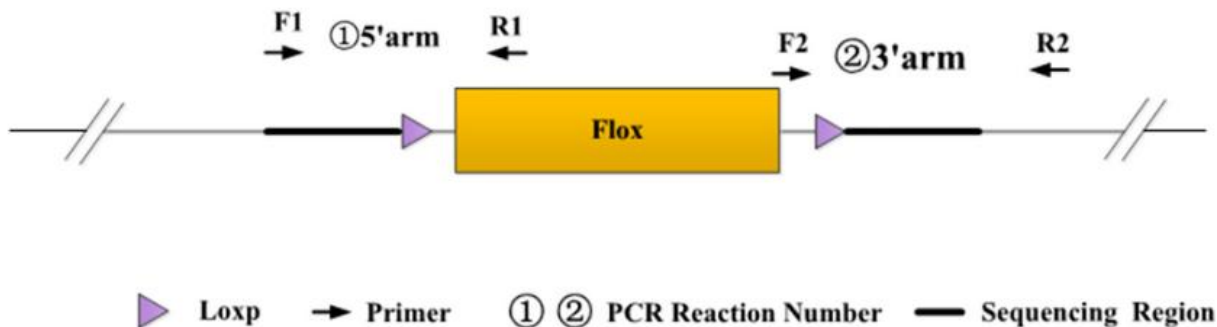


Genotyping Report

Strain ID	T052333	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Fam98a</i>		

1. Strategy of Genotyping



Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains none band.

Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a Targeted band.

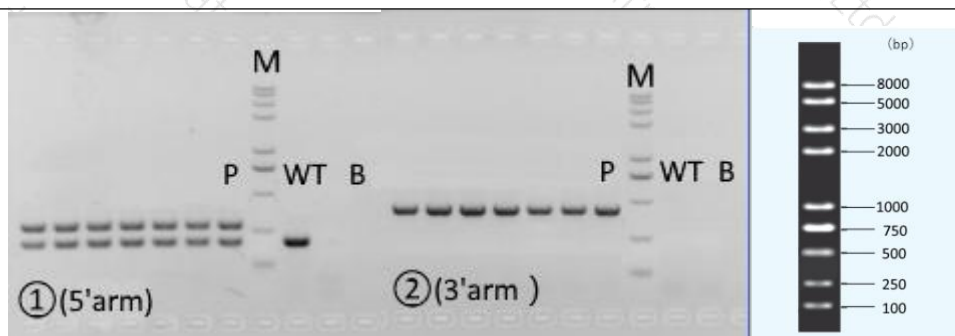
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.

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

2. Primer Information

PCR No.	Primer No.	PrimerName	Sequence	Band Size
①(5'arm)	F1	T052333-F1	ATGTGTGTGAGGCGAGCATTCT	WT:169bp Targeted:271bp
	R1	T052333-R1	GGAAACAGAGAAGAACCTTTGCCC	
②(3'arm)	F2	T052333-F2	CATCGCATTGTCTGAGTAGGTG	WT:0bp Targeted:407bp
	R2	T052333-R2	GCTCTACCTATGACAGGAAGAAGCAG	

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 $^{\circ}$ C	5min	20 \times
2	98 $^{\circ}$ C	30s	
3	65 $^{\circ}$ C * (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	
5	98 $^{\circ}$ C	30s	15 \times
6	55 $^{\circ}$ C *	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	

PCR program II the second choice

Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	35 \times
2	98 $^{\circ}$ C	30s	
3	58 $^{\circ}$ C *	30s	
4	72 $^{\circ}$ C	45s*	
5	72 $^{\circ}$ C	5min	
6	10 $^{\circ}$ C	hold	

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.