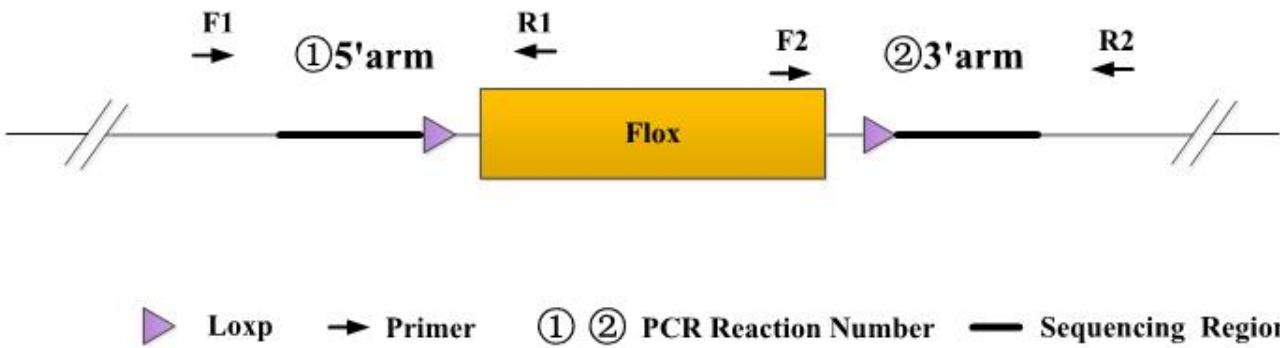




Genotyping Report

Strain ID	T039989	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name			<i>Knll</i>

1. Strategy of Genotyping



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.

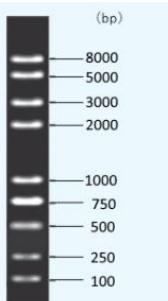
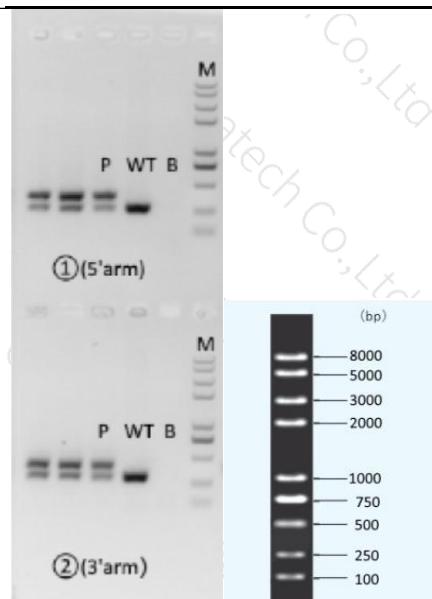
Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①(5'arm)	F1	T039989(P1)-F1	CATGTGCCACCACATCTGTTCCCT	WT: 267bp Targeted: 372bp
	R1	T039989(P1)-R1	ATTCTTAGTACCAAAGAGGTGGCCT	
②(3'arm)	F2	T039989(P1)-F2	ATCCAGGGCTACACAAAGAACCC	WT: 287bp Targeted: 393bp
	R2	T039989(P1)-R2	CAGACTGACCTCAAACTCAGAGATCC	

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

PCR Reaction Component

Seg.	reaction component	Volume (μl)
1	2 × Rapid Taq Master Mix (Vazyme P222)	12.5
2	ddH ₂ 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 °C	5min	
2	98 °C	30s	20×
3	65 °C * (-0.5 °C/cycle)	30s	
4	72 °C	45s*	15×
5	98 °C	30s	
6	55 °C *	30s	
7	72 °C	45s*	
8	72 °C	5min	



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9	10°C	hold	
PCR program II the second choice			
Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	35x
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.