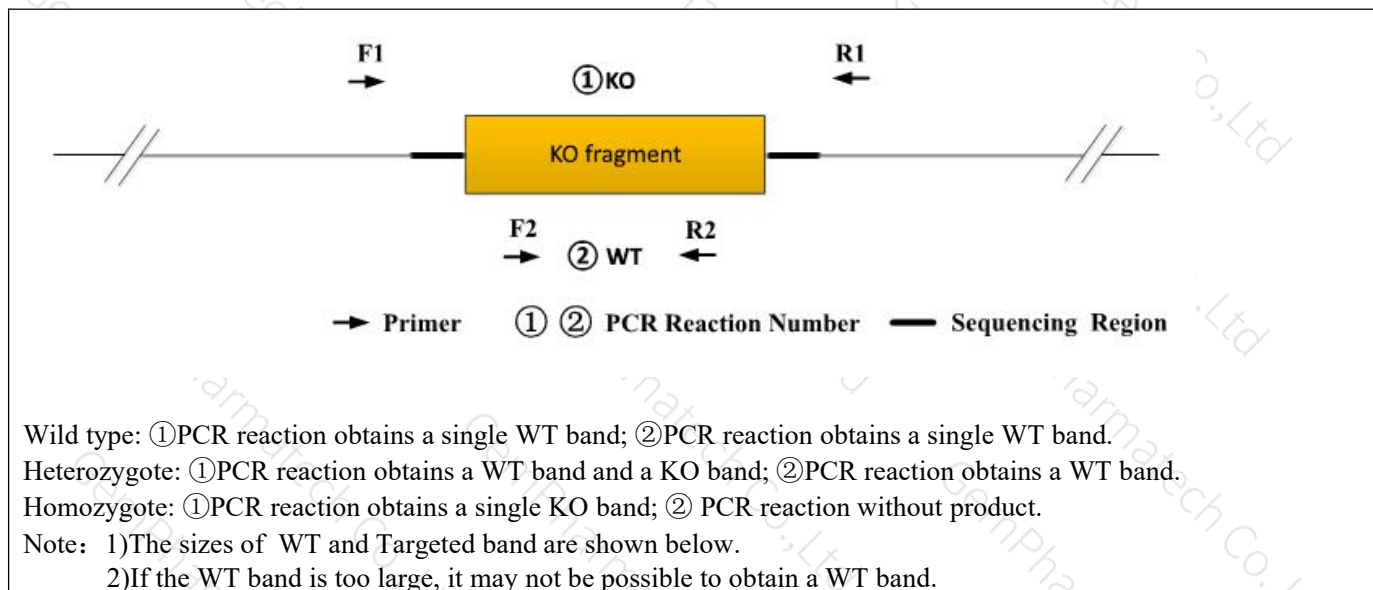


Genotyping Report

Strain ID	T028666	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Cacnal1f</i>		

1. Strategy of Genotyping

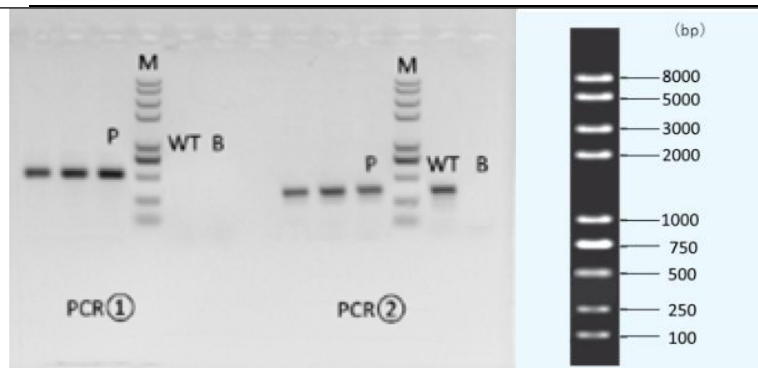


2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
PCR①	T028666-F1A	TGCAGATCAGGAACATACAGCTAGTG	WT: 5220bp Target:559bp
	T028666-R1A	GAGTGTCTCATTACTAGCTCAGGGCC	
PCR②	T028666-F2	CTGCTAACCACAACCTTGGTGAGG	WT: 360bp
	T028666-R2	GATGATGAAGTCGAGCAGGTTCC	

3. Gel Image

cctcactttgatcctaattccaagcccaagt-----4661bp-----ggcaggaaagattgcaagtttggtgaggccctgagcta



Note: P: Positive control; 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	ddH2O		9.5
3	Primer A(10pmol/μl)		1
4	Primer B(10pmol/μl)		1
5	Template(≈100ng/μl)		1
PCR program ① 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	20×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program ② 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.