

Electronic Supplementary Information

Highly sensitive detection of telomerase using telomeres-triggered isothermal exponential amplification-based DNzyme biosensor

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Materials. All oligonucleotides were synthesized by TaKaRa Bio. Inc. (Dalian, China), and their sequences were listed in Table 1. The Bst 2.0 WarmStart DNA polymerase (8,000 U.mL⁻¹), nicking endonuclease Nt. BspQI (10,000 U.mL⁻¹), deoxynucleotide triphosphates (dNTPs, 200 μM) were purchased from New England Biolabs (Beverly, MA, USA). SYBR Green I (10000×) was purchased from Xiamen Bio-Vision Biotechnology (Xiamen, China). TWEEN 20, ethylene glycol tetraacetic acid (EGTA), ethylenediaminetetraacetic acid (EDTA), trizma hydrochloride (pH 7.9 and pH 8.3), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt (HEPES), dimethyl sulfoxide (DMSO), sodium chloride (NaCl), magnesium chloride (MgCl₂), potassium chloride (KCl), and lead (II) nitrate were obtained from Sigma-Aldrich. Co (St. Louis, MO, USA). TRAPeZe[®] 1× CHAPS lysis buffer was purchased from Millipore (Bedford, MA, USA). Telomerase inhibitors, 2-[(*E*)-3-naphthalen-2-ylbut-2-enoylamino] benzoic acid (BIBR1532) and N,N-bis(2,3-dihydroxybenzoyl)-1,3-phenylenediamine (MST-312) were purchased from

Boehringer Ingelheim (Biberach, Germany) and Calbiochem (Gibbstown, NJ, USA), respectively.

Other chemicals were of analytical grade and used without further purification. Ultra-pure water obtained from a Millipore filtration system was used throughout.

Table 1. Sequences of the Oigonucleotides^a

Note	Sequence (5'-3')
reverse primer	CCC TTA CCC TTA CCC TTA CCC TAA
molecular beacon	CCA CCA CAT TCA AAT TCA CCA ACT ATrA GGA AGA GAT GTT ACG AGG CGG TGG TGG
telomerase substrate primer	ACC AAC TAT TTC GAC CGG CTC GGA GAA GAG ATG <u>TGA AGA GCA</u> CCA ACT ATT TCG ACC GGC TCG GAG AAG AGA TGT <u>GAA GAG CAA</u> TCC GTC GAG CAG AGT T
synthetic telomerase product (TPC5)	ACC AAC TAT TTC GAC CGG CTC GGA GAA GAG ATG <u>TGA AGA GCA</u> CCA ACT ATT TCG ACC GGC TCG GAG AAG AGA TGT <u>GAA GAG CAA</u> TCC GTC GAG CAG <i>AGT TAG GGT TAG GGT TAG GGT TAG GGT TAG GG</i>

^aThe boldface regions in MB indicate the stem of MB, rA denotes adenosine ribonucleotide at that position while all others are deoxyribonucleotides. The underlined letters symbolize the recognition sequence of nicking endonuclease Nt. BspQI. The italic bold letters in the TPC5 indicate the telomeric repeats.

Preparation of telomerase extracts. HEK293T and HeLa cells were cultured in Dulbecco's

modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), and MRC-5 cells were cultured with 15% fetal bovine serum and 1% L-glutamine in DMEM supplemented with 50 U.mL⁻¹ penicillin and 50 mg.mL⁻¹ streptomycin in a humidified chamber containing 5% CO₂ at 37 °C. The cells were removed from the substrate by trypsinization, washed twice with phosphate buffered saline (pH 7.4), and pelleted at 2,000 rpm for 10 min at 4 °C. About 1 × 10⁶ million cells were resuspended in 200 μL of ice-cold 1× CHAPS lysis buffer, incubated on ice for 30 min, and then centrifuged at 12,000× g for 20 min at 4 °C. After centrifugation, aliquots of the supernatant were rapidly frozen and stored at -80 °C. For the heat pretreatment control, 20 μL of active cell extracts were heated at 85 °C for 10 min prior to the detection.

Telomeres-induced isothermal exponential amplification. The isothermal exponential reaction system (20 μL) contains 1 μL of cell extracts, 100 nM telomerase substrate primer / 100 nM TPC5, 100 nM reverse primer, 30 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 100 mM KCl, 1 mM EGTA, 0.05% (vol/vol) Tween 20, dNTPs (200 mM each), 1 U Bst 2.0 WarmStart DNA polymerase, and 5 U Nt.BspQI NEase. After 20 min telomerase extension at 37 °C, the telomeres-induced exponential isothermal amplification reaction was performed at 55 °C for 30 min.

Real-time fluorescence detection and gel electrophoresis. The real-time fluorescence measurements of telomeres-induced isothermal exponential amplification reaction were performed in a BIO-RAD CFX connectTM Real-Time system (Singapore) with 1× SYBR Green I as the fluorescent indicator, and the fluorescence intensity was monitored at intervals of 30 s. The 12% nondenaturing polyacrylamide gel electrophoresis (PAGE) was carried out in 1× TBE buffer (9

mM Tris-HCl, pH 7.9, 9 mM boric acid, 0.2 mM EDTA) with 1× SYBR Green I as the fluorescent indicator. The gel was scanned by a Kodak Image Station 4000 MM (Rochester, NY, USA).

Catalytic and molecular beacon (CAMB) fluorescence detection. The CAMB fluorescence detection system (50 μL) included 10 μL of the products of isothermal exponential amplification reaction, 80 μM Pb²⁺, 200 nM MB, and the buffer solution (25 mM HEPES and 50 mM NaCl, pH 7.0), and were incubated at room temperature for 50 min. The F-4600 spectrometer (Hitachi, Japan) was used to measure the fluorescence intensity at 518 nm upon excitation at 495 nm.

Inhibition assay. The cells were cultured in serum-including medium and incubated for 6 h prior to the addition of inhibitor. After incubation with various concentrations of inhibitor MST-312 for 72 h, the cells were collected and the telomerase activity of 1000 HEK293T cell equivalence was measured. Inhibition of telomerase activity by various concentrations of inhibitor BIBR1532 was performed in the presence of cell extracts equivalent to 1000 HEK293T cells at room temperature for 1 h. The fluorescent signals in response to different-concentration telomerase inhibitors were obtained by the F-4600 spectrometer, and the relative activity (RA) of telomerase was quantitatively calculated according to the following equation¹:

$$RA = \frac{I_i - I_o}{I_t - I_o} \times 100\%$$

Where I_o , I_t and I_i are the fluorescence intensity in the absence of telomerase, in the presence of telomerase, and in the presence of both telomerase and the inhibitor, respectively. The IC₅₀ value of inhibitor was obtained from the curve-fitting equation.

Real-time fluorescence monitoring of telomeres-triggered isothermal exponential amplification reaction. Real-time fluorescence measurement with SYBR Green I as the fluorescent dye is used to monitor the telomeres-induced isothermal exponential amplification reaction. As shown in Fig. S1A, in the presence of 1×10^{-8} M TPC5 or telomerase extracts equivalent to 1000 HEK293T cells, the real-time fluorescence intensity increases in a sigmoidal fashion as the amplification probe are converted from single-stranded to partially or completely double-stranded DNAs, eventually reaching a plateau once all the single stranded probes have been converted into dsDNAs. Moreover, the signal in the presence of TPC5 or telomerase extracts can be well distinguished from the negative control without the cell extracts and with the heat-inactivated extracts. To further confirm the specificity of the proposed method, we characterized the amplification products using non-denaturing polyacrylamide gel electrophoresis (PAGE). As shown in Fig. S1B, two well-defined bands of DNAzyme (Fig. S1B, lane1, 33 nt and 41 nt) are observed in the presence of TPC5. In contrast, no distinguishable band is observed in the negative control in the presence of TS primer without telomerase (Fig. S1B, lane 2).

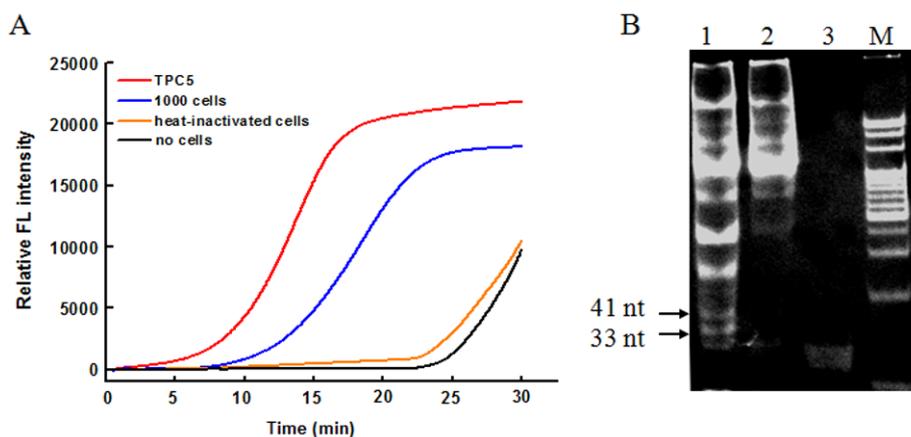


Fig. S1 (A) Real-time fluorescence monitoring of the exponential isothermal amplification reaction: amplification curves of 1×10^{-8} M TPC5 (red), telomerase extracts equivalent to 1000

HEK293T cells (blue), heat-inactivated telomerase extracts equivalent to 1000 cells (orange), and the control without cell extracts (black). (B) Nondenaturing PAGE analysis of EXPAR amplification products: lane 1, in the presence of 1×10^{-7} M TPC5; lane 2, in the presence of 1×10^{-7} M TS primer without telomerase; lane 3, the synthetic DNAzymes (33nt); lane M, the DNA ladder marker.

Optimization of experimental condition. The detection sensitivity of the proposed method is influenced by the experimental condition including the amount of polymerase, nicking endonuclease, the concentrations of the cofactor Pb^{2+} and fluorescence-labeled molecular beacon, and catalytic time of DNAzyme. We employed synthetic telomerase product TPC5, which corresponds to TS primer extended with five telomeric repeats (TTAGGG), as a model to optimize the experimental condition. The cooperation of two enzymes, Bst2.0 WarmStart DNA polymerase and Nt.BspQI nickase, has a crucial effect on the efficiency of EXPAR. High background amplification by thermophilic polymerases in the absence of either templates or priming DNA strands can be accelerated in the presence of nicking endonucleases.^{2,3} The precise mechanism for the enzymatic interaction, cooperation or competition with one another remains unclear, while the reaction might be simply modulated by changing the ratio of polymerase and nicking endonuclease.⁴ With a fixed amount of Nt.BspQI nickase (5 U), the effect of polymerase is firstly investigated (Fig. S2A). The ratio (F/F_0) of fluorescence signal (F and F_0 are the fluorescence intensity in the presence of TPC5 and in the absence of TPC5, respectively) increases with the increasing amount of Bst2.0 WarmStart polymerase from 0.2 U to 1 U, followed by the decrease beyond the polymerase amount of 1 U. We further investigate the influence of Nt.BspQI nickase

upon the fluorescence signal at a fixed amount of Bst 2.0 WarmStart DNA polymerase (1 U). As shown in Fig. S2B, the maximum value of F/F_0 is obtained when the Nt.BspQI amount is 5 U. Thus, 1 U Bst 2.0 WarmStart DNA polymerase and 5 U Nt.BspQI nickase are used in the subsequent research. To achieve the best performance for CAMB system, the concentrations of cofactor Pb^{2+} and fluorescence-labeled MB, and the catalytic time of the DNAzyme are optimized, respectively. As shown in Fig. S2, the maximum value of F/F_0 is obtained when the Pb^{2+} concentration is 80 μM (Fig. S2C), the concentration of MB is 200 nM (Fig. S2D), and the catalytic time of DNAzyme is 50 min, respectively (Fig. S2E). Therefore, the optimal conditions of CAMB system are set to be 80 μM Pb^{2+} , 200 nM MB and catalytic time of 50 min.

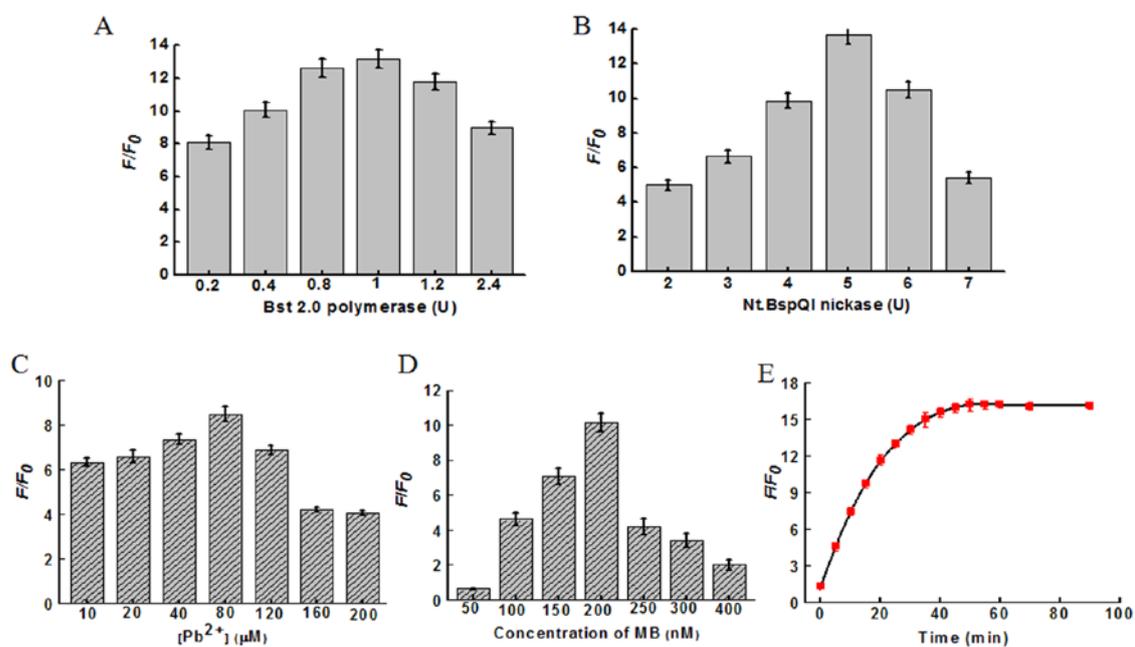


Fig. S2 (A) Variance of the value of F/F_0 with the amount of Bst 2.0 WarmStart DNA polymerase at a fixed amount of Nt.BspQI nickase (5 U). (B) Variance of the value of F/F_0 with the amount of Nt.BspQI nickase at a fixed amount of Bst 2.0 WarmStart DNA polymerase (1 U). (C) Variance of the value of F/F_0 with the concentration of Pb^{2+} . (D) Variance of the value of F/F_0 with MB concentration. (E) Variance of the value of F/F_0 with the catalytic time. F and F_0 are the

fluorescence intensities in the presence of 1×10^{-8} M TPC5 and in the absence of TPC5, respectively. Error bars show the standard deviations of three experiments.

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