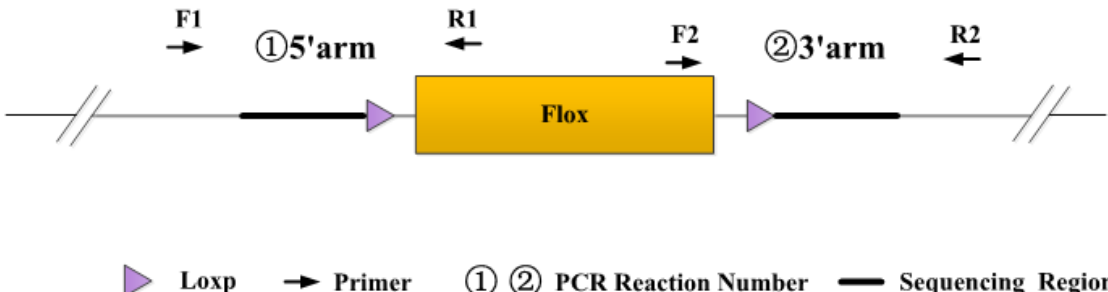





## Genotyping Report

Strain ID	T011065	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Ces4a</i>		

### 1. Strategy of Genotyping



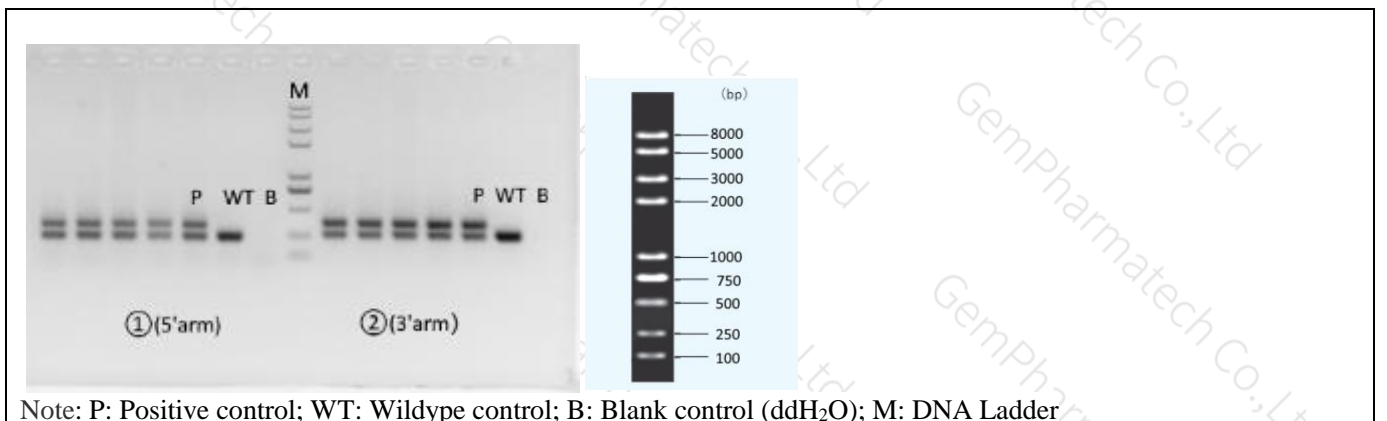
Legend:  Loxp     Primer    ① ② PCR Reaction Number     Sequencing Region

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.	Sequence	Band Size
①(5'arm)	T011065-F1	TGTGCTGCCTTGCTGGCTT	WT:242bp Targeted:346bp
	T011065-R1	ACTTGGACTGCCAAGTGTCGC	
②(3'arm)	T011065-F2	CTTTCAGACAAAGATAATGATGCACAG	WT:258bp Targeted:359bp
	T011065-R2	AAAGCTGTTCTGTTCTCAGAAGG	

### 3. Gel Image & Conclusion





#### 4. PCR Condition

PCR Reaction Component			
Seg.	reaction component		Volume ( $\mu$ l)
1	2 $\times$ Taq Master Mix , Dye Plus, (Vazyme P112-03)		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( $\approx$ 100ng/ $\mu$ l)		1
PCR program			
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	
6	55 $^{\circ}$ C	30s	20 $\times$
7	72 $^{\circ}$ C	45s	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	

Reviewer:Ting Sun

Date:2021-6-9