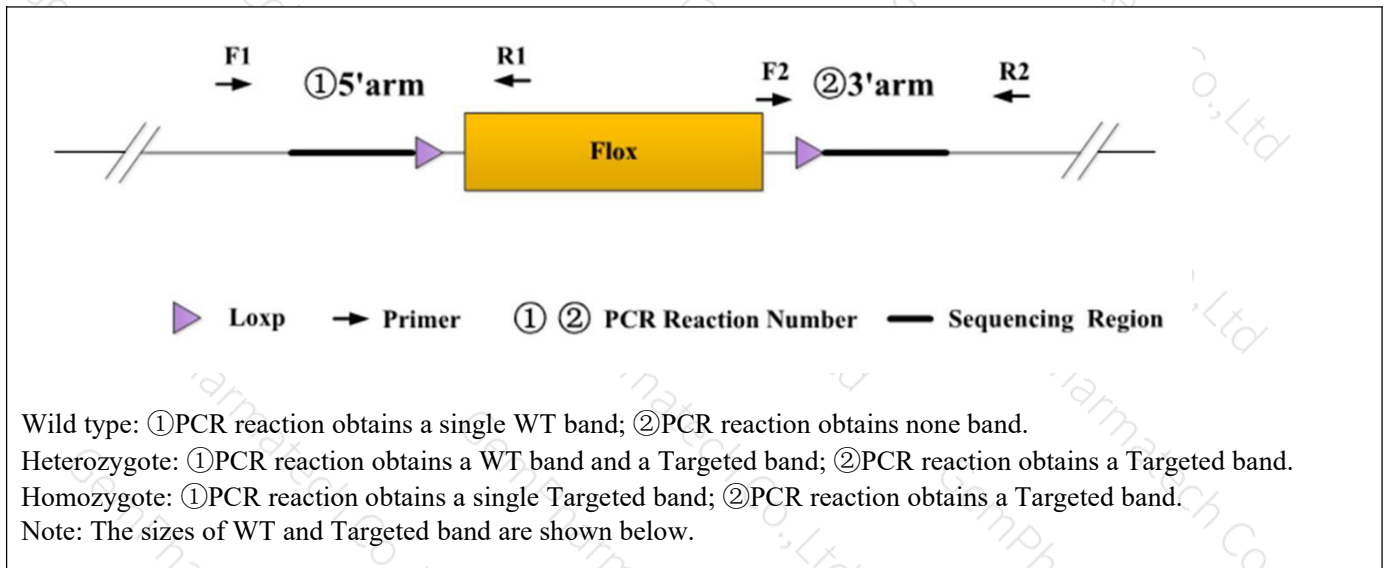


Genotyping Report

Strain ID	T017888	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	<i>Insr</i>		

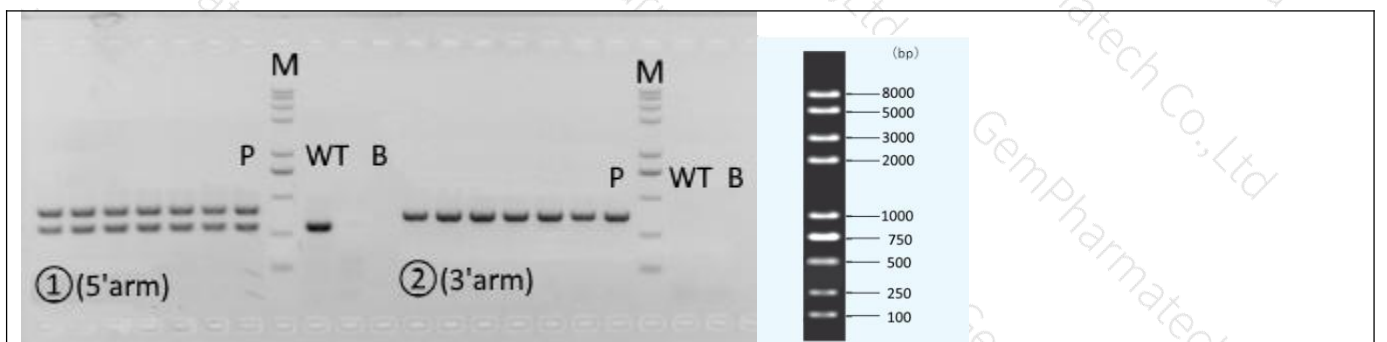
1. Strategy of Genotyping



2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T017888-F1	GAAAGGACTACACATTGGACAGCTC	WT:287bp Targeted:392bp
	T017888-R1	TTCCTCTGCTGTGTGCCTAATC	
②(3'arm)	T017888-F2	ATCGCATTGTCTGAGTACGTG	WT:0bp Targeted:323bp
	T017888-R2	GCAGCACTGTATCTCTTTGGACATG	

3. Gel Image & Conclusion



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°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.

