Multiplex CRISPR/Cas9-based genome engineering enhanced by Drosha-mediated sgRNA-shRNA structure

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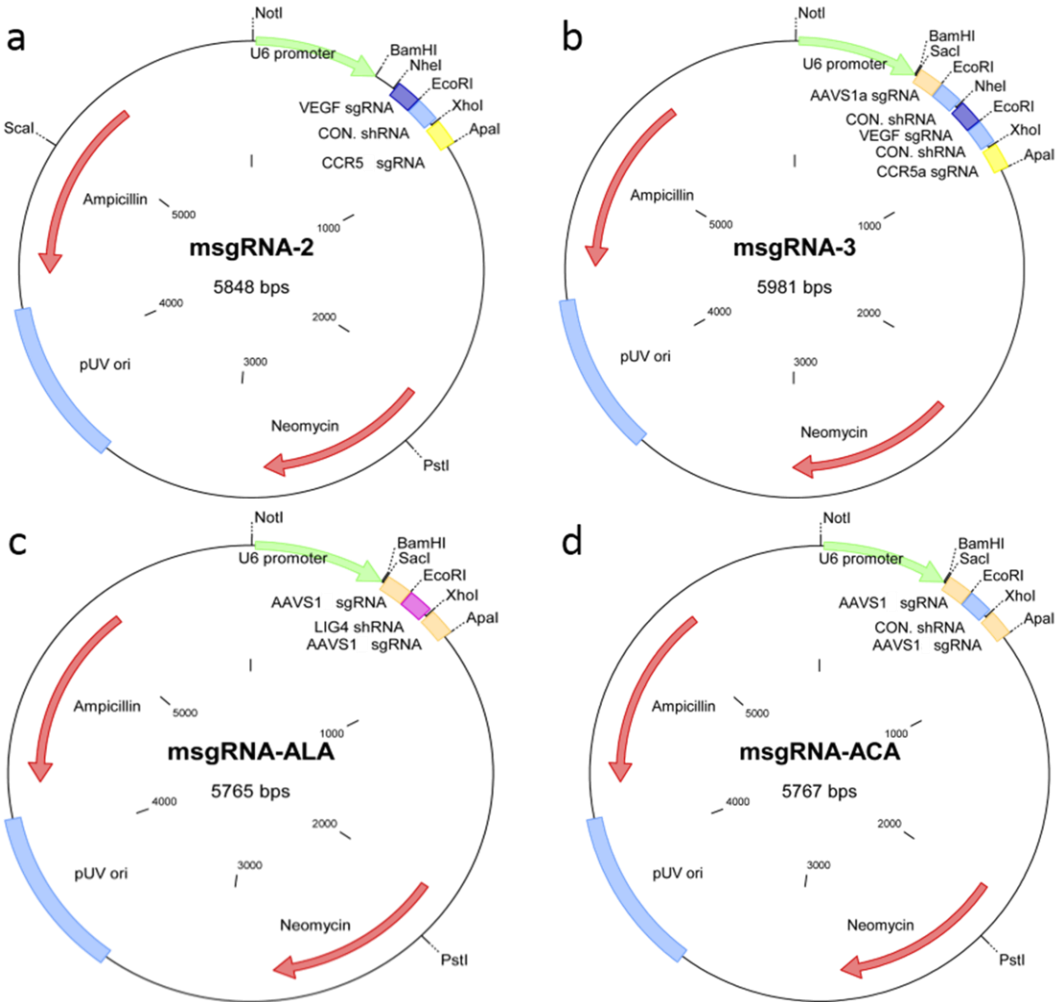
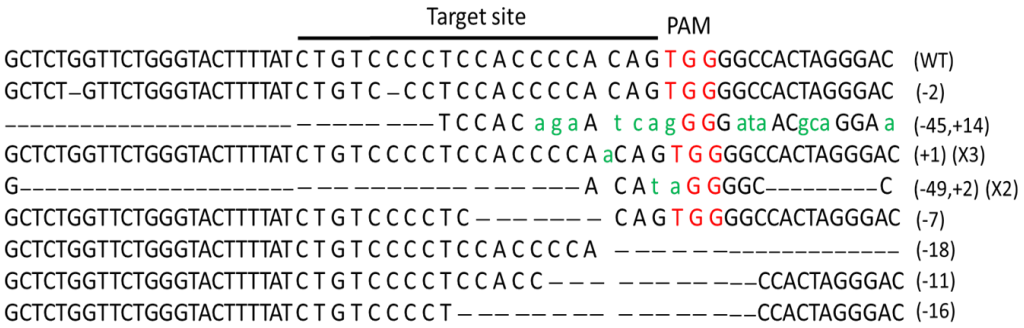
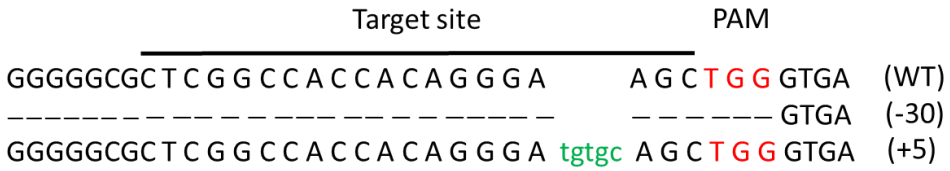
Supplementary Information



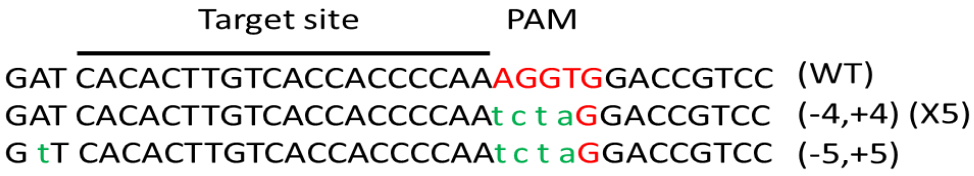
Supplementary Figure 1 Plasmid maps for the constructs: (a) msgRNA-2, (b) msgRNA-3, (c) msgRNA-ALA and (d) msgRNA-ACA



(a) AAVS1 site (11/12, 91.67%)

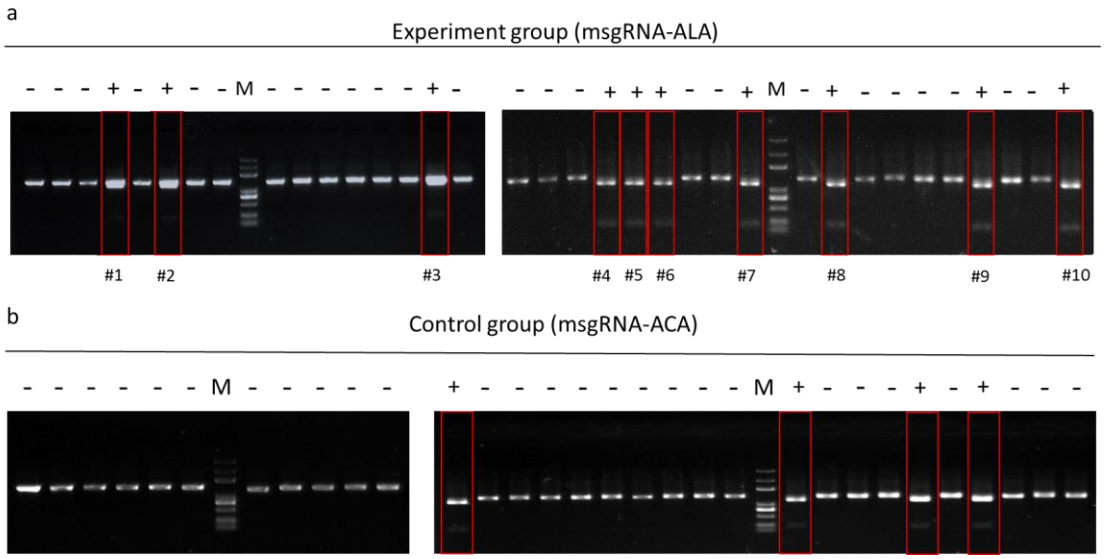


(b) VEGF site (2/9, 22.22%)

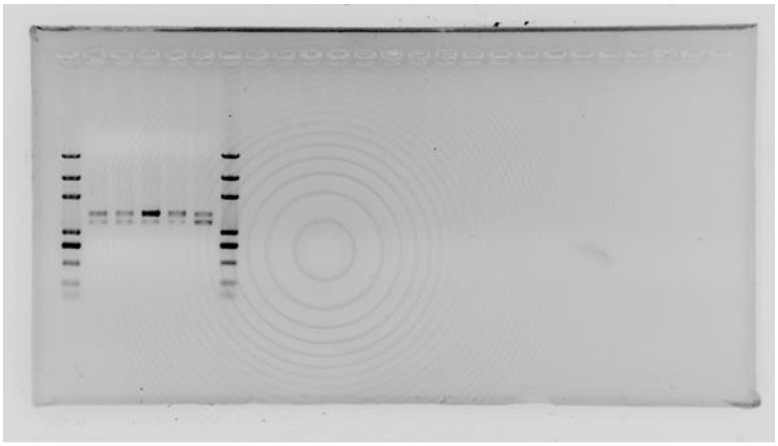


(c) CCR5 site (6/7, 85.71%)

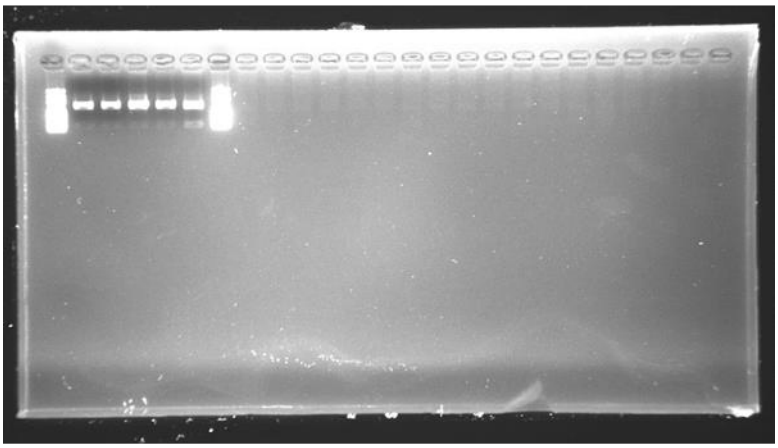
Supplementary Figure 2 The indels within the detected "T-A clones" from the cell pool targeted by msgRNA-3. (a) AAVS1 site, (b) VEGF site, (c) CCR5 site



Supplementary Figure 3 The results for the detection of genome edited positive clones by *Eco*RⅠ-digesting assay. The PCR product was supposed to be 1397 bp in length and could be digested into two fragments (1194 bp and 203 bp) if the genome DNA had been successfully edited by HDR-based repair. The PCR product would be partially digested if the clone was heterozygotes with only one allele edited. Percentage of digestion positive clones within detected clones was calculated to evaluate the precise genome editing efficiency. The results demonstrated 10 positive clones out of 35 cell clones (10/35) for the msgRNA-ALA group, while 4 out of 31 clones (4/31) for the msgRNA-ACA control. However, the pictures and the bands were not clear for confirming whether one or both alleles were edited. Further repeat detection of these positive clones demonstrated that they were all heterozygotes with one allele edited (refer to Fig.4e in the manuscript).



a



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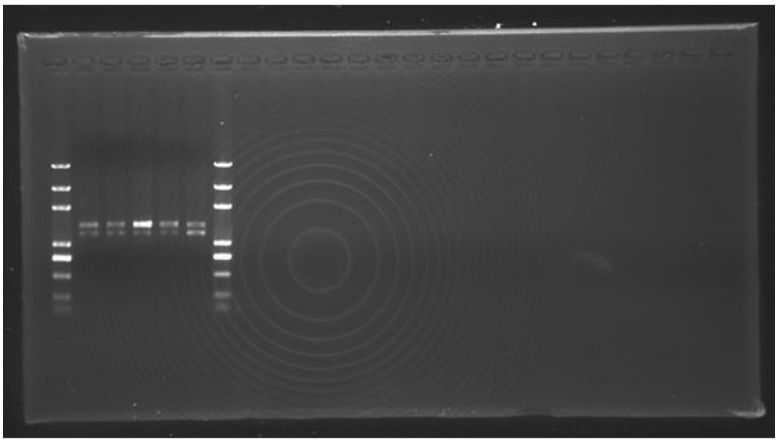


c

Supplementary Figure 4 Full-length gel images of representative results for the detection of positive clones by EcoR Ⅰ digestion. (a) Full-length gel of Fig. 4e in the manuscript. (b)

Overexposure after a shorter electrophoresis period for a clear vision of the 203bp band. (c) Normal-length exposure after a longer electrophoresis period for a clear vision of the 1397 bp and 1194 bp bands.

Supplementary Table 1 Transfection groups set for the DsRed-eGFP surrogate reporter assay

	Cas9 vector	msgRNA	Reporter	Group
Molar ratio	1	1.5	0.8	
VEGF target groups	pll3.7-U6-CMV-hStCas9	msgRNA-2	pRG.VEGF	Experiment group
	pll3.7-U6-VEGF-CMV-hStCas9	pcDNA3.1(+)	pRG.VEGF	Positive control
	pll3.7-U6-CMV-hStCas9	pcDNA3.1(+)	pRG.VEGF	Negative control
CCR5 target groups	pll3.7-U6-CMV-hStCas9	msgRNA-2	pRG.CCR5	Experiment group
	pll3.7-U6-CCR5-CMV-hStCas9	pcDNA3.1(+)	pRG.CCR5	Positive control
	pll3.7-U6-CMV-hStCas9	pcDNA3.1(+)	pRG.CCR5	Negative control

Supplementary Table 2 Transfection groups set for the multiplex genome targeting assay

	Cas9 vector	msgRNA	Reporter	Group
Molar ratio	1	1.5	0.8	
AAVS1 target groups	pll3.7-U6-CMV-hStCas9	msgRNA-3	pRPG.AAVS1	Experiment 1
	pll3.7-U6-AAVS1-CMV-hStCas9	pcDNA3.1(+)	pRPG.AAVS1	Positive control 1
VEGF target groups	pll3.7-U6-CMV-hStCas9	msgRNA-3	pRPG.VEGF	Experiment 2
	pll3.7-U6-VEGF-CMV-hStCas9	pcDNA3.1(+)	pRPG.VEGF	Positive control 2

CCR5 target groups	pll3.7-U6-CMV-hStCas9	msgRNA-3	pRPG.CCR5	Experiment 3
	pll3.7-U6-CCR5-CMV-hStCas9	pcDNA3.1(+)	pRPG.CCR5	Positive control 3

Supplementary Table 3 Diplex and triplex targeted positive clones detected in the multiplex genome targeting assay

Groups	**Number of diplex and triplex targeted positive clones/Detected cell clones**			
	AAVS1 & VEGF	VEGF & CCR5	AAVS1 & CCR5	AAVS1 & VEGF & CCR5
Experiment 1	1/12	0/12	2/12	1/12
Experiment 2	1/10	1/10	1/10	1/10
Experiment 3	0/10	0/10	1/10	1/10
General frequency	6% (2/32)	3% (1/32)	13% (4/32)	9% (3/32)

Supplementary Table 4 The overall mutated clones for each targeted locus in the multiplex genome targeting assay

Groups	**Mutated clones/Detected clones**			**Remarks**
	AAVS1	VEGF	CCR5	
Experiment 1	11/12	2/12	4/12	pRPG.AAVS1 as reporter
Experiment 2	6/10	3/10	5/10	pRPG.VEGF as reporter
Experiment 3	9/10	1/10	2/10	pRPG.CCR5 as reporter
General frequency	81% (26/32)	19% (6/32)	34% (11/32)	
Positive control 1	100% (6/6)	-	-	pRPG.AAVS1 as reporter
Positive control 2	-	100% (8/8)	-	pRPG.VEGF as reporter

Positive control 3	-	-	38% (5/13)	pRPG.CCR5 as reporter

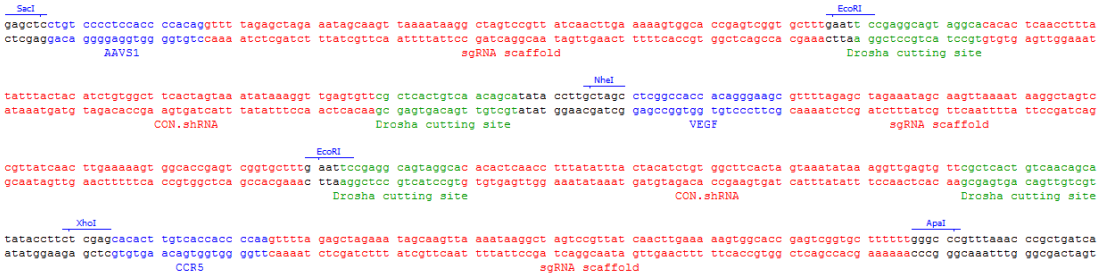
Supplementary Table 5 Primers used in Quantitative RT-PCR assay

Primer name	Sequence	Remarks
qP-LIG4.F	GCCCGAGGCCAGTTAAACGAGAAG	For *LIG4* gene in the
qP-LIG4.R	GTGGTTCTTATGAAGAGCATCATG	qRT-PCR assay
ACTB forward	ATTGCCGACAGGATGCAGA	For *β-actin* gene in the
ACTB reverse	GAGTACTTGCGCTCAGGAGGA	qRT-PCR assay

Supplementary Sequence 1 The DNA sequence designed for the msgRNA-2 construct (between *Nhe*I and *Apa*I cutting site).

NheI EcoRI
gctagcctcg gccaccacag ggaagcgttt tagagctaga aatagcaagt taaaataagg ctagtccgtt atcaacttga aaaagtggca ccgagtcggt gctttgaatt
cgatcggagc cggtggtgtc ccttcgcaaa atctcgatct ttatcgttca attttattcc gatcaggcaa tagttgaact ttttcaccgt ggctcagcca cgaaacttaa
VEGF sgRNA scaffold
XhoI
ccgaggcagt aggcacacac tcaacctta tatttactac atctgtggct tcactagtaa atataaaggt tgagtgttcg ctcactgtca acagcatata ccttctcgag
ggctccgtca tccgtgtgtg agttggaaat ataaatgatg tagacaccga agtgatcatt tatatttcca actcacaagc gagtgacagt tgtcgtatat ggaagagctc
drosha cutting site CON. shRNA drosha cutting site
ApaI
cacacttgtc accaccccaa gttttagagc tagaaatagc aagttaaaat aaggctagtc cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttgggcccgt
gtgtgaacag tggtggggtt caaaatctcg atctttatcg ttcaatttta ttccgatcag gcaatagttg aacttttca ccgtggctca gccacgaaaa aacccgggca
CCR5 sgRNA scaffold

Supplementary Sequence 2 The DNA sequence designed for the msgRNA-3 construct (between *Sac*I and *Apa*I cut sites).


SacI
EcoRI
AAVS1
sgRNA scaffold
Drosha cutting site
NheI
CON.shRNA
Drosha cutting site
VEGF
sgRNA scaffold
EcoRI
Drosha cutting site
CON.shRNA
Drosha cutting site
XhoI
ApaI
CCR5
sgRNA scaffold