Electronic Supplementary Information

One-pot Detection of Telomerase Activity with High Sensitivity and Specificity via RNA FRET Probes and RNase H-assisted Signal Cycling Amplification

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1. Materials and apparatus

The CHAPS lysis buffer was purchased from Millipore Co., Ltd (USA). 3′-azido-3′-deoxythymidine (AZT) and Peroxidase from horseradish was purchased from Sigma-Aldrich. RNase H, 5 × Hybrid RNA Degeneration Buffer, the oligonucleotides, dNTPs, RNase inhibitor, RNase-free water, 6 × nucleic acid sample loading buffer and 20 bp DNA ladder were obtained from TaKaRa Biotechnology Co. Ltd. (Dalian, China). Tween-20, potassium chloride (KCl), Magnesium chloride ($MgCl_2$), ethylene glycol tetraacetic acid (EGTA), 4S Red Plus nucleic acid stain, Trihydroxymethyl aminomethane (Tris), BSA solution (20 mg/mL) and glucose oxidase were obtained from Sangon Biotech Co. Ltd. (Shanghai, China). All the reagents were of analytical grade and were used as received without further purification. The HeLa, A549, CEM, MCF-7, and MRC-5 cell lines were purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China). The oligonucleotides were purified by HPLC and their sequences were as follows:

Telomerase substrate (TS) primer: 5′ - AAT CCG TCG AGC AGA GTT - 3′

RNA probe: 5′ - FAM - rArArT rCrCrG rTrCrG rArGrC rArGrA rGrTrT – BHQ1 - 3′

Synthetic telomerase extension product (TEP): 5′ - AAT CCG TCG AGC AGA GTT TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG - 3′

The fluorescence intensity was detected by using F-7000 fluorescent instrument (Hitachi). The real-time fluorescence measurements were performed with the StepOne Real-Time PCR system (Applied Biosystems, USA). The polyacrylamide gel electrophoresis (PAGE) was visualized by using the Gel Doc^{TM} EZ Imager (Bio-Rad).

2. Cell culture and telomerase extraction

The HeLa, MRC-5 cell lines were cultured in 5 mL MEM Medium (GBICO, Cat. 41500034) containing 10% (v/v) fetal calf serum (GBICO, Cat. 10099141), 1.5 g/L $NaHCO_3$, 0.11 g/L Sodium Pyruvate. The MCF-7 cell lines were cultured in 5 mL MEM Medium (Invitrogen, Cat. 11090081) containing 10% (v/v) fetal calf serum, 1% GlutaMAX (Invitrogen, Cat. 35050061), 1% Non-essential Amino Acids (Invitrogen, Cat. 11140050). The CEM cell line was cultured in 5 mL RPMI-1640 Medium (GIBCO, Cat. 31800022) containing 10% (v/v) fetal bovine serum, 1.5 g/L $NaHCO_3$, 2.5 g/L glucose, 0.11 g/L Sodium Pyruvate. The A549 cell line was cultured in 5 mL F-12K Medium (Invitrogen, Cat. 21127022) containing 10% (v/v) fetal bovine serum. All of the cell lines were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

The cultured HeLa cells or other cells were washed three times with the cold D-PBS buffer (10 mM sodium phosphate buffer, 0.1 M NaCl, pH 7.4 @25°C) after trypsinization (0.2% trypsin, 1 mM EDTA, Invitrogen). After cell counting, 1×10^6 HeLa cells or other cells were collected respectively into a 1.5 mL EP tube. Then the cells were pelleted by centrifuging at 2000 rpm for 10 min. Each cell pellet containing 10^6 cells was suspended in the 200 μL cold CHAPS lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 0.5% CHAPS, 10% glycerol). The lysate was incubated on ice for 30 min (5000 cells/μL) and then centrifuged for 30 min at 12000 rpm at 4 °C. The supernatant containing telomerase was collected carefully and stored at -80°C as the telomerase extracts (5000 cells/μL). Telomerase activity assay was performed by diluting telomerase extracts (5000 cells/μL) to the corresponding cell number using cold CHAPS lysis buffer.

3. Standard protocols of telomerase assay

The reaction mixtures for the telomerase activity assay were prepared separately as part A and part B solution. Telomerase extracts were diluted in cold CHAPS lysis buffer with the respective number of cells; the extracts (1.0 μL) were added to 4.0 μL part A containing extension reaction buffer, 2 μM telomerase substrate (TS) primer, dNTPs, RNase inhibitor with the volume of 5.0 μL. Part B consisted of RNA probe, RNase inhibitor, RNase H, Hybrid RNA Degeneration Buffer. Then part A and part B were mixed together. The reaction was performed in a volume of 10.0 μL containing extension reaction buffer (20 mM Tris-HCl, 1.5 mM $MgCl_2$, 63 mM KCl, 1 mM EGTA, 0.05% Tween-20), 1 μM telomerase substrate (TS) primer, 0.25 μM dNTPs, 4 U/μL RNase inhibitor, 1 μM RNA probe, 0.6 U/μL RNase H, 1 × Hybrid RNA Degeneration Buffer. The mixture solution was incubated for 120 min at 37 °C and finally heated to 95 °C for 5 min to inactivate telomerase activity. For the detection of synthetic telomerase extension product (TEP), TS was replaced by TEP without the telomerase extracts. For the blank control experiment, telomerase extract or TEP was replaced by the CHAPS buffer.

Finally, 190 uL PBS buffer was added to the reaction products, and the fluorescence spectra of the reaction products were recorded with an F-7000 fluorescence spectrophotometer under the excitation of 480 nm.

For telomerase activity inhibitor assay, the experiments were carried out in the same procedures as those for telomerase assay stated above, except the involvement of a fixed telomerase extracts equivalent to 800 HeLa cells and varied concentration of AZT (0 – 30 mM) in the reaction mixture.

For real-time fluorescence detection of telomerase activity, part A and part B were mixed together, followed by being placed in the StepOne Real-Time PCR system.

4. Optimization of the amount of RNase H

Our new strategy for telomerase activity assay relied on the hydrolysis of RNase H that can digest RNA probes hybridized with telomerase extended products. Thus the amount of RNase H was an important parameter for the telomerase activity assay. The effect of the amount of RNase H at different concentrations was investigated by detection of telomerase extracts from 100 HeLa cells. As shown in Figure S1 (A-D), with increasing the amount of RNase H from 1 U to 6 U, the digestion efficiency of RNase H increased gradually, accompanied by the enhancement of fluorescence intensity of reaction in the presence of telomerase extracts. More than 6 U, the fluorescence intensity tends to be stable (Figure S1 E). And the fluorescence intensity of the blank remained almost unchanged. Taking into consideration cost and detection sensitivity, 6 U was selected as the optimum amount of RNase H for the telomerase assay (Figure S1 F).

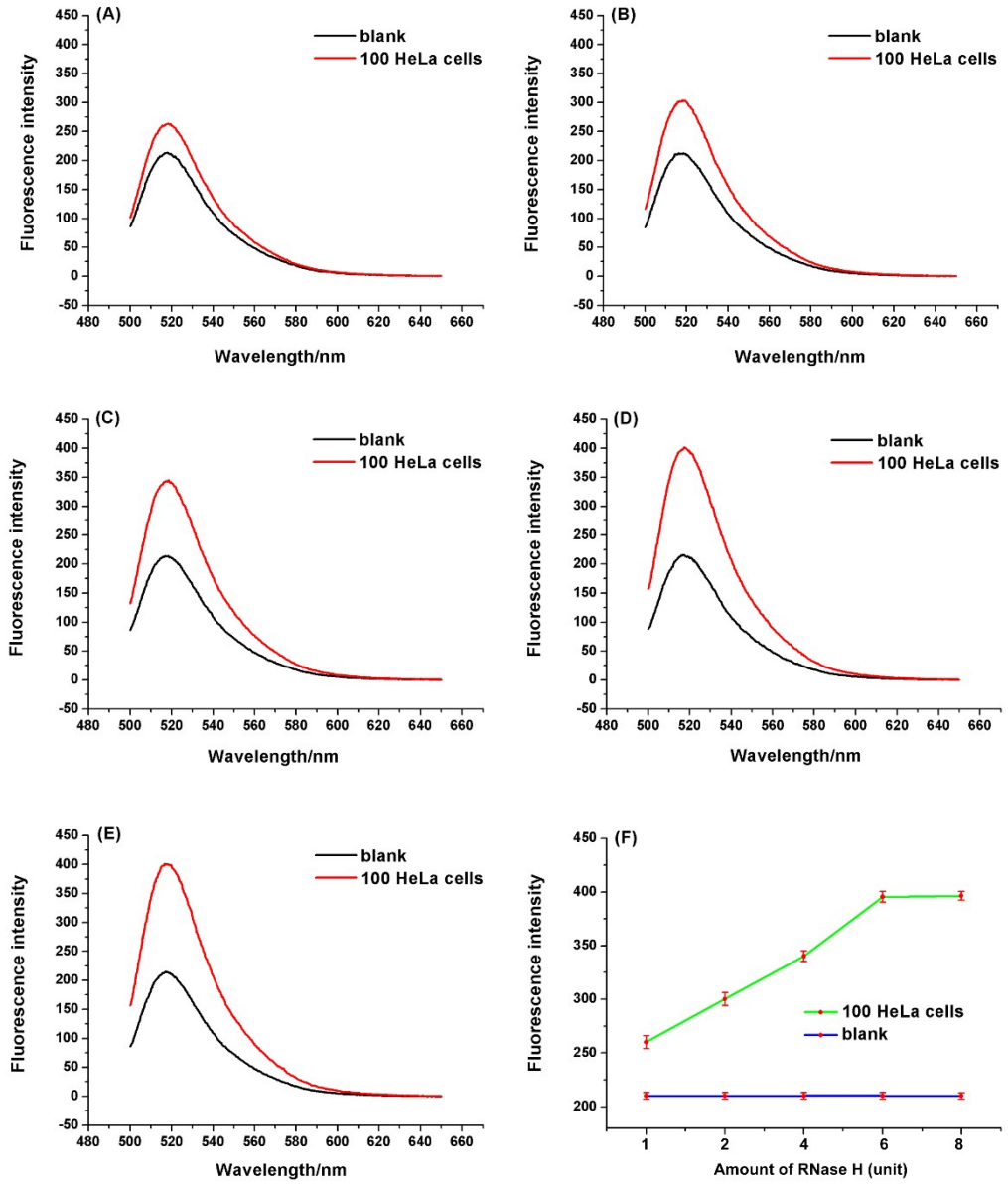

Figure S1. Effect of the amount of RNase H on the telomerase assay. Telomerase activity equivalent to 100 HeLa cells were detected according to the procedures described in the standard protocols of telomerase assay except for the amount of RNase H, which was 1 U (A), 2 U (B), 4 U (C), 6 U (D), 8 U (E). The blank was detected in the same procedure by replacing telomerase extracts with lysis buffer. (F) Fluorescence intensity obtained under different the amount of RNase H. Error bars represent the standard deviation of three repetitive tests.

5. Feasibility of the proposed one-pot strategy for the detection of telomerase activity in the crude lysates

We further investigated feasibility of the proposed one-pot strategy for the detection of telomerase activity in the crude lysates of 5, 10 and 20 cells, respectively. The cultured HeLa cells were washed three times with cold D-PBS

buffer after trypsinization, and then re-suspended in the cold D-PBS buffer at a concentration of 10^4 cells/μL. For the telomerase activity analysis of 20 cells, 10 cells or 5 cells, firstly, the cell suspension was serially diluted to 20 cells/μL, 10 cells/μL or 5 cells/μL in the cold D-PBS buffer, 1 μL diluted cell suspension (containing 20 cells, 10 cells or 5 cells) was transferred to the microslide, and then 20 cells, 10 cells or 5 cells were counted and manipulated by using Narishige micromanipulator system equipped on an Olympus IX53 inverted microscope with a monitor. Subsequently the 20 cells, 10 cells or 5 cells were dropped into 1 μL cold CHAPS lysis buffer, incubated for 30 min on ice. The cell lysates were then immediately used as the samples to detect the telomerase activity. As shown as in Figure S2, as low as 5 cells can also be well detected, which coincides with that for detection of the diluted extracts.

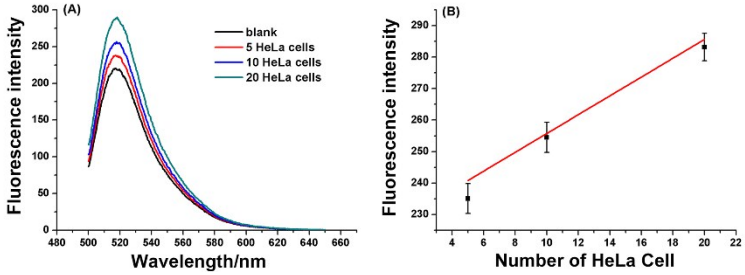

Figure S2. (A) Fluorescence spectra for detecting cell lysates from 5, 10 and 20 HeLa cells, respectively. The CHAPS buffer was used as the blank. (B) The linear plot of the fluorescence intensity at 520 nm versus the number of the HeLa cells. Error bar indicate standard deviation of three replicative tests.

6. Selectivity evaluation of the one-pot system for telomerase activity assay

We have investigated whether other proteins including of ProtoScript II reverse transcriptase, BSA, HRP and GOD could interfere with the detection of telomerase activity. It can be seen from Figure S3 that only the telomerase enhances a significant fluorescence signal. The fluorescence signals produced by ProtoScript II reverse transcriptase, bull serum albumin (BSA), horse radish peroxidase (HRP) and glucose oxidase (GOD) are almost the same with the blank control, clearly demonstrating the high selectivity of the proposed assay for telomerase activity detection.

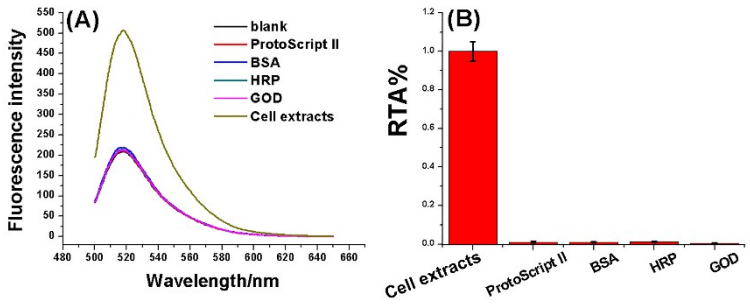

Figure S3. Selectivity evaluation of the one-pot system towards ProtoScript II reverse transcriptase, BSA, HRP and GOD. (A) Fluorescence spectra of the blank, ProtoScript II reverse transcriptase (100 U), BSA (0.1 μg), HRP (0.1 μg), GOD (100 U), HeLa cell extracts equivalent to 200 cells, respectively. (B) Relative detection of the different proteins. Error bars represent the standard deviation of three repetitive tests. The RTA of the HeLa cell extracts is normalized to be 1.0.

7. Inhibition assay of telomerase activity

To demonstrate the specificity and the potential application in telomerase activity inhibitor screening, the proposed method is further applied to the inhibition assay. 3′-azido-3′-deoxythymidine (AZT), a potent inhibitor of telomerase activity, is chosen for this study. The experiments were performed at a fixed HeLa cell extracts (equivalent to 800 cells) in the presence of AZT with different concentrations. As can be seen from Figure S4, fluorescence intensity decreased gradually with increasing concentration of AZT in the range 0.5-30 mM, clearly indicating the inhibition of telomerase activity. Taking advantage of simplicity and high sensitivity, the RNA probe and RNase H-assisted telomerase assay shows high specificity for detection of telomerase activity and great potential in high-throughput screening for telomerase activity inhibitors and for anticancer drug development.

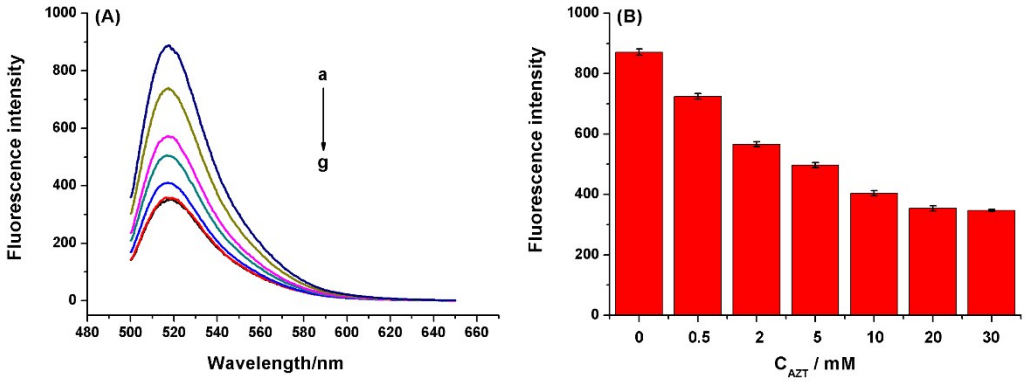

Figure S4. Inhibition assay of telomerase activity. Fluorescence spectra (A) and histogram (B) in response to telomerase extracts equivalent to 800 HeLa cells were incubated with different concentrations of AZT, the

fluorescence spectra from a to g refer to 0, 0.5, 2, 5, 10, 20, and 30 mM AZT, respectively. Error bars represent the standard deviation of three repetitive tests.

8. Comparison between the proposed one-pot assay and other reported methods for the detection of telomerase activity

Table S1. Comparison between the proposed one-pot assay and other reported methods for the detection of telomerase activity

Method	System	Steps of experiment	Detection limit	References
PCR-based assay	Telomeric repeat amplification protocol (TRAP)	Two	1 cell	13
PCR-based assay	TRAP based on primer-modified gold nanoparticles	Four	10 cells	15
Optical	Hemin/telomere-G-quadruplex/L-cysteine/aggregation AuNPs	Three	2700 cells	17
Fluorescence	Taqman probe and T7 exonuclease	Three	5 cells	21
Chemiluminescence	DNAzyme	Four	1000 cells	22
Electrochemical	Mimic hybridization chain reaction using spherical nucleic acids gold nanoparticles	Four	10 cells	24
Electrochemical	Conformation switching/nanoscaled porphyrinic Metal−Organic Frameworks	Four	100 cells	25
Fluorescence	Stem-loop primer-mediated exponential amplification	Two	1 cell	29
Electrochemical	T7 exonuclease and 5' methylene blue-labeled hairpin probe	Two	1 cell	30

Fluorescence	RNA FRET probe and RNase-H	One	5 cells	This work