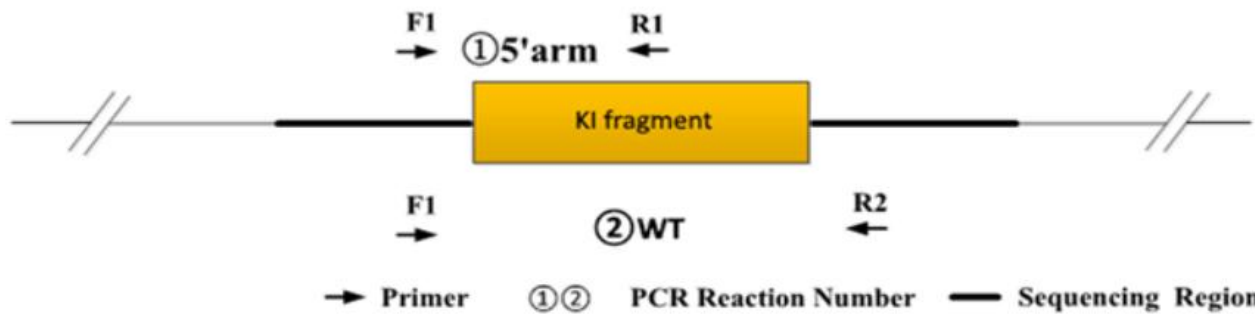


Genotyping Report

Strain ID	T053653	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Clu-P2A-CreERT2-P2A-EGFP</i>		

1. Strategy of Genotyping

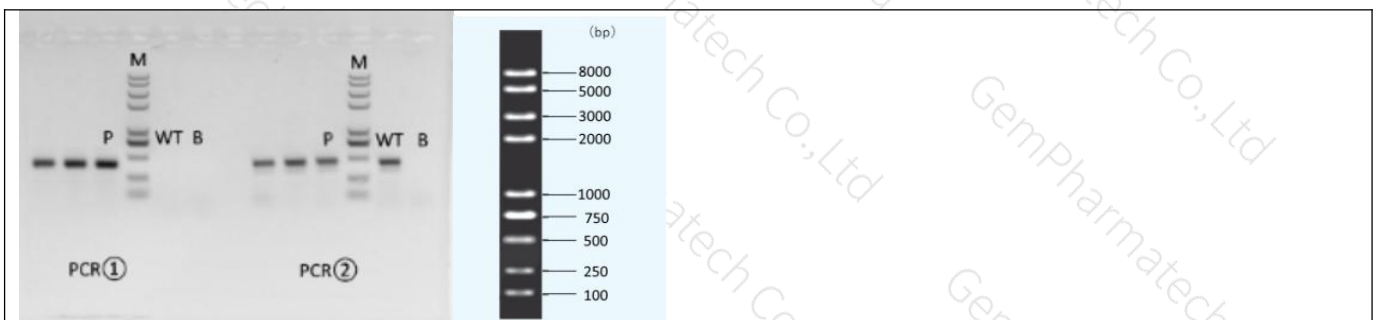


Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the targeted 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	T053653-F1	TGAAAGTCCTCAGGAGTCCAGA	WT:0bp Targeted:385bp
	R1	T053653-R1	CATGTCCATCAGGTTCTTGCGAAC	
②WT	F1	T053653-F2	TGAAAGTCCTCAGGAGTCCAGA	WT:468bp Targeted:3306bp
	R2	T053653-R2	AAGGGACACGTATCGCAAGG	

3. Gel Image & Conclusion



Note: P:Positive control; 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

PCR Reaction Component			
Seg.	reaction component	Volume (μl)	
1	2 × Rapid Taq Master Mix (Vazyme P222)	12.5	
2	ddH ₂ O	9.5	
3	Primer A(10pmol/μl)	1	
4	Primer B(10pmol/μl)	1	
5	Template(≈100ng/μl)	1	
PCR program ① priority selection			
Seg.	Temp.	Time	Cycle
1	95 °C	5min	
2	98 °C	30s	20×
3	65 °C* (-0.5 °C/cycle)	30s	
4	72 °C	45s*	
5	98 °C	30s	
6	55 °C*	30s	20×
7	72 °C	45s*	
8	72 °C	5min	
9	10 °C	hold	
PCR program ② the second choice			
Seg.	Temp.	Time	Cycle
1	95 °C	5min	
2	98 °C	30s	35×
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.