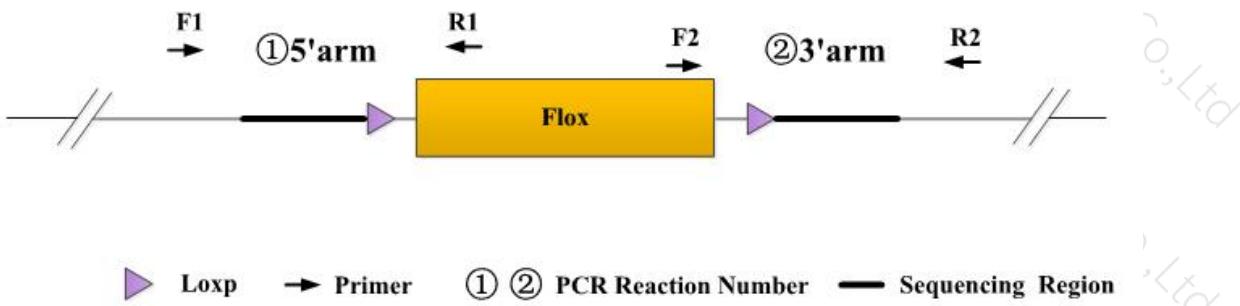




Genotyping Report

Strain ID	T015141	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name			<i>Hsd17b4</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.

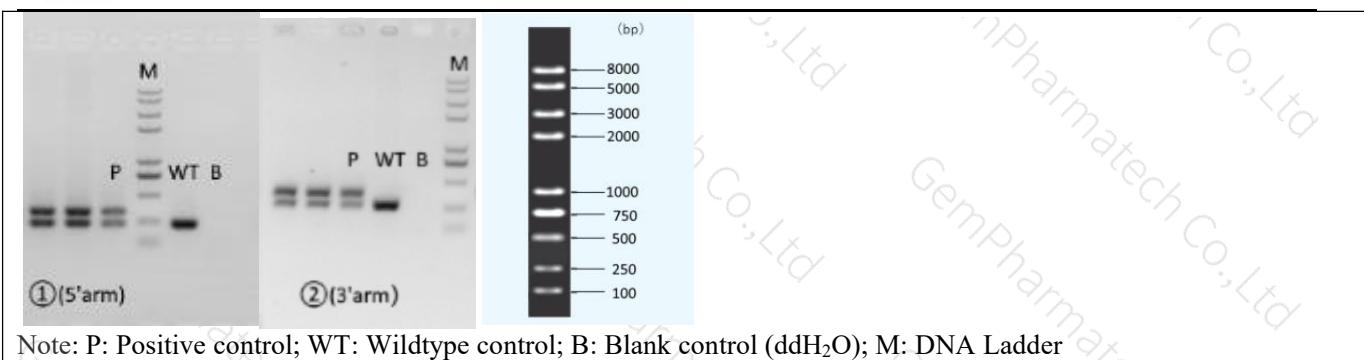
Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①(5'arm)	F1	JS05229-Hsd17b4-5wt-tF1	CATGAGGTTCAGCTGCCATTTC	WT:225bp Targeted:327bp
	R1	JS05229-Hsd17b4-5wt-tR1	GGGAAGCTGTGAGAAACACGC	
②(3'arm)	F2	JS05229-Hsd17b4-3wt-tF1	ACATTACAGACATCACCAAGATTCAGC	WT:267bp Targeted:370bp
	R2	JS05229-Hsd17b4-3wt-tR1	CTGTCTGGAAAAGGCTCAGATTCA	

3. Gel Image & Conclusion





Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH₂O); M: DNA Ladder

4. PCR Condition

PCR Reaction Component			
Seg.	reaction component	Volume (μl)	
1	2 × Taq Master Mix , Dye Plus, (Vazyme P112-03)	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

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	20×
6	55 °C	30s	
7	72 °C	45s	
8	72 °C	5min	
9	10 °C	hold	