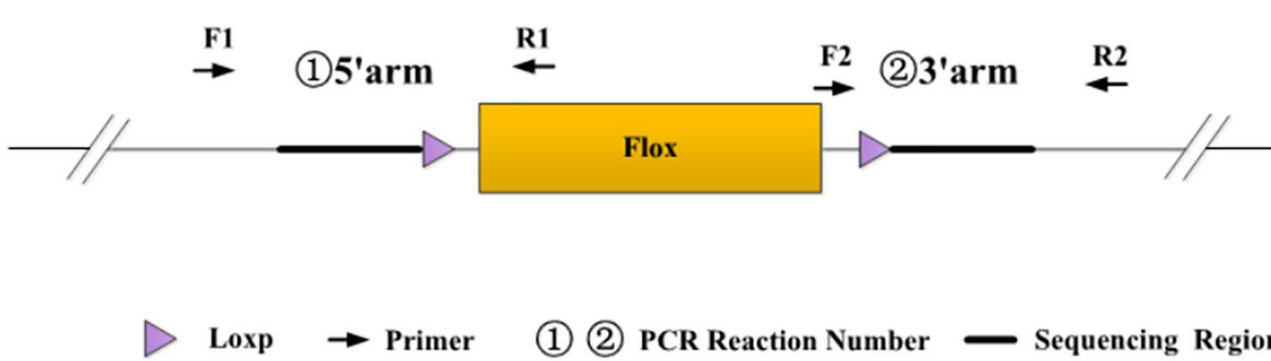


## Genotyping Report

Strain ID	T019400	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chen Chen	Gene Name	<i>Ly9</i>		

### 1. Strategy of Genotyping

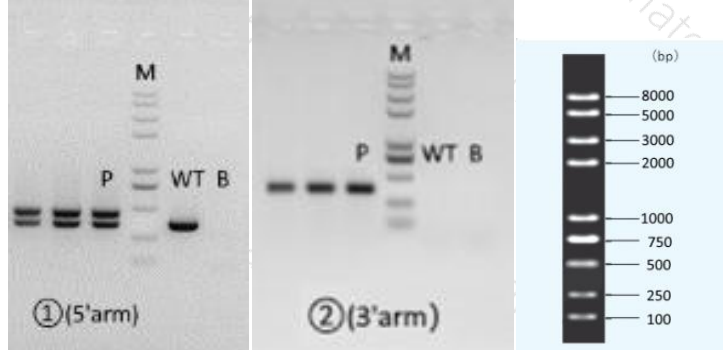


Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains none band.  
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a Targeted band.  
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.  
 Note: 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	JS09694-Ly9-5wt-tF1	AGTGGCATGGAACAGTCCCTC	WT: 356bp Targeted:458bp
	R1	JS09694-Ly9-5wt-tR1	GGGAAATTACTCAGGCTATGACTGGGT	
②(3'arm)	F2	ZMK2F4	CATCGCATTGTCTGAGTAGGTG	WT: 0bp Targeted:346bp
	R2	JS09694-Ly9-3wt-tR1	GTCCAGCCAAGTCACACAGTGTCAA	

### 3. Gel Image & Conclusion



Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control (ddH<sub>2</sub>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

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as GC%  $\geq$  60% or GC%  $\leq$  40%, recommend to use Vazyme P515.)

##### PCR Reaction Component

Seg.	Reaction Component	Volume ( $\mu$ l)
1	2 $\times$ Rapid Taq Master Mix(Vazyme P222) or 2 $\times$ Phanta Max Master Mix (Vazyme P515)	12.5
2	ddH <sub>2</sub> O	9.5
3	Primer A(10pmol/ $\mu$ l)	1
4	Primer B(10pmol/ $\mu$ l)	1
5	Template(20~80ng/ $\mu$ l)	1

##### PCR program I (priority selection)

Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	20 $\times$
3	65 $^{\circ}$ C*(-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	
5	98 $^{\circ}$ C	30s	15 $\times$
6	55 $^{\circ}$ C*	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	

##### PCR program II (the second choice)

Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	35 $\times$
3	58 $^{\circ}$ C*	30s	
4	72 $^{\circ}$ C	45s*	
5	72 $^{\circ}$ C	5min	
6	10 $^{\circ}$ C	hold	

Note\*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.