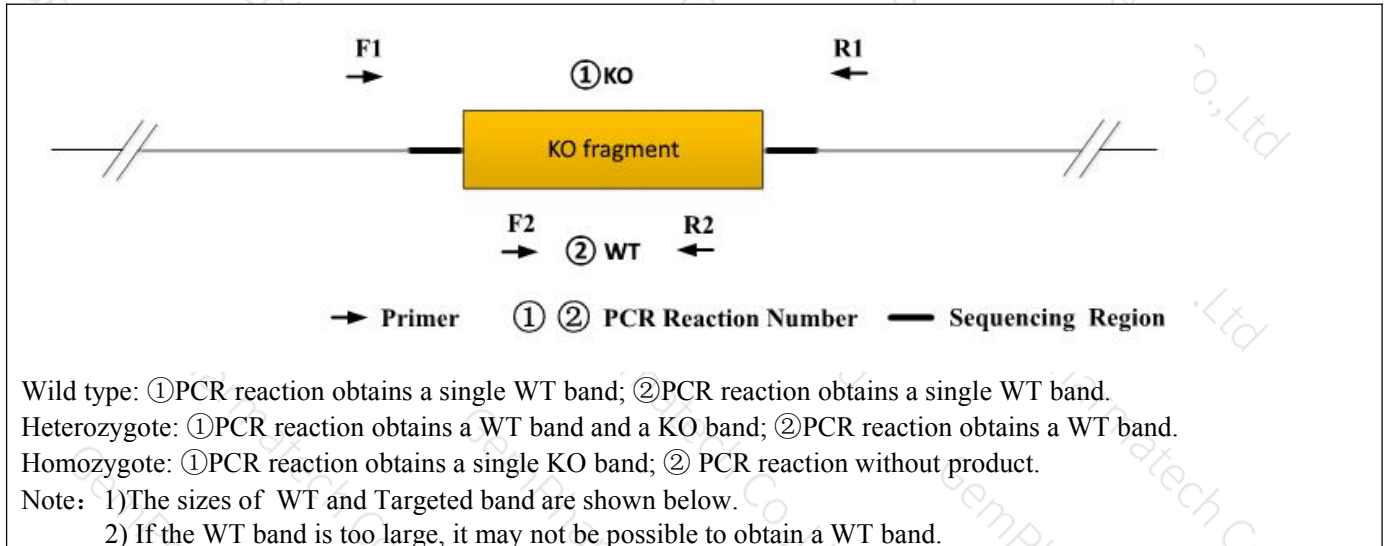


Genotyping Report

Strain ID	T052639	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chen Chen	Gene Name	<i>Tnfrsf14</i>		

1. Strategy of Genotyping

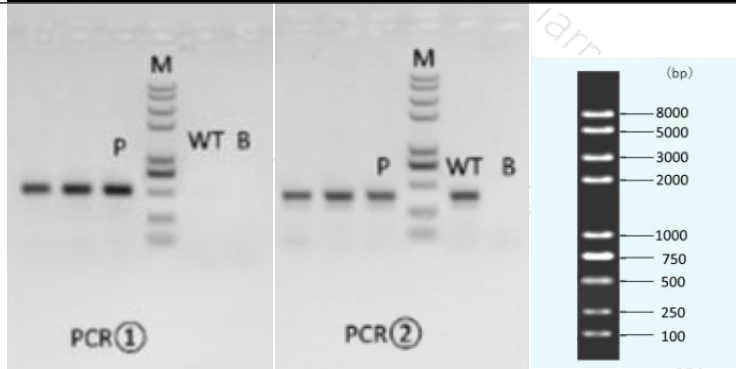


2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	T052639(P2)-F1	TACAGACAACACCTTCAGGCTGGT	WT:4446bp KO:517bp
	R1	T052639(P2)-R1	GGCTGAAGGAGCAGGGTAGAAAT	
PCR②	F2	T052639(P2)-F2	AGAGCCAGTTCTAAGGGATTTAGGAA	WT:388bp KO:0bp
	R2	T052639(P2)-R2	AATGCCTGAGCCTAAGTGCTCCA	

3. Gel Image

ccggcctccactcagacacactactatg---3982bp+53bp---
TTGGGGTAGATGCGGACTTTTGGAGGAAATGTGTCATGGGACTTTGGGGTTTcaacctgagagctgggatctctgaagat
ag



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	
2	98℃	30s	20×
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	
5	98℃	30s	
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	
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.