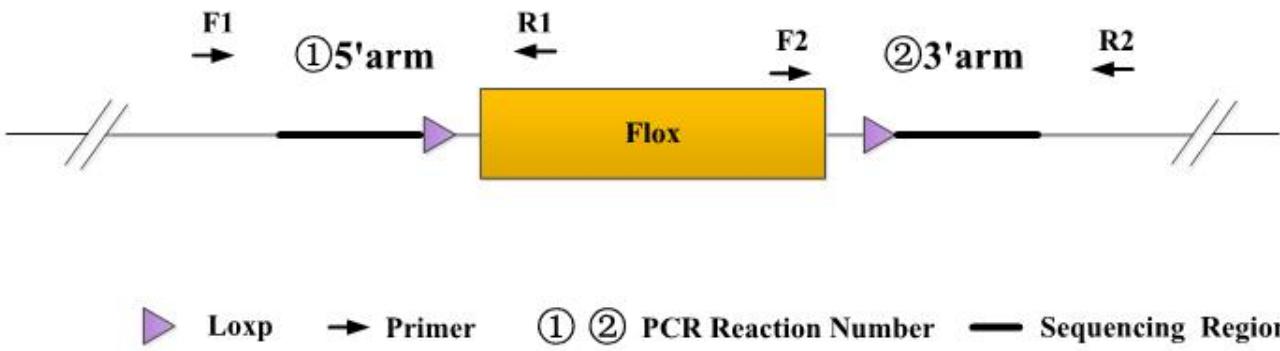




## Genotyping Report

Strain ID	T019440	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name			<i>Cadps2</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.

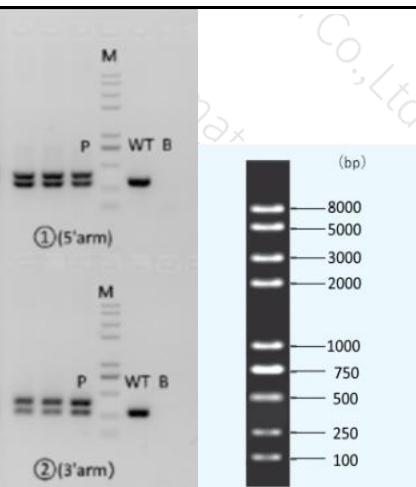
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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	JS09734-Cadps2-5wt-tF1	AGCCAAAAGCACTTGATAACCCT	WT:287bp Targeted: 391bp
	R1	JS09734-Cadps2-5wt-tR1	GCTGGTCCTTGCCTTAGCATT	
②(3'arm)	F2	JS09734-Cadps2-3wt-tF1A	AACCAGGAGCAAGGATACTTGCC	WT:283bp Targeted: 384bp
	R2	JS09734-Cadps2-3wt-tR1A	TGAGGGGTAGGAGAATGGAAAACTAA	

### 3. Gel Image & Conclusion



Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control ( $\text{ddH}_2\text{O}$ ); 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 ( $\mu\text{l}$ )
1	2 × Rapid Taq Master Mix (Vazyme P222)	12.5
2	ddH <sub>2</sub> O	9.5
3	Primer A(10pmol/ $\mu\text{l}$ )	1
4	Primer B(10pmol/ $\mu\text{l}$ )	1
5	Template(20~80ng/ $\mu\text{l}$ )	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*	
5	98 °C	30s	
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
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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.