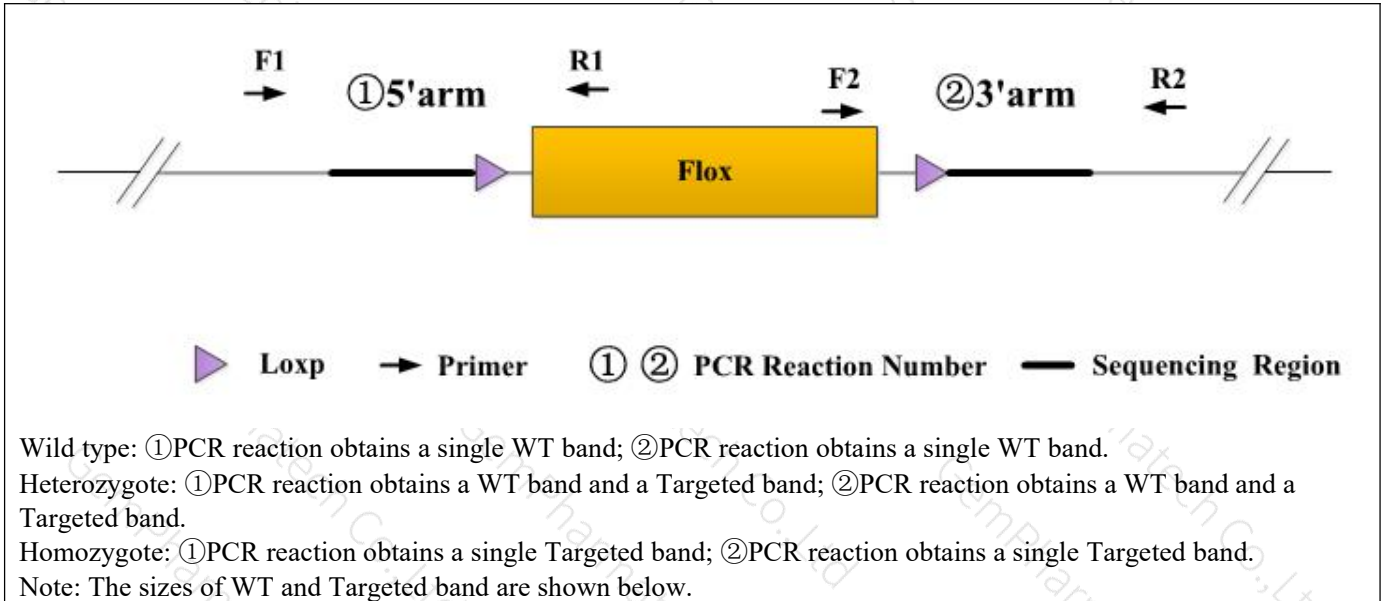


Genotyping Report

Strain ID	T018328	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Rreb1</i>		

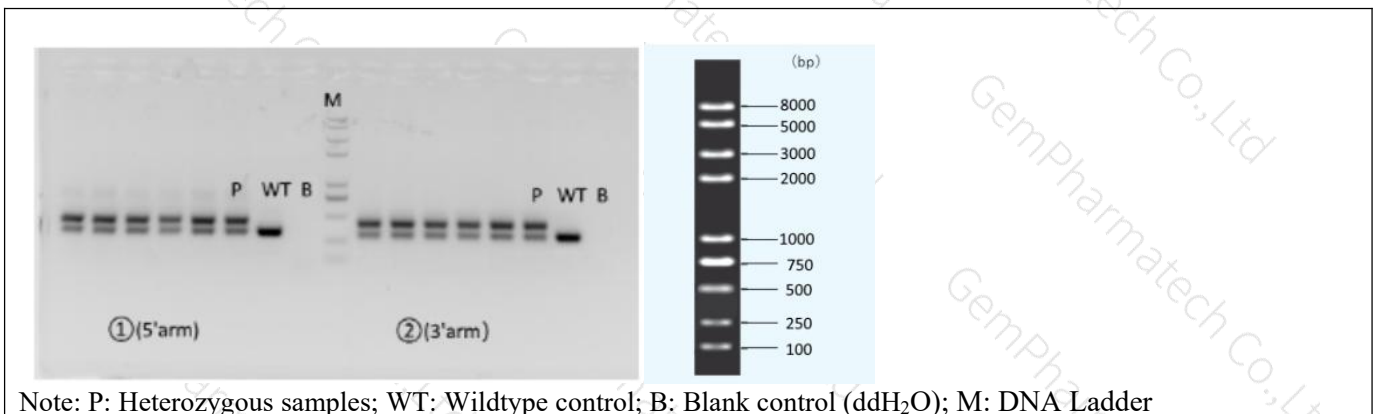
1. Strategy of Genotyping



2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T018328-F1	CACCTCGTGTAGAATCTTGAGTCTGG	WT: 318bp Targeted: 423bp
	T018328-R1	CAGGTTGGCCTGAAACCCTTTC	
②(3'arm)	T018328-F2	CTGGAGCCTTTGATAGTTAAGTGGC	WT: 292bp Targeted: 398bp
	T018328-R2	CAATCTGAGTCTCTTCTTCATCCCC	

3. Gel Image & Conclusion



- 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)		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°C	5min	
2	98°C	30s	20 \times
3	65°C* (-0.5°C/cycle)	30s	
4	72°C	45s*	
5	98°C	30s	15 \times
6	55°C*	30s	
7	72°C	45s*	
8	72°C	5min	
9	10°C	hold	
PCR program II the second choice			
Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	35 \times
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.

