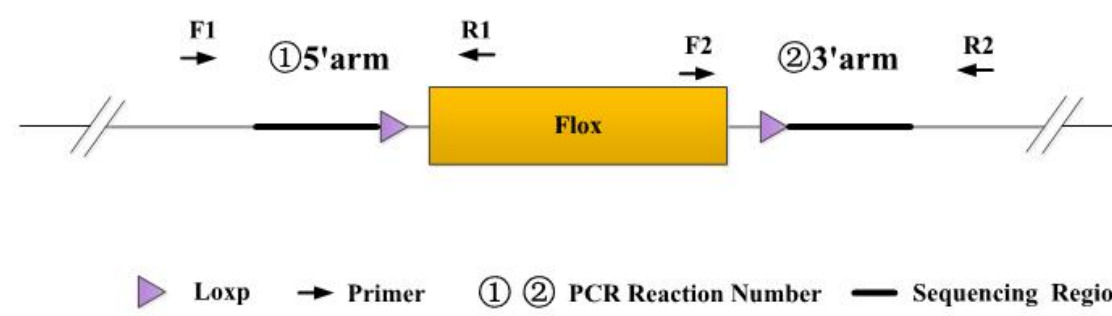


Genotyping Report

Strain ID	T012796	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Tnfsf4</i>		

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.	Sequence	Band Size
①(5'arm)	T012796-F1	AAGCCATCTCTCCTCCCTATGCA	WT: 281bp Targeted:386bp
	T012796-R1	AGAGAGAGAGAGGGAAATGCCACT	
②(3'arm)	T012796-F2	GGGGAATAGTAACCAAAGAAGTGCC	WT: 272bp Targeted:378bp
	T012796-R2	CATATAGGAACTTCTGGTGGTTGGC	

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% ≥ 60% or GC% ≤ 40%, recommend to use Vazyme P515.)

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(≈100ng/μl)	1	
PCR program ① 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*	
5	98°C	30s	
6	55°C*	30s	20×
7	72°C	45s*	
8	72°C	5min	
9	10°C	hold	
PCR program ② 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

