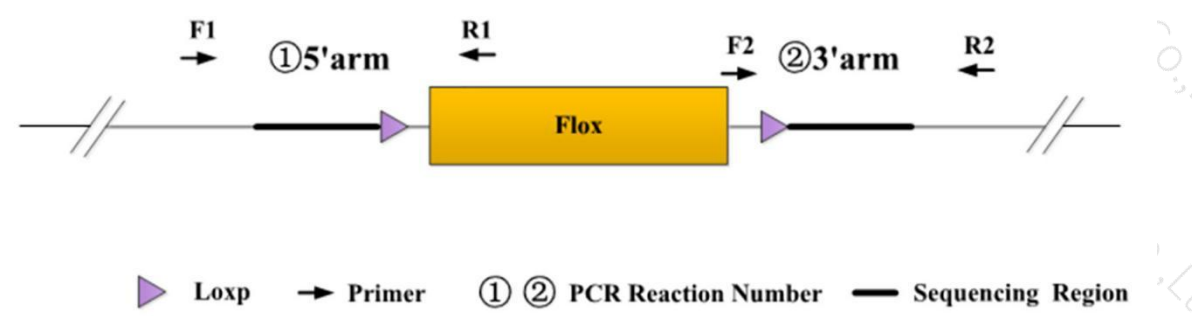


## Genotyping Report

Strain ID	T019565	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	<i>Robo4</i>		

### 1. Strategy of Genotyping



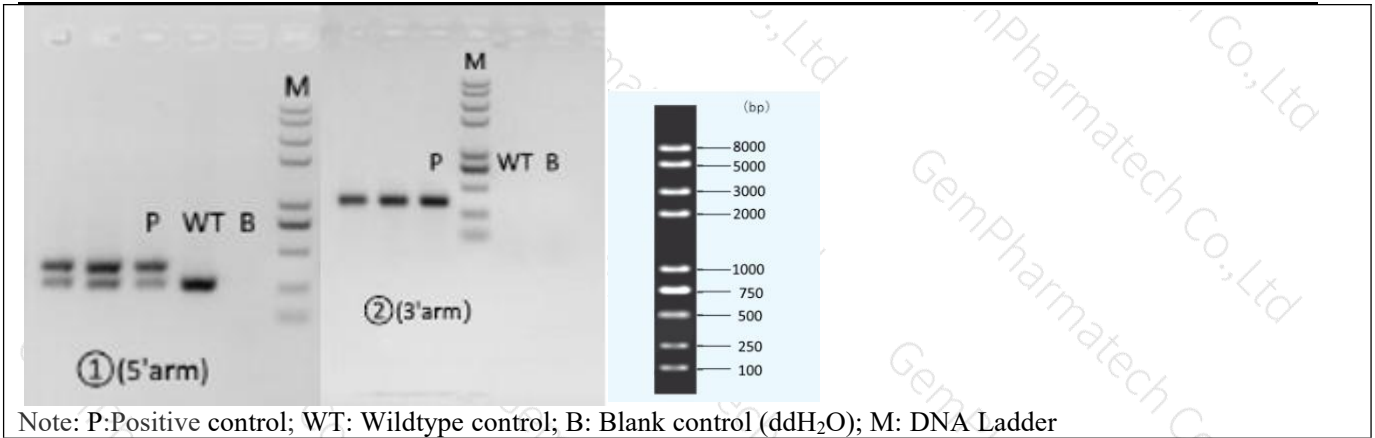
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains none band.  
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a Targeted band.  
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.  
 Note: 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	JS09859-Robo4-5wt-tF1	TGACACAGGTTAGAGTCATCTGGAGG	WT:259bp Targeted:363bp
	R1	JS09859-Robo4-5wt-tR1	AATAGGGAAAGAGCCTGGGAGCA	
②(3'arm)	F2	NEO-3F	TCTGAGGCGGAAAGAACCAG	WT:0bp Targeted:319bp
	R2	JS09859-Robo4-3wt-tR1	CAGGTATTCCTCAACCTCAGCCA	

### 3. Gel Image & Conclusion

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#### 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 <sub>2</sub> 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	20×
2	98 °C	30s	
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	

Reviewer:Ting Sun

Date:2020-5-12