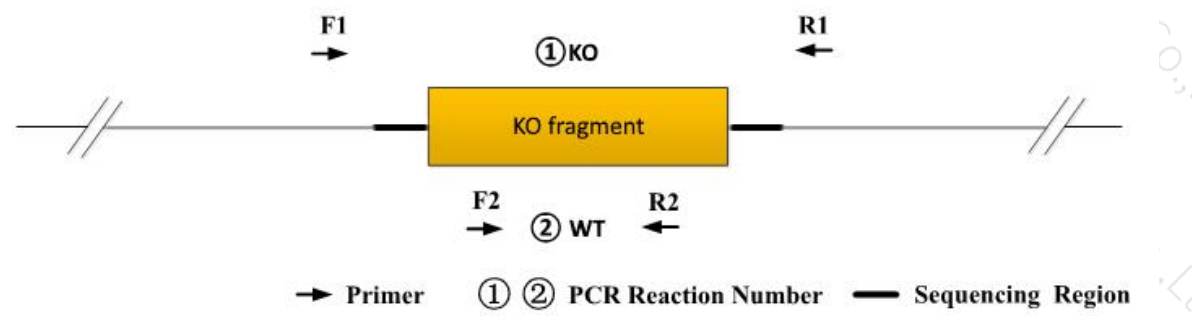


Genotyping Report

Strain ID	T007442	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	CREB1		

1. Strategy of Genotyping

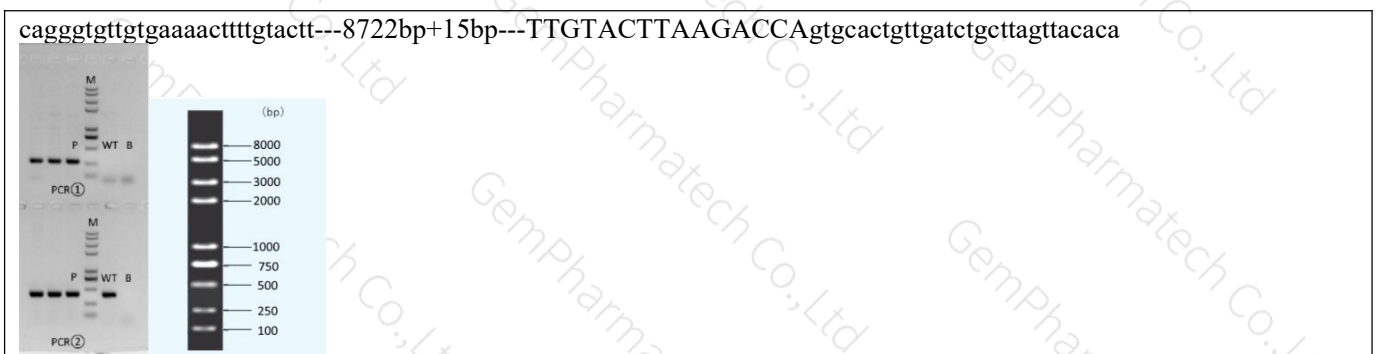


Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note: 1)The sizes of WT and Targeted band are shown below.
2)If the WT band is too large, it may not be possible to obtain a WT band.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	T007442-F1	AGCATGTGTTGTTCTGAACTTAGCAAG	WT:8969bp KO:262bp
	R1	T007442-R1	AGGTAGGTAACATCACAGTTGCACTC	
PCR②	F2	T007442-F2	ACATGAAGCCTAACTGCTGGCATAAC	WT:379bp KO:0bp
	R2	T007442-R2	AAGGGAAGAAACCGCTAATGAACC	

3. Gel Image



Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH₂O); M: DNA Ladder

① Control (WT) : It is an important reference mark for whether the PCR reaction is successful and whether the product band position and size meet the theoretical requirements.

② Control (B) : PCR amplification was performed without template in the PCR reagent to monitor whether the reagent was contaminated.

4. PCR Condition

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Rapid Taq Master Mix (Vazyme P222)		12.5
2	ddH ₂ O		9.5
3	Primer A(10pmol/μl)		1
4	Primer B(10pmol/μl)		1
5	Template(≈100ng/μl)		1
PCR program ① priority selection			
Seg.	Temp.	Time	Cycle
1	95 °C	5min	
2	98 °C	30s	20×
3	65 °C* (-0.5 °C/cycle)	30s	
4	72 °C	45s*	
5	98 °C	30s	
6	55 °C*	30s	20×
7	72 °C	45s*	
8	72 °C	5min	
9	10 °C	hold	
PCR program ② the second choice			
Seg.	Temp.	Time	Cycle
1	95 °C	5min	
2	98 °C	30s	35×
3	58 °C*	30s	
4	72 °C	45s*	
5	72 °C	5min	
6	10 °C	hold	

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.