SUPPORTING INFORMATION

One-day TALEN Assembly Protocol and a Dual-Tagging system for Genome Editing

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Table S1. Primers for TALE monomers amplification and colony PCR

Name	Sequence (5′→3′)	Usage
PCR-TAL-F	TATCATCATGCCTCCTCTAGAG	monomer amplification (universal primer)
PCR-TAL-R	TTGGTCATGGGTGGCTCGAGG	
PCR-TAL-F1	TATCATCATGCCTCCTCTAGAGGTCTCCCTATCTTAAAC CGGCCAACATACCCGTCTCCCCCTGAACCTGACCCCGG ACCAAGTGGTGGCTATCGCCAGC	First monomer amplification
PCR-TAL-R5	TTGGTCATGGGTGGCTCGAGGGTCTCCATA GAGTCTGTCTTTCCCCTTTCCCGTCTCCTGCACCG	Fifth monomer amplification
PCR-TAL-F6	TATCATCATGCCTCCTCTAGAGGTCTCCCTATCTTAAAC CGGCCAACATACCCGTCTCGTGCAGCGGC	Sixth monomer amplification
PCR-TAL-R10	TTGGTCATGG GTGGCTCGAG GGTCTCCATA GAGTCTGTCT TTCCCCTTTC CCGTCTCCCGCC	Tenth monomer amplification
PCR-TAL-F11	TATCATCATGCCTCCTCTAGAGGTCTCATGGCCTGACCC CGGACCAAGTGGTGGCTATCGCCAGC	Eleven monomer amplification
PCR-TAL-R14	TTGGTCATGG GTGGCTCGAG GGTCTCCTCG AAGTCTGTCT TTCCCCTTTC CCGTCTCCAACAGCCG	Fourteenth monomer amplification
PCR-TAL-F15	TATCATCATGCCTCCTCTAGAGGTCTCCTCGACTTAAAC CGGCCAACATACCCGTCTCCTGTTGCCGG	Fifteenth monomer amplification
PCR-TAL-F18	TATCATCATGCCTCCTCTAGAGGTCTCGCTATCGCCAGC	Eighteenth monomer amplification
PCR-TAL-R18	TTGGTCATGGGTGGCTCGAGGGTCTCTTCGAAGTCTGTC TTTCCCCTTTCCCGTCTCTCGTTGGTCAAC	Eighteenth monomer amplification
TAL-F	TTGGCGTCGGCAAACAGTGG	Colony PCR amplification and TALE sequencing
TAL-R	GGCGACGAGGTGGTCGTTGG	Colony PCR amplification and TALE sequencing

Table S2. Synthetic double-stranded DNA

Name	Encoded chain sequence (5′→3′)
$dsDNA_{10.5}$	TATCATCATGCCTCCTCTAGAGGTCTCCTCGACTTAAACCGGCCAACATACCCGTCTCTGG CGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATG GCCGAGACCCTCGAGCCACCCATGACCAA
$dsDNA_{17.5}$	TATCATCATGCCTCCTCTAGAGGTCTCCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGC CAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGAGACCCTCGAGCCACCCATG ACCAA

Table S3. Primers vector and homology arm preparation and clone identification

Name	Sequence (5′→3′)	Usage
FokI-F	CCGGAATTCCCTAGTGAAATCTGAATTGGAAG	FokI amplification
FokI-R	AAGGAAAAAAGCGGCCGCTTAAAAGTTTATCTCACCG	
5-RELA-hom-F	TGGACTTCTCAGCCCTGCTGAGTCAGATCAGCTCCGGA GGAGGTTCCGGTGGAGGTGG	RELA homology arm amplification
3-RELA-hom-SBP-R	TCAATCCCCTGCAACCCAGTGCTCTGGGGAGGGCATTAA AGGGCAAGGAGTGTGGCACC	
3-RELA-hom-AviTag-R	TCAATCCCCTGCAACCCAGTGCTCTGGGGAGGGCACTA ACGTGGCTTCTTCTGCCAAAG	
5-RELB-hom-F	GGGGCGGCCTCCTATCCCCGGGGCCTGAAGCCACGGGA GGAGGTTCCGGTGGAGGTGG	RELB homology arm amplification
3-RELB-hom-SBP-R	TTGCACGGCTCCTCCACCTCCCTCCCCACCCAGTGTTAA AGGGCAAGGAGTGTGGCACC	
3-RELB-homAviTag-R	TTGCACGGCTCCTCCACCTCCCTCCCCACCCAGTGCTAA CGTGGCTTCTTCTGCCAAAG	
5-CREL-hom-F	TGAGTGACTCCTTTCCATATGAATTTTTTCAAGTAGGAG GAGGTTCCGGTGGAGGTGG	CREL homology arm amplification
3-CREL-hom-SBP-R	CCCAGTTAGACAAATACAAAATGCTGCATCTATATTTAAA GGGCAAGGAGTGTGGCACC	
3-CREL-hom-AviTag-R	CCCAGTTAGACAAATACAAAATGCTGCATCTATATCTAAC GTGGCTTCTTCTGCCAAAG	
5-NFKB1-hom-F	ATTATGGGCAGGAAGGACCTCTAGAAGGCAAAATTGGA GGAGGTTCCGGTGGAGGTGG	NFKB1 homology arm amplification
3-NFKB1-hom-SBP-R	AGCTTCTGTCTTGTGGACAACGCAGTGGAATTTTATTAA AGGGCAAGGAGTGTGGCACC	
3-NFKB1-hom-AviTag-R	AGCTTCTGTCTTGTGGACAACGCAGTGGAATTTTACTAA CGTGGCTTCTTCTGCCAAAG	
5-NFKB2-hom-F	GGCTCTGCCACGGGCACCCCCAGCCTCAGGTGCACGGA GGAGGTTCCGGTGGAGGTGG	NFKB2 homology arm amplification
3-NFKB2-hom-SBP-R	GGTGTGGGGTGTTAAATAAGATTTGAAATAGGTGGTTAA AGGGCAAGGAGTGTGGCACC	
3-NFKB2-homAviTag-R	GGTGTGGGGTGTTAAATAAGATTTGAAATAGGTGGCTAA CGTGGCTTCTTCTGCCAAAG	
Lac-px-F	ACGGTCTCGCACCGGGTCTTCAGCGCCCAATACGCAAA CCGCCTC	Lac amplification
Lac-px-R	TCGGTCTCCAAACAGGTCTTCGCGTCCATTCGCCATTCA GGCTGCGC	
U6-test-F	GAGGGCCTATTTCCCATGATTC	NF-κB-targeting psgRNA-Cas9 positive clone identification by colony PCR
RELA-sgRNA-test-R	CTTAGGAGCT GATCTGACTC	
RELB-sgRNA-test-R	CTCCTCTGGCATCGCGGGGC	
CREL-sgRNA-test-R	TTTAAATCTT GATACCACCT	
NFKB1-sgRNA-test-R	CACCGTGTAA ACCAAAGCCC	
NFKB2-sgRNA-test-R	CTTCCCGGACCCCCTGTACA	

Table S4. Designed TALEN targets

Name	Sequence (5′→3′)
RELA-left-TALEN	CTGAGTCAGATCAGCTCC
RELA-right-TALEN	AGTGCTCTGGGGAGGGCA
RELB-left-TALEN	CCGGGGCCTGAAGCCACG
RELB-right-TALEN	CCCCACCCAGTGCCCCTC
CREL-left-TALEN	TATGAATTTTTTCAA GTA
CREL-right-TALEN	ATCAAGATTTAAAAGGAT
NFKB1-left-TALEN	CCTCTAGAAGGCAAAATT
NFKB1-right-TALEN	TTGGTTTACACGGTGTGG
NFKB2-left-TALEN	CCCCAGCCTCAGGTGCAC
NFKB2-right-TALEN	CCGGGAAGGGGGCTGGGG

Table S5. Designed sgRNA targets

Name	sequence (5′→3′)
RELA-sgRNA-PAM	GAGTCAGATCAGCTCCTAAGGGG
RELB-sgRNA-PAM	GCCCCGCGATGCCAGAGGAGGGG
CREL-sgRNA-PAM	AGGTGGTATCAAGATTTAAAAGG
NFKB1-sgRNA-PAM	GGGCTTTGGTTTACACGGTGTGG
NFKB2-sgRNA-PAM	TGTACAGGGGGTCCGGGAAGGGG

Table S6. Oligonucleotide used to prepare target-specific regions (20 bp) of sgRNA

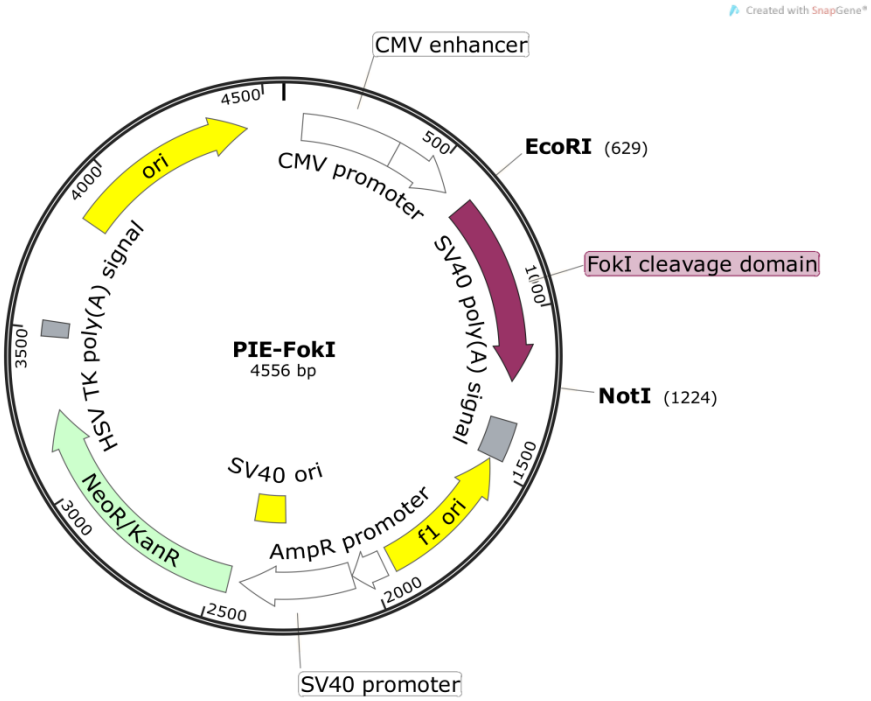
Gene	Name	Sequence (5′→3′)
RELA	RELA-sg-S	5′-AGGAAGACGGCACCGGGAGTCAGATCAGCTCCTAAGGTTTGGGTCTTCGA-3′
	RELA-sg-AS	5′-TCGAAGACCCAAACCTTAGGAGCT GATCTGACTCCCGGTGCCGTCTTCCT-3′
RELB	RELB-sg-S	5′-AGGAAGACGGCACCGGGCCCCGCGATGCCAGAGGAGGTTTGGGTCTTCGA-3′
	RELB-sg-AS	5′-TCGAAGACCCAAACCTCCTCTGGCATCGCGGGGCCCGGTGCCGTCTTCCT-3′
CREL	CREL-sg-S	5′-AGGAAGACGGCACCGGAGGTGGTATCAAGATTTAAAGTTTGGGTCTTCGA-3′
	CREL-sg-AS	5′-TCGAAGACCCAAACTTTAAATCTT GATACCACCTCCGGTGCCGTCTTCCT-3′
NFKB1	NFKB1-sg-S	5′-AGGAAGACGGCACCGGGGGCTTTGGTTTACACGGTGGTTTGGGTCTTCGA-3′
	NFKB1-sg-AS	5′-TCGAAGACCCAAACCACCGTGTAA ACCAAAGCCCCCGGTGCCGTCTTCCT-3′
NFKB2	NFKB2-sg-S	5′-AGGAAGACGGCACCGGTGTACAGGGGGTCCGGGAAGGTTTGGGTCTTCGA-3′
	NFKB2-sg-AS	5′-TCGAAGACCCAAACCTTCCCGGACCCCCTGTACACCGGTGCCGTCTTCCT-3′

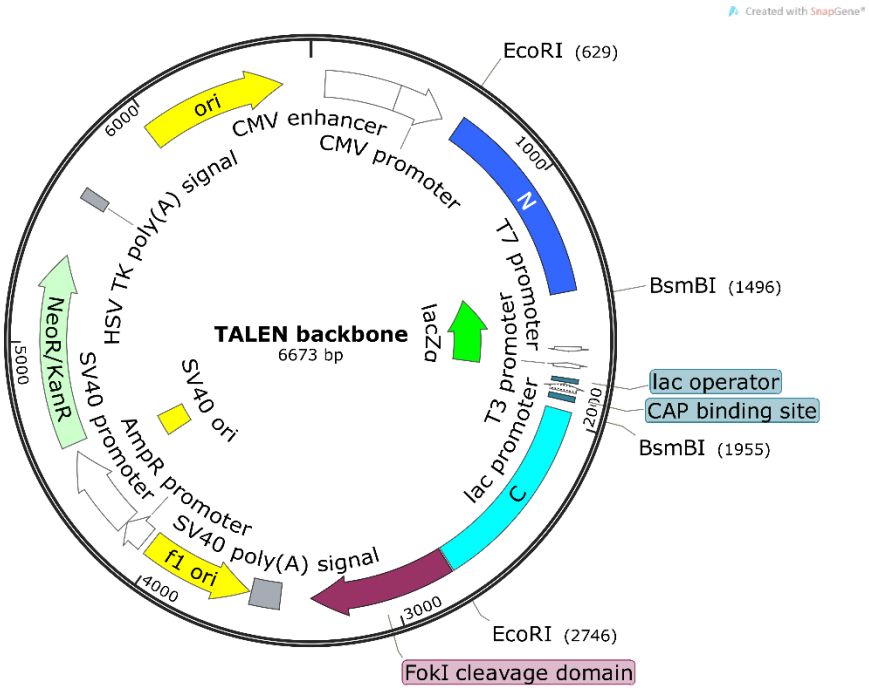
S, sense; AS, antisense

Table S7. Primers for verifying homologous recombination

Name	Sequence (5′→3′)	Usage
RELA-F	TGGACTTCTCAGCCCTGCTGAGTCAGATCAGCTCC	RELA editing detection with qPCR
IRES2-R	GACTCTTTCCACAACTATCCAACTCACAACGTGG	
RELB-F	GGGGCGGCCTCCTATCCCCGGGGCCTGAAGCCACG	RELB editing detection with qPCR
IRES2-R	GACTCTTTCCACAACTATCCAACTCACAACGTGG	
CREL-F	TGAGTGACTCCTTTCCATATGAATTTTTTCAAGTA	CREL editing detection with qPCR
IRES2-R	GACTCTTTCCACAACTATCCAACTCACAACGTGG	
NFKB1-F	ATTATGGGCAGGAAGGACCTCTAGAAGGCAAAATT	NFKB1 editing detection with qPCR
IRES2-R	GACTCTTTCCACAACTATCCAACTCACAACGTGG	
NFKB2-F	GGCTCTGCCACGGGCACCCCCAGCCTCAGGTGCAC	NFKB2 editing detection with qPCR
IRES2-R	GACTCTTTCCACAACTATCCAACTCACAACGTGG	

Vectors and their elements and sequences





CMV enhancer + CMV promoter + N-term of TALE + Repeating sequence area of TALE instead of LacZa + C-term of TALE + FokI cleavage domain

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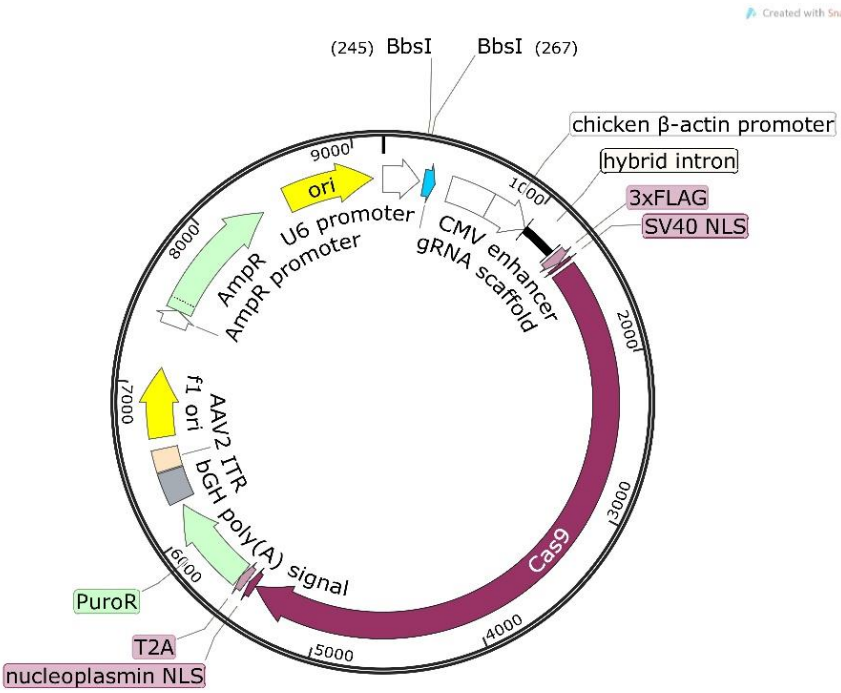

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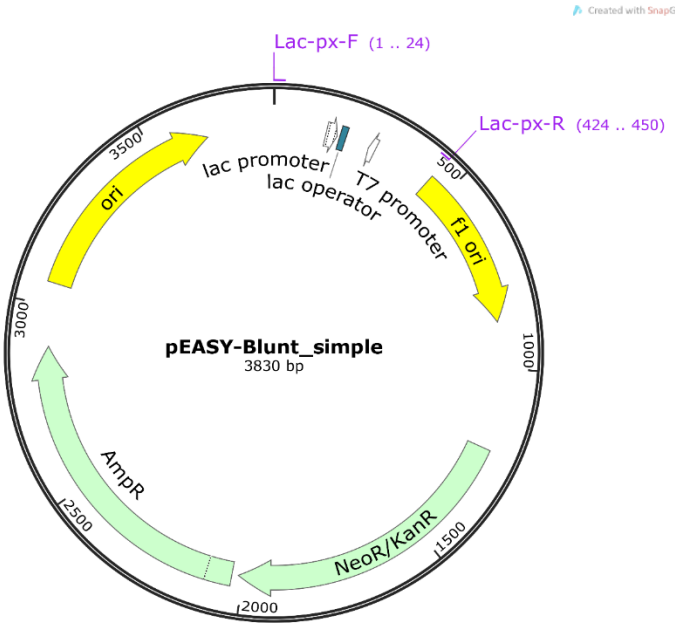

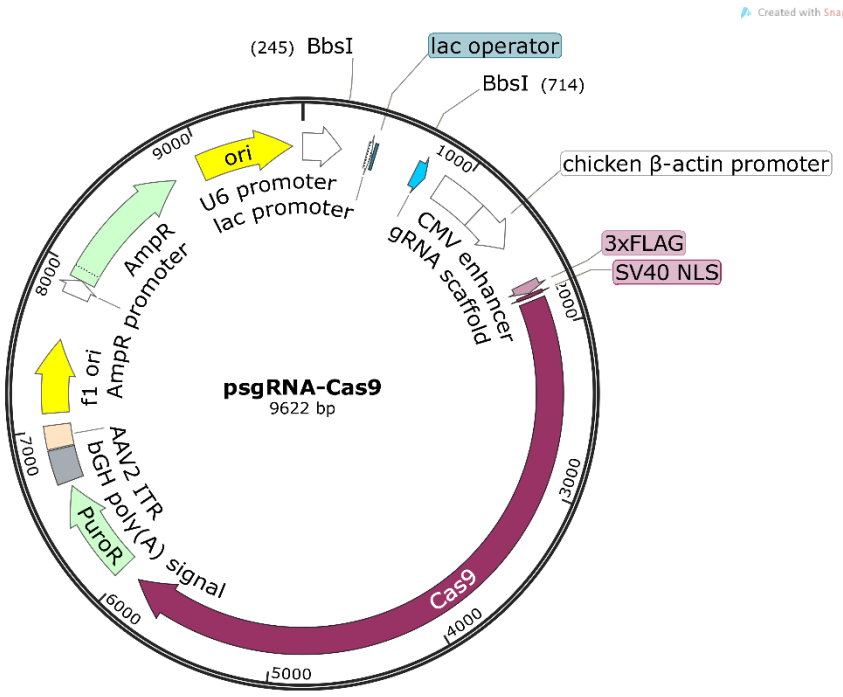

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+3×FLAG+SV40 NLS+Cas9+PuroR

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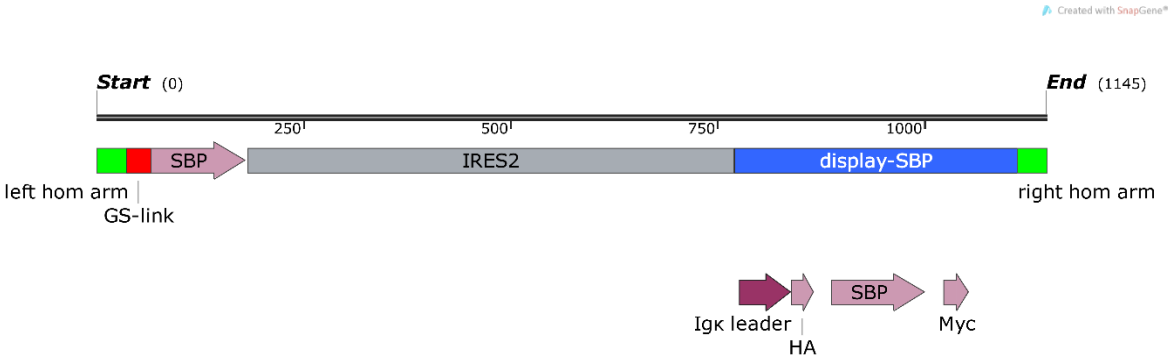
HDR donor: SBP-IRES2-displaySBP



Left hom arm+GSlink+SBP+IRES2+display-SBP+right hom arm

Left hom arm+ ggaggaggttccggtggaggtggttctgga+
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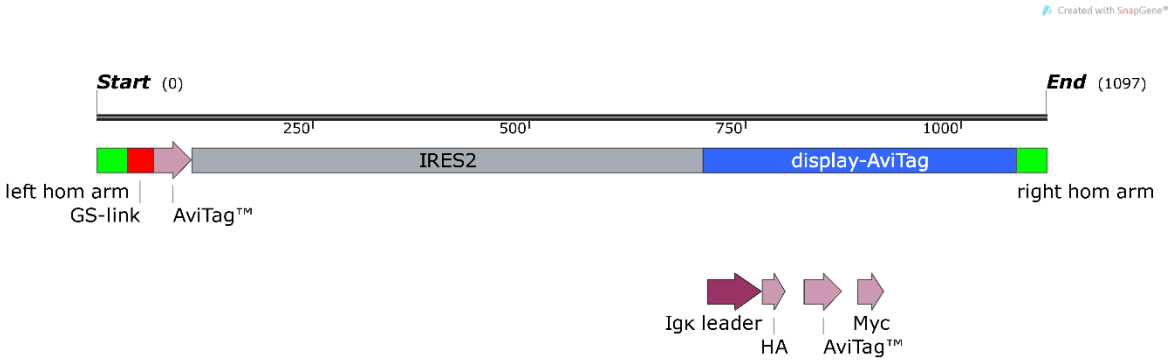
HDR donor: AviTag-IRES2-display AviTag



Left hom arm+ ggaggaggttccggtggaggtggttctgga+
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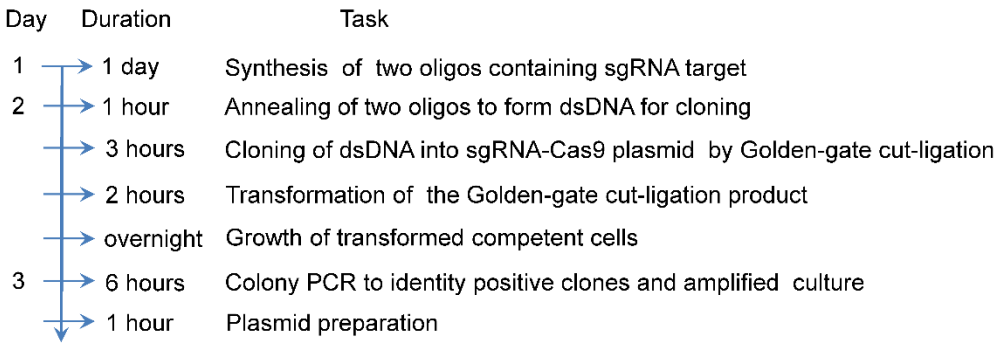
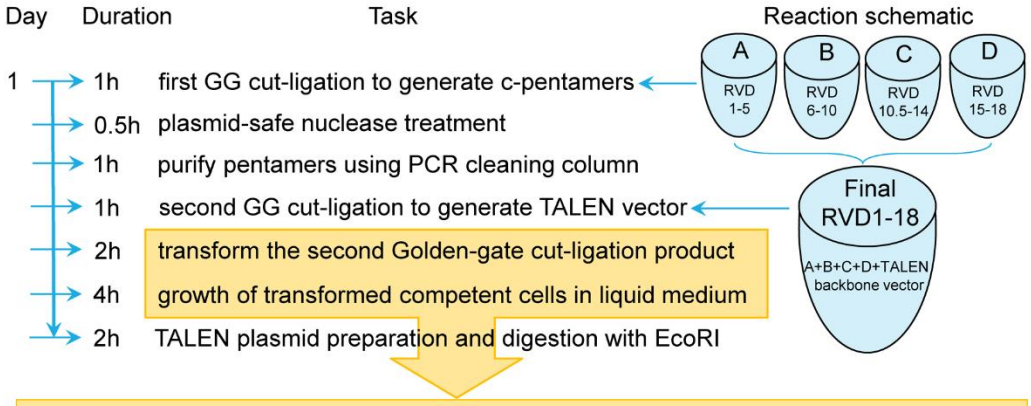


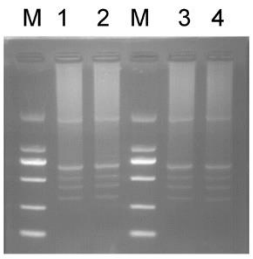
Figure S1. Pipeline for constructing custom CRISPR/Cas9-sgRNA expression vector (plasmid). Time used in each step was given. The whole procedure can be finished in three days.

A



1. At the end of the second Golden-gate cut-ligation reaction, the total of 20 μL reaction product was then used to transform the competent *E. coli* DH5α (Tiangen, China).
2. The transformed DH5α (200 μL) was added with 800 μL LB media and incubated at 37℃ for 1 h.
3. The total of 1 mL bacterial fluid was added into 5 mL LB media containing 100 μg/mL kanamycin and cultured at 37℃ for 4 h.

B



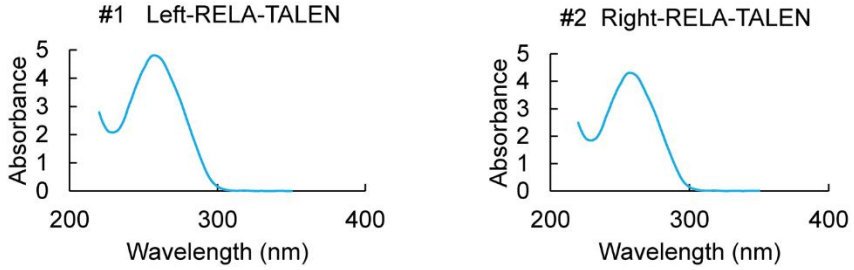
4. After 4-h cultivation, bacterial fluids were directly detected by PCR.

1 and 2, Left-RELA-TALEN;
3 and 4, Right-RELA-TALEN.
M: DL2000 marker
Note: Each bacterial fluid was detected two times.

C

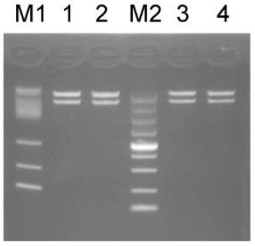
#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1		admin	2019-12-28 13:44:18	238.0	ng/μl	4.760	2.425	1.96	2.29	DNA	50.00

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
2		admin	2019-12-28 13:45:55	213.2	ng/μl	4.265	2.173	1.96	2.31	DNA	50.00



5. Plasmid DNA was extracted from all culture media (6 mL) with the EndoFree Plasmid kit. Plasmid DNA was quantified with NanoDrop. Totally 120 μL DNA solution was obtained for each TALEN. As a result, as total as 28.6 μg (Left-RELA-TALEN) and 25.6 μg (Right-RELA-TALEN) plasmid DNA was obtained for each TALEN.

D



6. The extracted TALEN plasmids were confirmed by EcoRI digestion.

1 and 2, Left-RELA-TALEN;
3 and 4, Right-RELA-TALEN.
M1: DL10000 marker
M2: DL 5000 marker
Note: Each plasmid DNA was detected two times.

The obtained 28.6 μg (Left-RELA-TALEN) and 25.6 μg (Right-RELA-TALEN) plasmid DNA are sufficient for the transfection of as many as 6.25×10^7 cells (125 wells of 24-well plate, 5×10^5 cells/well for transfection), from which as many as 6×10^6 cells positive cells (i.e. successfully edited cells) can be obtained according to the editing efficiency of 9.75% .

Figure S2. Preparation of TALEN expression plasmid just in one day. Steps 1–6 describes the whole process. (A) Pipeline for constructing custom TALEN with linear monomers and TALEN backbone plasmid. Time used in each step was given. The detail protocol for Step 1–3 were provided. (B) Detection of PCR products of bacterial fluids. (C) quantification of purified TALEN plasmid DNA. (D) Detection of purified TALEN plasmid DNA with agarose gel electrophoresis.

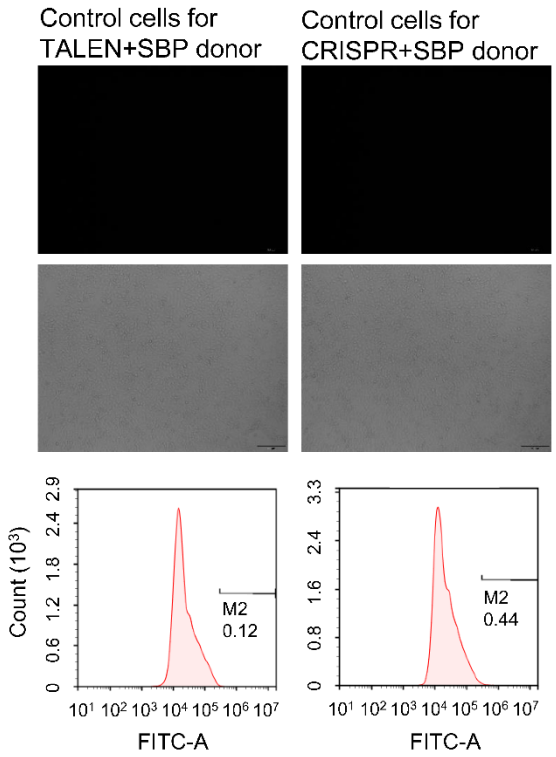


Figure S3. Editing five NF-κB genes with TALEN and CRISPR (control cells). The controls cells were just transfecte with lipofectin. The Cells were stained with Alexa Fluor® 488-conjugated Streptavidin and imaged with fluorescence microscope (up) and quantitative analzed with flow cytometry (down).

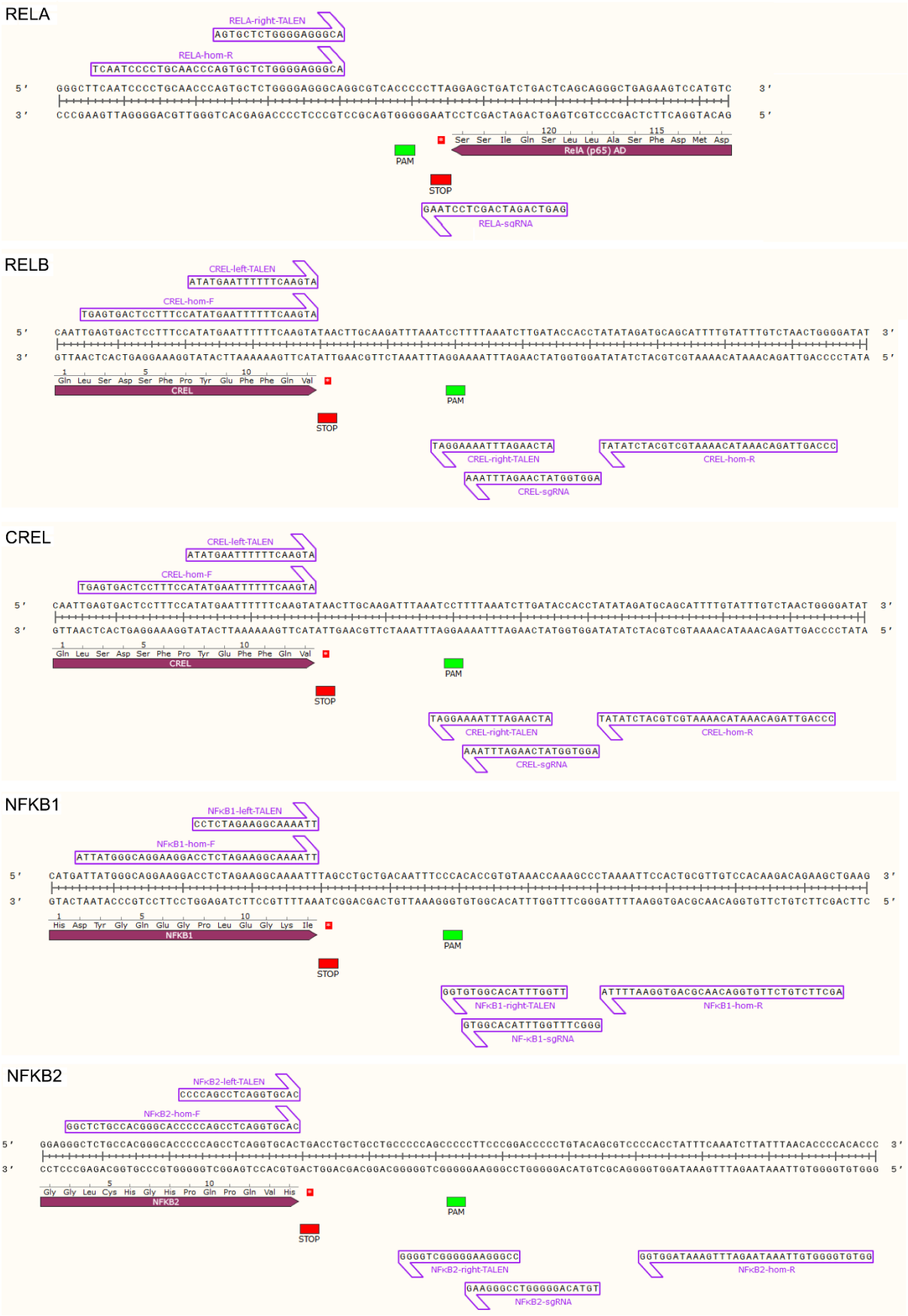


Figure S4. Locations of binding sites of all TALENs and CRISRP/Cas9-sgRNA