Electronic Supplementary Information

A novel and versatile nanomachine for ultrasensitive and specific detection of microRNAs based on molecular beacon initiated strand displacement amplification coupled with catalytic hairpin assembly with DNAzyme formation

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Table S1. Oligonucleotides used in the present work

Nucleic acid[a]	Sequence (5'- 3')
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
miRNA-17	CAAAGUGCUUACAGUGCAGGUAG
miRNA-222	AGCUACAUCUGGCUACUGGGUCUC
SM	UAGCUUAUCAGACUGAUGUUUA
DM	UAGCUUAUCAGACUGAUUUUUA
NC	AUUGAAUAUUCUUAUUAUAAUU
molecular beacon -21 (MB-21)	TGGAGTGTGACAATGGTGTTTGTTCCTCAGCTAGCTTAT TCAACATCAGTCTGATAAGCTAAAAA
molecular beacon-17 (MB-17)	TGGAGTGTGACAATGGTGTTTGTTCCTCAGCCAAAGTGC CTACCTGCACTGTAAGCACTTTGAAAA
molecular beacon-222 (MB-222)	TGGAGTGTGACAATGGTGTTTGTTCCTCAGCAGCTACAT GAGACCCAGTAGCCAGATGTAGCTAAAA
Hairpin probe 1 (H1)	GGGTAGGGCGGGTTGGGAACAAATGGAGTGTGACAAT GGTGTTTGTTCCCAACCC
Hairpin probe 2 (H2)	GGGTTGGGAACAAACACCATTGTCTTTGTTCCC

[a] SM, single-base mismatched miRNA-21; DM, double-base mismatched miRNA-21; NC, non-complementary mismatched miRNA-21; MB, molecular beacon with C6 SPACER protected 3'-end.

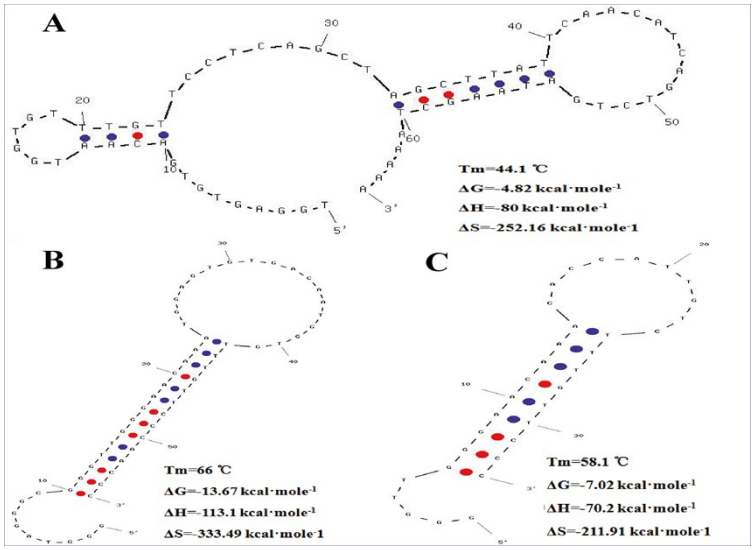

Fig. S1 Ideal structure of hairpin probes calculated by Oligo Analyzer 3.1: (A) ideal structure and parameters of MB, (B) ideal structure and parameters of hairpin H1, (C) ideal structure and parameters of hairpin H2.

Experimental

Ethanol precipitation procedure

The final mixture of the SDA reaction was added into 5 μL of 3 M sodium acetate (pH 5.2), 4 μL of Dr.GenTLE Precipitation Carrier, and 150 μL of ice absolute ethyl ethanol and vortexed. The mixture was centrifuged at 12000 rpm at 4 °C for 15 min and the supernatant was discarded. Then, the pellet was rinsed with 70 % ice ethanol and centrifuged again at 12000 rpm at 4 °C for 5 min. Finally, discard the supernatant and dry. The pellet was dissolved in the TNAK buffer.

Gel electrophoresis

The purified SDA mixture was analyzed by 12% native polyacrylamide gel electrophoresis (PAGE) in 1×TBE buffer (89 mM Tris-boric acid, 2 mM EDTA, pH 8.3) at 105 V constant voltage for 60 min at 4°C before staining with Goldview (Saibaisheng, China). The gels were visualized via gel image system (Bio-Rad Laboratories, USA).

The extraction of RNA from MCF-7 cells

The MCF-7 cells were added with 1 mLof TRIZOL, and then incubated for 5 minutes at room temperature. The samples were added with 0.2 mL of chloroform, and shaken vigorously for 15 seconds, then centrifuged at 12,000 g for 15 minutes at 4℃. The upper aqueous phase was transferred carefully without disturbing the interphase into fresh tube. The RNA was precipitated from the aqueous phase by mixing with 0.5 mL of isopropyl alcohol. The samples were incubated at room temperature for 10 minutes and centrifuged at 12,000 g for 15 minutes at 4^{o}C. Then the supernatant was removed completely, and the RNA pellet was washed with 1 mL of 70% ethanol, and then centrifuged at 7,500 g for 5 minutes at 4^{o}C, repeated once. All leftover ethanol was removed, and the RNA pellet was Air-dry for 5~10 minutes till the residual ethanol volatilized. The RNA samples were dissolved with DEPC water, and detected OD at 260 nm and 280 nm to determine sample concentration and purity. At the same time, the RNA quality was also detected by 0.8% agarose gel electrophoresis.

Table S2. The recoveries determined using the established machine in the real sample.

	Assay value	Accuracy (RSD)	Recovery ratio (%)
MCF-7 total RNA (400 ng)[a]	12.02 fM	9.70 %	-
miRNA-21 (500 fM)[b]	515.50 fM	7.04 %	100.70
miRNA-21 (5 pM)[b]	5.01 pM	2.5 %	99.96
miRNA-21 (5 nM)[b]	4.64 nM	4.5 %	92.80

[a] Based value [b] Spiking value