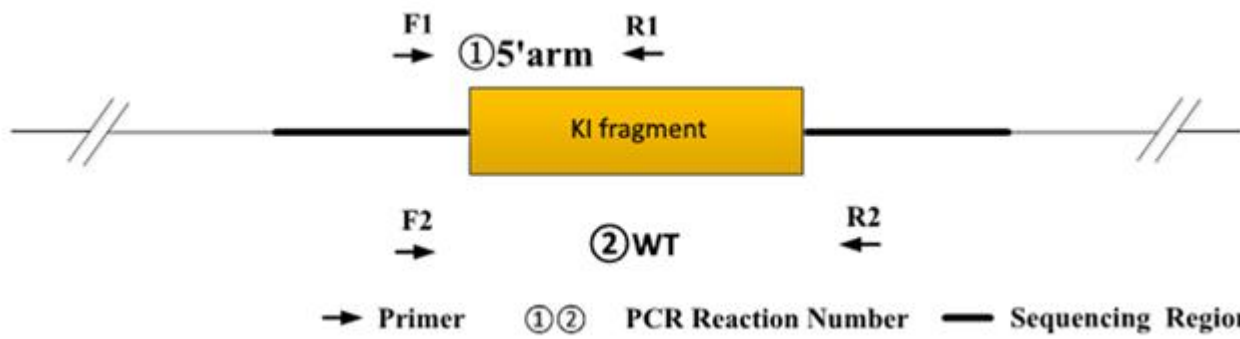


## Genotyping Report

Strain ID	T069084	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Binjie Jiao	Gene Name	<i>H11-Thy1-CreERT2</i>		

### 1. Strategy of Genotyping

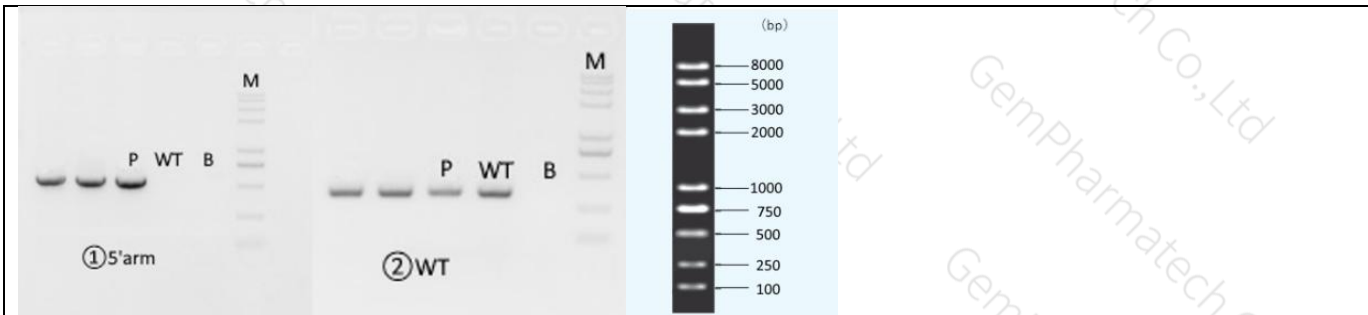


Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.  
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.  
Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the target band, it is likely to produce dominant amplification, the reaction is only used to judge whether there is a WT allele.

### 2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
① 5'arm	F1	H11-tF3	GGGCAGTCTGGTACTTCCAAGCT	WT:0bp Targeted:538bp
	R1	T069084-Thy1-ki-5tR1	CCAGTGCTGAGCCACTCTACT	
② WT	F2	H11-wt-tF1B	GGCCTCCAAGTCTTGACAGTAGATT	WT:569bp Targeted:9349bp
	R2	H11-wt-tR1B	TTGTGGGTCTTCCACCTTTCTTC	

### 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°C	5min	
2	98°C	30s	20 $\times$
3	65°C*(-0.5°C/cycle)	30s	
4	72°C	45s*	
5	98°C	30s	15 $\times$
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	35 $\times$
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.