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Supporting Information

T7 Exonuclease-Assisted Cyclic Enzymatic Amplification Method Coupled With Rolling Circle Amplification: A Dual-Amplification Strategy for Sensitive and Selective MicroRNA Detection

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Experimental Section

Materials and Reagents

T7 Exonuclease (T7 Exo) was purchased from Affymetrix, Inc. (Santa Clara, CA, USA). CircLigase™ ssDNA Ligase was purchased from Epicentre Biotechnologies (Madison, WI, USA). Phi29 DNA polymerase was purchased from New England Biolabs (Beijing, China). Exo III, HPLC-purified padlock probes and miRNAs were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). TaqMan probes, linear molecular beacons (LMBs) and target DNA were synthesized on a PolyGen Column 12 DNA synthesizer (Germany) and all DNA synthesis reagents were purchased from Glen Research (Sterling, VA, USA). All DNA/RNA sequences are listed in Table 1. Total RNA Kit was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was used to extract the total RNA from hepatoma cells and hepatocytes.

Fluorescence Measurements

Fluorescence measurements were carried out on a RF-5301-PC Fluorescence Spectrophotometer (Shimadzu, Japan). In the time scan mode, excitation and emission wavelengths were set at 490 and 520 nm, respectively, with 5 nm bandwidth. 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. The T7 Exo activity experiments were conducted in 20 mM Tris-HCl (pH 7.5) buffer containing 10 mM $MgCl_2$ and 20 mM NaCl (T7 Exo buffer), and the Exo III activity experiments were conducted in 50 mM Tris-HCl (pH 8.0) buffer containing 5 mM $MgCl_2$ (Exo III buffer).

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T7 Exo-Assisted Cyclic Enzymatic Amplification Method

To test the feasibility of T7 Exo for CEAM, CEAM was carried out in the presence of different concentrations of target cDNA in 10 μL T7 Exo buffer (20 mM Tris-HCl, 10 mM $MgCl_2$ and 20 mM NaCl, pH 7.5) containing T7 Exo (5 U/μL) and TaqMan probes (1 μM) for 1 hr at 37°C. After incubation, the samples were diluted to 200 μL with T7 Exo buffer and analyzed by spectrofluorometry.

RCA-CEAM Dual Amplification Method for Ultrasensitive Detection of miRNA

First, the padlock probes were ligated and circularized by ssDNA ligase. Then the RCA in the presence of different concentrations of target miRNA was carried out in 10 μL phi29 buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH4)_2SO_4$, 4 mM DTT, pH 7.5) containing circular template (200 nM), phi29 DNA polymerase (1 U/μL), dNTPs (0.25 mM), ribonuclease inhibitor (0.8 U/μL), and BSA (0.25 μg/μL) for 6 hr at 30°C. To test the RCA performance, the experiment was carried out by addition of 1×SybrGreen before the RCA reaction. After RCA, the samples were diluted to 20 μL with addition of 4 μL 5×T7 Exo buffer, 1 μM LMBs, 50 U T7 Exo and then incubated at 37°C for 1 hr. The samples were diluted to 200 μL with T7 Exo buffer and analyzed by spectrofluorometry.

Gel Electrophoresis for Monitoring T7 Exo Digestion of DNA

The experiment was carried out with different concentrations of DNA in 10 μL T7 Exo buffer (20 mM Tris-HCl, 10 mM $MgCl_2$ and 20 mM NaCl, pH 7.5) containing T7 Exo (5 U/μL) and TaqMan probes (10 μM) for 1 hr at 37 °C. Afterwards, a 20% denaturing polyacrylamide gel was prepared using 1 × TBE buffer (pH 8.3). The gel was run at 1W power for about 1 hr in 1 × TBE buffer, stained for 30 min by Stains All solution (500 mL formamide, 100 mL 10 × TBE, 400 mL H_2O, 200 mg Stains All), then illuminated with white light until the stained band appeared, and finally photographed with a digital camera.

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Table S1. Sequences used in this work.[a]

Name	Sequence
TaqMan probe	5’ - Dabcyl - AACTATA CAA CCTAC – FAM- 3’
Target cDNA	5' - TGA GGT AGT AGG TTG TAT AGT TTT AGT A - 3'
1mDNA	5' - TGA GGT AGT AGG TTC TAT AGT TTT AGT A - 3'
3mDNA	5'- TGA GGT AGT AGG TCC GAT AGT TTT AGT A -3'
Padlock probe	5’ - PO_4- CTA CTA CCT C AT TTG CAT TTC AGT TTA CGG TTT AGC ATT TCG CAA TTT TAA CTA TAC AAC - 3’
LMBs	5’- FAM- AT (Dabcyl) T TGC ATT TCA GTT TAC GGT - 3’
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’
Mir 122	5’- UGG AGU GUG ACA AUG GUG UUU G - 3’

[a] Underlined letters represent the mismatched site

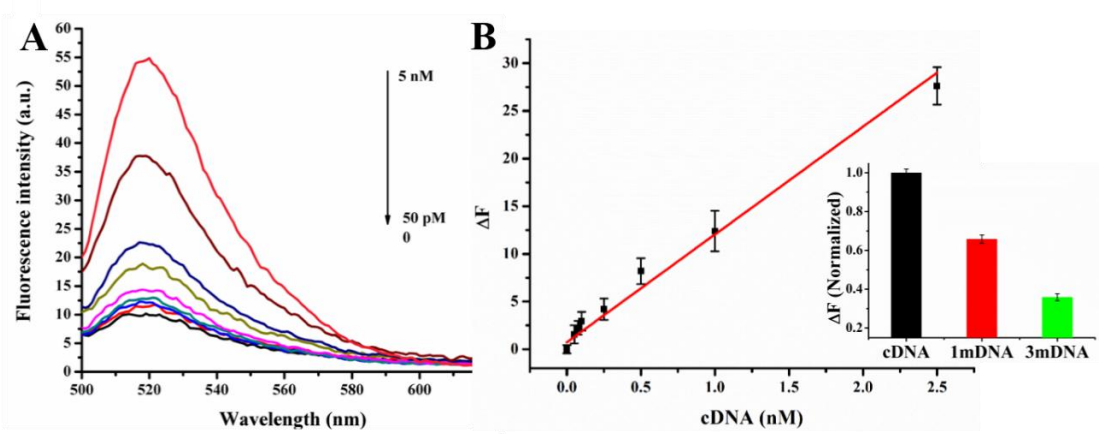

Fig. S1 (A) Detection of different concentrations (0, 50 pM, 75 pM, 100 pM, 250 pM, 500 pM, 1 nM, 2.5 nM, 5 nM) of target cDNA based on T7 Exo-assisted CEAM. (B) The relationship of the rate of fluorescence enhancement with target cDNA concentration. CEAM can differentiate the perfectly matched and mismatched DNA targets (insert of B, concentration: 2.5 nM).

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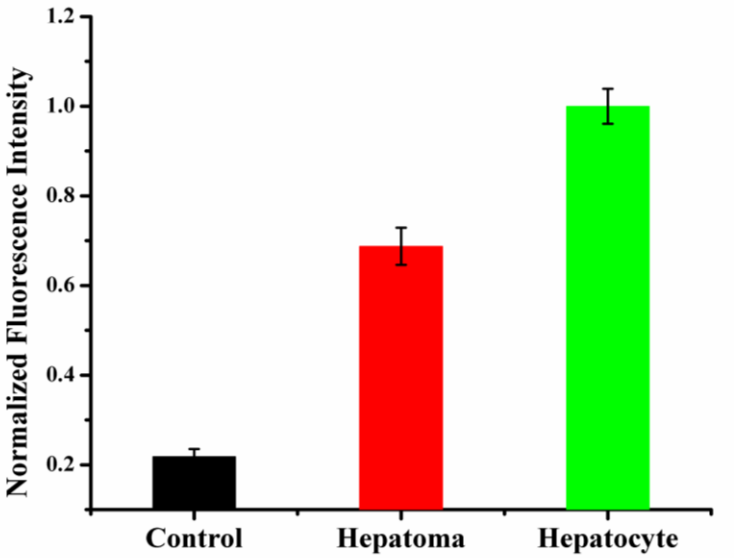

Fig. S2 The expression levels of let 7a in total RNA samples from hepatocytes and hepatoma cells. The "Control" is the background signal without RNA sample.