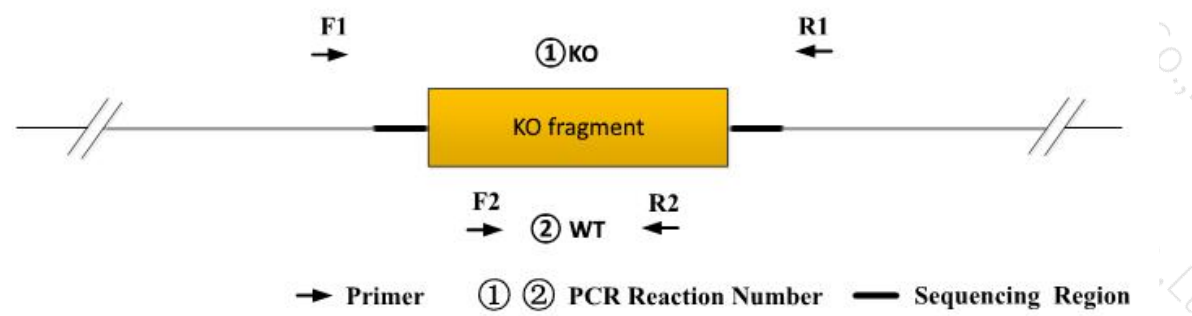


Genotyping Report

Strain ID	T035124	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chen Chen	Gene Name	<i>Pitpnb</i>		

1. Strategy of Genotyping



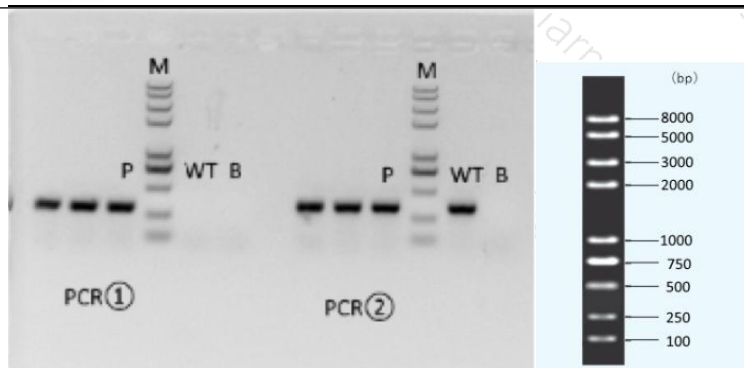
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note: 1)The sizes of WT and Targeted 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	JS26058-Pitpnb-5S1-tF1	GAGTTCTTAGAGATAAAATTTTGTGCC	WT: 1713bp KO: 323bp
	R1	JS26058-Pitpnb-3S1-tR1	TAACTATGCTAATGCCATATCAGGGA	
PCR②	F2	JS36058-Pitpnb-wt-tF1	ACCCAGCACTGTCACTTGGGTT	WT: 338bp KO:0bp
	R2	JS36058-Pitpnb-wt-tR1	TGCTAGACAGACGCTCTAGGCCTCA	

3. Gel Image

tagtcaaagttgaagagattggcactagtc---1390bp---tgatgggcactgggattgtgggtacaggtc
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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

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as GC% $\geq$ 60% or GC% $\leq$ 40%, recommend to use Vazyme P515.)

PCR Reaction Component

Seg.	reaction component	Volume (μ l)
1	2 $\times$ Rapid Taq Master Mix(Vazyme P222) or 2 $\times$ Phanta Max Master Mix (Vazyme P515)	12.5
2	ddH ₂ O	9.5
3	Primer A(10pmol/ μ l)	1
4	Primer B(10pmol/ μ l)	1
5	Template(20~80ng/ μ l)	1

PCR program I (priority selection)

Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	20 $\times$
2	98 $^{\circ}$ C	30s	
3	65 $^{\circ}$ C* (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	
5	98 $^{\circ}$ C	30s	15 $\times$
6	55 $^{\circ}$ C*	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	

PCR program II (the second choice)

Seg.	Temp.	Time	Cycle
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1	95℃	5min	
2	98℃	30s	35×
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.