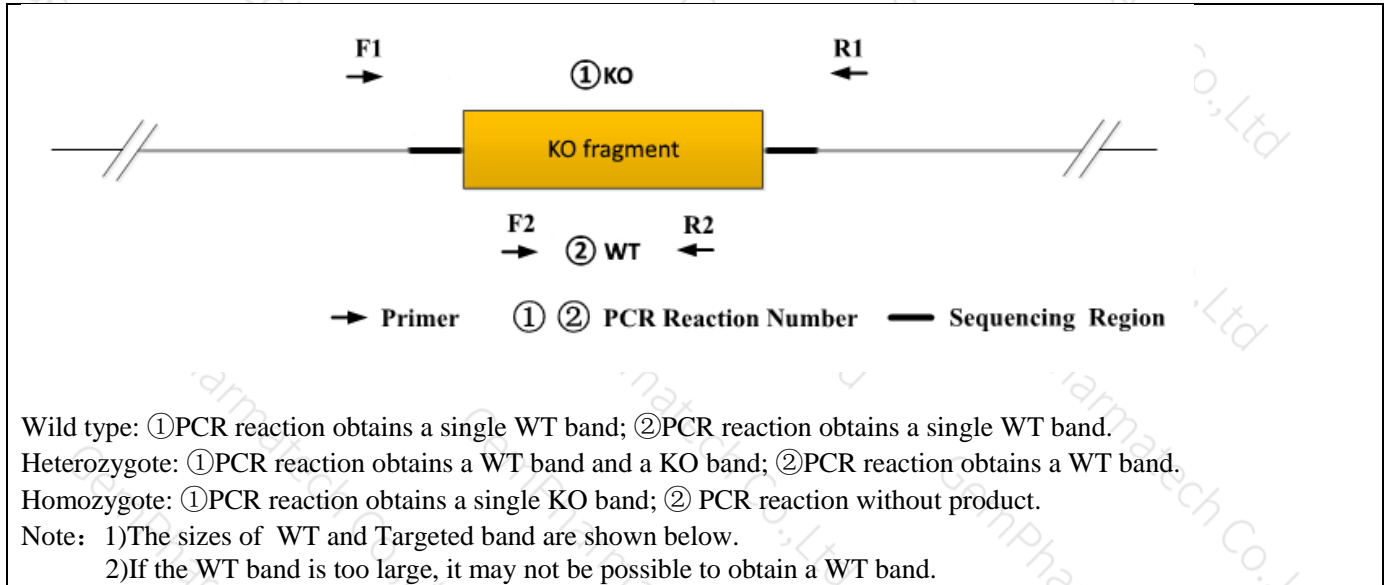


Genotyping Report

Strain ID	T048832	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	Cfap46		

1. Strategy of Genotyping

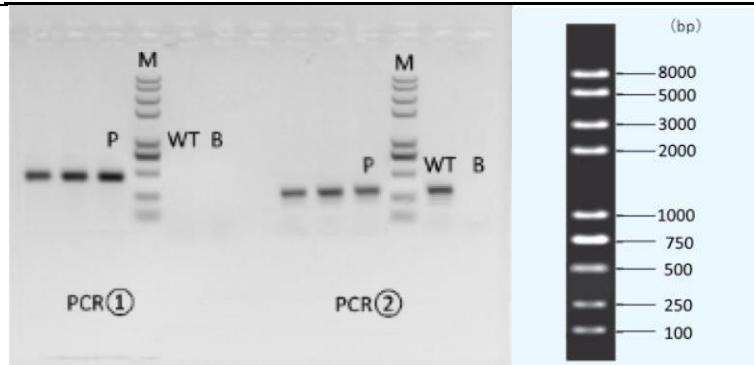


2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
PCR①	T048832-F1	GCCCACTGCTCATTGGTTGAT	WT: 6778bp
	T048832-R1	AAAGTTGGAGAGCCGCTGATG	Targeted: 479bp
PCR②	T048832-F2	TCGCTGAAAGCGTAAGTGGTTG	WT: 318bp
	T048832-R2	TCACCTTAGGAGTGGACATCCTGT	Targeted:0bp

3. Gel Image

gcaagggtcttggagacagttataaatgggccg---6299bp---ccgcgccgaagctgtggtctcttgtagtgagcctt



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	ddH ₂ O	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	
2	98℃	30s	20×
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	
5	98℃	30s	
6	55℃*	30s	20×
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program ② 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.