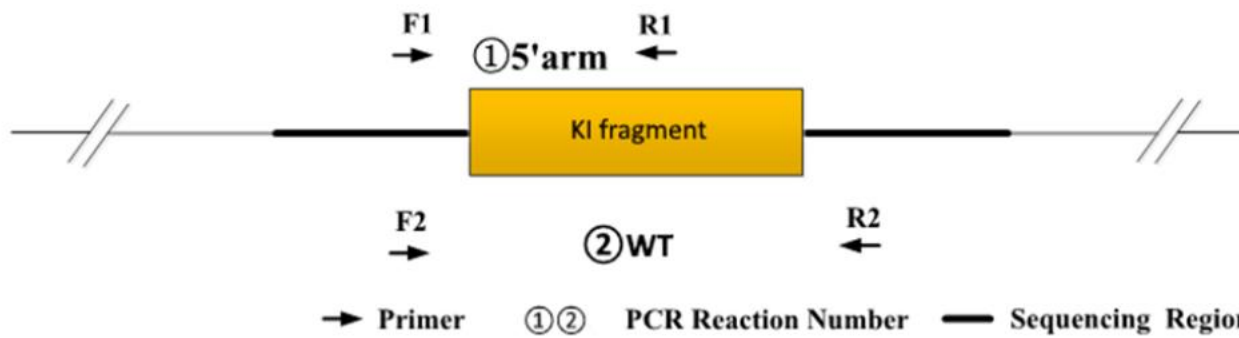


Genotyping Report

Strain ID	T005635	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>H11-MMTV-iCre</i>		

1. Strategy of Genotyping

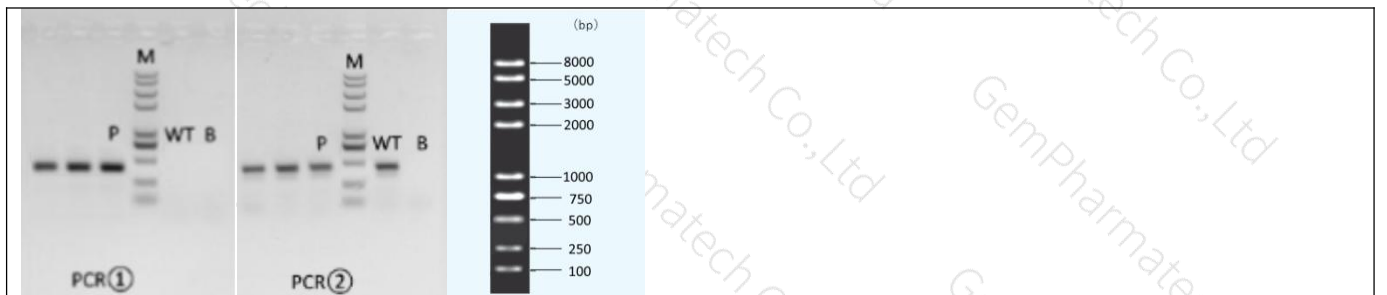


Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below. 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
①5'arm	F1	T005635-F1	GGGCAGTCTGGTACTTCCAAGCT	WT:0bp Targeted:332bp
	R1	T005635-R1	AGCAGAGCTATGCCAAGTTTGC	
②WT	F2	T005635-F2	CAGCAAAACCTGGCTGTGGATC	WT:412bp Targeted:0bp
	R2	T005635-R2	ATGAGCCACCATGTGGGTGTC	

3. Gel Image & Conclusion



Note: P:Heterozygous samples; WT: Wildtype control; B: Blank control (ddH₂O); 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 (μ l)
1	2 \times Rapid Taq Master Mix(Vazyme P222) or 2 \times Phanta Max Master Mix (Vazyme P515)		12.5
2	ddH ₂ O		9.5
3	Primer A(10 μ mol/ μ l)		1
4	Primer B(10 μ mol/ μ l)		1
5	Template(20~80ng/ μ 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	
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.