Supplementary Materials

Detailed experimental procedures

(1) Establishing BFP stable cell line

The pBFP-N1 plasmid involving a BFP gene [1] was digested by a restriction enzyme EcoO109I, and was introduced to human K562 cells by electroporation using Neon® Transfection system (Life Technologies). The conditions of electroporation were Square wave, Pulse Voltage 1450 V, Pulse Width 10 ms, Pulse Number 3, 500 ng of plasmid DNA, and 1×10^5 K562 cells. At 24 hours after the transfection, these cells were plated on a 96-well plate containing culture medium (RPMI1640 with 10%FBS) supplemented with 0.6 mg/ml G418 to select stably-transfected cells. After being incubated for approximately 2 weeks at 37 ºC in 5 % CO2, the cells were observed on a fluorescent microscopy DMI6000B (Leica Microsystems). Several clones showing blue fluorescence were picked up, and the BFP gene copy number in them was analysed by Southern blotting. The cell line containing a single copy of BFP gene was used in this study.

1. H. Katada, H.S. Chen, N. Shigi, and M. Komiyama, *Chem. Commun.*, 2009, 43, 6545-6547.

(2) Clipping of internal fragments from human genome.

The cells, in which a BFP gene was stably integrated into the genome (see (1)), were cultured at 37°C and 5% CO_2 in RPMI-1640 medium (Sigma-Aldrich) containing 2 mM L-glutamine and 10% fetal bovine serum. The cells were harvested and washed by PBS(-). The whole human genomic DNA was isolated from these cells by using a DNeasy Blood & Cell Tissue Kit (QIAGEN). The mixture was incubated at 50 °C for 4 h to form a double-duplex invasion complex under the conditions that [human genomic DNA] = 15 μg in 250 μL, [each of pcPNAs] = 500 nM, and [HEPES] = 5 mM (pH 7.0). Then, S1 nuclease (TAKARA) and NaCl was added to the reaction mixture (the final concentrations of S1 nuclease and NaCl were 18 U/μL and 300 mM, respectively). [$ZnSO_4$] = 5 μM (see text for details). The scission reaction was carried out at 37 °C for 1 h or at 50 °C 30 min, and the reaction was stopped by adding EDTA to a final concentration of 20 mM.

The whole products were purified by the extraction with phenol:chloroform:isoamyl alcohol mixture (25/24/1 v/v/v), followed by ethanol precipitation. The DNA was resuspended in 200 μL solution containing 100 U of *Nsi*I (from NEB). After incubating the mixture at 37 °C for 16 h, the whole products were purified again by phenol:chloroform:isoamyl alcohol extraction/ethanol precipitation. The purified DNA was resuspended in 25 μL of ddH_2O, and subjected to 1% agarose gel electrophoresis. After the gel was stained by GelStar (TAKARA), genomic DNA fragments were transferred from the gel via capillary action to a 0.45-μm pore size, positively charged Nylon Membrane (Roche) using 20×SSC buffer. The membrane was UV-crosslinked on a 2×SSC wetted 3-MM paper, rinsed in ddH2O and air-dried. The Southern blotting was achieved using two probes which bind to the upper scission fragment (Probe1) and the lower fragment (Probe2). Hybridization was performed at 50 °C overnight using Roche's Dig Easy Hybridization solution. Washing, blocking and detection were accomplished according to manufacturer's directions using the Roche Wash and Block Buffer Set. The phosphorimaging was carried out with the use of CDP-Star on a LAS-4000 EPUV mini (FUJIFILM).

The probes 1 and 2 were synthesized by using PCR DIG Probe Synthesis Kit (Roche). pBFP-n1 was used as a PCR template. The PCR primers used are as follows.

Probe1(568 bp): pBFP-241F: 5'-AAGTACGCCCCCTATTGACGT-3'
pBFP-808R: 5'-TCAGCTTGCCGTAGGTGGCAT-3'
Probe2(426 bp): pBFP-973F: 5'-ATCTTCTTCAAGGACGACGGC-3'
pBFP-1398R: 5'-TTACTTGTACAGCTCGTCCATG-3'

(3) Clipping of telomere from each of human chromosomes

The whole human genomic DNA was isolated from cultured Flp-in 293 cells by using a DNeasy Blood & Cell Tissue Kit (QIAGEN). Invasion reactions were performed with [human genomic DNA] = 3 μg in 50 μL, [each of pcPNAs] = 200 nM, and [HEPES] 5 mM (pH 7.0). The mixture was incubated at 50 °C for 4 h to form a double-duplex invasion complex. Then, S1 nuclease (TAKARA) and NaCl was added to the reaction mixture (the final concentrations of NaCl and S1 nuclease were 18 U/μL and 300 mM, respectively). [Zn(II)] = 5 μM. The scission reaction was carried out at 37 °C for 45 min, and stopped by adding 150 μL of AE buffer (QIAGEN; 10 mM Tris-Cl, 0.5 mM EDTA (pH 9.0)).

The mixture was desalted and concentrated by ultrafiltration (Amicon Ultra-0.5 10kDa Ultracel; Millipore) and washed 100 μL of AE buffer three times, and was analysed by the 1% pulsed-field gel electrophoresis (CHEF Mapper™ System, BIO-RAD) at 14 °C with the use of Pulsed Field Certified Agarose (BIO-RAD). Then, the products were detected by Southern hybridization with a telomere probe (TeloTAGGG Telomere Length Assay; Roche).

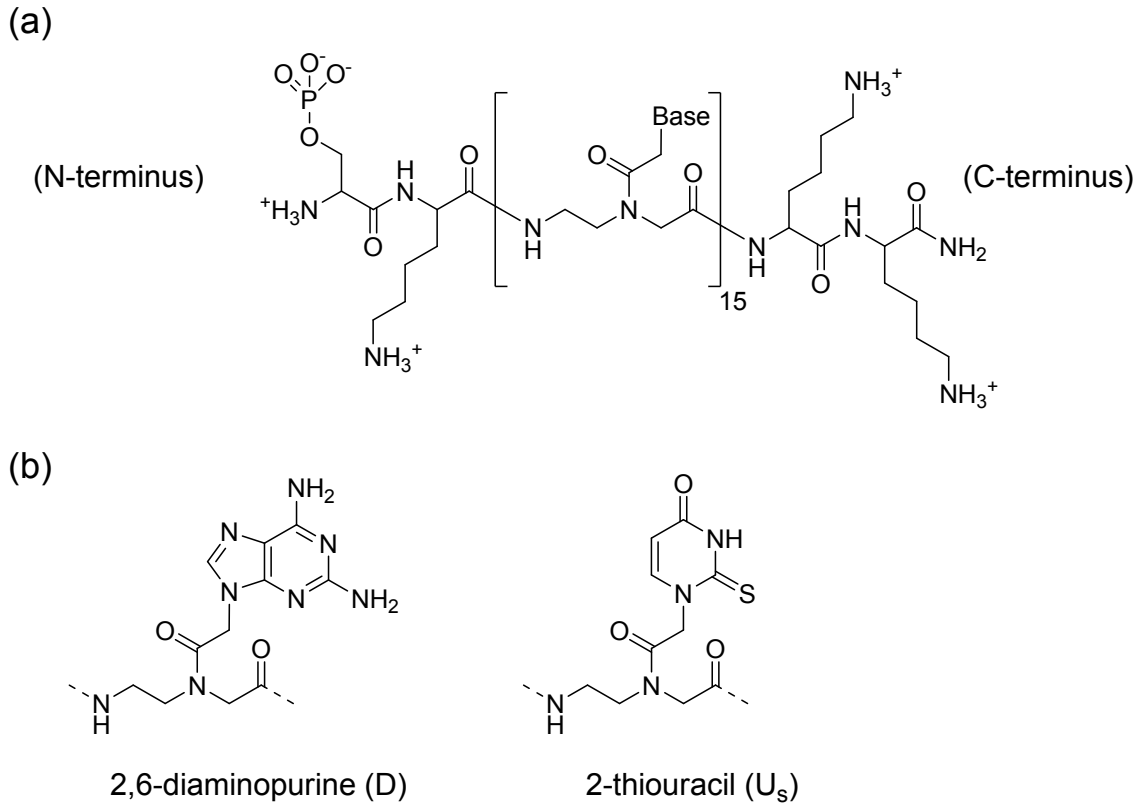

Fig. S1 (a) Structure of pcPNA with three lysine residues and one phosphoserine. In place of conventional nucleobases A and T, 2,6-diaminopurine (D) and 2-thiouracil (U_s) [shown in (b)] are used together with G and C. When D and U_s form a pair as A and T do, the 2-NH_2 group of D and the 2-S of U_s are located nearby and significant steric repulsion between them occurs. As the result, pcPNA/pcPNA duplex is destabilized, resulting in promotion of the double-duplex invasion.

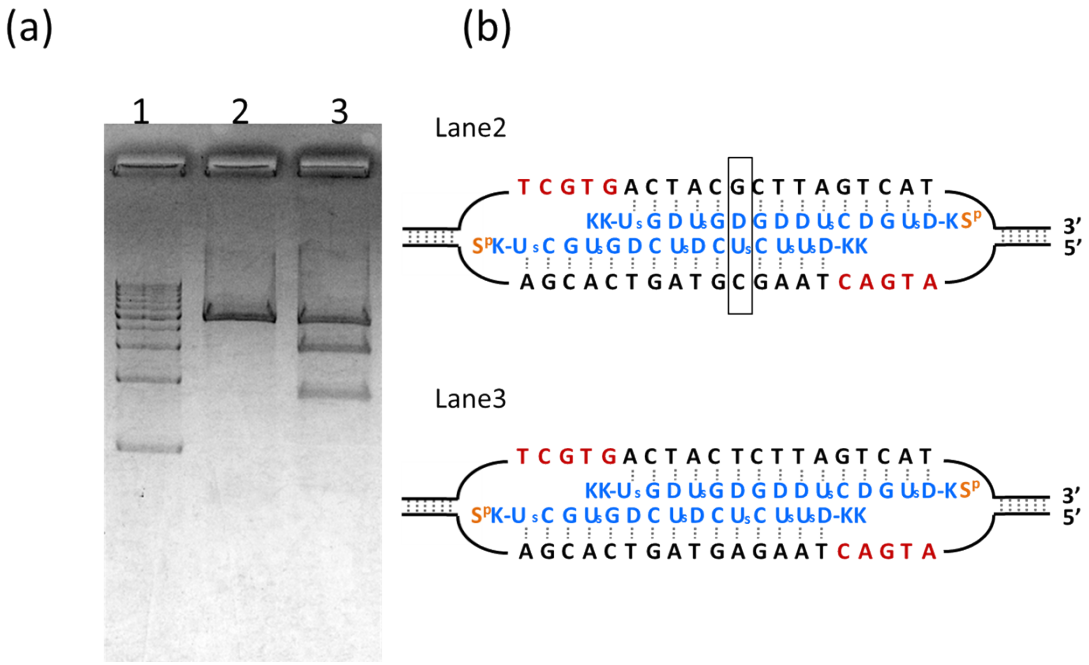

Fig. S2 (a) Site-selective scission of DNA substrate (4,733 bp) involving a BFP gene by the combination of S1 nuclease and a pair of pcPNA. Lane 1, 1 kbp markers; lane 2, scission of DNA substrate involving a mismatch between each pcPNA and the corresponding DNA strand; lane 3, site-selective scission of fully matching DNA substrate. The fully matching and mismatching DNA substrates, as well as the pcPNAs, are presented in (b). Note that the DNA sequence at the invasion site in lane 3 is the same as the one used in Fig. 2. These DNA substrates were treated by the S1 nuclease-pcPNA combination under the conditions described below and analysed by gel electrophoresis. When the scission by the combination occurred at the target site, two fragments of sizes 1.7 kbp and 3.0 kbp should be formed as observed in lane 3. In contrast, these two fragments were never formed in lane 2, where one T/A pair in the DNA was changed to G/C pair and a mismatch was introduced between each pcPNA and the corresponding DNA strand as shown by the square in (b). High mismatching-recognition activity of the S1 nuclease-pcPNA combination has been confirmed.

In the DNA scission, substrate DNA (80 ng in 20 μL) and pcPNAs (50 nM each) were first incubated at pH 7.0 (5 mM HEPES) and 50 °C for 1 h. Then, S1 nuclease (TAKARA) and NaCl were added to the final concentrations of 18 U/μL and 300 mM, respectively, and the enzymatic reaction was carried out at 37 °C for 45 min. The fully matching substrate in lane 3 (a linear DNA of 4,733 bp) was prepared by cutting pBFP-N1 plasmid with a restriction enzyme *Stu*I. The DNA substrate in lane 2 involving the mismatches was prepared by cutting the corresponding plasmid, obtained from pBFP-N1 using QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies), with *Stu*I. The sequences of these substrates were confirmed by sequencing experiments.

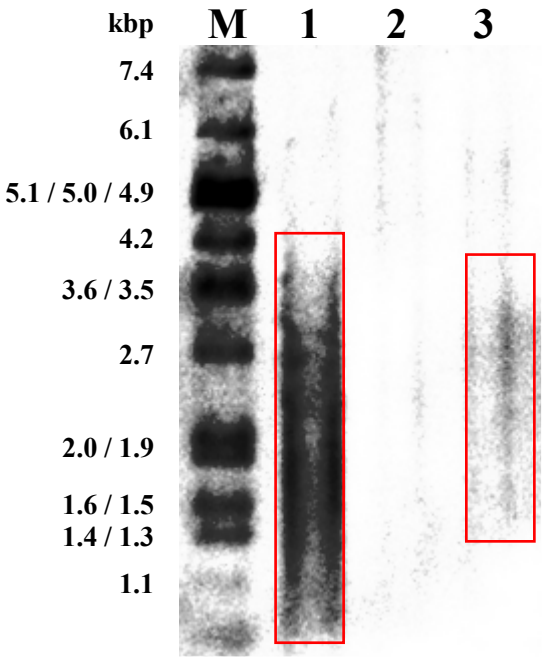

Fig. S3 Use of two non-designated pcPNA strands for the S1 nuclease-scission of human genome. Lane M, marker; lane 1, conventional method (by using TeloTAGGG Telomere Length Assay; Roche); lane 2, with pcPNAs which are not complementary with the DNA; lane 3, with pcPNAs for Xp/Yp. In lane 2, the whole human genomic DNA from K562 cells was treated by S1 nuclease in the presence of a pair of non-designated pcPNAs (the sequences are $KKGCDU_SU_SDU_SGCU_SGDGU_SGKS^p$ and $KKGDU_SDU_SCDCU_SCDGCDU_SKS^p$). In contrast with site-selective scission in lane 3 (designated pcPNA pair), no scission occurred, confirming the proposed mechanism in which single-stranded portions are formed through the double-duplex invasion.