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Supporting information

A. Experimental section

Materials

Nanopure water (18.2 MΩ; Millipore, USA) was used in all experiments. SYBR Green I (SG) was purchased from Lonza Rockland Inc., USA. All other chemicals were purchased from Sigma-Aldrich and were used without further purification. All oligonucleotides used in this study were synthesized by Bioneer Corporation (Daejeon, South Korea). The sequences used were as follows: TS primer: AAT CCG TCG AGC AGA GTT; chemically synthesized telomerase product (CST): AAT CCG TCG AGC AGA GTT TTA GGG TTA GGG TTA GGG TTA GGG.

Cell culture and telomerase extract preparation

Various cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, and the cells were maintained at 37°C in a humidified atmosphere (95% air and 5% CO_2).

The telomerase extracts were prepared according to the previously reported protocol with slight modifications.[S1] Briefly, HeLa cells were first collected in the exponential phase of growth and counted. Next, 1×10^6 cells were transferred into an RNase-free 1.5-mL EP tube and washed twice with ice-cold PBS by centrifugation at 1,800 rpm for 5 min. After discarding the supernatant, the cells were resuspended in 150 μL of ice-cold RIPA lysis buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS), incubated for 30 min on ice, and centrifuged at 12,000 rpm for 30 min at 4°C. Finally,

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the supernatant was collected and either used immediately for the telomerase assay or frozen at -80°C. The telomerase extracts from other cells were prepared using a similar protocol.

Tumor tissue and telomerase extract preparation

Frozen tumor tissue samples were weighed, homogenized in buffer containing 20 mM Tris-HCl pH 8.3, 1.5 mM $MgC1_2$, 63 mM KCl, and centrifuged at 12,000 rpm for 10 min at 4°C. The tissue extract supernatants were analyzed for protein content by Bradford assay. The tumor tissues used in this study were provided by the Biobank of Chungnam university hospital, a member of Korea Biobank and the protocol was approved by the Institutional Review Board (IRB) of KRIBB (KRIBB-IRB-20130503-01).

Telomerase extension reaction

Telomerase extracts were first diluted in lysis buffer with the required number of cells, and the extracts (5 μL) were added to 50 μL of the telomerase extension reaction buffer (20 mM Tris-HCl pH 8.3, 1.5 mM $MgC1_2$, 1 mM EGTA, 63 mM KCl, 0.05% Tween 20, 1 mM dNTPs, and 200 nM TS primer). The solution was then incubated at 37°C for 2 hours. In the inhibition experiments, different volumes of 2, 6-diaminoanthraquinone solution were also added to the telomerase extension reaction buffer to achieve the desired final concentrations.

Fluorescence detection of telomerase activity.

After the telomerization reaction, the resulting solution was diluted with H_2O to 95 μL, and 5 μL SG 400X was then added to the diluted solution. After 5 min, the fluorescence intensity of the solution was recorded at room temperature in a quartz cuvette (with a path length of 10 mm and an inner width of 1 mm) on an LS 55 fluorescent spectrometer (Perkin Elmer,

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UK). The excitation wavelength was 490 nm, the emission wavelengths were in the range of 510 to 700 nm, the slit widths of both excitation and emission were at 5 nm, and the scanning speed was 600 nm/min. In some experiments, the fluorescence intensity at 530 nm was used for more detailed calculations.

B. Supporting data

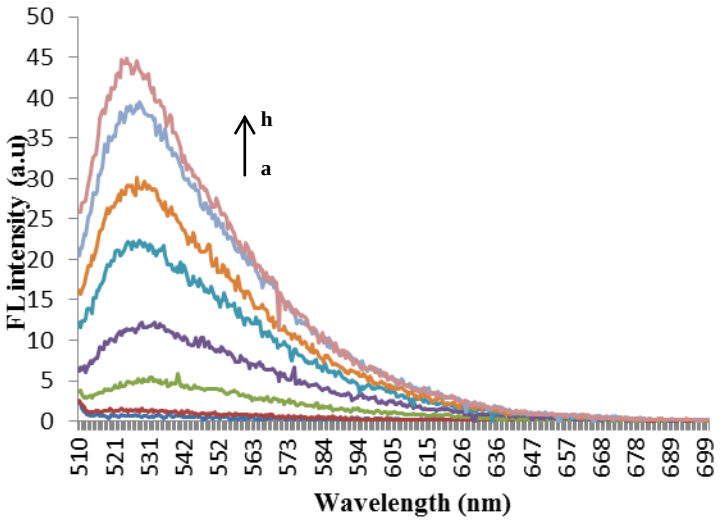

Figure S1. Fluorescence intensity changes correspond with different concentrations of the chemically synthesized telomerase product. The arrow from a to h represent the solution of 5 µL SG with water, TS primer 200 nM, CST 2 nM, CST 5 nM, CST 10 nM, CST 20 nM, CST 50 nM, CST 100 nM, respectively.

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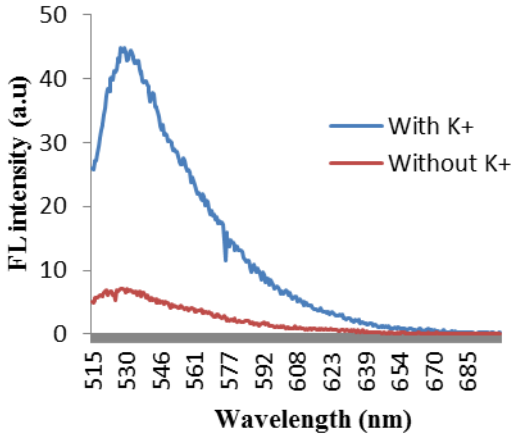

Figure S2. A comparison in fluorescence intensity between with and without the addition K^+ into the solution of CST at the same concentration (100 nM). KCl (50 mM) was added into the K^+-solution, followed by incubation at room temperature for more 30 min. Then, 5 µL of SG was added and fluorescence intensities were recorded after 5 min.

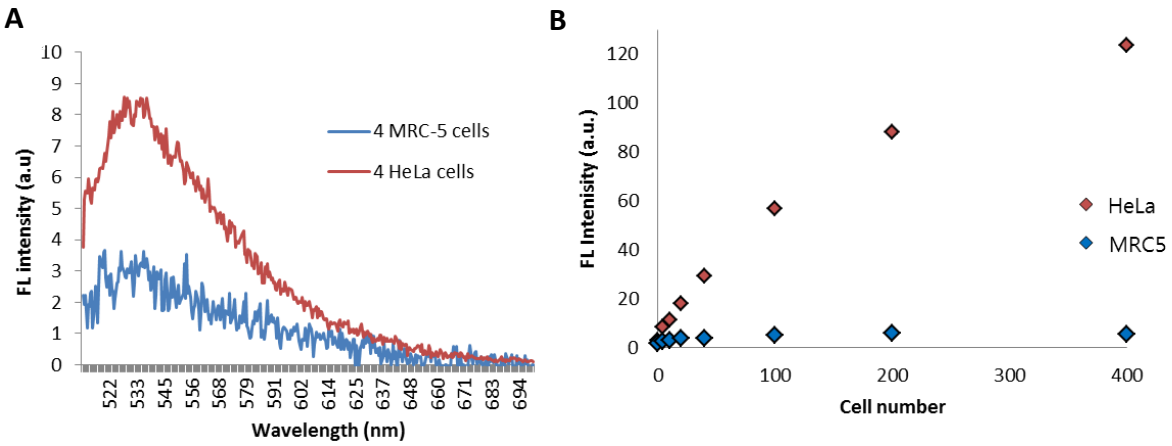

Figure S3. Analysis of telomerase activity in HeLa cells and MRC5 cells: (A) telomerase activity of cell extracts equivalent to 4 cells from MRC-5 and HeLa and (B) fluorescence intensities plotted as a function of different number of cells from 0 to 400 cells.

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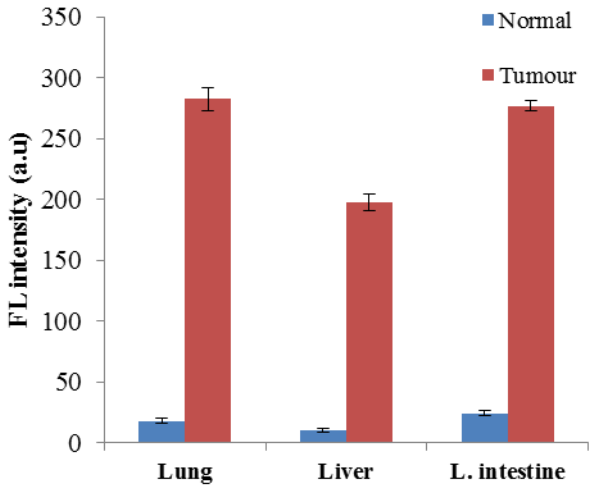

Figure S4. Telomerase activity in various tumour tissues. Telomerase assays were performed using extracts containing 10 μg of total protein from surgically resected lung, liver and large intestine cancer tissues and matched adjacent noncancerous tissues (normal).

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Table S1. A comparison in sensitivity between the proposed method and other reported assays for the detection of telomerase activity.

Detection method	**Strategy**	**Sensitivity**
Scanometric [S2]	Bio-barcode	10 cells
Gel analysis or RT PCR [S3]	Primer-modified AuNP	10 cells
Fluorescence [S4]	Exonuclease III-aided	30 cells
Chemiluminescence [S5]	DNAzyme	100 cells
Impedance intensity [S6]	Electrochemical	1000 cells
Colourimetric [S7]	Primer-modified AuNP	1 cell
Electrochemical [S8]	Bio-barcode with $[Ru(NH_3)_6]^{3+}$	10 cells
Fluorescence [S9]	T7 exonuclease-assited	5 cells
Electrochemiluminescence [S10]	Porphyrin-functionalized graphene	10 cells
Real - time detection [S11]	Exponential isothermal amplification	1cell
Fluorescence [S12]	DNAzyme-based	0.1 - 1μg protein
Transmission loss spectrum [S13]	Silicon microring resonator-based biosensor	10 cells
Fluorescence [S14]	DNAzyme-based	200 cells
Fluorescence [S15]	Cascade isothermal signal amplification	3 cells
Fluorescence (this paper)	Sybr Green intercalating	4 cells

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