Supplementary Information

Homologous Recombination in Human Cells using Artificial Restriction DNA Cutter

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Experimental details

pBFP-N1 was constructed by introduction of the five amino acid mutations (T65S, Y66H, Q80R, I167T, L231H) into pEGFP-N1 (Clontech) using QuikChange site-directed mutagenesis (Stratagene) according to the manufacturer's protocols. The sequences of primers are as follows; for T65S and Y66H (5'-GGCCCACCCTCGTGACTACTCTTAGT CATGGTGTACAGTGCTTCAGCCGCTAC-3', 5'-GTAGCGGCTGAAGCACTGTACACCATGACTAAGAGTAGT CACGAGGGTGGGCC-3'); for Q80R (5'-CCGACCACATGAAGCGCCACGACTTCTTCAAG-3', 5'-CTTGAAGA AGTCGTGGCGCTTCATGTGGTCGG-3'); for I167T (5'-CAAGGTGAACTTCAAGACCCGCCACAACATCGAG-3', 5'-CTCGATGTTGTGGCGGGTCTTGAAGTTCACCTTG-3'); for L231H (5'-GCCGCCGGGATCACTCACGGC ATGGACGAGCTG-3', 5'-CAGCTCGTCCATGCCGTGAGTGATCCCGGCGGC-3').

The adenovirus vector for BFP expression was prepared using ViraPower Adenovirus Expression system (Invitrogen) according to the manufacturer's instructions. The CMV-driven BFP expression cassette was PCR-amplified using pBFP-N1 as a template and a set of primers (5'-CACCGCTCACATGTTCTTTC-3', 5'-GTTTGGACAAACCACAAC TAG-3'), and cloned into the pENTR/D-TOPO vector. The insert in the pENTR was switched into the adenovirus vector, pAd/PL-DEST, through the Gateway technique.

pQE30-EGFP, which was used as a PCR template in preparing the EGFP donor fragment, was constructed as follows. First, pEGFP-N1-mut that encodes EGFP with three amino acid substitutions (Q80R, I167T, L231H) was constructed as above. Next, the full-length coding region of EGFP mutant was amplified from pEGFP-N1-mut using the following two primers containing *Bam*HI site and *Hind*III site, respectively (primer-*Bam*HI;. 5'-CGCGGATCCGTGAGCAAGG GCGAGGAG-3', primer-*Hind*III; 5'-GCCGCTCGAGAAGCTTTTACTTGTACAGCTCGTC-3'). Then, the product was digested with *Bam*HI and *Hind*III and cloned into pQE30Xa plasmid (QIAGEN), which was digested with *Bam*HI and *Hind*III. Using the same primer set (primer-*Bam*HI and primer-*Hind*III) and pQE30-EGFP plasmid prepared above as a template, the donor fragment (742 bp) containing an entire EGFP gene was prepared by PCR amplification.

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 synthesis, purification, and characterization of pcPNA strands were described elsewhere.[8b] The ARCUT reactions were carried out at 37ºC and pH 7.0 (5 mM HEPES buffer) for 66 h under the following conditions. The cleavage of pBFP-N1 (8 nM): [each of pcPNAs] = 100 nM, [Ce^{IV}/EDTA] = 100 μM, and [NaCl] = 100 mM. The cleavage of pAd/PL-DEST-BFP (0.9 nM): [each of pcPNAs] = 50 nM, [Ce^{IV}/EDTA] = 50 μM, and [NaCl] = 100 mM. The reactions were stopped by adding ethylenediaminetetramethylenephosphonic acid to a final concentration of 500 μM and purified by QIAquick PCR purification kit (QIAGEN).

The human cells were maintained in DMEM supplemented with 10% fetal bovine serum at 37ºC with 5% CO_2. For the

homologous recombination of plasmid DNA, the 293T cells were seeded at 1.3×10^5 cells per 35 mm dish. On the next day, ARCUT-treated (or untreated) pBFP-N1 and the donor EGFP gene fragment (in mole ratio of 1:6.5, 2 μg in total) were introduced into these 293T cells using Fugene 6 transfection reagent (Roche) according to the manufacturer's protocol. After 48 h incubation, the expressions of EGFP and BFP were analyzed by a fluorescence microscope (DMI 6000B, Leica), and the numbers of EGFP positive cells and transfected cells were counted. After the extraction of the total DNA, the plasmid DNA was isolated by agarose gel electrophoresis, digested by *Dpn*I, and then directly transformed into *E. coli*. Several colonies were picked up and cultured in LB media. The plasmid DNA was purified with QIAprep spin Miniprep Kit (QIAGEN), and its sequence was determined by an ABI PRISM 3130 genetic analyzer. For the adenovirous vector recombination, the 293T cells were seeded on a 96-well plate at 3.5×10^4 cells per well. Next day, ARCUT-treated pAd/PL-DEST-BFP was introduced into these cells, together with the donor fragment (in mole ratio of 1:15, 320 ng in total), using Lipofectamine 2000 (Invitrogen).

Supplemental Figures

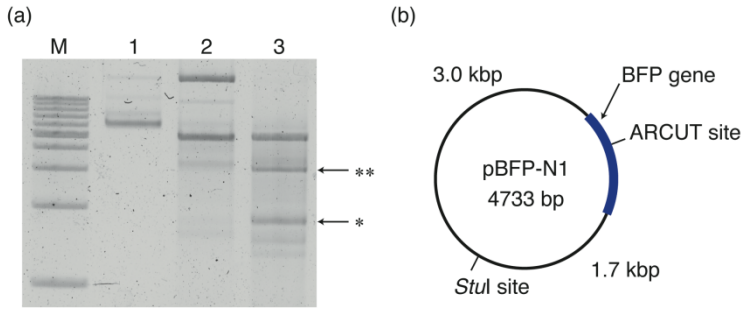

Fig. S1 (a) Agarose gel electrophoresis patterns for ARCUT cleavage of pBFP-N1. Lane M, 1 kbp DNA ladder; Lane 1, control (no treatment); Lane 2, after ARCUT treatment; Lane 3, *Stu*I digest of the product in Lane 2. The bands corresponding to 1.7 kbp (*) and 3.0 kbp (**) were formed by the site-specific cleavage. (b) The map of pBFP-N1 plasmid. The lengths of the fragments obtained by consecutive scissions by ARCUT and *Stu*I (in lane 3) are also shown.

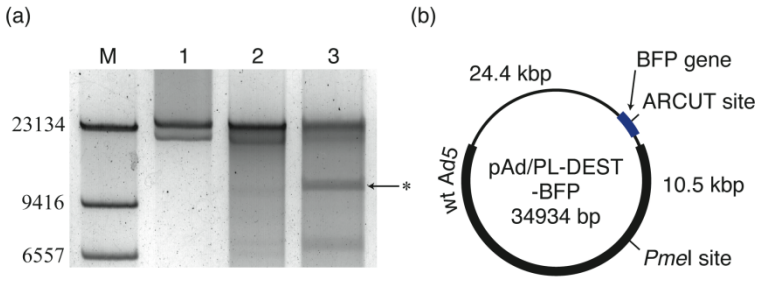

Fig. S2 (a) Agarose gel electrophoresis patterns for ARCUT cleavage of pAd/PL-DEST-BFP. Lane M, lambda/*Hind*III ladder; Lane 1, control (no treatment); Lane 2, after ARCUT treatment; Lane 3, *Pme*I digest of the product in Lane 2. The band corresponding to 10.5 kbp (*) was formed by the site-specific cleavage. (b) The map of pAd/PL-DEST-BFP vector. The lengths of the fragments obtained by consecutive scissions by ARCUT and *Pme*I (in lane 3) are also shown.