SUPPLEMENTARY INFORMATION

Orthogonal genetic regulation in human cells using chemically induced CRISPR/Cas9 activators

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SUPPLEMENTARY TEXT

Selection of efficient gRNAs for activating endogenous human genes

Previous studies have shown that the use of different gRNA targeting sites resulted in varying gene activating efficiencies. Furthermore, synergistic effects can be achieved when multiple gRNAs targeting the same gene are co-transfected into the cell[1, 2]. It has not been fully understood how the DNA sequence and position of a target site affect the efficiency of dCas9 activators, especially for the less characterized $dCas9_{NM}$ activators. To select for efficient gRNAs, we designed four gRNAs for each of three endogenous human genes: *ASCL1*, *TTN*, and *RHOXF2* (Table S1), by simply searching for PAMs (NGG for $dCas9_{SP}$, NNNNGATT or NNNNGCTT for $dCas9_{NM}$) closest to the transcription start site of each gene. Another four gRNAs targeting *IL1RN* were selected from a previous study[1]. When dCas9-VPR alone was transfected into HEK293T cells without gRNA, gene activation was not observed (Fig. S3). We then transfected HEK293T cells with dCas9-VPR, together with either single or all gRNAs targeting the same gene. For both CRISPR/Cas systems, we observed varying gene activating efficiencies among different gRNAs. For *ASCL1* and *IL1RN*, all the single gRNAs were able to activate target gene expression on their own (Fig. S3A). For *ASCL1*, the highest activation efficiency was observed when all four gRNAs were transfected into the cells. However, the synergistic effect was not obvious since the sum of activation efficiencies of all four gRNAs was higher. The gRNA 130bp upstream of the transcription start site (SP-ASCL1-130, Table S1) gave the highest efficiency among the four gRNAs targeting *ASCL1*. For *IL1RN*, all four gRNAs gave comparable activation efficiencies. An obvious synergistic effect was observed when all four gRNAs were transfected into the cells, which is consistent with the previous study[1]. For *TTN* and *RHOXF2*, some gRNAs failed to activate target gene expression (Fig. S3B). For *TTN*, only gRNA NM-TTN-68 (Table S1) activated gene expression. A reduced activation efficiency was observed when all four gRNAs were transfected into the cells, possibly due to the dilution of the effective gRNA. For *RHOXF2*, two of the four gRNAs (NM-RHOXF2-148 and NM-RHOXF2-302, Table S1) activated target gene expression. A slight synergistic effect was observed when all four gRNAs were transfected.

SUPPLEMENTARY FIGURES

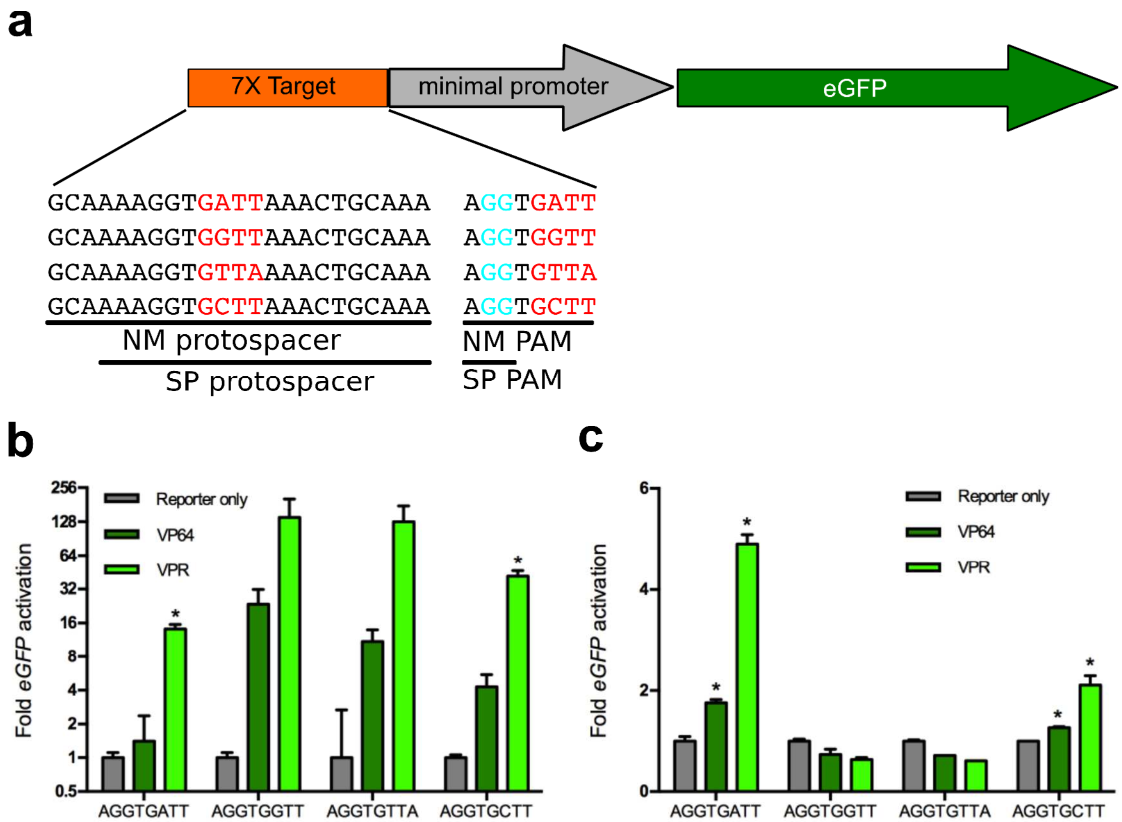

Fig S1. Comparison of VP64 and VPR in activating an eGFP reporter gene. (**a**) eGFP reporters with four different targeting sites. (**b**) Fold *eGFP* activation by dCas9$_{SP}$-VP64 and dCas9$_{SP}$-VPR. eGFP expression level of reporter only control was normalized to 1. (**c**) Fold *eGFP* activation by dCas9$_{NM}$-VP64 and dCas9$_{NM}$-VPR. eGFP expression level of reporter only control was normalized to 1. Experiments were performed with two biological replicates. Error bars represent standard error of the mean. *, $P < 0.05$.

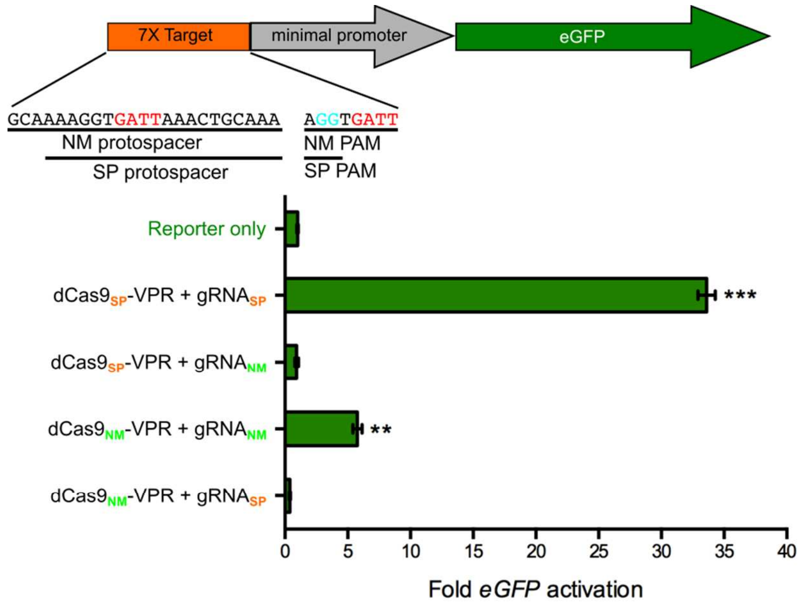

Fig S2. Orthogonality of dCas9$_{SP}$-VPR and dCas9$_{NM}$-VPR. Upper panel shows the reporter gene with dCas9 binding sites. Lower panel shows *eGFP* activation with different dCas9-VPR/gRNA combinations. n = 2 biological replicates. All error bars represent standard error of the mean. All significance levels were determined versus reporter only control. **, $P < 0.01$. ***, $P < 0.001$.

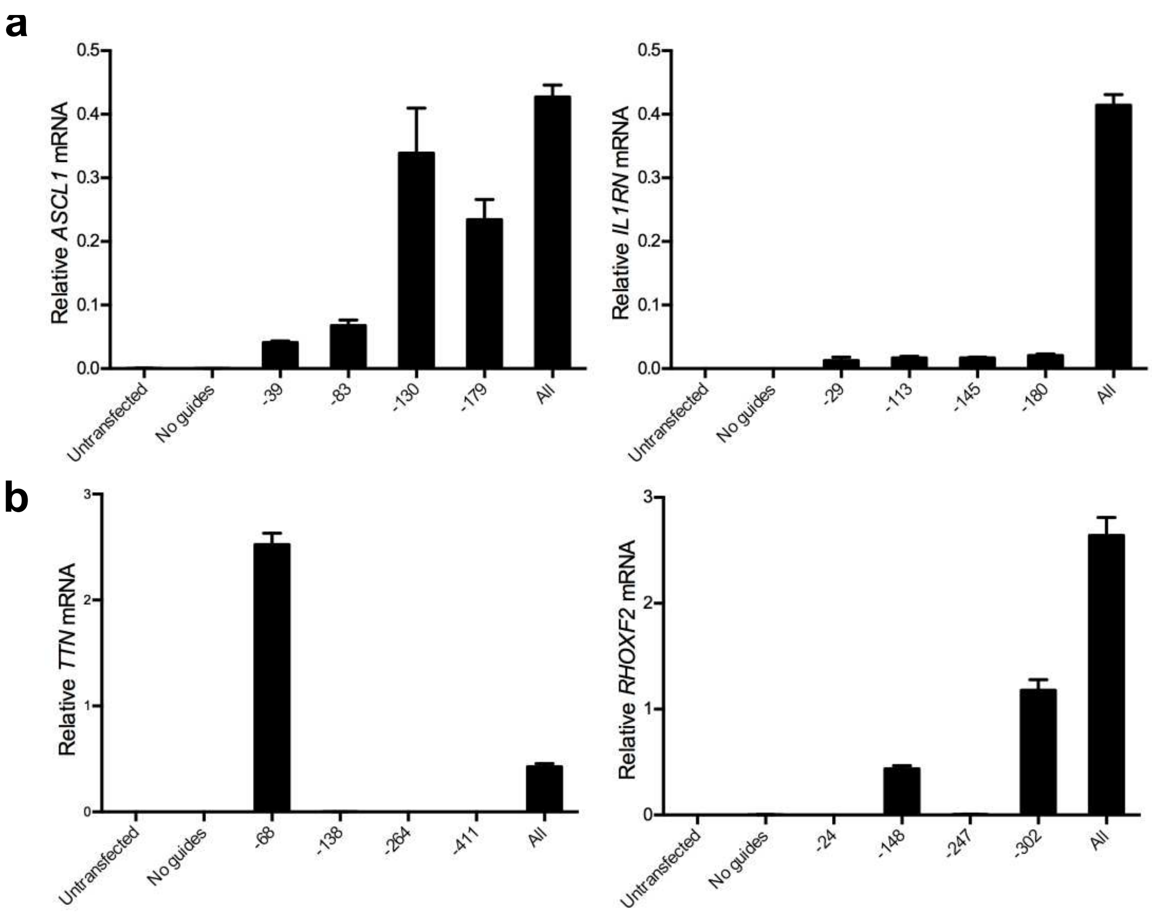

Fig S3. (**a**) Gene activation profiles of *ASCL1* and *IL1RN* gRNAs using dCas9$_{SP}$-VPR. (**b**) Gene activation profiles of *TTN* and *RHOXF2* gRNAs using dCas9$_{NM}$-VPR. Experiments were performed with three biological replicates. Error bars represent standard error of the mean.

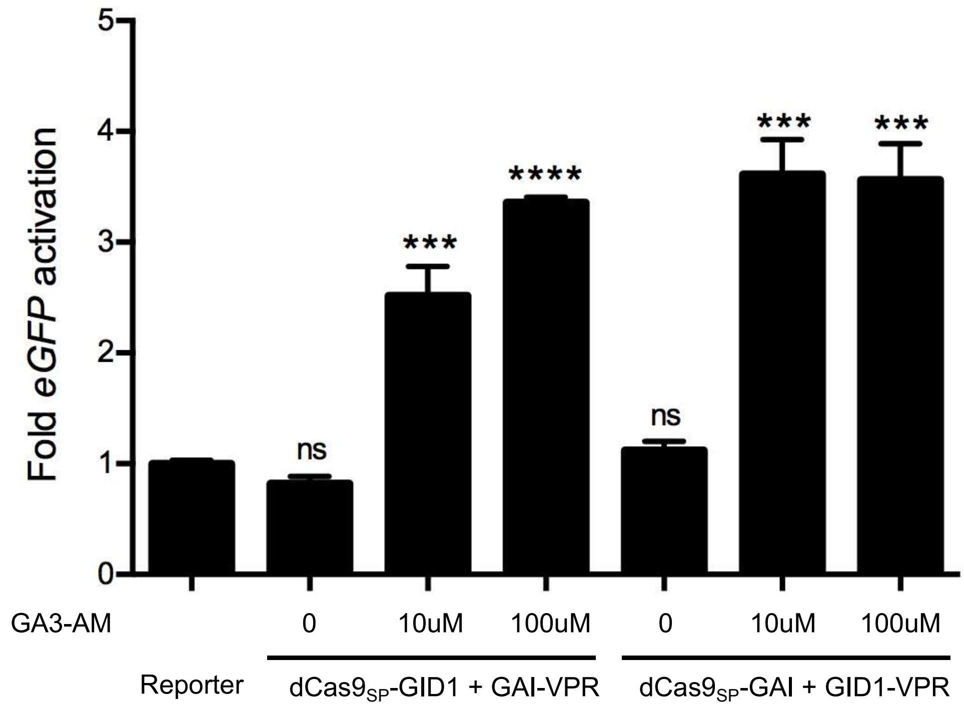

Fig S4. Determining optimal GA_3-AM concentration and protein design for gene activation. Experiments were performed with two biological replicates. Error bars represent standard error of the mean. Significance was determined versus Reporter control. ***, $P < 0.001$. ****, $P < 0.0001$. ns, not significant.

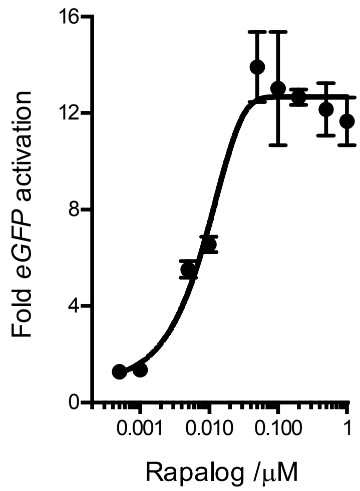

Fig S5. Dose response curve of rapalog concentration for gene activation. dCas9$_{SP}$-2×FKBP and FRB-VPR were used. Experiments were performed with two biological replicates. Error bars represent standard error of the mean.

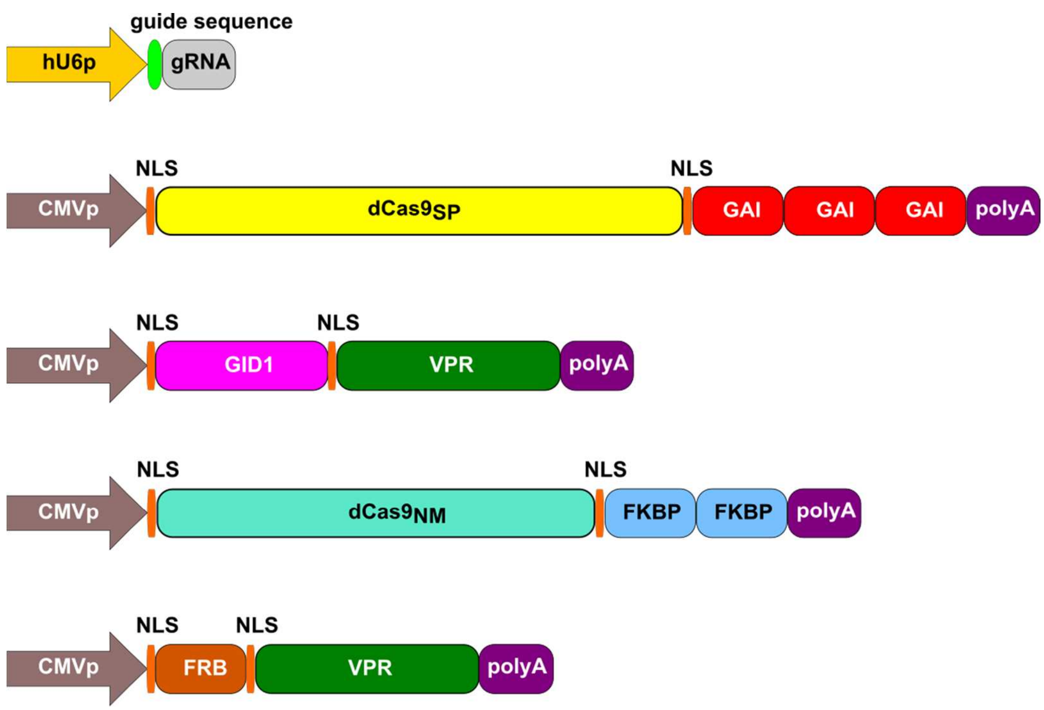

Fig S6. Expression cassettes of gRNAs and proteins used in this study.

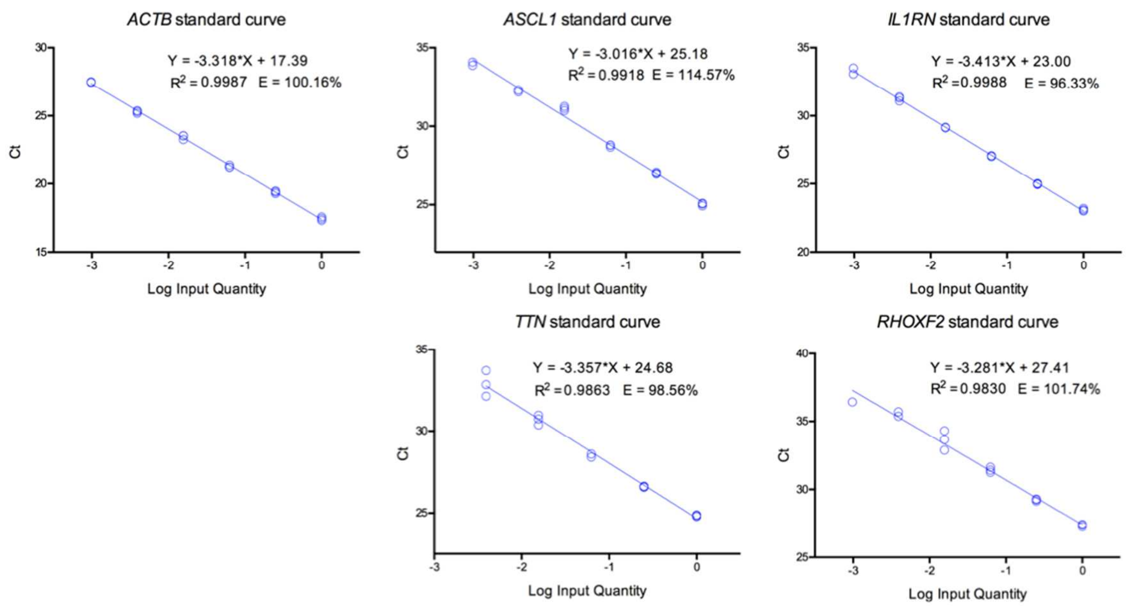

Fig S7. qRT-PCR standard curves for *ACTB*, *ASCL1*, *IL1RN*, *TTN* and *RHOXF2*.

SUPPLEMENTARY TABLES

Table S1. All gRNA sequences used in this study. SP means gRNA for $dCas9_{SP}$. NM means gRNA for $dCas9_{NM}$. The number at the end of gRNA targeting an endogenous gene means the distance in bp between the transcription start site and the base 5' to the PAM sequence. All gRNA targeting sequences were selected by searching for PAM sequences within 500bp upstream of the transcription start site, except for *IL1RN* gRNAs, which were reported previously[2]. SP-CCR5 was used to target pGL3-Basic-9×Seq2-hCMVmp-eGFP. NA, not applicable.

gRNA	Guide sequence (5'->3')	PAM (5'->3')	Strand
SP-ASCL1-39	tgggtgtcccattgaaaagg	cgg	+
SP-ASCL1-83	gcggggagtgggggggcgagg	cgg	+
SP-ASCL1-130	ggtaagaggagggggggggag	tgg	+
SP-ASCL1-179	ggggagaggagaggaggagg	ggg	+
SP-IL1RN-29	ttgtactctctgaggtgctc	tgg	+
SP-IL1RN-113	gcatcaagtcagccatcagc	cgg	+
SP-IL1RN-145	tgagtcaccctcctggaaac	tgg	+
SP-IL1RN-180	tacgcagataagaaccagtt	tgg	+
NM-TTN-68	cccacatccttaggcagggccct	gggtgatt	-
NM-TTN-138	gtagtgtcacataccacctgttc	ttctgatt	-
NM-TTN-264	tcacacctctttaaacctgcatt	ttcggatt	+
NM-TTN-411	gagataaatttcctcatctgctg	tcatgatt	+
SP-TTN-68	acatccttaggcagggccct	ggg	-
NM-RHOXF2-24	gcctgctgaggtgcgcatgcgtc	aggggctt	-
NM-RHOXF2-148	tgggagggggagtaggatggggt	gcgtgctt	-
NM-RHOXF2-247	gcaagcccattttatggcgataa	gggagatt	-
NM-RHOXF2-302	gtctccaggagcaggagcttggg	cctggatt	-
SP-RHOXF2-144	gtgggagggggagtaggatg	ggg	-
SP-RHOXF2-304	caggagcaggagcttgggcc	tgg	-
SP-CCR5	aaaggtcgagaaactgcaaa	agg	NA
SP-AGGTGATT	aaaggtgattaaactgcaaa	agg	NA
SP-AGGTGGTT	aaaggtggttaaactgcaaa	agg	NA
SP-AGGTGTTA	aaaggtgttaaaactgcaaa	agg	NA
SP-AGGTGCTT	aaaggtgcttaaactgcaaa	agg	NA
NM-AGGTGATT	gcaaaaggtgattaaactgcaaa	aggtgatt	NA
NM-AGGTGGTT	gcaaaaggtggttaaactgcaaa	aggtggtt	NA
NM-AGGTGTTA	gcaaaaggtgttaaaactgcaaa	aggtgtta	NA
NM-AGGTGCTT	gcaaaaggtgcttaaactgcaaa	aggtgctt	NA

Table S2. Primers for qRT-PCR.

Gene	Primers	Sequence	Amplicon size	Reference
ACTB	ACTB-for	5'-catgtacgttgctatccaggc	250bp	[3]
	ACTB-rev	5'-ctccttaatgtcacgcacgat		
ASCL1	ASCL1-for	5'-ggagcttctcgacttcacca	125bp	[1]
	ASCL1-rev	5'-aacgccactgacaagaaagc		
IL1RN	IL1RN-for	5'-ggaatccatggagggaagat	100bp	[1]
	IL1RN-rev	5'-tgttctcgctcaggtcagtg		
TTN	TTN-for	5'-tgttgccactggtgctaaag	204bp	[3]
	TTN-rev	5'-acagcagtcttctccgcttc		
RHOXF2	RHOXF2-for	5'-ttttccaacgcgagcagttc	154bp	This study
	RHOXF2-rev	5'-ggcagcatgtttcttgccat		

SUPPLEMENTARY NOTES

DNA sequences of proteins and gRNAs used in this study

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$dCas9_{SP}$
GAI

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GID1
VPR
HA tag

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3×FLAG
NLS
$dCas9_{NM}$

FKBP

>FRB-VPR
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ACGCTTCTTGA

3×FLAG
NLS
FRB
VPR
HA tag

>$gRNA_{SP}$
NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTA
TCAACTTGAAAAAGTGGCACCGAGTCGGTGC

20bp guide sequence

>$gRNA_{NM}$

NNNNNNNNNNNNNNNNNNNNNNNGTTGTAGCTCCCTTTCTCATTTCGGAAACGAAATGAGAACCG
TTGCTACAATAAGGCCGTCTGAAAAGATGTGCCGCAACGCTCTGCCCCTTAAAGCTTCTGCTTTA
AGGGGCATCGTTTA

23bp guide sequence

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