Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

A Simple "Add and Measure" FRET-based Telomeric Tandem Repeat Sequence Detection and Telomerase Assay Method

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Supporting Information

CD spectroscopy : A duplex formation of the telomeric DNA and FAM-modified ODN was confirmed by CD (circular dichroism) measurement. CD spectra of DNA at a double strand concentration of 10 μM were recorded in a 1 mm path length quartz cell at 4°C using a J-820 spectropolarimeter (Jasco Co., Ltd., Hachioji, Japan). Before CD measurements were taken, the samples were heated to 90°C for 10 min, gently cooled to 4°C at rate of 0.5°C/min, and incubated at 4°C overnight.

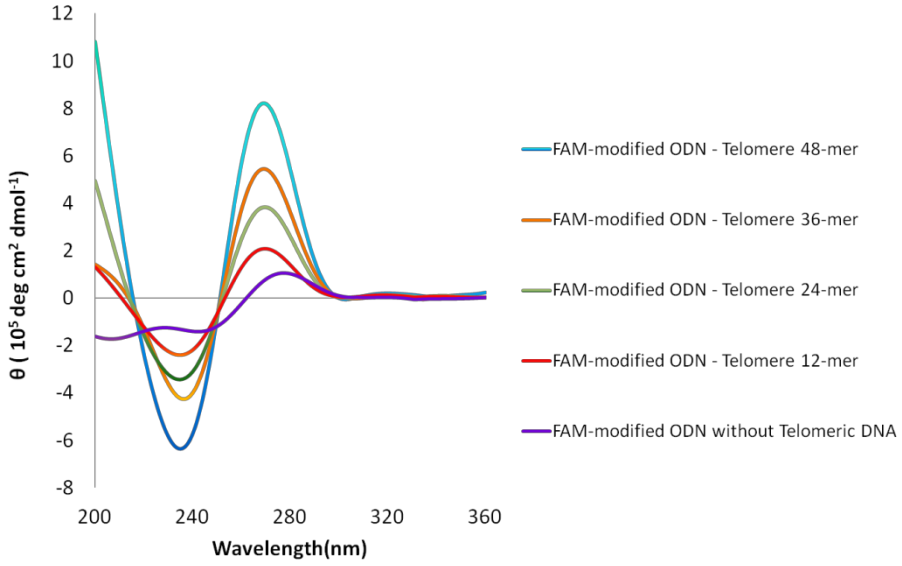

Figure S1. CD spectra of telomeric DNA and FAM-modified ODN at a double strand concentration of 10 μM in 100 mM LiCl and 50 mM Tris-HCl buffer (pH 8.4), 4°C.

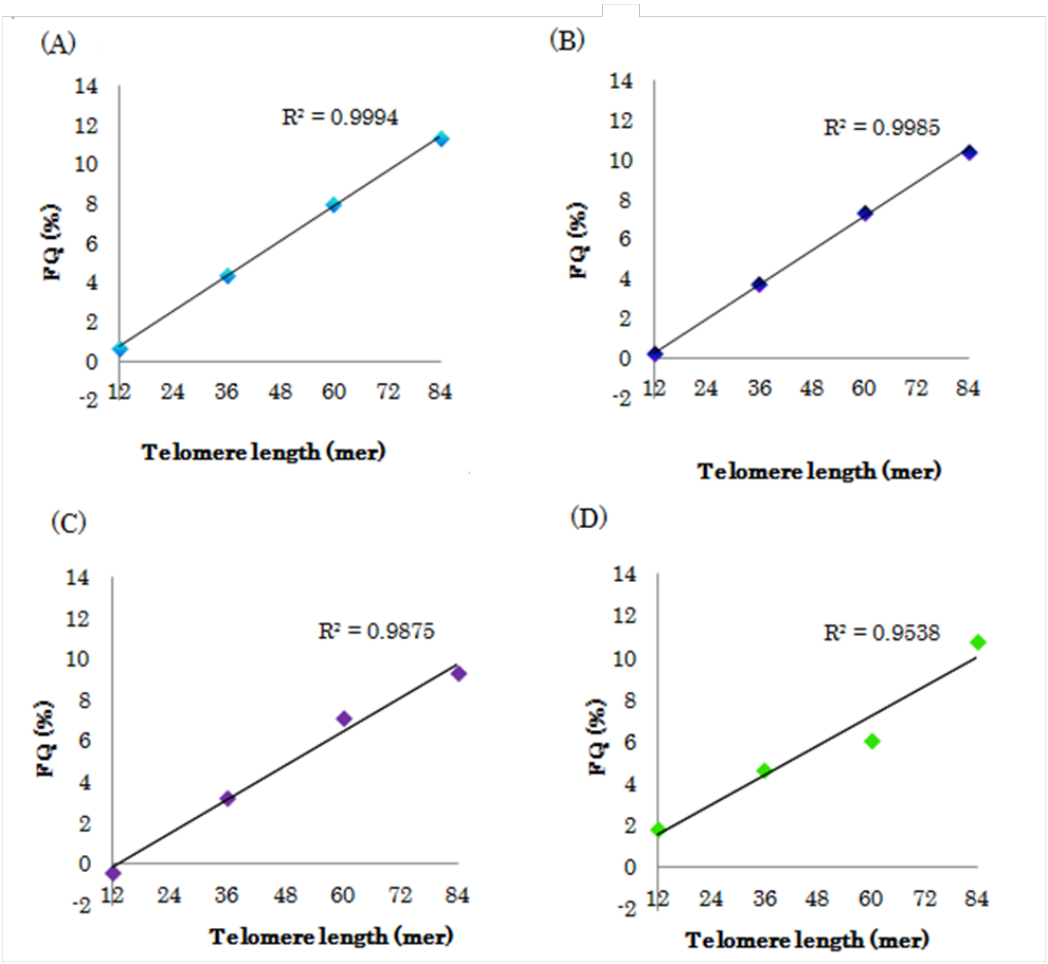

Figure S2. Relationships between the FQ values and telomere length at several concentrations. Each sample contains 1 μM EB and (A) 50 nM FAM probe and 1 nM telomere strand, (B) 5 nM FAM probe and 0.1 nM telomere strand, (C) 0.5 nM FAM probe and 10 pM telomere strand, (D) 0.25 nM FAM probe and 5 pM telomere strand. Excitation and emission wavelength are (A), (B) and (C) λex/λem 494 nm/516 nm, (D) λex/λem 480 nm/516 nm

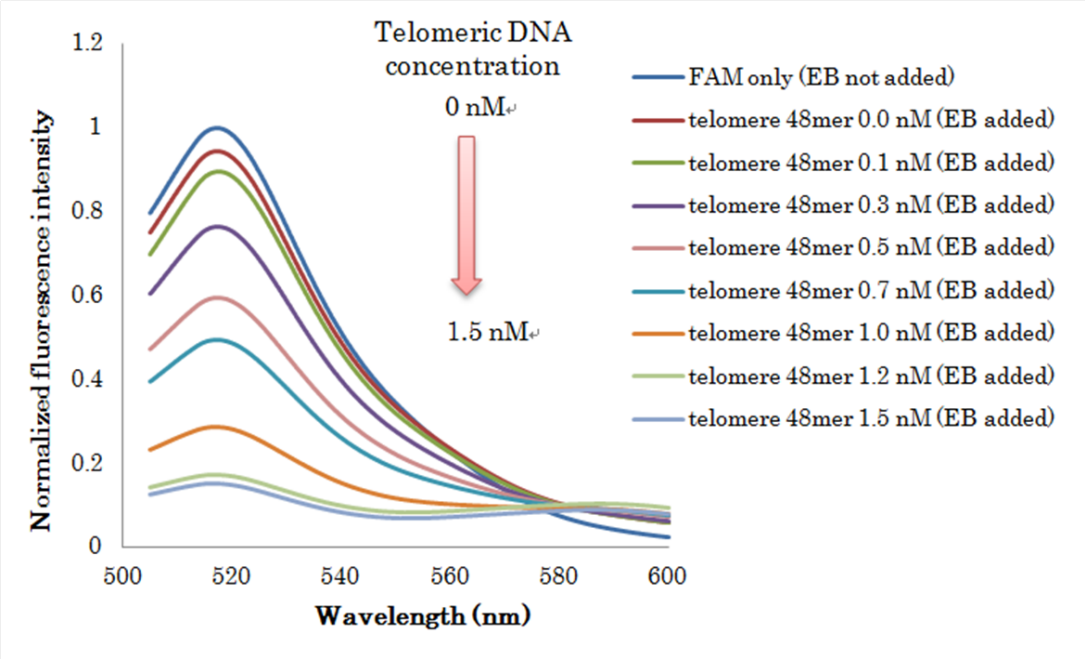

Figure S3. Normalized fluorescence spectra in various concentrations of telomeric DNA (48-mer). Concentrations of FAM-modified ODN and EB were 5 nM and 1 μM, respectively.

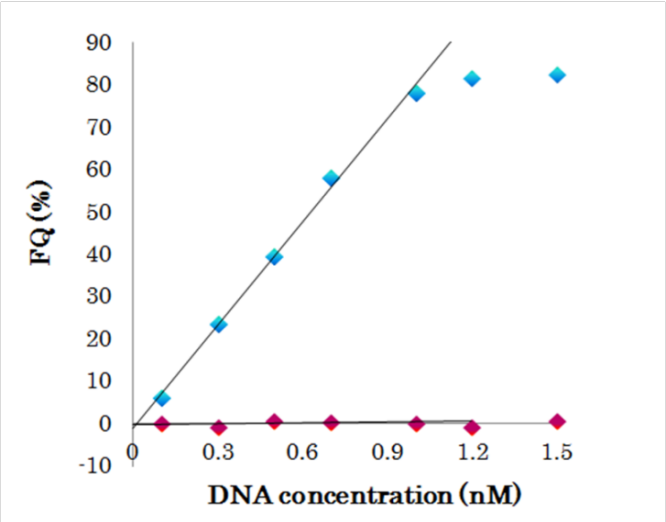

Figure S4. Relationships between the FQ values (λex/λem 494 nm/516 nm) and the concentration of 48-mer telomeric DNA (pink diamond) or 48-mer non-telomere sequence (5'-(TGA GTG TGA GTG)$_4$-3') (light blue diamond) at 4°C. Sequence of FAM-CC(complementary control) probe DNA is 5'-FAM-CAC TCA CAC TCA-3'. Each sample contains 5 nM FAM-CC probe DNA and 1 μM EB.

Table S1. Mixing ratio of various length telomere strands

Sample	12-mer	24-mer	36-mer	48-mer	60-mer	72-mer	84-mer	Average (mer)
A	80%	20%						14.4
B	50%	30%	20%					20.4
C	20%	30%	50%					27.6
D		40%	30%	20%	10%			36.0
E		20%	30%	20%	30%			43.2
F			20%	20%	30%	20%	10%	57.6
G			10%	10%	10%	10%	60%	72.0

* 12-84 mer telomeric DNA were adjusted 1 μM

Table S2. The amount of included telomeric DNA indicated by 12-mer telomere unit concentrations in 2.0 mL of sample solutions

Sample	TL Ave. (mer) *1	Volume (μL) *2	Telomere units (nM)	FQ (%)
A	14.4	0.1	0.0600	1.75
B	20.4	0.3	0.255	3.47
C	27.6	0.6	0.690	7.64
D	36.0	0.9	1.35	18.0
E	43.2	1.2	2.16	26.8
F	57.6	1.4	3.36	49.8
G	72.0	1.6	4.80	63.6

*1 : average length of the mixture of telomeres

*2 : each sample was diluted by the buffer in fluorescence cuvette, and total volume was 2.0 mL