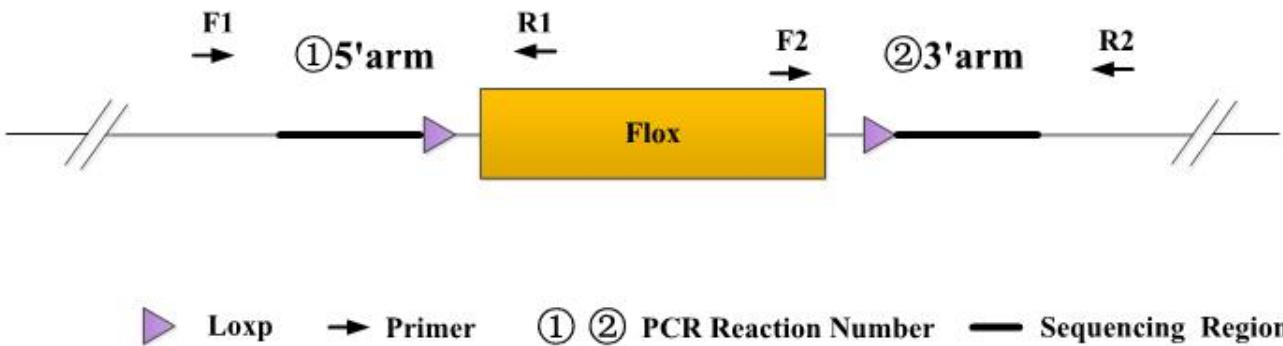




Genotyping Report

Strain ID	T062903	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chen Chen	Gene Name			<i>Slc35a2</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.

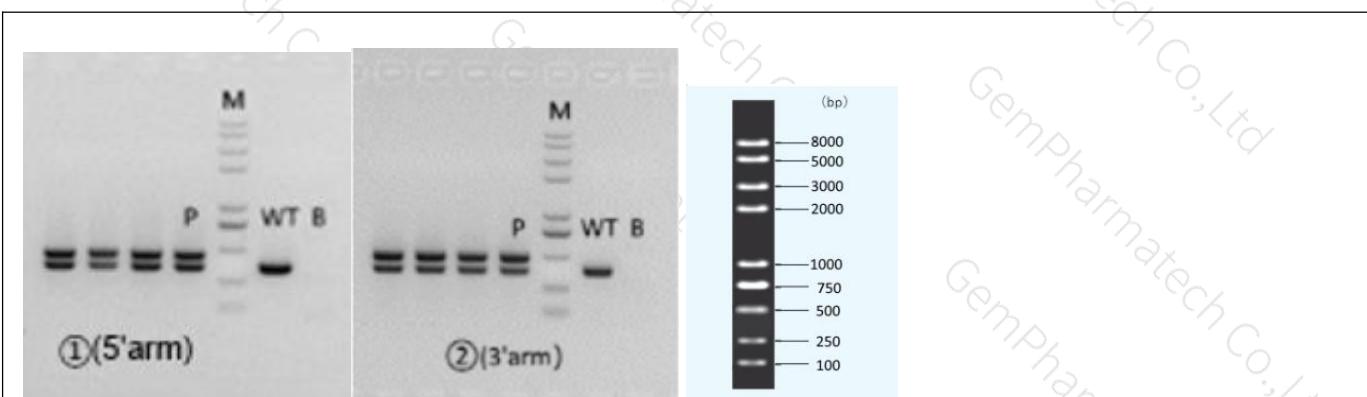
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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	T062903(P1)-F1	GTTTGGGTGGAAATGGACCAT	WT: 344bp Targeted: 449bp
	R1	T062903(P1)-R1	GGGGTGCTTGCCTAAGAGATGTGTAT	
②(3'arm)	F2	T062903(P1)-F2	AAGCAGGACGACTTAGGGCTCAA	WT: 403bp Targeted: 509bp
	R2	T062903(P1)-R2	CACCACGCCTGGATTAGCATCT	

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

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(20~80ng/μl)	1

PCR program I priority selection

Seg.	Temp.	Time	Cycle
1	95 °C	5min	
2	98 °C	30s	
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
1	95 °C	5min	
2	98 °C	30s	
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.



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