

Electronic Supplementary Information

**Dendrimer-like polymeric DNAs as a chemiluminescence probe for
amplified detection of telomere DNA on a solid-phase membrane**

*Ahmed F. M. El-Mahdy, Takayuki Shibata, Tsutomu Kabashima, Masaaki Kai**

Faculty of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Nagasaki University,
Nagasaki 852-8521, Japan.

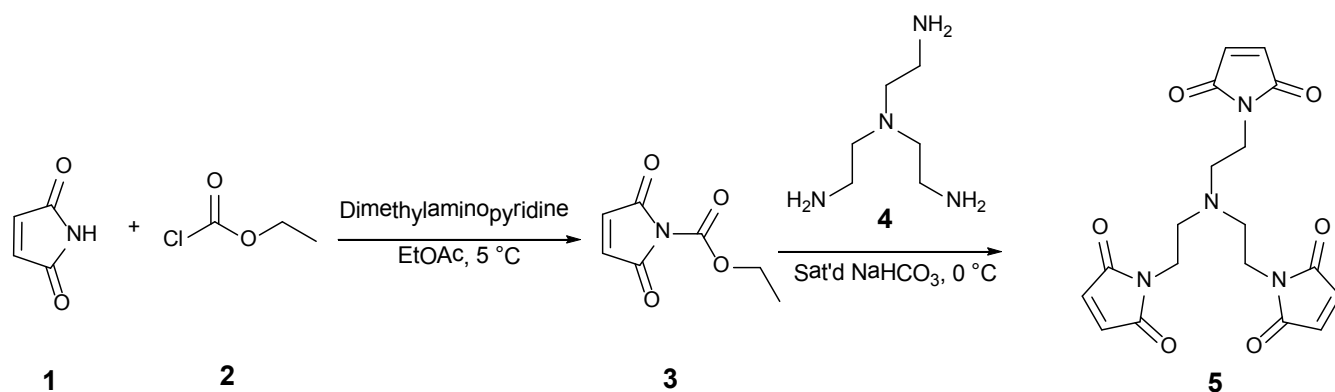
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1. Chemicals

All chemicals were obtained of analytical grade and were used as received without further purification. The water was purified using a Millipore water-purification system. Thiol-blocked sense DNA (HO-(CH₂)₆-S-S-(CH₂)₆-5'-TTAGGGTTAGGGTTAGGG-3'), thiol-blocked antisense DNA (HO-(CH₂)₆-S-S-(CH₂)₆-5'-CCCTAA-CCCTAACCTAA-3'), 5'-biotin-label antisense ssDNA (biotin-5'-CCCTAACCTAACCTAA-3') and telomere DNA (5'-TTAGGG(TTAGGG)₈TTAGGG-3') were acquired from Sigma Genosys Japan (Ishikari, Japan). Tetra-*n*-propyl ammonium hydroxide (TPA) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and its pH was adjusted to 8.5 with conc. H₃PO₄. A LuminGLO[®] kit (Streptavidin-horseradish peroxidase (HRP) and CL substrates) was obtained from KPL Inc. (Gaithersburg, MD, USA). NAP-10 columns were obtained from GE Healthcare (Buckinghamshire, UK). Nylon membranes (0.45 μm) were obtained from Atto (Tokyo, Japan).

2. Synthesis of tris(2-maleimidoethyl)amine (TMEA) (5)



Scheme S1. Synthetic route of TMEA (5)

TMEA was synthesized as previously reported,^[2] but with a slight modification as follows (scheme S2). Maleimide (**1**, 2.0 g, 20 mmol) was dissolved in 20 mL of ethylacetate in a 100-mL round-bottom flask, and the solution was cooled to 5 °C. 4-Dimethylaminopyridine (2.77 g, 22 mmol) was added to the solution. Ethylchloroformate (**2**, 1.96 mL, 20 mmol) in 10 mL of ethylacetate was added dropwise, and the mixture was allowed at room temperature while stirring for 1h. The solution was diluted with 100 mL of ethylacetate and washed with 100 mL of H₂O containing saturated sodium chloride. The organic layer was separated, dried over MgSO₄, and filtered. The solvent was removed under reduced pressure to give oil. The resulting *N*-(ethoxycarbonyl)maleimide (**3**) was purified on silica gel column chromatography with methylene chloride : ethylacetate (66:34, v/v) to yield the product as oil (2.8 g, 80 %). ¹H-NMR (400 MHz, D₃-CDCl₃, 25 °C, TMS) (Fig. S1): δ 6.75 (2H, d, *J* = 0.6 Hz), 4.35 (2H, q, *J* = 2.8 Hz), 1.3 (3H, t, *J* = 2.8 Hz). MS *m/z* (ESI+) 191.1 [M +Na]⁺.

Tris(2-aminoethyl)amine (**4**, 100 mg, 0.68 mmol) was dissolved in 5 mL of water and THF (1:1, v/v) with saturated NaHCO₃. At 0 °C the solution was mixed with *N*-(ethoxycarbonyl)maleimide (**3**, 703 mg, 4.13 mmol). To the mixture, 20 mL of water and THF (1:1, v/v) with saturated NaHCO₃ was added, and then stirred for 4 h. After that, extraction was carried out with ethylacetate (3 x 100 mL) at 0 °C and the organic layer was washed with 100 mL of saturated sodium chloride aqueous solution. The organic layer was dried over MgSO₄, and then the solvent was removed under reduced pressure. The desired compound, TMEA (**5**) was obtained as a light yellow solid (150 mg, 57 %, mp = 98-100 °C). ¹H-NMR (400 MHz, D₆-DMSO, 25 °C, TMS) (Fig. S2): δ 6.7 (6H, s), 3.52 (6H, t, *J* = 6.6 Hz), 2.71 (6H, t, *J* = 6.6 Hz). ESI-MS (Fig. S3): *m/z* = 386.12 [M +Na]⁺.

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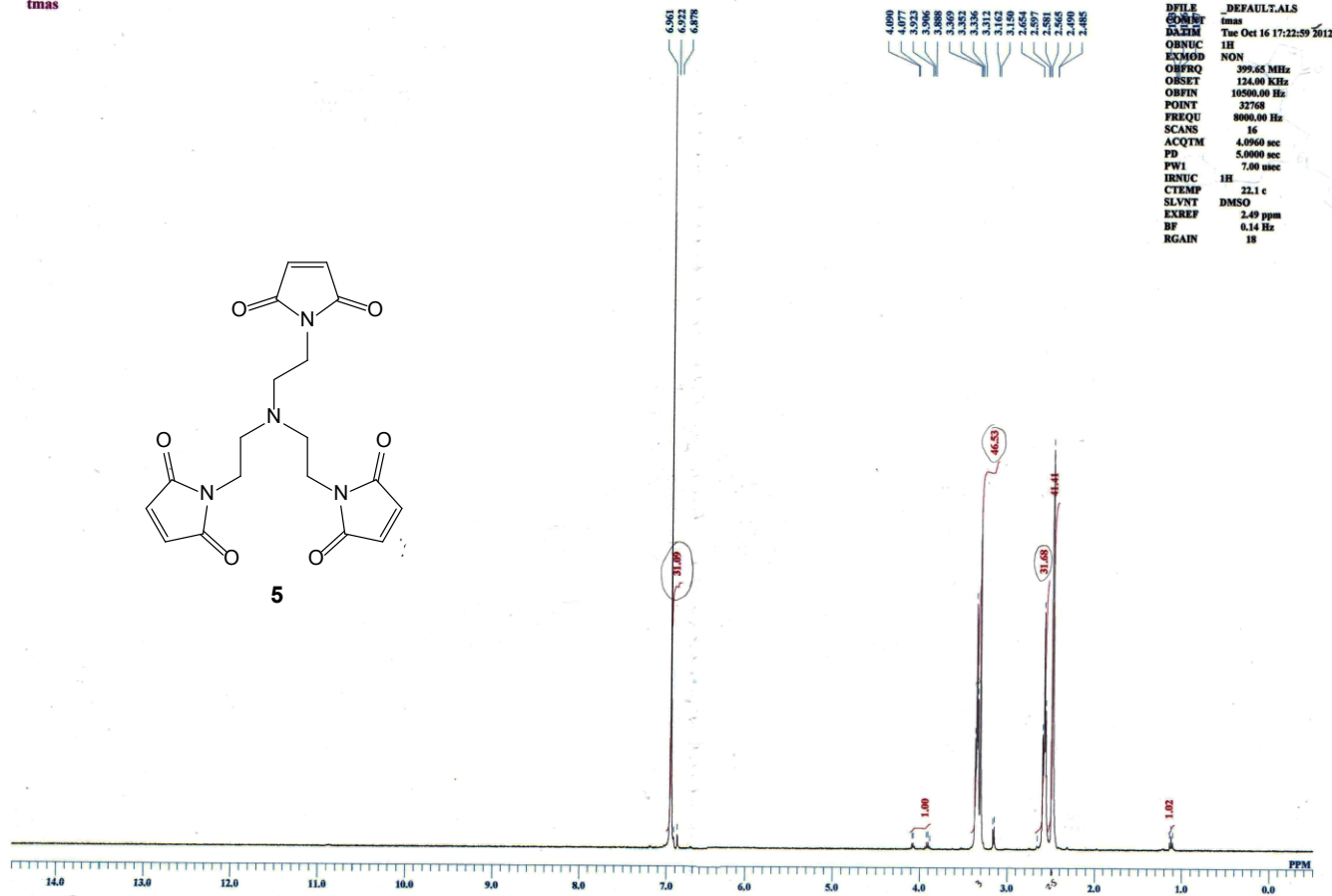


Fig. S2 ¹H-NMR spectrum of TMEA (5)

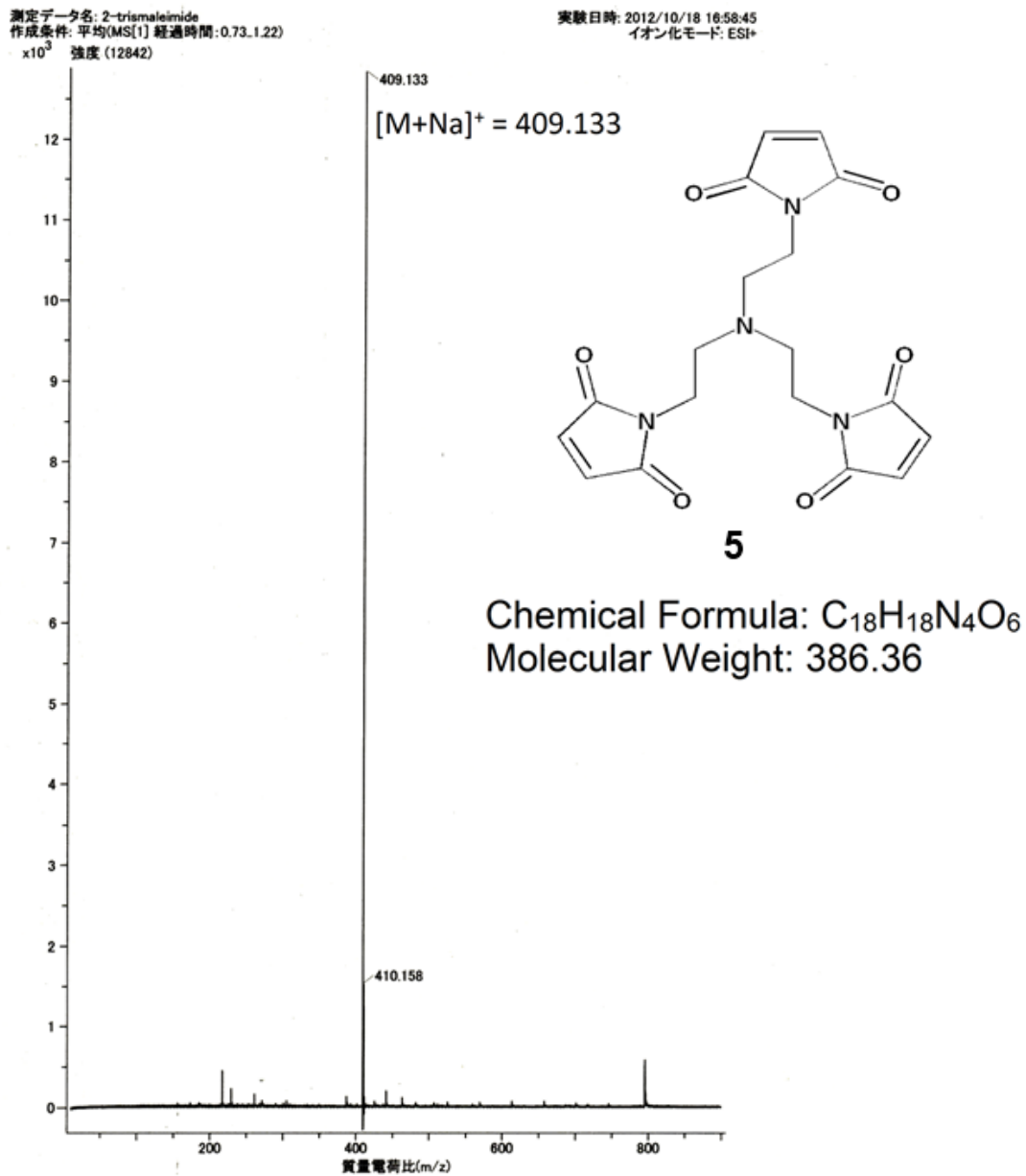


Fig. S3 Mass spectrum of TMEA (**5**)

3. Synthesis of YY-DNAs (Scheme 2 in text)

Sense and antisense ssDNAs possessing a thiol group at 5'-end of telomere were prepared as follows: 125 μL of 100 mM DTT in sodium phosphate buffer (pH 8.5) was mixed with 63.5 nmol of thiol-blocked sense (372.23 μg) or antisense (351.07 μg) DNA at the 5'-end, and incubated at room temperature for 1 h to produce free thiol group. After incubation, the excess DTT and other byproducts were removed by using a NAP-10 column and the concentration was determined by measuring the absorbance at 260 nm. For the preparation of Y-DNA and Y-cDNA, 180 nmol of the sense or antisense ssDNA possessing thiol group at 5'-end in 100 μL of 1x PBS (pH 7.4) was reacted with 60 nmol of TMEA dissolved in 6 μL of DMSO. After overnight incubation at room temperature, the residual TMEA and DMSO were eliminated through the NAP-10 column. Equal amounts of each resulting product of Y-DNA and Y-cDNA were assembled by incubation in 1x PBS buffer (pH 7.4) at 37 °C for 1 h to yield the probe of YY-DNAs. The products of Y-DNA, Y-cDNA and YY-DNAs were analyzed by 20 % polyacrylamide gel electrophoresis (PAGE) (Fig. 1). The gel was stained with a fluorescent dye, SYBR-Gold (InvitrogenTM, Eugene, USA). Each band intensity was calculated by a densitometry using Image J software (National Institute of Health, USA).

4. Bright-field optical microscopy and dynamic light scattering of YY-DNAs

Dendrimer-like polymeric DNAs (30 μL) was placed onto the microscope glass and then the YY-DNAs polymeric spheres were observed with a microscope (EVOS digital) equipped with x 40. The image was acquired with Micron (EVOS) digital imaging software. The observation was performed at room temperature.

The spheres diameter of the YY-DNAs was measured by a dynamic light scattering (DLS) technique. The YY-DNAs dispersed into 1x PBS buffer, pH 7.4 at the concentration of 0.5 mg/mL. The DLS measurement was performed at 25 $^{\circ}\text{C}$ using a Zetasizer Nano ZS (Malvern Instruments, Worcs, UK) at 90 $^{\circ}$ to the incident beam (623 nm wavelength from 4 mW He-Ne laser tubes) in 1-cm length quartz cuvette. Data fitting was carried out using a multimodal algorithm supplied by Malvern Instruments and then the hydrodynamic diameter was identified using the Einstein-Stokes equation.

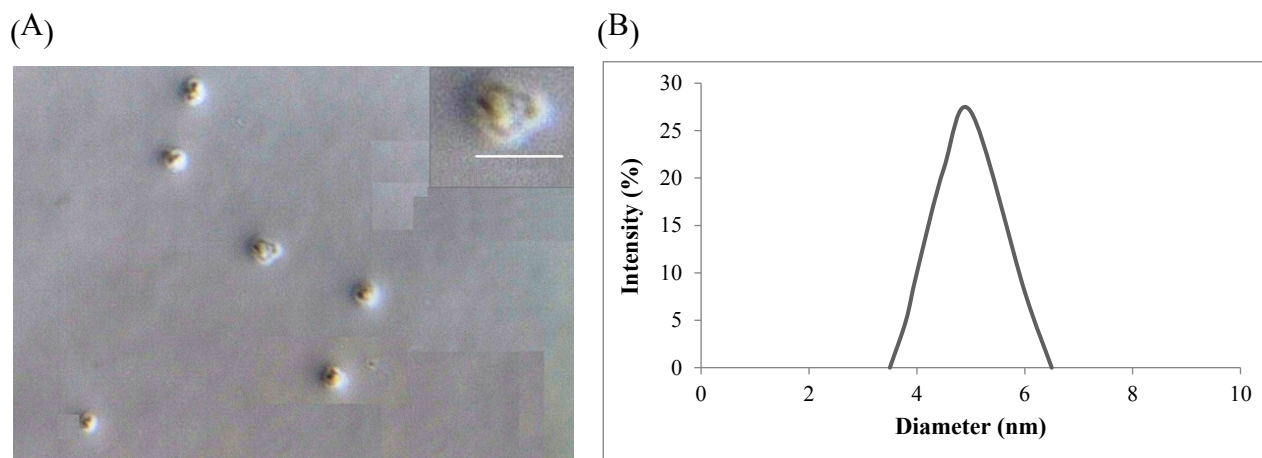


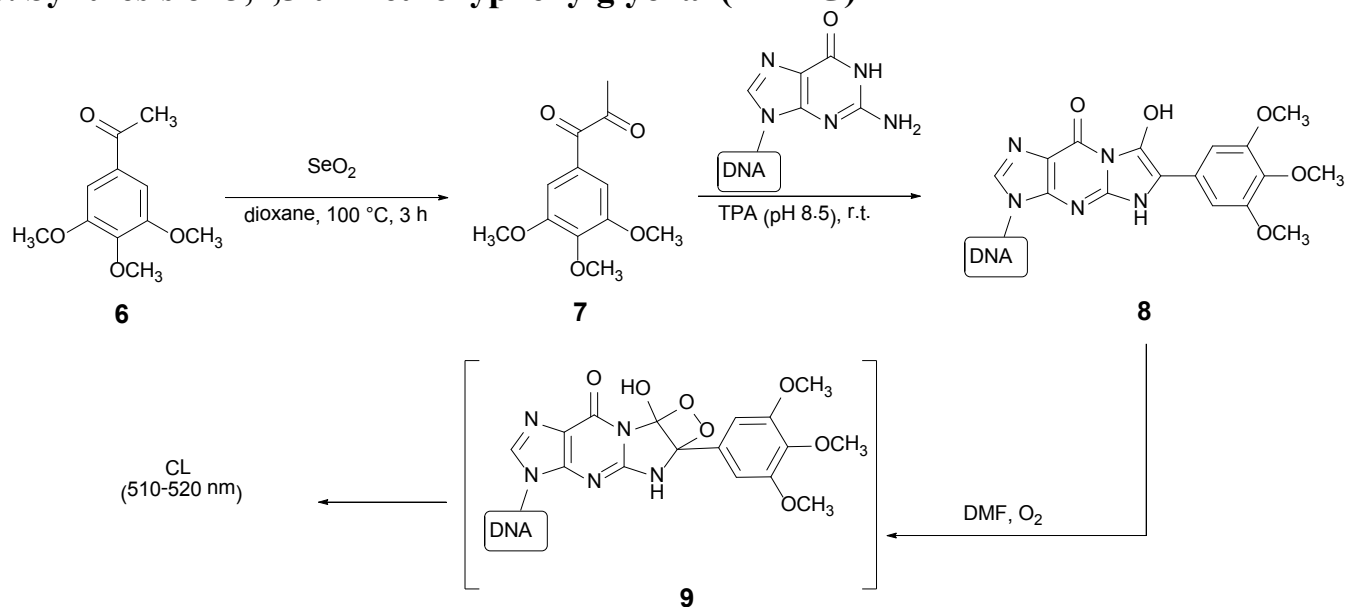
Fig. S4 (A) Bright-field optical microscopy image of YY-DNAs. Scale bar, 6 μm . (B) Size distribution of polymeric YY-DNAs spheres obtained by using dynamic light scattering technique.

5. CL detection of Y-DNA, Y-cDNA, YY-DNAs and biotin-cDNA on a nylon membrane (Fig. 2 in text)

A nylon membrane was spotted with the products of Y-DNA (2.9-46 pmol), Y-cDNA (2.9-46 pmol) and YY-DNAs (0.08-1.4 pmol) and dried in vacuo for 20 min. The membrane was immersed into 0.1 M tetra-*n*-propylammonium hydroxide (TPA, pH 8.5) for approximately 10 s and then immediately dipped into 30 mM TMPG solution in DMF for approximately 15 s at room temperature before CL detection for 2 min by a charge-coupled device (CCD) camera running an operating system of Atto densitography cool saver (ver. 2.0) (light capture AE-6972 device, Atto Co., Tokyo, Japan). The data of CL imaging were analyzed with an Atto CS analyzer (ver. 2.0).

5'-Biotin labeled cDNA (18 mer) of 2.9, 5.7, 11.5, 23 and 46 pmol/spot were spotted on a nylon membrane. After drying in vacuo at 42 °C for 20 min, the membrane was blocked with 2 mL of Church's buffer at 42 °C for 2 h. The membrane was then washed with 2 mL of 1x SSC buffer containing 1 % SDS at 30 °C for 5 min, and 2 mL of H₂O at 30 °C for 5 min. The membrane was then immersed with gentle shaking for 20 min at room temperature into 2 mL of 1/500x streptavidin-HRP in 1x SSC buffer containing 1 % SDS. After washing with 2 mL of 1x SSC buffer containing 1 % SDS solution and 2 mL of H₂O at 30 °C for 5 min, the membrane was immersed into LuminGLO[®] solution for 60 s before CL detection for 2 min with a CCD camera.

6. Synthesis of 3,4,5-trimethoxyphenylglyoxal (TMPG) (7)



Scheme S2. Synthesis of TMPG (7) and its CL reaction with guanine in DNA

TMPG was synthesized according as previously described,^[1] but with a slight modification as follows. To a stirred solution of selenium dioxide (45 mmol) in 40 mL of dioxane was added 3,4,5-trimethoxyacetophenone (6, 50 mmol) at $40\text{ }^\circ\text{C}$. The mixture was refluxed for 2 h, and selenium dioxide (45 mmol) was then added to the reaction mixture. After reflux for 3 h, the mixture was filtered to remove insoluble selenium. The filtrate was mixed with 240 mL of H_2O and then kept at $4\text{ }^\circ\text{C}$ for approximately 15 h. The formed precipitate was recrystallized from water to give pure TMPG (7) as a colorless needle (m.p. $101\text{-}102\text{ }^\circ\text{C}$, yield = 60-70 %). TMPG was unstable in DMF, and thus could be prepared just before use. TMPG could be reacted with guanine of a nucleic acid in 0.1 M TPA aqueous solution (pH, 8.5) at room temperature to form derivative 8 which undergoes oxidation in DMF at room temperature to yield an unstable CL derivative 9 for the generation of intensive CL light.^[1]

7. CL detection of telomere DNA with YY-DNAs (Fig. 3 in text)

Telomere DNA was spotted on a nylon membrane (0.5, 1, 2.5, 5 and 10 pmol/spot), and then the membrane was dried in vacuo at 42 °C for 20 min. The membrane was blocked with 2 mL of Church's buffer at 42 °C for 2 h. After blocking, the membrane was washed with 2 mL of 1x SSC buffer containing 1 % SDS at 30 °C for 5 min, and 2 mL of H₂O at 30 °C for 5 min. The membrane was then submerged into 2 mL of a hybridization buffer (1x SSC buffer containing 1 % SDS) containing 6 µg of YY-DNAs at 42 °C for 16 h. After washing with 2 mL of 1x SSC buffer containing 1 % SDS at 30 °C for 5 min, and 2 mL of H₂O at 30 °C for 5 min, the membrane was immersed into 0.1 M TPA (pH 8.5) for approximately 10 s and successively dipped into 30 mM TMPG in DMF for approximately 15 s at room temperature, and immediately the CL intensity was detected for 2 min with CCD camera.

8. Conditions of the direct hybridization of telomere DNA with YY-DNAs

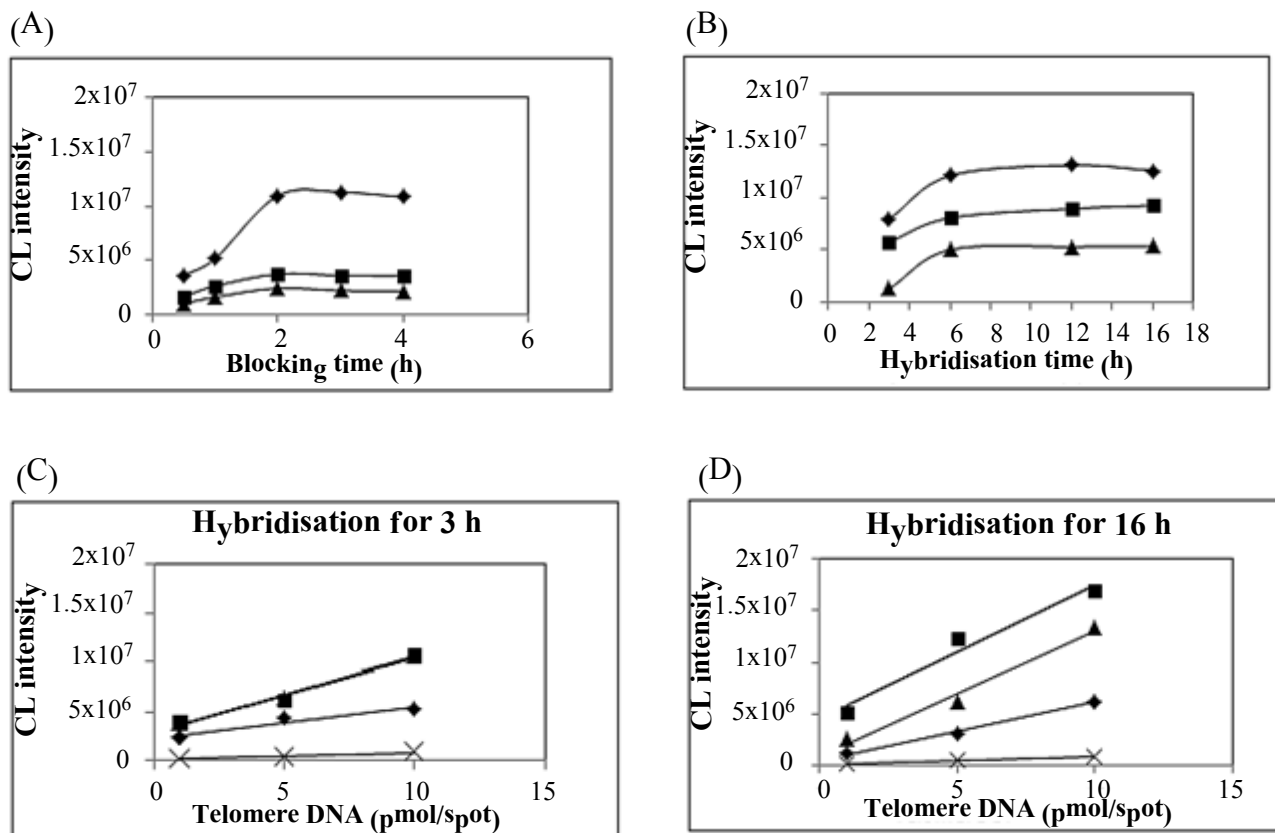


Fig. S5 Effects of (A) blocking time and (B) hybridization time on the direct hybridization of (▲) 1 pmol, (■) 5 pmol and (◆) 10 pmol of telomere DNA per spot on a nylon membrane with YY-DNAs. The effect of the amount of telomere DNA spotted onto the membrane on the hybridization for (C) 3 h and (D) 16 h at (◆) 37 °C, (■) 42 °C and (▲) 60 °C. The blank (X) was the same way, but without the probe of YY-DNAs. The protocol was explained in Experimental section in text.

9. Enzymatic CL-detection using a biotin-labeled cDNA probe

In order to compare with our detection method, an enzymatic CL-detection of telomere DNA blotted on the same nylon membrane was carried out by the hybridization with antisense DNA possessing a biotin group at 5'-end, and followed by binding the biotin group with streptavidin-HRP, as follows. A nylon membrane was spotted with telomere DNA (60 mer) of 0.5, 1, 2, 5 and 10 pmol/spot. After drying in vacuo at 42 °C for 20 min, the membrane was blocked with 2 mL of Church's buffer at 42 °C for 2 h. The membrane was then washed with 2 mL of 1x SSC buffer containing 1 % SDS at 30 °C for 5 min, and 2 mL of H₂O at 30 °C for 5 min. Hybridization solution (1x SSC and 1 % SDS, 2 mL) containing 2 µg of biotin-labeled cDNA probe was added to the membrane and incubated at 42 °C for 16 h. The hybridization solution was removed and then the membrane was washed with 2 mL of 1x SSC buffer containing 1 % SDS and 2 mL of H₂O at 30 °C for 5 min. The membrane was then immersed with gentle shaking for 20 min at room temperature into 2 mL of 1/500x streptavidin-HRP in 1 x SSC buffer containing 1 % SDS. After washing with 2 mL of 1 x SSC buffer containing 1 % SDS solution and 2 mL of H₂O at 30 °C for 5 min, the membrane was immersed into LuminGLO[®] solution for 60 s before CL detection for 2 min with a CCD camera.

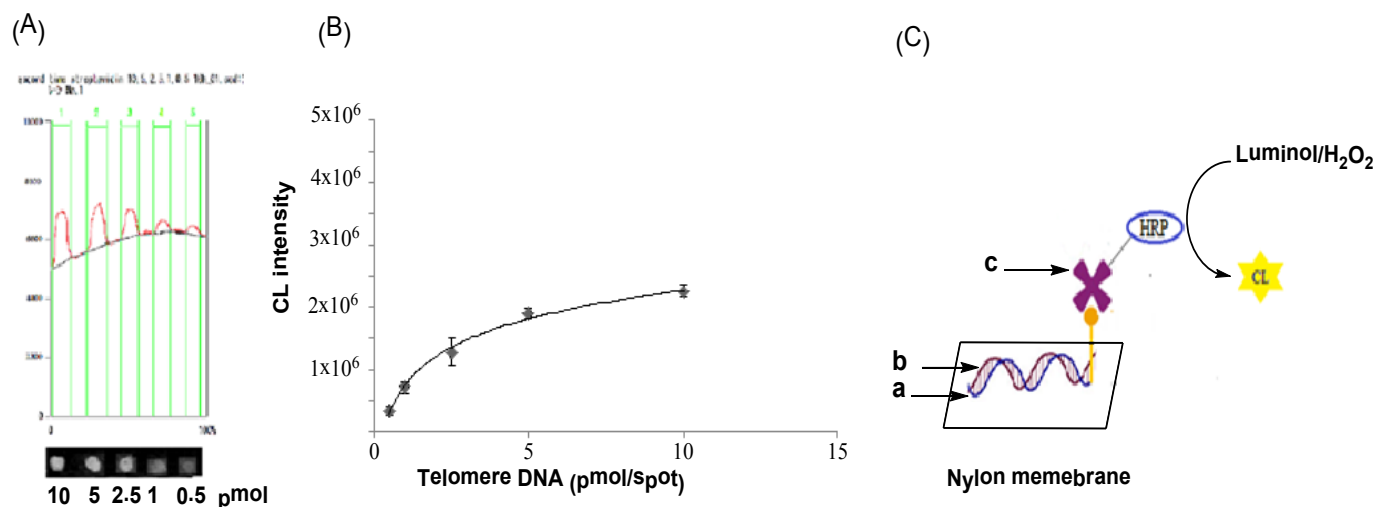


Fig. S6 CL detection of the telomere DNA on a nylon membrane by a commercial available biotin-avidin-HRP method. (A) CL intensities and image of the telomere DNA (0.5-10 pmol). (B) Calibration curve ($n = 3$) of the telomere DNA in the range of 0.5-10 pmol per spot on the nylon membrane. (C) Its schematic principle: **a**, telomere DNA; **b**, biotin-labeled antisense DNA; **c**, the CL detection system with biotin, streptavidin and HRP.

As shown in Fig. S6, the CL-imaging signals indicated a logarithmic relationship upto 10 pmol of telomere DNA adsorbed on the membrane, since the calibration curve was $y = 6.5 \times 10^5 \log(x) + 7.5 \times 10^5$, $R^2 = 0.9834$ (y and x indicate the CL intensity and the telomere DNA amounts on the membrane, respectively). By this method the detection limit of the telomere DNA at S/N = 3 ratio was approximately 250 fmol (4.5 ng). Thus, the CL signals from our synthesized probe of YY-DNAs was enhanced approximately 5 folds as compared to that obtained by the biotin-avidin-HRP method.

10. Comparison with other methods

Table S1: Sensitivity of other detection methods for telomere DNA

Method	Label	Detection limit	Reference
Colorimetric	Gold nanoparticles	5.7 pM	3
FRET	Gold nanorods	58 nM	4
PCR	³² P labeled- probe	35 ng	5
PCR	Fluorescence-labeled primer	0.3 ng	6
CL detection	Magnetic beads TMPG reaction	5 nM	7
Electrochemical genosensor	Cobalt cations	100 nM	8

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