Supplementary information

Multiplex genome engineering in human cells using all-in-one CRISPR/Cas9 vector system

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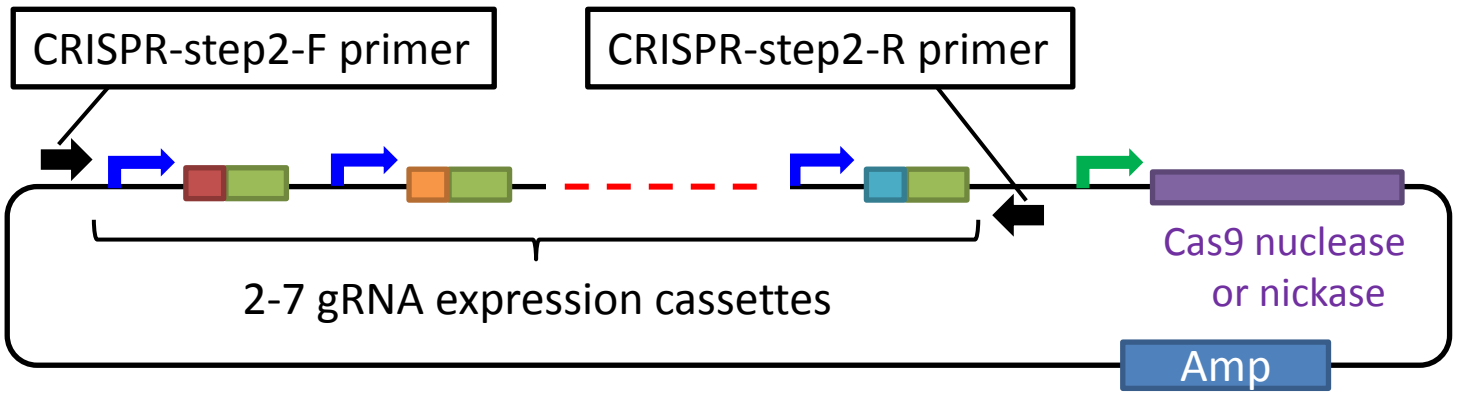
A



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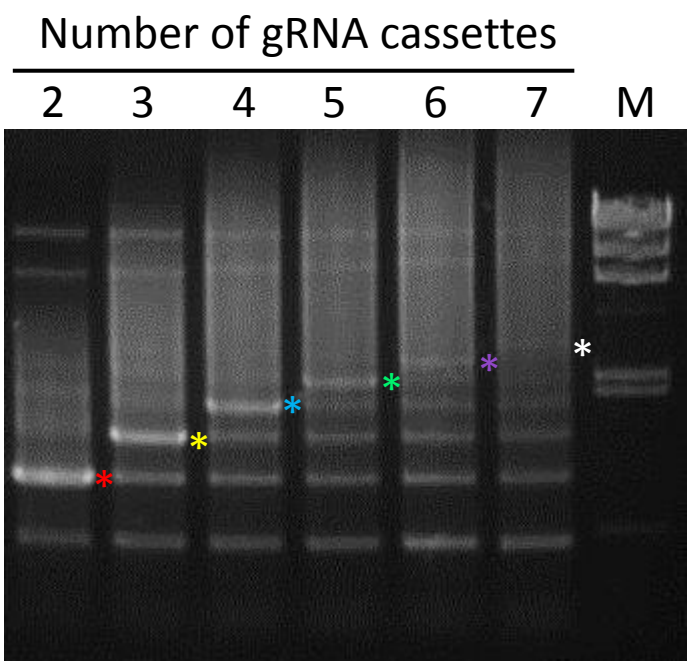
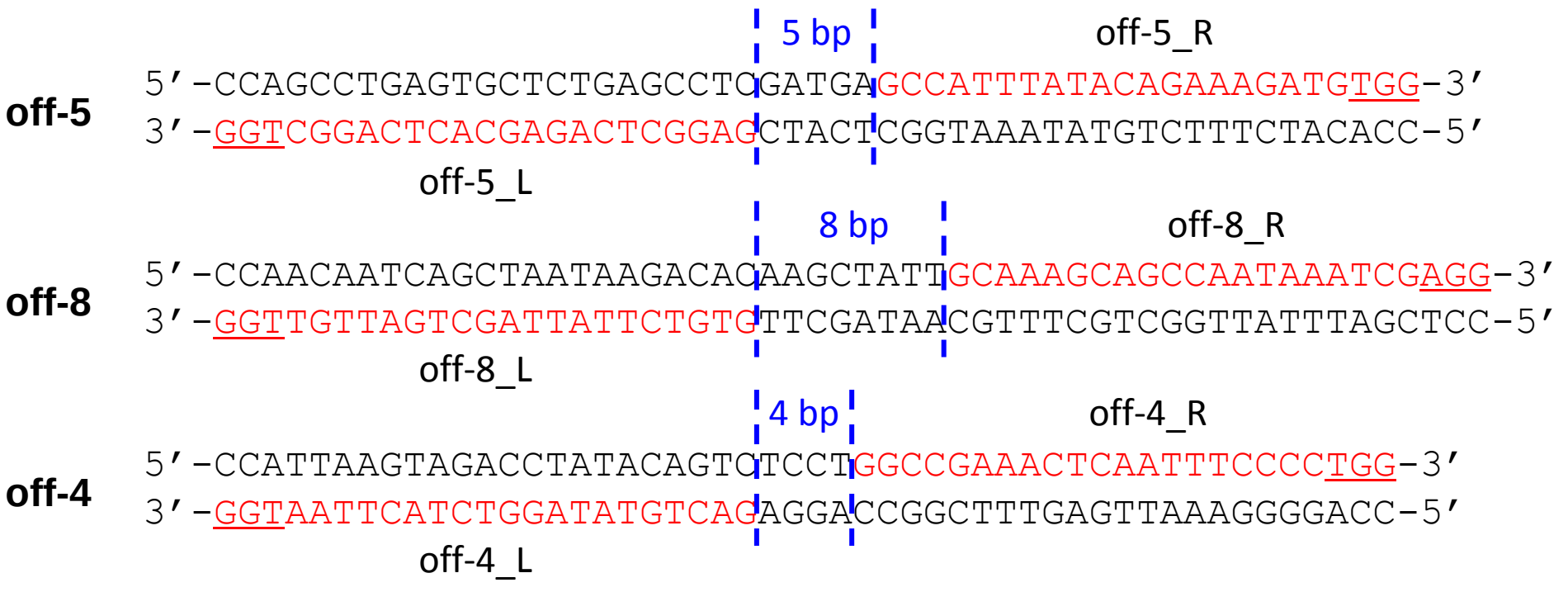


Supplementary Figure S1. PCR screening of gRNA cassette-assembled clones. (A) Schematic illustration of PCR screening. CRISPR-step2-F and CRISPR-step2-R primers were used for amplification. (B) Gel image of PCR screening. In this figure, plasmid DNAs were used as templates. Red, yellow, blue, green, purple and white asterisks indicate expected sizes of amplicons from 2-, 3-, 4-, 5-, 6- and 7-gRNA-assembled clones, respectively. M, λHindIII marker.

A

HPRT1_A	5′-TTATGCTGAGGATTTGGAAAGGG-3′ 3′-AATACGACTCCTAAACCTTTCCC-5′
HPRT1_B	5′-CCTATGACTGTAGATTTTATCAG-3′ 3′-GGATACTGACATCTAAAATAGTC-5′
ATM	5′-TGAATTGGGATGCTGTTTTTAGG-3′ 3′-ACTTAACCCTACGACAAAAATCC-5′
APC	5′-CCGGTCGCCCCTTTGCCCGCTTC-3′ 3′-GGCCAGCGGGGAAACGGGCGAAG-5′
CDH1	5′-TGACTTGCGAGGGACGCATTCGG-3′ 3′-ACTGAACGCTCCCTGCGTAAGCC-5′
AXIN2	5′-TATGTTGGTGACTTGCCTCCCGG-3′ 3′-ATACAACCACTGAACGGAGGGCC-5′
CFTR	5′-CCTATGATGAATATAGATACAGA-3′ 3′-GGATACTACTTATATCTATGTCT-5′

B



Supplementary Figure S2. Schematic design of CRISPR/Cas9 target sequences used in this study. (A) Design of CRISPR/Cas9 nucleases, related to Fig. 2. Red letters indicate target sequences including PAMs. PAM sequences are underlined. (B) Design of CRISPR/Cas9 nickases, related to Fig. 3. Red letters indicate target sequences including PAMs. PAM sequences are underlined. Blue lines and letters indicate offsets between two gRNA target sequences.

Supplementary Table S1. Summary of plasmid construction for establishing all-in-one vector system.

	Vector			Insert		
Plasmid name	Template	Forward primer (5'->3')	Reverse primer (5'->3')	Template	Forward primer (5'->3')	Reverse primer (5'->3')
pX330S	pX330	AAGCATTGGTAACTGTCAGACCAAGTTTACTC	AAGTGCCACCTGACGTCTAAGAAACC	pFUS_A30A	CGTCAGGTGGCACTTCCAGCCAGGACAGAAATGCCTC	CAGTTACCAATGCTTTTATTTGCCGACTACCTTGGTGATCTC
pX330S-2	pX330S	TGAGACCCGTTACATAACTTACGGTAAATGGCCCG	TGAGACCTGAGCAAAAGGCCAGCAAAAGG	pX330	TTTGCTCAGGTCTCACTCTAGAGGCATGTGAGGGCCTATTTCCCATGATTC	ATGTAACGGGTCTCATAGAGCCATTTGTCTGCAGAATTGGCG
pX330S-3					TTTGCTCAGGTCTCATCTAGAGGCATGTGAGGGCCTATTTCCCATGATTC	ATGTAACGGGTCTCACTAGAGCCATTTGTCTGCAGAATTGGCG
pX330S-4					TTTGCTCAGGTCTCACTAGAGGCATGTGAGGGCCTATTTCCCATGATTC	ATGTAACGGGTCTCATCTAGAGCCATTTGTCTGCAGAATTGGC
pX330S-5					TTTGCTCAGGTCTCATAGAGGCATGTGAGGGCCTATTTCCCATGATTC	ATGTAACGGGTCTCACTCTAGAGCCATTTGTCTGCAGAATTGGC
pX330S-6					TTTGCTCAGGTCTCAAGAGGCATGTGAGGGCCTATTTCCCATGATTC	ATGTAACGGGTCTCACCTCTAGAGCCATTTGTCTGCAGAATTGG
pX330S-7					TTTGCTCAGGTCTCAGAGGCATGTGAGGGCCTATTTCCCATGATTC	ATGTAACGGGTCTCAACCTCTAGAGCCATTTGTCTGCAGAATTGG
pX330A-1x2	pX330	AGGTACCCGTTACATAACTTACGGTAAATGGC	CCATTTGTCTGCAGAATTGGCGC	pFUS_A30A	TCTGCAGACAAATGGCTCTAGAGACCGGCGCCGCTACAG	ATGTAACGGGTACCTCTAGAAGAGACCACCGGTGGAAAGCG
pX330A-1x3						ATGTAACGGGTACCTCTAGAGAGACCACCGGTGGAAAGCG
pX330A-1x4						ATGTAACGGGTACCTCTAAGAGACCACCGGTGGAAAGCG
pX330A-1x5						ATGTAACGGGTACCTCTAGAGACCACCGGTGGAAAGCG
pX330A-1x6						ATGTAACGGGTACCTCAGAGACCACCGGTGGAAAGCG
pX330A-1x7						ATGTAACGGGTACCTAGAGACCACCGGTGGAAAGCG

Supplementary Table S2. Oligonucleotides and vectors for the insertion of gRNA target sequences.

Target locus	Vector	Sense oligonucleotide (5'->3')	Antisense oligonucleotide (5'->3')
*HPRT1*_A	pX330A-1x7	CACCGTTATGCTGAGGATTTGGAAA	AAACTTTCCAAATCCTCAGCATAAC
*HPRT1*_B	pX330S-2	CACCGCTGATAAAATCTACAGTCAT	AAACATGACTGTAGATTTTATCAGC
ATM	pX330S-3	CACCGTGAATTGGGATGCTGTTTTT	AAACAAAAACAGCATCCCAATTCAC
APC	pX330S-4	CACCGAAGCGGGCAAAGGGGCGAC	AAACGTCGCCCCTTTGCCCGCTTC
CDH1	pX330S-5	CACCGTGACTTGCGAGGGACGCATT	AAACAATGCGTCCCTCGCAAGTCAC
AXIN2	pX330S-6	CACCGTATGTTGGTGACTTGCCTCC	AAACGGAGGCAAGTCACCAACATAC
CFTR	pX330S-7	CACCGTCTGTATCTATATTCATCAT	AAACATGATGAATATAGATACAGAC
off-5_L	pX330A_D10A-1x6	CACCGAGGCTCAGAGCACTCAGGC	AAACGCCTGAGTGCTCTGAGCCTC
off-5_R	pX330S-2	CACCGCCATTTATACAGAAAGATG	AAACCATCTTTCTGTATAAATGGC
off-8_L	pX330S-3	CACCGTGTCTTATTAGCTGATTGT	AAACACAATCAGCTAATAAGACAC
off-8_R	pX330S-4	CACCGCAAAGCAGCCAATAAATCG	AAACCGATTTATTGGCTGCTTTGC
off-4_L	pX330S-5	CACCGACTGTATAGGTCTACTTAA	AAACTTAAGTAGACCTATACAGTC
off-4_R	pX330S-6	CACCGGCCGAAACTCAATTTCCCC	AAACGGGGAAATTGAGTTTCGGCC

Supplementary Table S3. Primers used for genomic cleavage assays.

Target locus	Forward primer (5'->3')	Reverse primer (5'->3')
*HPRT1*_A	TCCTGTAATGCTCTCATTGAAACAGC	GCTGCTGATGTTTGAAATTAACACAAG
*HPRT1*_B	TTTCTGTAGGACTGAACGTCTTGCTC	ATCTCACTGTAACCAAGTGAAATGAAAGC
ATM	GCGCCTGATTCGAGATCCTGAAAC	AAATGCCAAATTCATATGCAAGGCATAATG
APC	CTCGATGCTGTTCCCAGGTACTGTTG	GCCCAGTGGCCAGAAGTACGAG
CDH1	GCACCTGTGAGCTTGCGGAAGTCAG	TCCGCTCCTCAGGACCCGAACTTTC
AXIN2	CCAACCCATCTTCGTTCCGCCTGG	TGAGAGAGACAGAGAGACCACGCCG
CFTR	GGGAGAACTGGAGCCTTCAGAG	TGAAGGGTTCATATGCATAATCAAAAAG
off-5	CGAGAAGTACCTAAAAATAAAGCACCTACTGCTG	TTCATTTGATTCTTTAGGCTGCTCTGATTC
off-8	GGAACTCCTTACTGTTTTTCACGAAATGATTC	GTTGCTGCCCCTCTGTCTGGTATG
off-4	TGAAGGTCAAACAGCCACCACTTCTC	GAACCTGAGGACTTAGTTGAAGCAGTACTAGGG

Supplementary Table S4. Primers used for detecting chromosomal deletions.

Target locus	Forward primer (5'->3')	Reverse primer (5'->3')
HPRT1	TCCTGTAATGCTCTCATTGAAACAGC	ATCTCACTGTAACCAAGTGAAATGAAAGC
APC	CGAGAAGTACCTAAAAATAAAGCACCTACTGCTG	GAACCTGAGGACTTAGTTGAAGCAGTACTAGGG