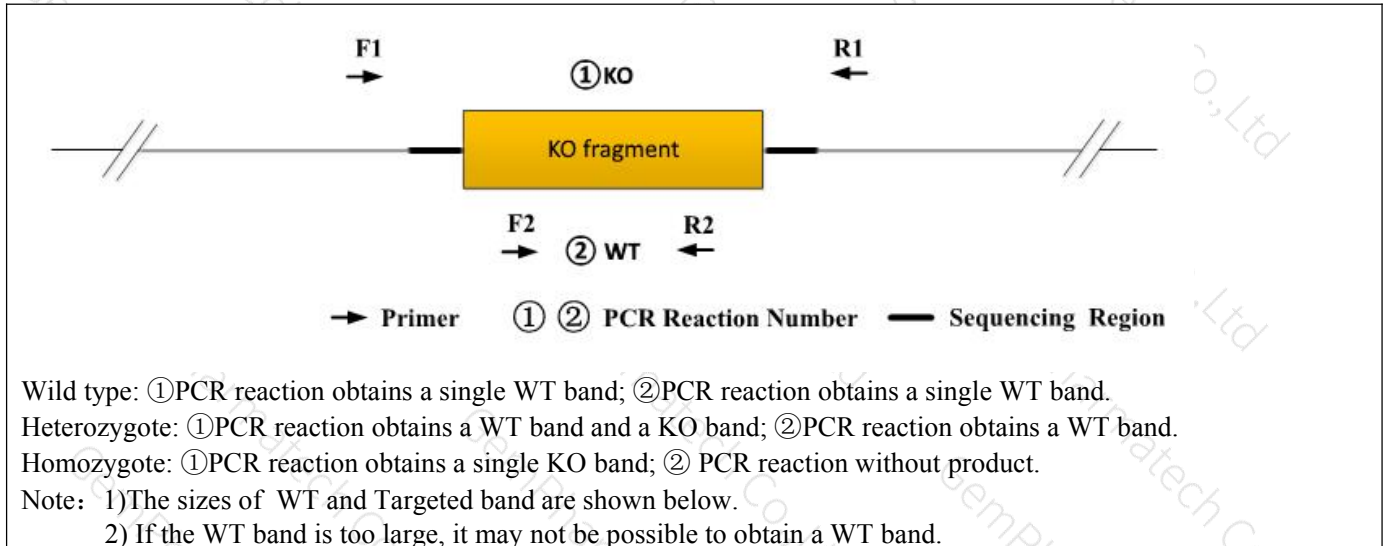


Genotyping Report

Strain ID	T028604	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Yuting Liu	Gene Name	<i>S100g</i>		

1. Strategy of Genotyping

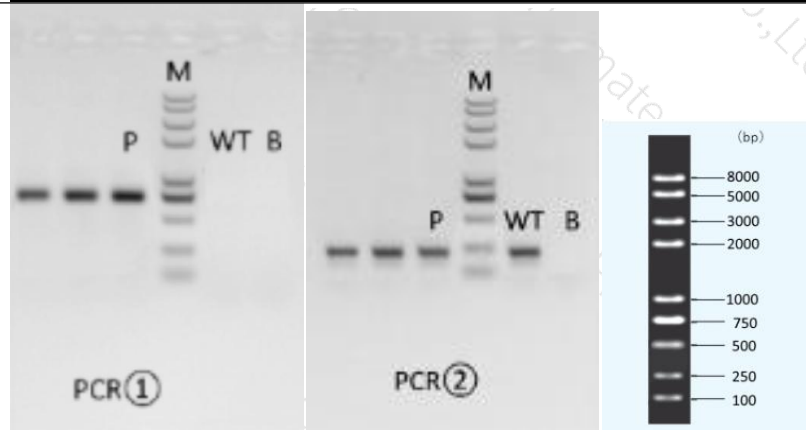


2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	T028604(P1)-F1A	TATAAAAGAGCCCCTCCTGGCTC	WT: 3689bp KO: 793bp
	R1	T028604(P1)-R1A	CTGCGTGGCTCCTTGCTCTTTA	
PCR②	F2	T028604(P1)-F2	AGCCTTCTAAGCAGTTGGGATTAC	WT: 234bp KO:0bp
	R2	T028604(P1)-R2	TAGGATCGGAATCCGGGTCTTTT	

3. Gel Image

cttattctccccctgctcttccaagga---2915bp+19bp---GGGGATCGCAGGAAAACCatagagttaaggctaccctggctacatga



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

(Generally recommend to use Vazyme P222; if the sequences contain special structures such as GC% \geq 60% or GC% \leq 40%, recommend to use Vazyme P515.)

PCR Reaction Component

Seg.	reaction component	Volume (μ l)
1	2 \times Rapid Taq Master Mix(Vazyme P222) or 2 \times Phanta Max Master Mix (Vazyme P515)	12.5
2	ddH ₂ O	9.5
3	Primer A(10pmol/ μ l)	1
4	Primer B(10pmol/ μ l)	1
5	Template(20~80ng/ μ l)	1

PCR program I (priority selection)

Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	20 \times
3	65 $^{\circ}$ C* (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	
5	98 $^{\circ}$ C	30s	
6	55 $^{\circ}$ C*	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	

PCR program II (the second choice)

Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	35×
3	58°C*	30s	
4	72°C	45s*	
5	72°C	5min	
6	10°C	hold	

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.