Supporting Information

Backbone-Modified Molecular Beacons for Highly Sensitive and Selective Detection of MicroRNA Based on Duplex-Specific Nuclease Signal Amplification‡

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Experimental Section

Materials

HPLC-purified RNA, RNase inhibitor, and DEPC-treated water were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). Duplex-specific nuclease (DSN) was obtained from Newborn Co. Ltd. (Shenzhen, China). The FAM/BHQ1-labeled molecular beacons (MB) were synthesized and purified by Sangon Biotech Co.,Ltd. (Shanghai, China). All DNA/RNA sequences are listed in Table S1. Cell lysate from breast cancer cell MDA-MB-231 was obtained by the reported procedure.[1]

Fluorescence measurements

Fluorescence measurements were carried out on a FluoroMax-4P Fluorescence Spectrophotometer (Horiba, Germany). The excitation and emission wavelengths were set at 490 and 520 nm, respectively, with a 3 nm bandwidth and 0.3s integration time. The emission spectra were obtained by exciting the samples at 490 nm and scanning the emission from 500 to 650 nm in steps of 1 nm/s. All experiments were conducted in the 1×DSN buffer containing 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$ and 1 mM DTT. The amplified detection of miRNA was performed in 200 μL solution with 50 nM MB, 0.2 U DSN and different concentrations of target miRNA at 65℃ for 40 min and the fluorescence intensities were detected afterwards. The miRNA concentrations in samples ranged from 0.5 pM to 50 nM.

Gel electrophoresis

A 20% non-denaturing PAGE analysis of the products from the cyclic enzymatic amplification reaction was carried out in 1×TBE (pH 8.3) at 1W power for about 1.5 hr. After Stains-All staining, gel images were obtained using a Canon camera.

Table S1. Sequences of oligonucleotides for this work

Name	Sequence
Molecular Beacon (MB)	**5'- FAM-CGA GTC AAC TAT ACA ACCT ACT ACC TCA GACTCG-BHQ1-3' (2-OMe-RNA are marked with underline)**
Let-7a	**5' - UGA GGU AGU AGG UUG UAU AGU U -3'**
Let-7e	**5'- UGA GGU AGG AGG UUG UAU AGU -3'**
Let-7f	**5'-UGA GGU AGU AGA UUG UAU AGU U-3'**
Let- 7i	**5'-UGA GGU AGU AGU UUG UGC UGU U-3'**
Mir 122	**5'- AAC GCC AUU AUC ACA CUA AAU A -3'**

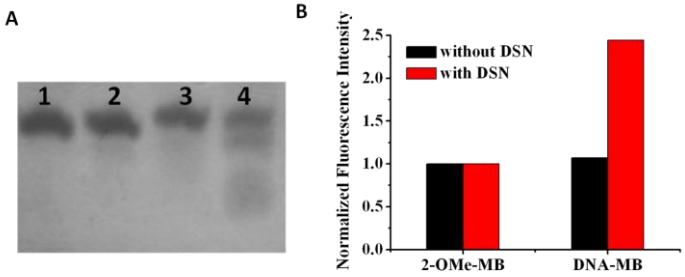

Figure S1. (A) Denatured PAGE analysis of 2-OMe-MB and DNA-MB with and without DSN. Lane 1: 2-OMe-MB; lane 2: 2-OMe-MB+DSN; lane 3: DNA-MB; lane 4: DNA-MB+DSN. (B) Comparison of 2-OMe-MB and DNA-MB towards DSN cleavage.

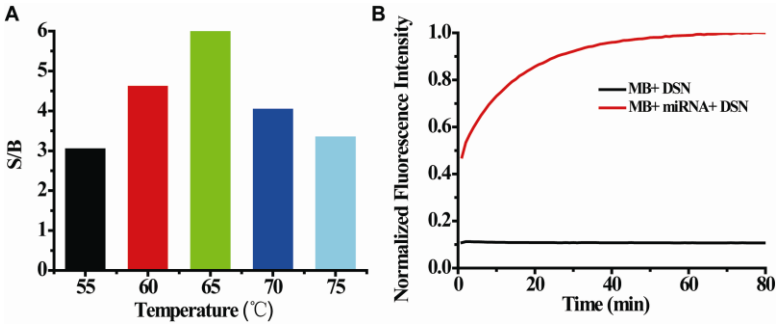

Figure S2 (A) The performance of the assay was evaluated by the signal-to-background (S/B) ratio at different temperatures. The concentration of miRNA is 5 nM. (B) Time course of MB + DSN and MB + miRNA + DSN. The concentration of miRNA is 25 nM.

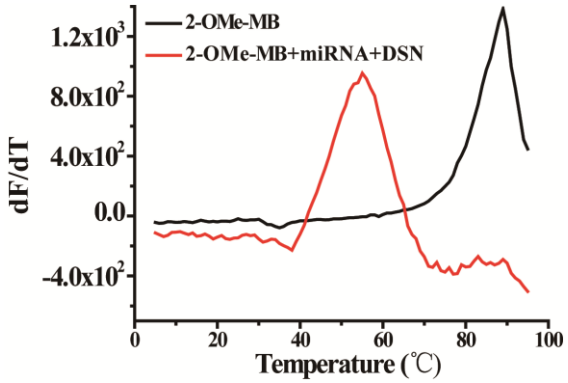

Figure S3 Melting temperature measurement of 2-OMe-MB and the product of the digestion reaction (2-OMe-MB + miRNA + DSN).

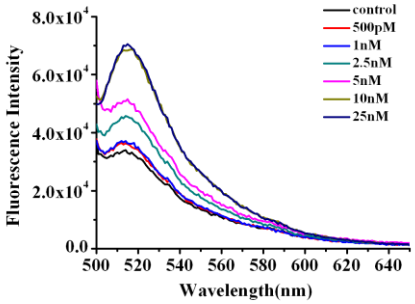

Figure S4. Fluorescence emission spectra of different concentrations of miRNA target in the absence of DSN.

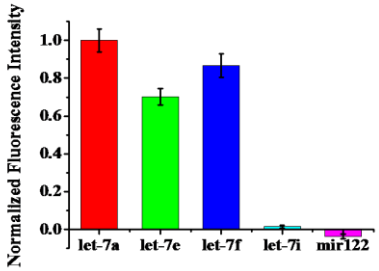

Figure S5. Selectivity of DSN-Taqman probe-based signal amplification method for let-7a over let-7e, let-7f, let-7i and mir122.

Table S2. Comparison of different analysis assays for miRNA

Assay	Sensitivity	Selectivity	Time
Current Method (MB probe)	**0.5 pM**	**one-base difference**	**40 min**
Northern Blotting (LNA probe)	**~0.4 nM**	**two-base difference**	**3 hr**
Stem-loop RT-PCR	**1 aM**	**one-base difference**	**>2 hr with complexity**
RCA	**10 fM**	**one-base difference**	**8 hr**
EXPAR	**15 aM**	**poor selectivity**	**30 min**
DSNSA	**0.1 pM**	**four-base difference**	**30 min**

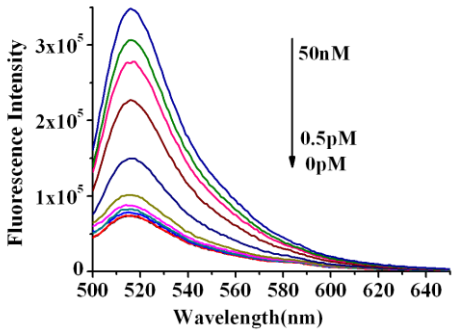

Figure S6. Fluorescencce emission spectra of MB solutions with different concentrations of miRNA in cell lysate. The concentrations of miRNA were 0, 0.5pM, 5pM, 50pM, 250pM, 500pM, 2.5nM, 5nM, 10nM, 25nM, and 50nM

References:

1. B. C. Yin, Y. Q. Liu and B. C. Ye, *J Am Chem Soc*, 2012, **134**, 5064.