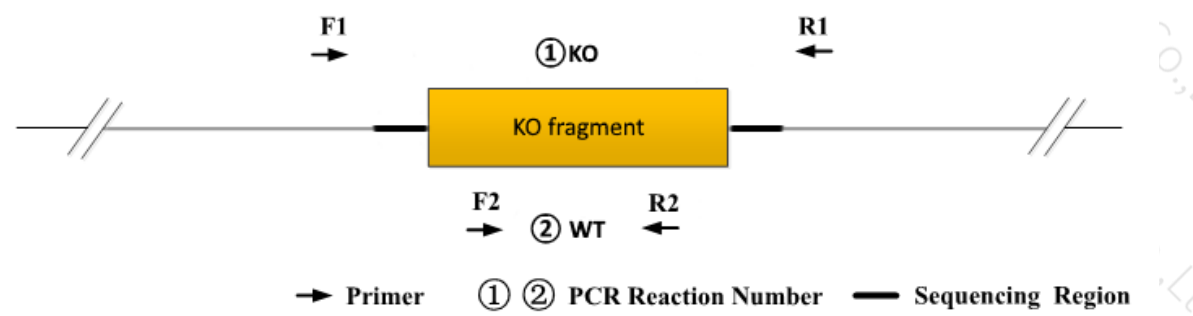


Genotyping Report

Strain ID	T045429	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	Ascl4		

1. Strategy of Genotyping



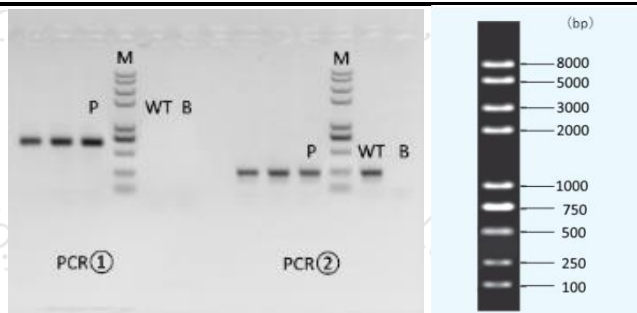
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ②PCR reaction without product.
Note: 1)The sizes of WT and Targeted 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.	Sequence	Band Size
PCR①	T045429-F1	CCTGCCTCAGCTTCAACATC	WT: 8503bp Targeted: 744bp
	T045429-R1	ACGTGGAAGTGGCTATGTAGACC	
PCR②	T045429-F2	GGGACTGGCTGTGTATAGAATTGTC	WT: 261bp Targeted: 0bp
	T045429-R2	TGAGATCTTGGGCAGTGTCTGAG	

3. Gel Image

cagacacagatgttttctatttcaacctgccatt---7967bp+208bp--- TTTAGAGGAAGCTTGGCTTGAGAGGGATGGAGTGGAGGAGCACGGACTTTGGTGAGCACTCTCTAGGCG CCATCTCCTCACAAGGCCCTTCCTGCCTGCCTATCGCATTGTCTGAGTACGTGATAACTTCGTATAGCAT ACATTATACGAAGTTATGGGCCAACAGAATTCGATATCCTGCAGGGACTAACAGAAGAACGCGTTGTGcg



tgattcagcgctatcttttccttagaa

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.