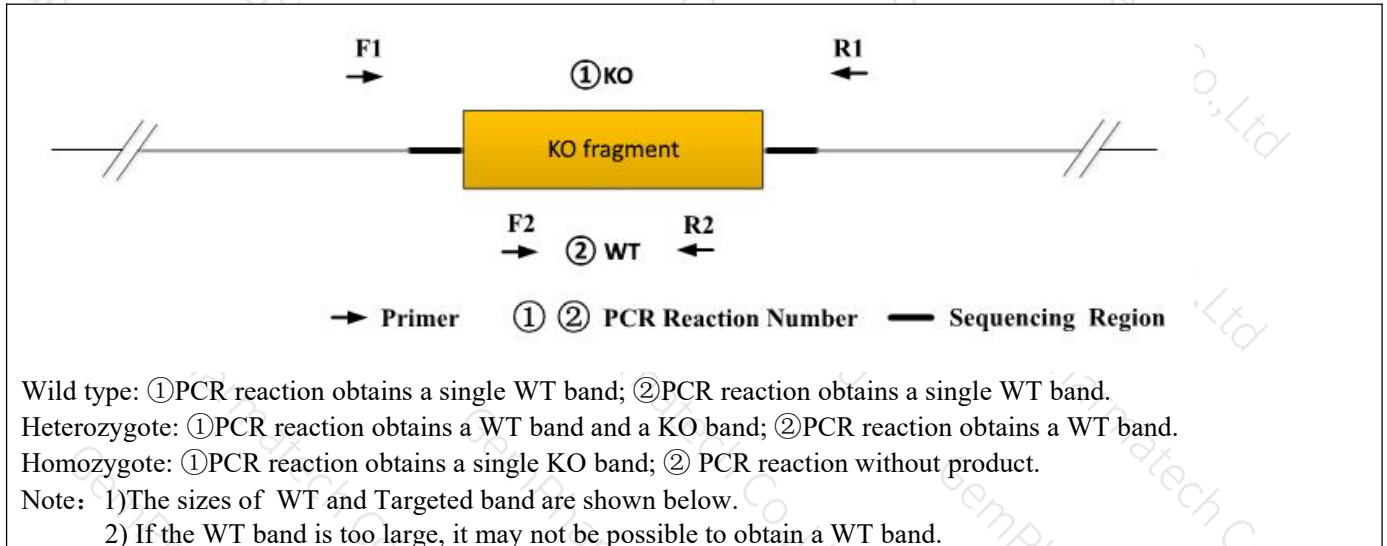


Genotyping Report

Strain ID	T016707	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	<i>Kpna2</i>		

1. Strategy of Genotyping

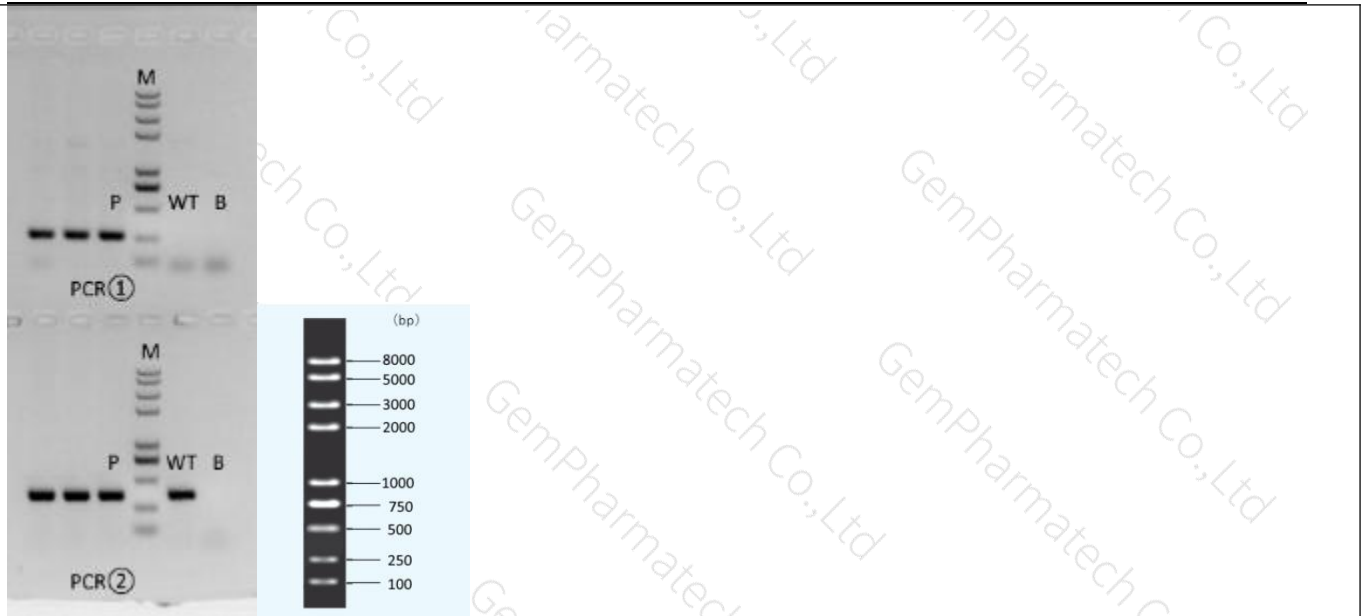


2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR① GC: 39%	F1	JS06027-Kpna2-5wt-tF1	GGGATTGAATCCTAGAGCTTCACACTC	WT:9241 bp KO: 287bp
	R1	JS06027-Kpna2-3wt-tR1	CCCTAAGACAGCCTGGACTACATAGAG A	
PCR②	F2	JS16027-Kpna2-wt-tF1	CTCTTCATCACTCATTGGCCATCT	WT: 304bp KO:0bp
	R2	JS16027-Kpna2-wt-tR1	CACAGTGTACCCACTGGTTCAATAGG	

3. Gel Image

tatgtgggggaggattttatgtgcacct-----8954bp-----gagatgctcacaatgaactctggtcttatt



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*	15×
5	98℃	30s	
6	55℃*	30s	
7	72℃	45s*	hold
8	72℃	5min	
9	10℃	hold	

PCR program II (the second choice)			
Seg.	Temp.	Time	Cycle
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.