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Supplemental Information

A Simple Cloning-free Method to Efficiently

Induce Gene Expression Using CRISPR/Cas9

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Supplementary information:



Supplementary figure 1: Design of sgRNA expression cassette for SpCas9.

gBLOCK_F_primer

My	c tag	X	U6 promoter >>>				
	20	40		60	80	100	
GACTGTAAACACA CTGACATTTGTGT	AAGATATTAGTAG	CAAAATACGTGACGTAGA GTTTTATGCACTGCATCT	AAGTAATAATTTCTT	GGGTAGTTTGCAGTTT CCCATCAAACGTCAAA	TAAAATTATGTTT	TAAAATGGACTATCATATG ATTTTACCTGATAGTATAC	
70			U6 promoter			2	
12	a	149	160	189		200	
TTACCGTAACTTG AATGGCATTGAAC	GAAAGTATTTCGAT		CTTGTGGAAAGGACG/ GAACACCTTTCCTGC	AAACACCNNNNNNNN TTTGTGGNNNNNNNNN	INNNNNNNNNN INNNNNNNNNN INNNNNNNNNNNNNN	tttagtactctggaaacag aaatcatgagacctttgtc	
TTACCGTAACTTG AATGGCATTGAAC	AAAGTATTTCGAT	TTCTTGGCTTTATATAT MAGAACCGAAATATATA U6 promoter	CTTGTGGAAAGGACG/ GAACACCTTTCCTGC	AAACACCNNNNNNNN TTTGTGGNNNNNNNN SaCass	INNNNNNNNNN INNNNNNNNNN SgRNA	tttagtactctggaaacag aaatcatgagacctttgtc a	
TTACCGTAACTTG AATGGCATTGAAC	SAAAGTATTTCGAT CTTTCATAAAGCTA 24	TTCTTGGCTTTATATAT MAAGAACCGAAATATATA U6 promoter 10	CTTGTGGAAAGGACG GAACACCTTTCCTGC GAACACCTTTCCTGC	AAACACCNNNNNNNN TTTGTGGNNNNNNNN SaCass 1 280	INNNNNNNNNN INNNNNNNNNNN SgRNA 300	tttagtactctggaaacag aaatcatgagacctttgtc 320	
TTACCGTAACTTG AATGGCATTGAAC 220 atctactaaaaaca	AAAGTATTTCGAT TTTCATAAAGCTA 24 baggcaaaatgccg	AAGAACCGAAATATATA U6 promoter 10 gtgtttatctcgtcaact	CTTGTGGAAAGGACG/ GAACACCTTTCCTGC 260 tgttggcgagatttt	AAACACCNNNNNNNN TTTGTGGNNNNNNNN SaCass 280 tGTTTTAGCGTAATCT	INNNNNNNNNN INNNNNNNNNNN SgRNA 300 GGAACATCGTATG	tttagtactctggaaacag aaatcatgagacctttgtc 320 GGTA	
TTACCGTAACTTG AATGGCATTGAAC 220 atctactaaaaaca tagatgattttgt	GAAAGTATTTCGAT CTTTCATAAAGCTA 24 Daggcaaaatgccg ctccgttttacggc	AAGAACCGAAATATATA WAGAACCGAAATATATA U6 promoter W0 gtgtttatctcgtcaact cacaaatagagcagttga	CTTGTGGAAAGGACG/ GAACACCTTTCCTGC 260 tgttggcgagatttt acaaccgctctaaaaa	AAACACCNNNNNNNN TTTGTGGNNNNNNNN SaCass 280 tGTTTTAGCGTAATCT aCAAAATCGCATTAGA	INNNNNNNNNNN INNNNNNNNNNN SgRNA 300 IGGAACATCGTATG ACCTTGTAGCATAC	tttagtactctggaaacag aaatcatgagacctttgto 320 GGTA CCAT	
TTACCGTAACTTG AATGGCATTGAAC 220 atctactaaaaaca tagatgattttgt	GAAAGTATTTCGAT CTTTCATAAAGCTA 24 Baggcaaaatgccg Ctccgttttacggc saCas9 s	AGAACCGAAATATATA WAGAACCGAAATATATA U6 promoter W0 gtgtttatctcgtcaact cacaaatagagcagttga	CTTGTGGAAAGGACG/ GAACACCTTTCCTGC 260 tgttggcgagatttt acaaccgctctaaaa	AAACACCNNNNNNNN TTTGTGGNNNNNNNN SaCass 280 tGTTTTAGCGTAATCT aCAAAATCGCATTAGA gBI	INNNNNNNNNNN SgRNA 300 GGGAACATCGTATGG CCTTGTAGCATACC OCK_R_primer HA tag	tttagtactctggaaacag aaatcatgagacctttgto 320 GGTA CCAT	

Supplementary figure 2: Design of sgRNA expression cassette for SaCas9.



Supplementary figure 3: Kinetics of *ASCL1* gene activation using dSpCas9VPR in HEK293A. Results are displayed as the mean of technical triplicates \pm SEM.



Supplementary figure 4: Assessment of multiple sgRNAs for dSpCas9VPR to induce gene activation in rat fibroblast R12 cells. Results are displayed as the mean of three technical repeats \pm SEM.



Supplementary figure 5: Lipofectamine 3000 allows efficient transfection in A) rat Müller glial cells rMC1 and B) rat fibroblasts R12.



Supplementary figure 6: Efficient gene activation using dSpCas9VPR in mouse embryonic fibroblasts. qPCR analysis of gene activation for *Nkx2.5*, results are displayed as the mean of three independent biological repeats \pm SEM

Cas9 activator	Species	Genes/sgRNA	Number of genes	Success rate
dSpCas9VPR	Human	Total genes tested	15	100%
		Gene activation with first sgRNA	13	86.7%
		Gene activation with second sgRNA	1	6.7%
		Gene activation with third sgRNA	1	6.7%
dSaCas9VPR	Human	Total genes tested	1	100%
		Gene activation with first sgRNA	1	100%

Supplementary table 1: Success rate of designing sgRNAs for SpCas9 and SaCas9 in human cells