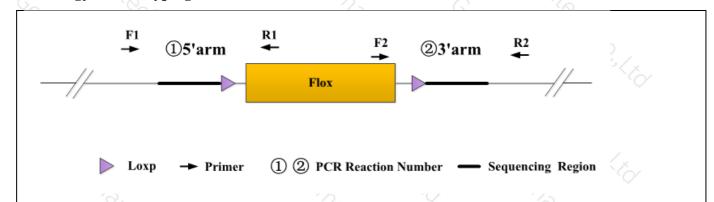
Genotyping Report

Strain ID	T040768	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name);<	Ipcef1	6

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.

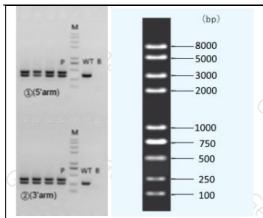
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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

2. Primer Information

1 10			7/1	
PCR No.	Primer No.	Sequence	Band Size	
①(5'arm)	T040768-F1	CAAAATCCTGGAGGCTAGGGA	WT: 318bp Targeted:423bp	
	T040768-R1	ATATGGGACCATACGTGCCACA		
②(3'arm)	T040768-F2	CAAGCATCCAGAGGAGGGTAATG	WT: 285bp	
	T040768-R2	TCACCTAGCCCTCGTTGAAGAG	Targeted:391bp	

3. Gel Image & Conclusion



Note: P: Positive control; WT: Wildype 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

4. I CK CU	nution		(A)	
PCR Reaction	n Component	760/	73,	
Seg.	reaction	reaction component		
1	2 × Rapid Taq Master Mix(Va	2 × Rapid Taq Master Mix (Vazyme P222)		
2	ddH2O	73× , (A)	9.5	
3	Primer A(10pmol/μl)	CX	1 7 ×	
4	Primer B(10pmol/μl)	<u> </u>	G 1 C	
5	Template(≈100ng/μl)	3/x.	70 1 C	
PCR program	n ① priority selection	7	19/2 3/X	
Seg.	Temp.	Time	Cycle	
1	95℃	5min	, C/2	
2	98℃	30s	20×	
3	65℃* (-0.5℃/cycle)	30s		
4	72℃	45s*	9/2	
5	98℃	30s	20×	
6	55℃*	30s	G	
7	72℃	45s*	, (A)	
8	72℃	5min	9/2	
9	10℃	hold	79%	
PCR program	1 ② the second choice	6		
Seg.	Temp.	Time	Cycle	



1	1/2/12/2	95℃	19 ₁₂	5min		(S)	,
2	· 1921	98℃	9×	30s		35×	Ó
3 (Z. ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	58℃*	5	30s	C ₂	AX.	
4	70/	72℃	G C	45s*	³ 70.	7 _C	
5	73/2	72℃	70	5min	7	24 3/x	
6	, Jax	10℃	, 9 ¹²	hold		(J) (Q	<i>></i>

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