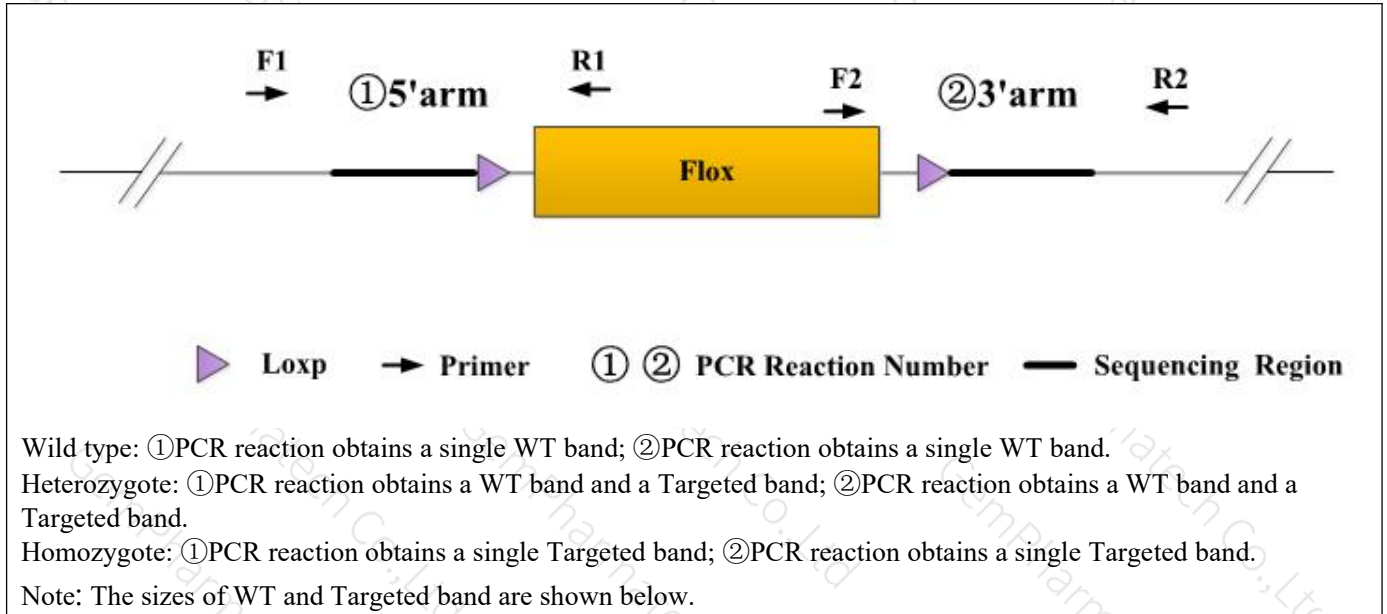


## Genotyping Report

Strain ID	T018641	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>C1qb</i>		

### 1. Strategy of Genotyping

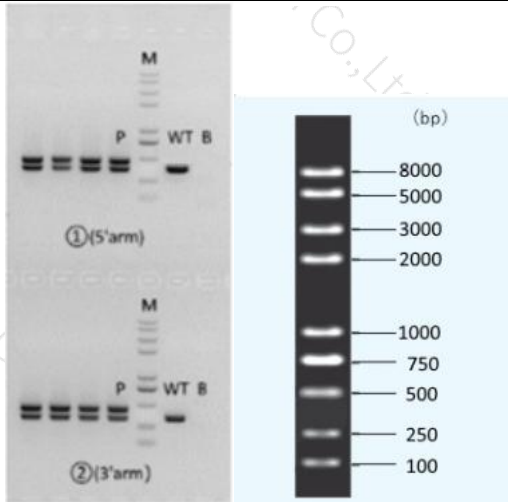


### 2. Primer Information

PCR No.	Primer Name	Primer No.	Sequence	Band Size
①(5'arm)	F1	JS08935-C1qb-5wt-tF1	TGCTGAGAACTGAACTCAGTTCC	WT:406bp Targeted:510bp
	R1	JS08935-C1qb-5wt-tR1	CTGCATCCACCTATGCAGCA	
②(3'arm)	F2	JS08935-C1qb-3wt-tF1A	AGTAGCTCTCTGGTGTCTCCTGGAA	WT:369bp Targeted:470bp
	R2	JS08935-C1qb-3wt-tR1A	TGACACCAGGCAGGTTGCTT	

### 3. Gel Image & Conclusion

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Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control (ddH<sub>2</sub>O); 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

PCR Reaction Component			
Seg.	reaction component	Volume (μl)	
1	2 × Rapid Taq Master Mix (Vazyme P222)	12.5	
2	ddH <sub>2</sub> O	9.5	
3	Primer A(10pmol/μl)	1	
4	Primer B(10pmol/μl)	1	
5	Template(20~80ng/μl)	1	
PCR program I priority selection			
Seg.	Temp.	Time	Cycle
1	95℃	5min	20×
2	98℃	30s	
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	
5	98℃	30s	15×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	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.