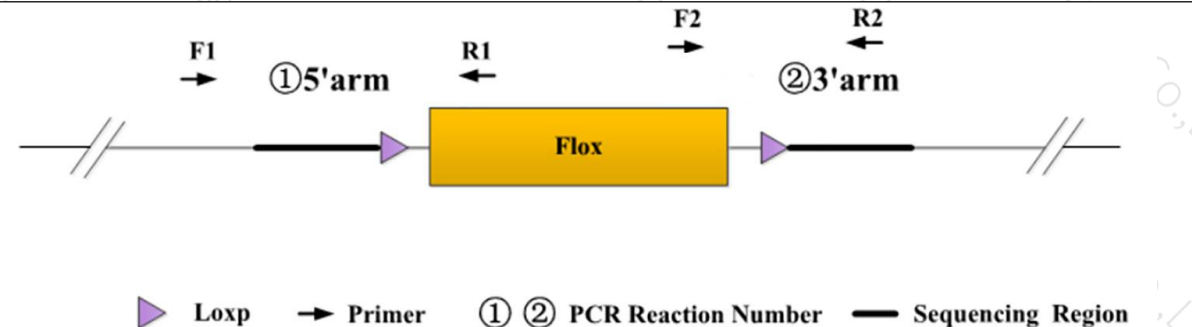


Genotyping Report

Strain ID	T052002	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Xinyuan Liang	Gene Name	C5ar1		

1. Strategy of Genotyping



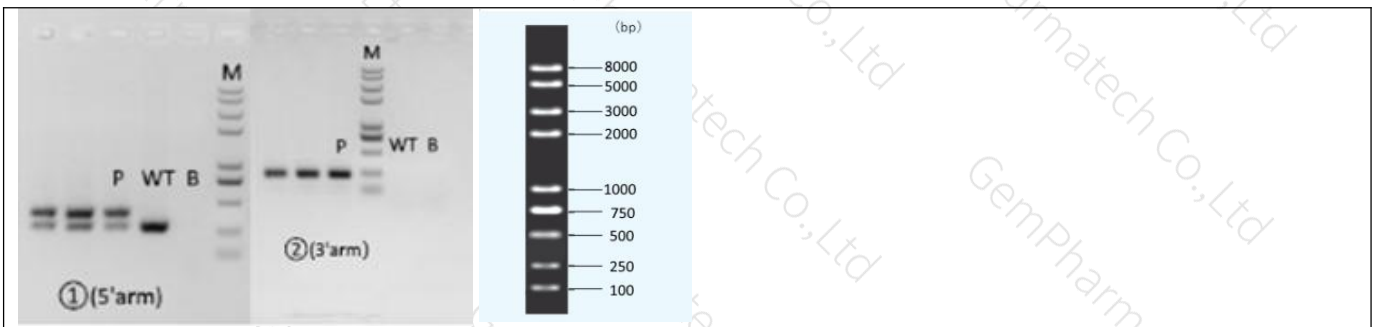
▶ Loxp \rightarrow Primer ① ② PCR Reaction Number — Sequencing Region

Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.
 Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
① (5'arm)	F1	T052002-F1A	ACATCAGCTGGGAAGTGATGA	WT: 281bp Targeted: 386bp
	R1	T052002-R1A	TGTGAAAGCCAGGGGATAAC	
② (3'arm)	F2	T052002-F2	TATCGGTCACATCTGTATTGCCAC	WT: 0bp Targeted: 233bp
	R2	LAR3 (G-C)	CACAACGCGTTCTTCTGTTAGTCC	

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	20 \times
2	98°C	30s	
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	35 \times
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.