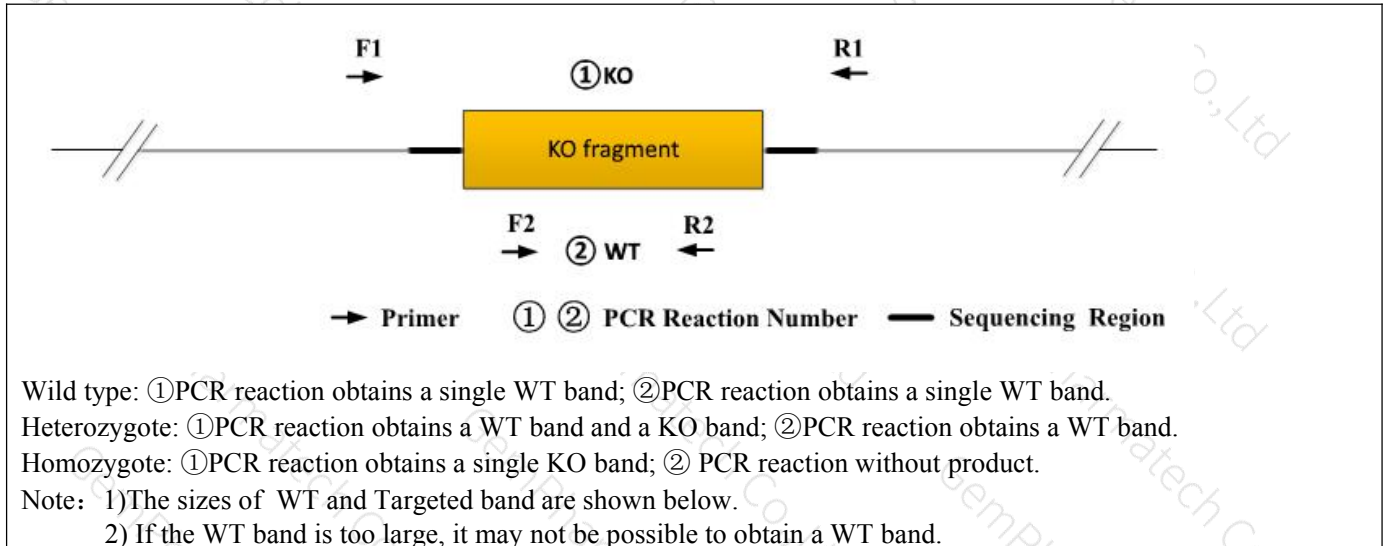


Genotyping Report

Strain ID	T030148	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Man Xu	Gene Name	<i>Bcam</i>		

1. Strategy of Genotyping

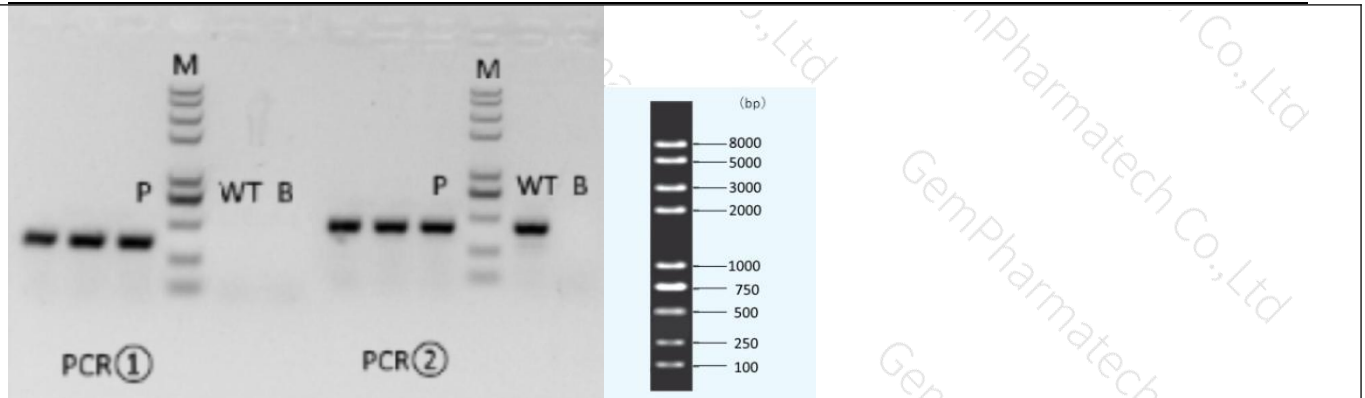


2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	JS21081-Bcam-5wt-tF1	TTGTCCTGTTTGCTGGAGGC	WT:3049bp KO:383bp
	R1	JS21081-Bcam-3wt-tR1	GGTAGGGAAGTGGCTCTCAGAAG	
PCR②	F2	JS31081-Bcam-wt-F1A	AGCATTACGTGCTGGAATGGTTC	WT:400bp KO:0bp
	R2	JS31081-Bcam-wt-R1A	CCAAGTGCTATTATGTTGACTCCTAGC	

3. Gel Image

actttaagcctacaagtctgagaggccgagccagtg---2666bp---gtttggggttgcatgaaatagggtcttatgaag



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.