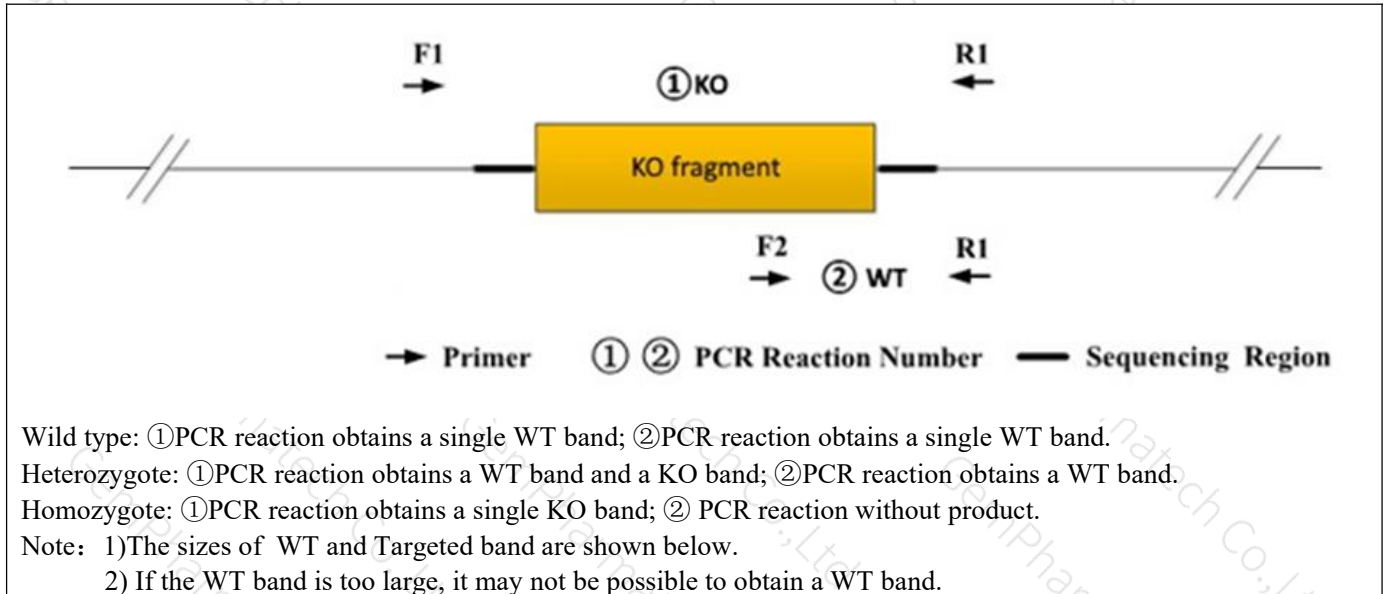


Genotyping Report

Strain ID	T004369	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	Smarca2		

1. Strategy of Genotyping



2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	3513-Smarca2-KO-tF1	CAGAGACAGGTTAAAGATTGCATG	WT: 1771bp KO: 675bp
	R1	3513-Smarca2-KO-tR1	TTGGTGTTTTCCCACAAAGAGG	
PCR②	F2	3513-Smarca2-3S-intF1	GTGCTTAGAATACACCGATCAAAAG	WT: 490bp KO:0bp
	R1	3513-Smarca2-KO-tR1	TTGGTGTTTTCCCACAAAGAGG	

3. Gel Image

tttacaggaacttatcagcagtttgcaaagtgagaccttggg-----1096bp-----cacgggccagtttgagcagaactcagaagcttgctgcttcgc



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	20 \times
2	98 $^{\circ}$ C	30s	
3	65 $^{\circ}$ C* (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	15 \times
5	98 $^{\circ}$ C	30s	
6	55 $^{\circ}$ C*	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	



9	10°C	hold	
PCR program II (the second choice)			
Seg.	Temp.	Time	Cycle
1	95°C	5min	35×
2	98°C	30s	
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.