Supporting Information

A Chemistry-Based Method to Detect Individual Telomere Length at a Single Chromosome Terminus

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Synthesis of pcPNAs : The pcPNA strands were synthesized by Boc-chemistry, purified by the reversed-phase HPLC, and characterized by MALDI-TOF MS (Table S1 and Figure S1).

Characterization of pcPNA strands: All PNAs were purified and characterized by MALDI-TOF mass spectrometry (Bruker Autoflex) using a 3,5-dimethoxy-4-hydroxycinnamic acid (matrix; #85429, Aldrich) dissolved in a 3:7 mixture of ACN and water containing 0.1% TFA.

Table S1. MALDI-TOF MS characterization of pcPNA strands used in this study

	Sequence[a)]	Calcd.	Found.
pcPNA1	H_2N-K K D D D C G U_sC G G G D C U_sU_sD K P-H	4699.82	4699.19
pcPNA2	H_2N-K K D C U_sD D U_sD D G U_sC C C G D K P-H	4740.82	4739.63
pcPNA3	H_2N-K K G D C U_sD U_sC C D G G U_sG D D K P-H	4740.82	4739.98
pcPNA4	H_2N-K K D G D C $U_sU_sU_s$C D C C U_sG G D K P-H	4678.75	4678.48
pcPNA5	H_2N-K K G C D U_sU_sD U_sG C U_sG D G U_sG K P-H	4736.71	4736.96
pcPNA6	H_2N-K K G D U_sD U_sC D C U_sC D G C D U_sK P-H	4677.76	4677.95

a) U_s, D, K and P represent 2-thiouracil, 2,6-diaminopurine, L-lysine and L-phosphoserine (activator of the scission), respectively. Written N to C terminal for all the pcPNAs.

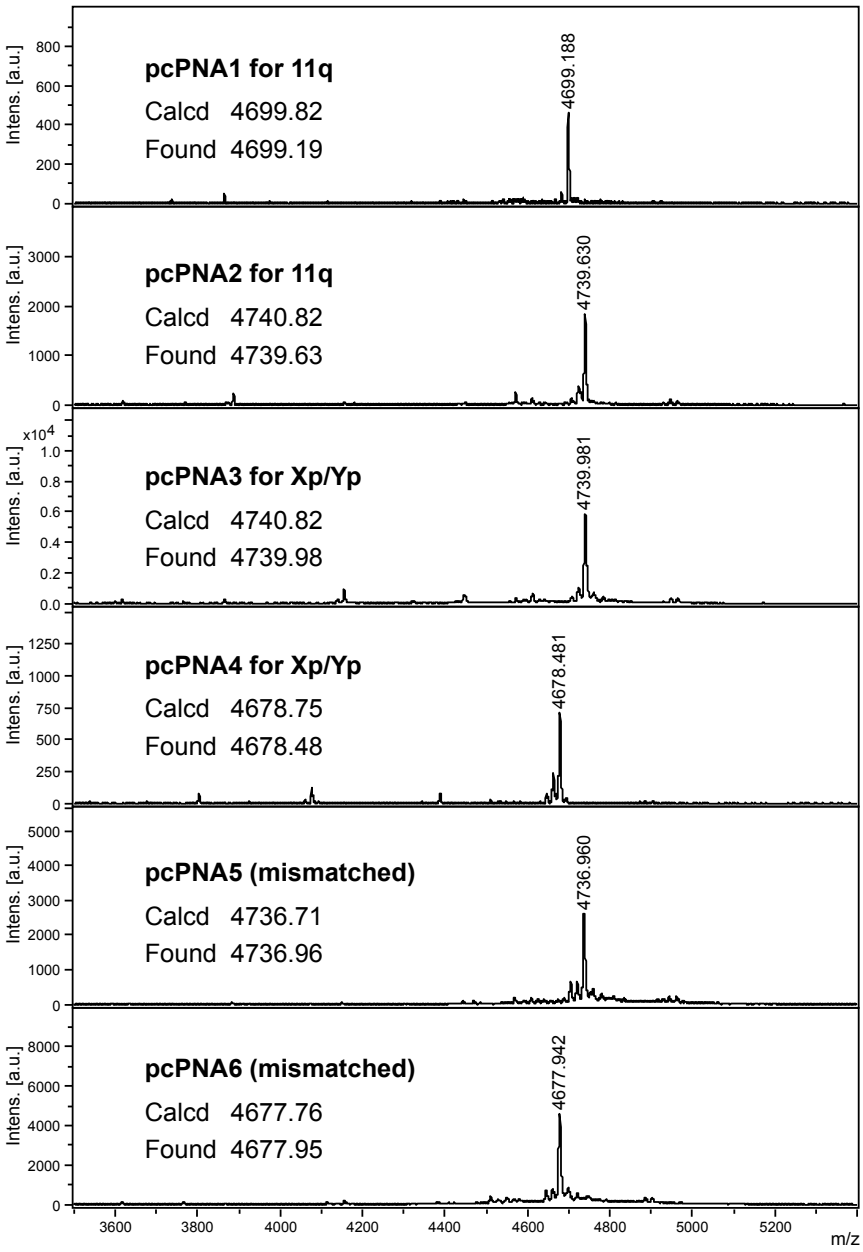

Figure S1. MALDI-TOF MS spectra of pcPNA strands used in this study.

(a)

Xp/Yp (+): 5'-CGA AGC TTC CAC AGT ATG GAA AGG GAC CCC ATT GGG TTG CCA CTG CTG GCT CAG GCA GTC TGC TTT TAT TCT CTA ATC TGC TCC CAC CCA CAT CCT GCT GAT AGG TCC ACT TTC AGA-3' (117-mer)

Xp/Yp (−): 5'-TCT GAA AGT GGA CCT ATC AGC AGG ATG TGG GTG GGA GCA GAT TAG AGA ATA AAA GCA GAC TGC CTG AGC CAG CAG TGG CAA CCC AAT GGG GTC CCT TTC CAT ACT GTG GAA GCT TCG-3' (117-mer)

Primer Xp/Yp FW: 5'-CGA AGC TTC CAC AGT ATG GAA AGG-3' (24-mer)
Primer Xp/Yp REV: 5'-TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TCT GAA AGT GGA CCT ATC AGC AGG-3' (84-mer)

11q (+): 5'-GAC CCG GGG GAC ACC GCA AAG GCG GAG CAG GTT TCT CAG CAC AGA CCT TGG AGG CAC GGC CTT CGT TTG GGA CAA TTC GGG GCC GCA TCG ACG GTG AAT AAA ATC CTT CCT CTT TGC AGC CCT GAA TAA TCA GGG TCA GAG ATC AG-3' (146-mer)

11q (−): 5'-CTG ATC TCT GAC CCT GAT TAT TCA GGG CTG CAA AGA GGA AGG ATT TTA TTC ACC GTC GAT GCG GCC CCG AAT TGT CCC AAA CGA AGG CCG TGC CTC CAA GGT CTG TGC TGA GAA ACC TGC TCC GCC TTT GCG GTG TCC CCC GGG TC-3' (146-mer)

Primer 11q FW: 5'-GAC CCG GGG GAC ACC GCA AAG-3' (21-mer)
Primer 11q REV: 5'-TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CTG ATC TCT GAC CCT GAT TAT TCA G-3' (88-mer)

(b)

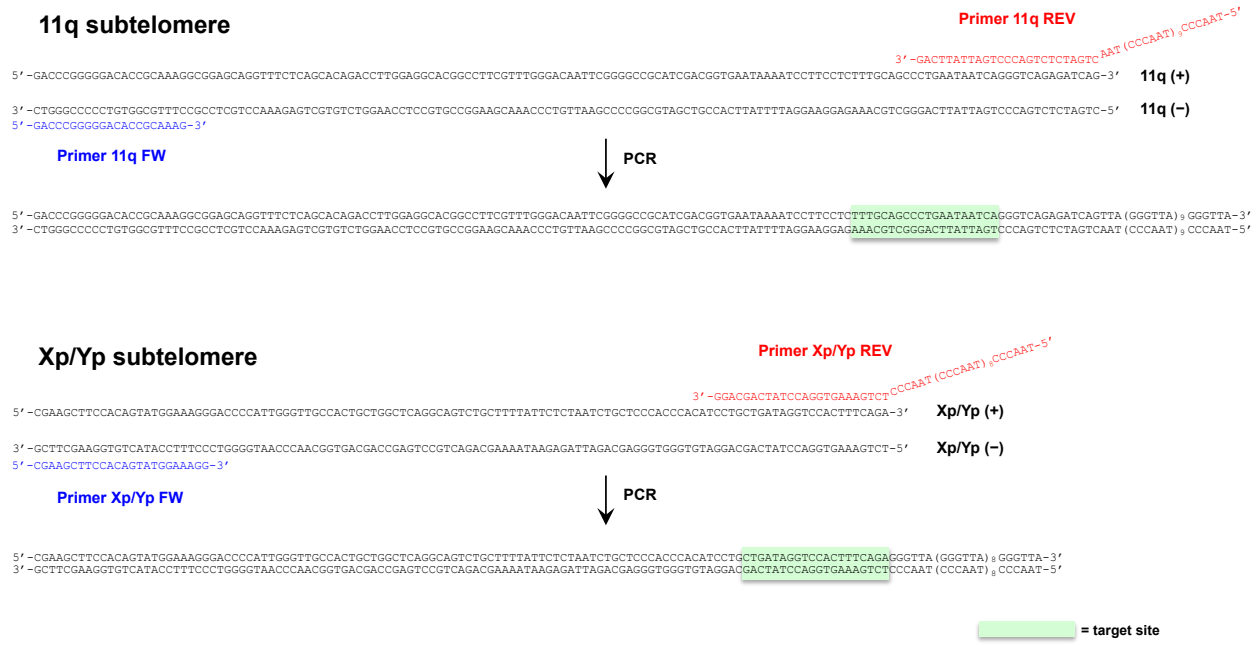

Figure S2. (a) Sequences of PCR products of Xp/Yp and 11q chromosome subtelomere regions used in the scission experiment of ARCUT and primer sequences used in PCR. (b) Schematic representation of the sequences of PCR products and primers, target site of ARCUT also was shown.

Gel-shift assay

Gel-shift assay was achieved by 5%-nondenaturing polyacrylamide gel electrophoresis in TBE buffer at 25 °C. The mixture of double-stranded DNA and PNA strands was incubated for 1.5 h at 50 °C and pH 7.0 (5 mM HEPES buffer; [NaCl] = 0 M). Then, loading buffer containing bromophenol blue (0.05%) and glycerol (30%) in 0.5×TBE buffer was added, and the mixture was subjected to 5%-nondenaturing polyacrylamide gel electrophoresis at 25 °C. The bands were stained with GelStar (from Cambrex), and analyzed on a FUJIFILM FLA-3000G imaging analyzer.

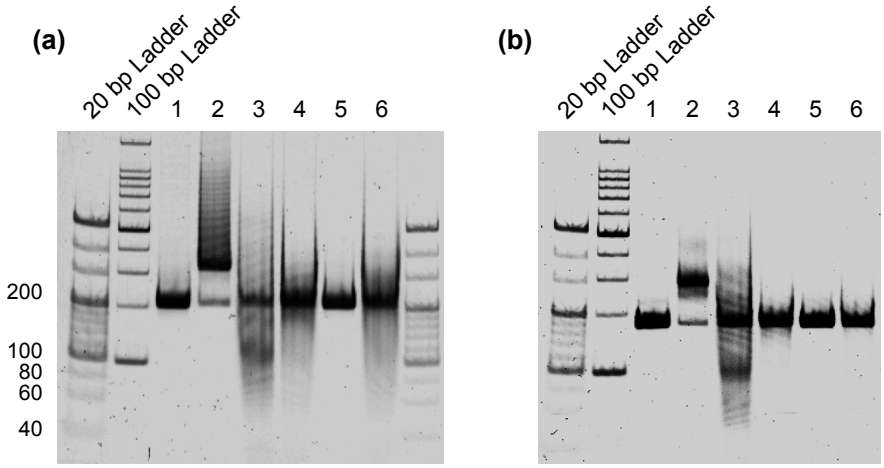

Figure S3. Site-selective scissions of short dsDNAs (PCR products) for **(a)** 11q chromosome and **(b)** Xp/Yp chromosome by ARCUT. Lane 1: dsDNA only, lane 2: dsDNA + pcPNAs (invasion complex), lane 3: lane 2 + Ce(IV)/EDTA treatment, lane 4: lane 3 – pcPNAs, lane 5: dsDNA + mismatched pcPNAs, lane 6: lane 5 + Ce(IV)/EDTA treatment. Conditions: [dsDNA (209-bp for 11q chromosome or 177-bp for Xp/Yp chromosome)] = 50 nM, [each of pcPNAs] = 250 nM, [Ce(IV)/EDTA] = 100 μM, and [NaCl] = 100 mM. pcPNA 1 and 2 for 11q, pcPNA 3 and 4 for Xp/Yp.

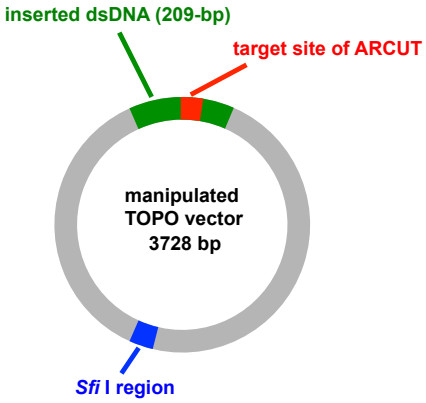

Figure S4. A graphical plasmid map of manipulated TOPO vector. The inserted dsDNA (209-bp) is highlighted in green. The target site for ARCUT is highlighted in red. *Sfi* I region is highlighted in blue.

Site-selective scission of human genomic DNA by ARCUT

The Ce(IV)/EDTA solution was prepared by mixing an aqueous solution of $Ce(NH_4)_2(NO_3)_6$ (20 mM) and EDTA·4Na (20 mM) in HEPES buffer and then adjusting the pH to 7.0 with a small amount of NaOH. The ARCUT reactions were carried under the following conditions.

(I) The cleavage of the short dsDNA (209-bp for 11q chromosome or 177-bp for Xp/Yp chromosome) :

[dsDNA] = 50 nM, [each of pcPNAs] = 250 nM, [Ce(IV)/EDTA] = 100 μM, and [NaCl] = 100 mM. The mixture was incubated at 50 °C for 2 h to form a double-duplex invasion complex. Then, the Ce(IV)/EDTA complex was added and the scission reaction was carried out at 50 °C for 16 h. The reactions were stopped by adding ethylenediaminetetramethylenephosphonic acid (EDTP) to a final concentration of 500 μM.

(II) The cleavage of the human genomic DNA:

The whole human genomic DNA as substrate was isolated from cultured Flp-in 293 cells (#R750-07, Invitrogen) by using a Blood & Cell Culture DNA Maxi Kit (#13362, QIAGEN). This cell line is hypotriploid, and involves three X chromosomes in each cell. [human genomic DNA] = 4 μg, [each of pcPNAs] = 200 nM, [Ce(IV)/EDTA] = 100 μM, and [NaCl] = 100 mM. The mixture was incubated at 50 °C for 4 h to form a double-duplex invasion complex. Then, the Ce(IV)/EDTA complex was added and the scission reaction was carried out at 50 °C for 16 h. The reactions were stopped by adding ethylenediaminetetramethylenephosphonic acid to a final concentration of 500 μM. The mixture was desalted by illustra™ MicroSpin™ S-300 HR Columns (#27-5130-01,GE Healthcare).

TRF analysis

We used TeloTAGGG Telomere Length Assay (#12209136001, Roche) to detect the telomere fragments. We digested human genomic DNA with *Hinf*I and *Rsa*I and resolved 1.5 μg of DNA by 0.7% Tris-acetate-EDTA for agarose gel electrophoresis. In case of site-selective scission by ARCUT, we performed the reaction described above. The mixture was performed the pulsed-field gel electrophoresis (CHEF Mapper™ System, BIO-RAD) with 1% pulsed Field Certified Agarose (#162-0137, BIO-RAD) at 14 °C. TRFs were detected by Southern hybridization with a DIG probe containing a TTAGGG repeat and phosphorimaging (LAS-4000 EPUV mini, FUJIFILM) with CDP-*Star* ready-to-use (Roche).

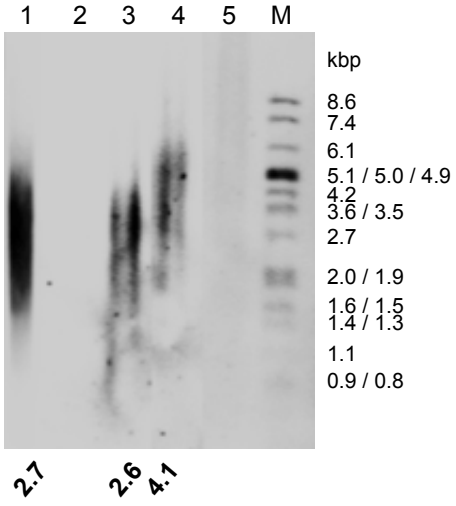

Figure S5. Southern blot analysis of the TRFs of 11q and Xp/Yp chromosome in HEK 293 cells. The mean single telomere lengths are shown below in kilobases. The whole human genomic DNA as substrate was isolated from cultured cell lines. Conditions: [human genomic DNA] = 4 μg, [each of pcPNAs] = 200 nM, [Ce(IV)/EDTA] = 100 μM, and [NaCl] = 100 mM. Lane M: marker, lane 1: traditional method, lane 2: without pcPNAs, lane 3: 11q targeting, lane 4: Xp/Yp targeting, lane5: mismatched pcPNAs.

hTERT gene knockdown

HeLa cells, transfected with hTERT specific siRNAs (Sigma-Aldrich, sequences are below) by using Lipofectamine RNAiMAX (Invitrogen), were cultured for 3 weeks. The detailed protocol was described in a previous paper.[1,2]

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5′-GAGCAAGUUGCAAAGCAUUTT-3′
3′-TTCUCGUUCAACGUUUCGUAA-5′
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Figure S6. Sequences of siRNAs used in this study.

References

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(2) Dong. X.; Liu, A.; Zer, C.; Feng, J.; Zhen, Z.; Yang, M.; Zhong, L. *BMC Cancer* **2009**, *9*, 133.