Electronic Supplementary Information

Ultrasensitive detection of long non-coding RNAs based on duplex-specific nuclease-actuated cyclic enzymatic repairing-mediated signal amplification

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EXPERIMENTAL SECTION

Materials and Reagents

All DNA oligonucleotides, lncRNA, miRNA (Table S1), deoxyadenosine triphosphate (dATP), RNase-free water and RNase inhibitor were purchased from TaKaRa Bio. Inc. (Dalian, China). Duplex-specific nuclease (DSN) was obtained from Evrogen Joint Stock Company (Moscow, Russia). Terminal transferase (TDT) and apurinic/apyrimidinic endonuclease (APE1) were obtained from New England Biolabs (Ipswich, MA, USA). The streptavidin-coated magnetic beads (Dynabeads^{TM} M-280 streptavidin) were obtained from Invitrogen (California, CA, USA). The silver staining kit was obtained from Tiandz, Inc. (Beijing, China). Human lung adenocarcinoma cell line (A549 cells), human cervical carcinoma cell line (HeLa cells) and human colon cancer cells (SW480 cells) and human breast cancer cell line (MCF-7 cells) were bought from Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). All other reagents were of analytical grade and used as received without further purification.

Table S1. Sequences of the Oligonucleotides [a]

note	sequence (5′-3′)
HOTAIR capture probe	CTT CTA GAC CTA ATA TAA GCA TAT TAT AGA GTT GCT TTT-biotin
MALAT1 capture probe	CGG AGC AGC ACG GGC TGT CTG CTT GGG AAA TCT TAT TTT -biotin
signal probe (15 nt)	FAM -TTT TTT TTX TTT TTT T-BHQ1
signal probe (17 nt)	FAM-TTT TTT TTT XTT TTT TTT-BHQ1
signal probe (19 nt)	FAM-TTT TTT TTT TXT TTT TTT TT-BHQ1
signal probe (21 nt)	FAM-TTT TTT TTT TTX TTT TTT TTT T-BHQ1

lncRNA HOTAIR	GCA ACU CUA UAA UAU GCU UAU AUU AGG UCU AGA AG
lncRNA MALAT1	UAA GAU UUC CCA AGC AGA CAG CCC GUG CUG CUC CG
let-7a	UGA GGU AGU AGG UUG UAU AGU U

[a] The X in the signal probe indicates the apurinic/apyrimidinic (AP) site mimic (the tetrahydrofuran analogue).

Conjugation of capture probes with the streptavidin-coated magnetic beads

To fabricate the magnetic beads (MBs)-capture probe conjugates, 100 μL of streptavidin-coated MBs solution (10 mg/mL) was transferred into a centrifuge vial and washed twice to remove the preservative. The supernatant was removed by magnetic separation, and the MBs were re-suspended in Tris-HCl buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 2 M NaCl) with a final concentration of 5 μg/μL. Then 200 μL of 1 μM biotinylated capture probes was mixed with 200 μL of 5 μg/μL MBs solution and incubated in the dark for 10 min on a roller mixer at room temperature. Subsequently, the mixture was washed three times to remove the uncoupled capture probes, and the remained capture probe-MB conjugates were dispersed in 50 μL of 1× DSN buffer as the stock solution for subsequent use.

Cell culture and extraction of total RNA

The MCF-7 cells, HeLa cells, SW480 cells and A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (Invitrogen, USA) at 37 °C with 5% CO_2. The total RNA was obtained by miRNeasy mini kit (Qiagen, German) according to the

manufacturer's procedure, and its concentration was determined by the NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, U.S.A.).

lncRNA Assay

Different concentrations of synthetic lncRNA / total RNA sample were added into hybridization solution containing the freshly prepared capture probe-functionalized MB, 20 U of RNase inhibitor, 1× TdT buffer (10 mM $Mg(Ac)_2$, 20 mM Tris-Ac, 50 mM KAc, pH 7.9), followed by incubation at room temperature for 20 min to form the DNA/RNA heteroduplexes. After the magnetic separation, 0.2 U of DSN, 20 U of RNase inhibitor, 1× TdT buffer were added to the reaction mixture with a total volume of 20 μL, and incubated at 55 °C for 40 min. After the magnetic separation, the supernatants were inactivated at 95 °C for 5 min. Then 20 μL of supernatants were incubated with 400 nM signal probes, 500 μM dATP, 4 U of APE1, 8 U of TdT, 1× TdT buffer, 0.25 mM $CoCl_2$ with a total volume of 30 μL at 37 °C for 90 min, followed by inactivation at 80 °C for 20 min.

Gel Electrophoresis Analysis

The 12% nondenaturating polyacrylamide gel electrophoresis (PAGE) analysis was carried out in 1× TBE buffer (9 mM Tris-HCl, pH 8.0, 9 mM boric acid, 0.2 mM EDTA) at a 110 V constant voltage for 50 min at room temperature. The gel was stained with a silver staining kit and visualized by a Kodak Image Station 4000 MM (Rochester, NY, USA).

Measurement of Fluorescence Spectra

The fluorescence signals of the mixture were measured by Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) with an excitation wavelength of 490 nm. The emission spectra were scanned from 510 to 650 nm, and the emission intensities at 520 nm were used for data

analysis.

SUPPLEMENTARY RESULTS

Optimization of Experimental Conditions

We optimized the experimental conditions including the length of signal probes and the concentration of signal probes. An ideal signal probe not only generates a low background fluorescence signal, but also keeps the polyA/signal probe hybrids stable under the reaction condition, and thus the length of signal probe should be optimized. As shown in Fig. S1A, the F/F_0 value improves with the increase of signal probe length from 15 to 17 nt, followed by the decrease beyond the length of 17 nt (F and F_0 are the fluorescence intensity in the presence and absence of lncRNA HOTAIR, respectively). Therefore, a 17-nt signal probe is used in the subsequent experiments.

We optimized the concentration of signal probes as well. The high-concentration signal probes will result in the high hybridization efficiency of polyA/signal probe hybrids and consequently the high cleavage efficiency of APE1 and the generation of a high fluorescence signal, but the high-concentration signal probes might cause a high background signal due to the presence of more unquenched fluorophores. As shown in Fig. S1B, the F/F_0 value enhances with the increasing concentration of signal probes from 100 to 400 nM, and reaches the highest value at 400 nM (F and F_0 are the fluorescence intensity in the presence and absence of lncRNA HOTAIR, respectively). Thus, 400 nM signal probe is used in the subsequent research.

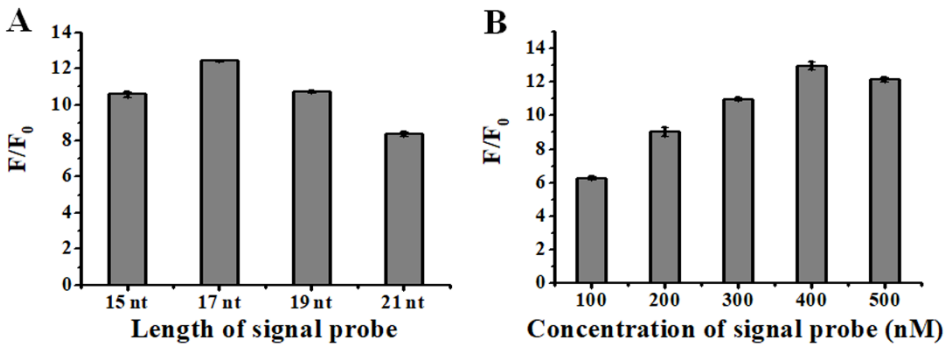

Fig. S1 (A) Variance of the F/F_0 value with different lengths of signal probes. (B) Variance of the F/F_0 value with different concentrations of signal probes. F and F_0 are the fluorescence intensity in the presence and absence of HOTAIR lncRNA, respectively. The concentration of HOTAIR lncRNA is 1 pM. Error bars show the standard deviation of three experiments.

Multiplex Detection of LncRNA in the Total RNA Sample.

We measured lncRNA HOTAIR and lncRNA MALAT1 in the total RNA sample. For the detection of lncRNA HOTAIR in the total RNA sample, the total RNA sample were first added into the hybridization solution containing HOTAIR specific capture probe-functionalized MB, and subsequently the separated target lncRNA HOTAIR was analyzed by the proposed method. After magnetic separation, the supernatant containing the non-target RNA mixture except for the HOTAIR was detected by using the HOTAIR specific capture probes as well. As shown in Fig. S2, in the presence of lncRNA HOTAIR-specific capture probes, only the HOTAIR can be separated from the mixture and generates a distinct fluorescence signal, while the non-target RNAs in the total RNA sample cannot generate a distinct fluorescence signal. Similarly, in the presence of lncRNA MALAT1-specific capture probes, only the target MALAT1 can be separated from the mixture and generates a distinct fluorescence signal, while the non-target RNAs in the total RNA sample cannot generate a distinct fluorescence signal (Fig. S2). These results demonstrate that the

target lncRNA-specific capture probes can only separate the target lncRNA from total RNA sample and DSN has the capability to discriminate the perfect matched DNA/RNA duplexes from single-stranded DNAs and single-stranded RNAs. Therefore, our method can be used for multiplexed detection of various lncRNAs in the total RNA sample.

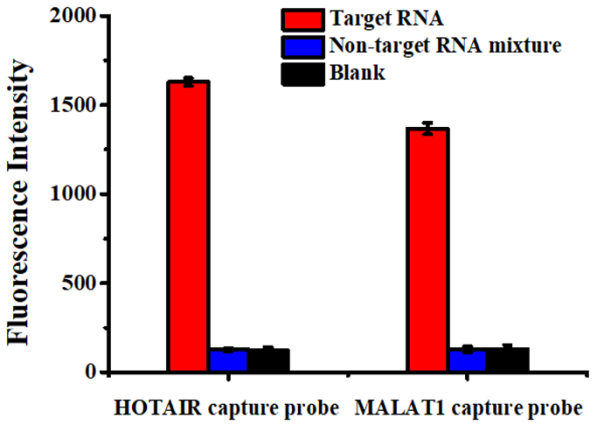

Fig. S2 Multiplexed detection of target lncRNA HOTAIR and lncRNA MALAT1 in MCF-7 cells. The total RNA extracted from 100000 MCF-7 cells was used in this experiment. Error bars show the standard deviation of three experiments.

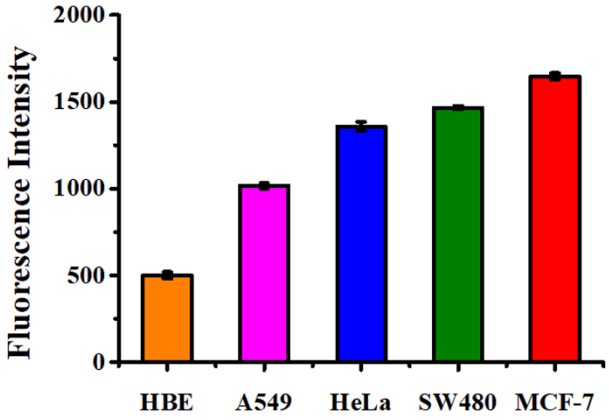

Fig. S3 Measurement of lncRNA HOTAIR in MCF-7 cells, SW480 cells, HeLa cells, A549 cells and HBE cells. The total RNA extracted from 100000 different cell lines was used in this experiment. Error bars show the standard deviation of three experiments