 (8 pmol) + incubation temperature (45°C) + incubation time (120 min). The detection procedure was carried out as described in the Experimental Section.

Optimization of incubation temperature and time

First of all, we chose 37°C for the incubation temperature. As shown in Figure S5, although the DSN kit instructions recommend 60°C as the best, according to the optimization result, we found that the background-subtracted sample fluorescence intensity continuously decreased from 45°C to 60°C, and the fluorescence intensity reached a peak at 37°C. Secondly, as shown in Figure S6, after the incubation time of 120 min, the fluorescence intensity tended to be stable and did not rise, so we chose the reaction time of 120 min and the temperature as 37°C.

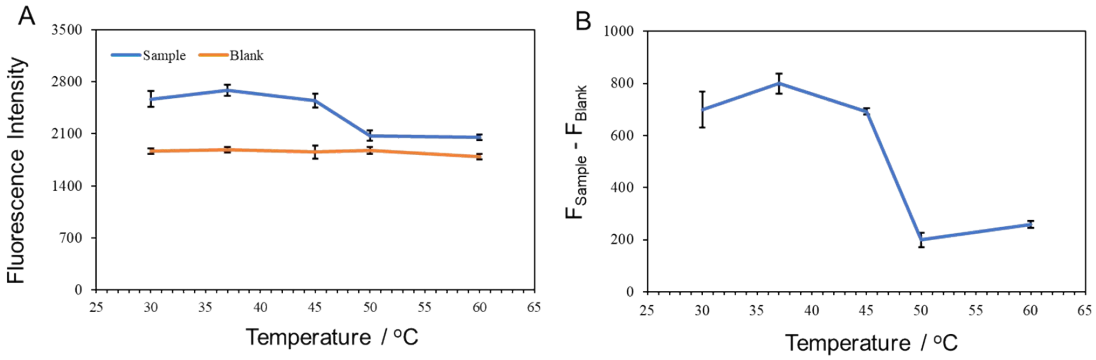

Figure S5. (A) Influence of incubation temperature on the fluorescence intensity. (B) Influence of incubation temperature on the difference value of fluorescence intensity. Experimental conditions: miR-146b (0.2 pmol) + DSN (0.2U) + SA-MBs (40 μg) + BTR-a probe (6.25 pmol) + HP1 (8 pmol) + HP2 (8 pmol) + HP3 (8 pmol) + incubation time (120 min). The detection procedure was carried out as described in the Experimental Section.

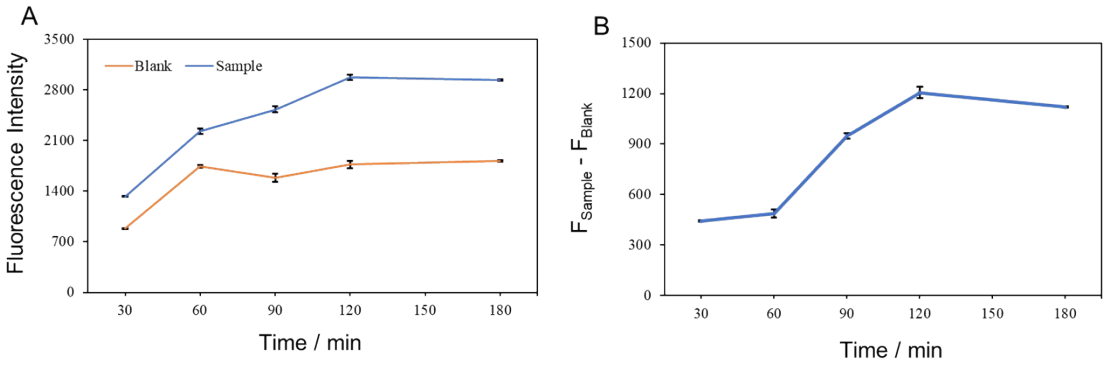

Figure S6. (A) Influence of incubation time on the fluorescence intensity. (B) Influence of incubation time on the difference value of fluorescence intensity. Experimental conditions: miR-146b (0.2 pmol) + DSN (0.2U) + SA-MBs (40 μg) + BTR-a probe (6.25 pmol) + HP1 (8 pmol) + HP2 (8 pmol) + HP3 (8 pmol) + incubation temperature (37°C). The detection procedure was carried out as described in the Experimental Section.

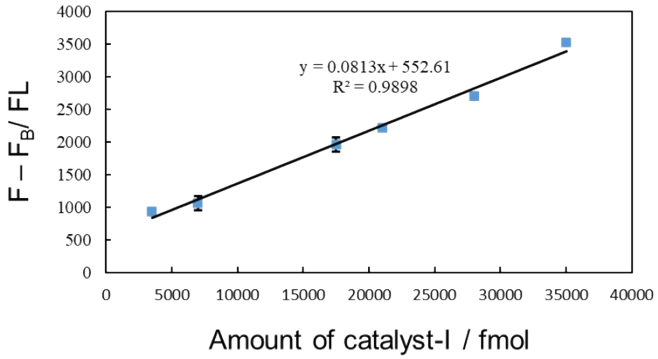

Figure S7. Calibration curve for detecting catalyst-I using the CHA method. F_B represents the fluorescence intensity of the blank group. F represents the fluorescence intensity of catalyst-I. Experimental conditions: catalyst-I (35000-3500 fmol) + HP1 (8 pmol) + HP2 (8 pmol) + HP3 (8 pmol) + incubation temperature (room temperature).

References

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