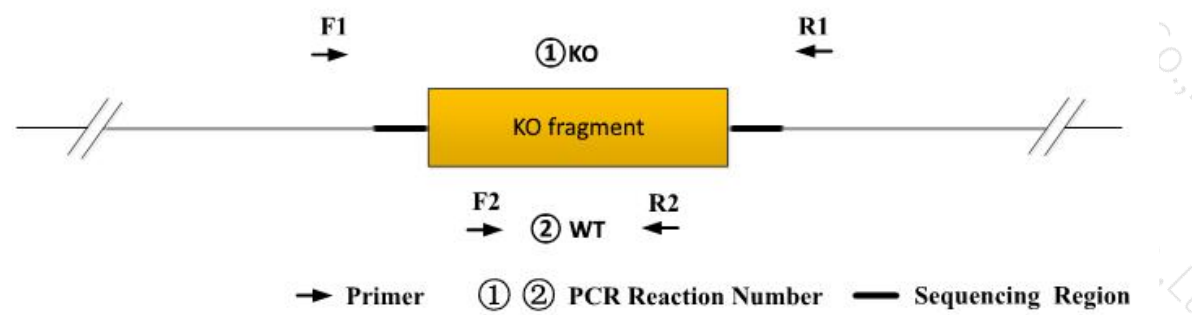


Genotyping Report

Strain ID	T013715	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Cd8a</i>		

1. Strategy of Genotyping



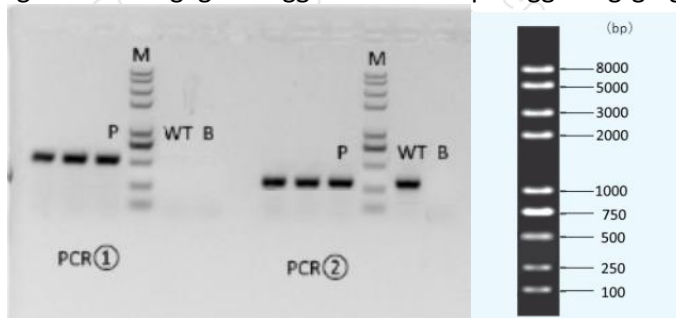
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
 Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
 Note: 1)The sizes of WT and Targeted 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	GJS0720221112352-01-Cd8a-KO-tF1	GGGAACCCAAGAACCTATCTACAAG	WT:2207bp KO:512bp
	R1	GJS0720221112352-01-Cd8a-KO-tR1	GGAGTGAATGGTTTGTGAGGTGG	
PCR②	F2	GJS0720221112352-01-Cd8a-WT-tF1	CTGGGTGGGATTGGAAGTGAT	WT:277bp KO:0bp
	R2	GJS0720221112352-01-Cd8a-WT-tR1	CAAGAAGGAGCCGTTTCCTAGC	

3. Gel Image

tgcatctcactctgagttcccagccct---1695bp---aggaccagtgtagtggttctttcttgaag



Note: P: Heterozygous samples; 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(20~80ng/μl)		1
PCR program I (priority selection)			
Seg.	Temp.	Time	Cycle
1	95℃	5min	20×
2	98℃	30s	
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	
5	98℃	30s	15×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program II (the second choice)			
Seg.	Temp.	Time	Cycle
1	95℃	5min	35×
2	98℃	30s	
3	58℃*	30s	
4	72℃	45s*	
5	72℃	5min	
6	10℃	hold	

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