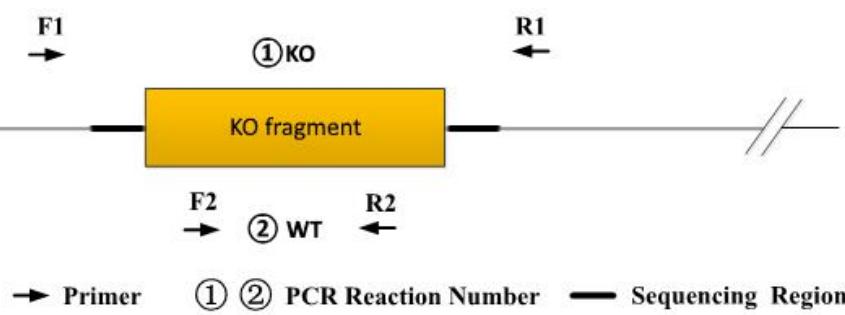




Genotyping Report

Strain ID	T044742	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name			<i>Parp12</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; ②PCR reaction without product.

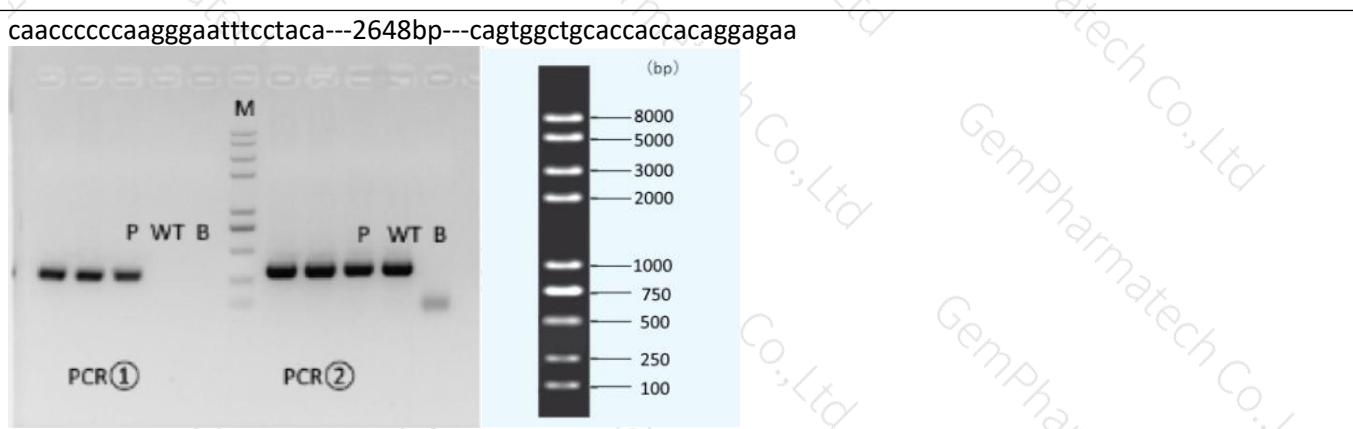
Note: 1)The sizes of WT and Targeted band are shown below.

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	JS38369-Parp12-KO-tF1	GGAGTCTCAAAGCTGGTGAATG	WT:2920bp KO:272bp
	R1	JS28369-Parp12-3wt-tR1	CAGCCAACATTCCACTATGGATG	
PCR②	F2	JS38369-Parp12-wt-tF1	GCCTACACTACAACAAAGGTGATGG	WT:327bp KO:0bp
	R2	JS38369-Parp12-wt-tR1	CCAGCAGCACTTCATTAGC	

3. Gel Image





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 °C	5min	20×
2	98 °C	30s	
3	65 °C * (-0.5 °C/cycle)	30s	
4	72 °C	45s*	
5	98 °C	30s	
6	55 °C *	30s	
7	72 °C	45s*	
8	72 °C	5min	
9	10 °C	hold	

PCR program II (the second choice)			
Seg.	Temp.	Time	Cycle
1	95 °C	5min	35×
2	98 °C	30s	
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.



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