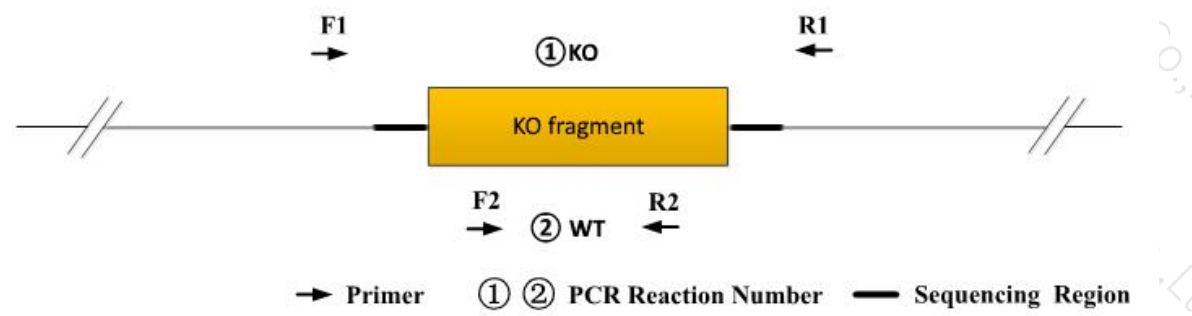


## Genotyping Report

Strain ID	T012535	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	<i>Nt5e</i>		

### 1. Strategy of Genotyping

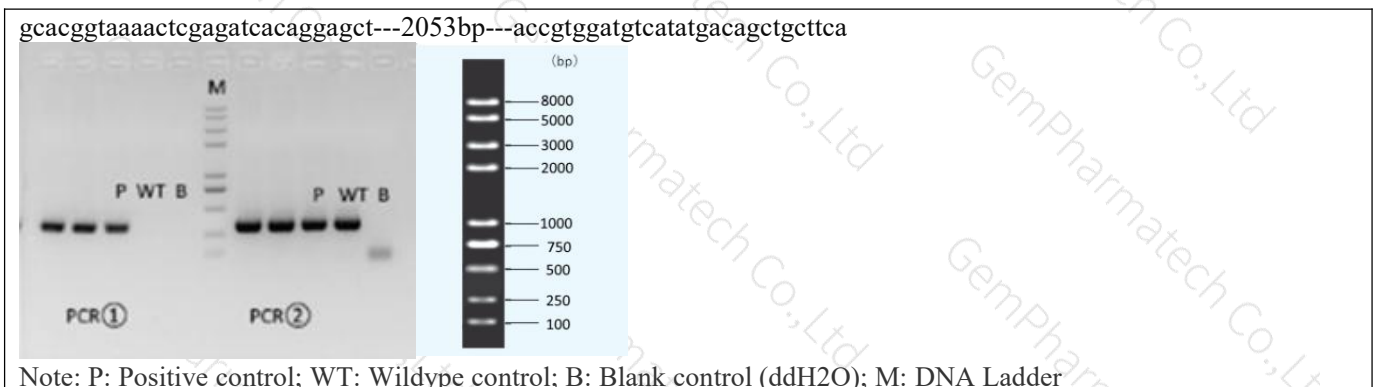


Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.  
Note: 1)The sizes of WT and Targeted band are shown below.  
2)If the WT band is too large, it may not be possible to obtain a WT band.

### 2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
PCR①	T012535-F1	CTGGTTTTGGTGGCTTCTGAAGCT	WT:2340bp KO:287bp
	T012535-R1	GGAATGCCAGGGATGATCTGATTC	
PCR②	T012535-F2	GCTTGGGAATAGATGTGGAAAAGC	WT:290bp KO:0bp
	T012535-R2	TCATGTGTACGTGCTCCGAGCA	

### 3. Gel Image





#### 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	20 $\times$
6	55 $^{\circ}$ C	30s	
7	72 $^{\circ}$ C	45s	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	