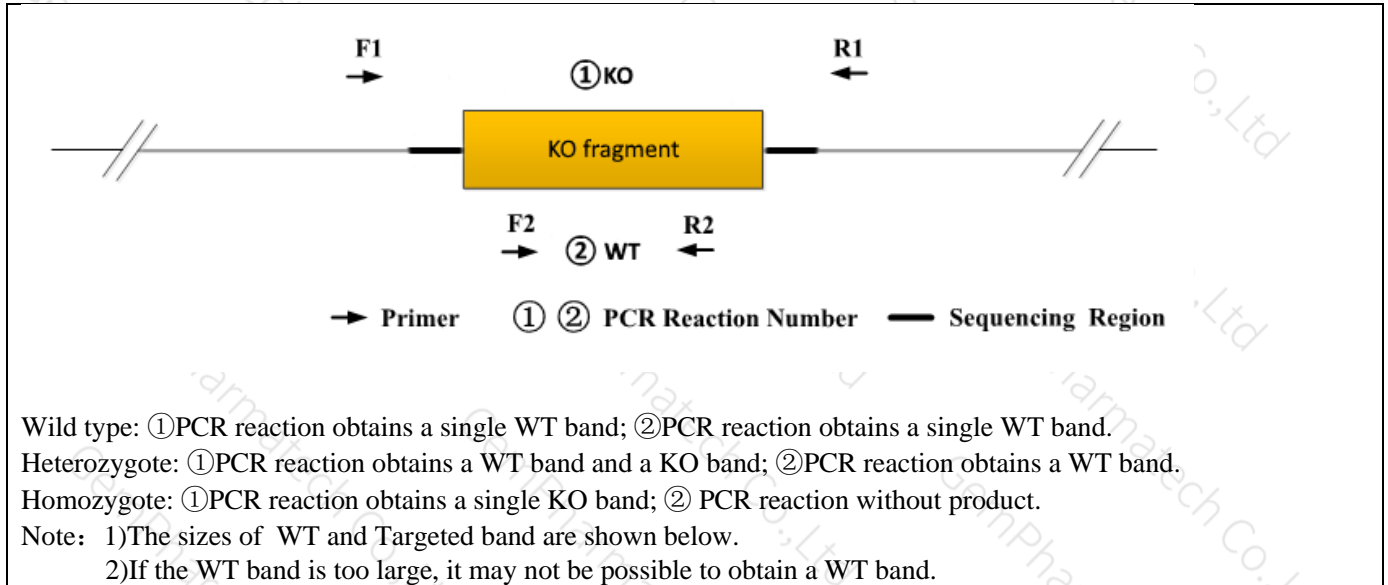


Genotyping Report

Strain ID	T028015	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Yin Chen	Gene Name	Ranbp2		

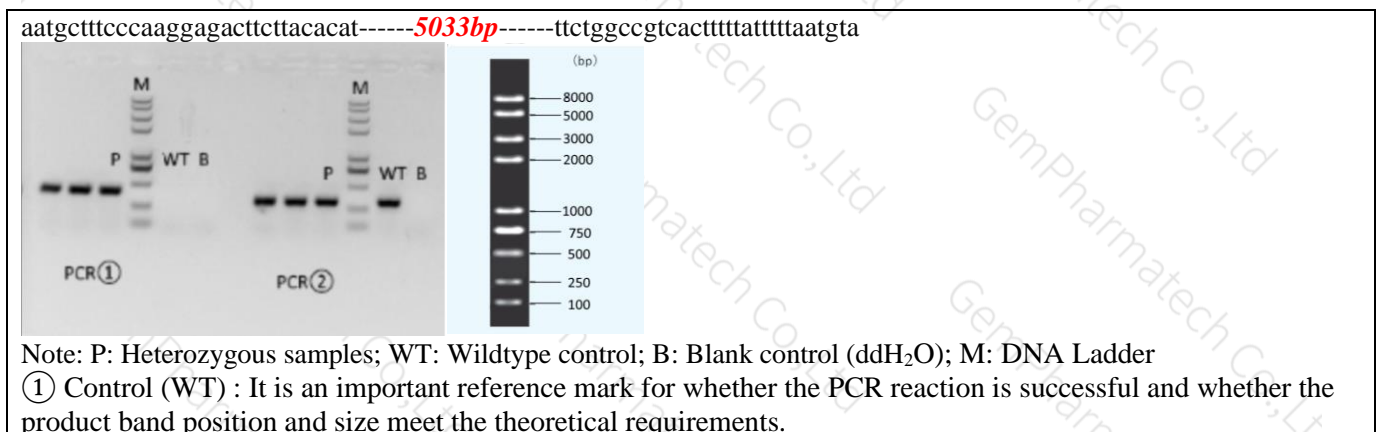
1. Strategy of Genotyping



2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	JS08948-Ranbp2-5wt-tF1	CAGGGCTATACAGAGAAACCCTGTC	WT:5430bp KO: 397bp
	R1	JS08948-Ranbp2-3wt-tR1	GGGTAGTAGGGTAAACATGAAAACCTTG	
PCR②	F2	JS18948-Ranbp2-wt-F1	CTATGTAGGCCAAGCTGGTCTTGAAC	WT:309bp KO:0bp
	R2	JS18948-Ranbp2-wt-R1	GAGCACCCACATGAATGTAGGTACAC	

3. Gel Image



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 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	35 \times
3	58 $^{\circ}$ C*	30s	
4	72 $^{\circ}$ C	45s*	
5	72 $^{\circ}$ C	5min	
6	10 $^{\circ}$ C	hold	

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

