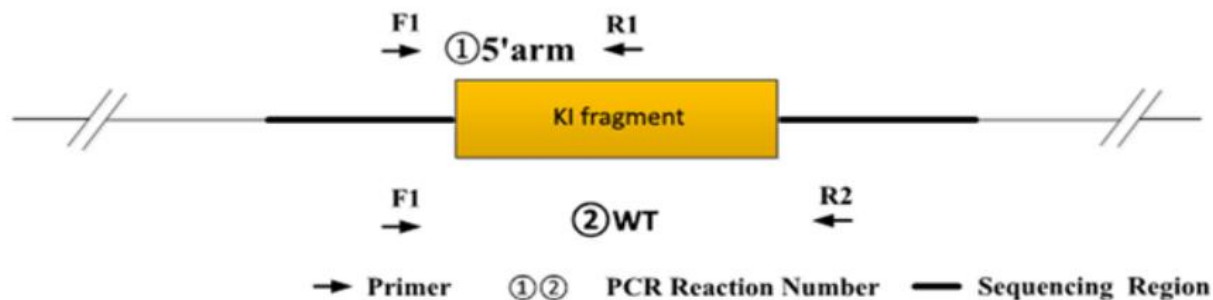


Genotyping Report

Strain ID	T052778	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Rosa26-CAG-LSL-Myc-Pawr-IRES-EGFP-PolyA</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.

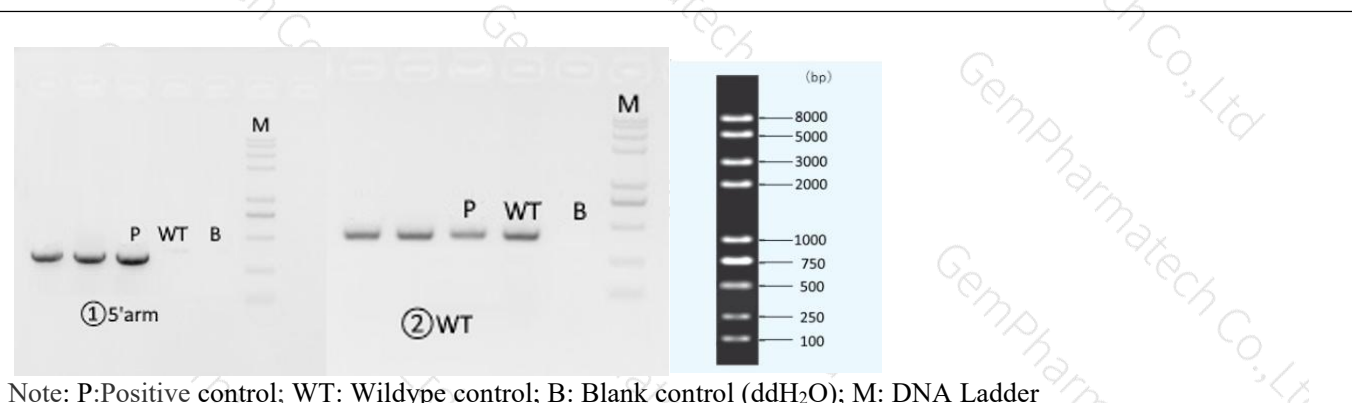
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.

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	T052778-F1	CCCAAAGTCGCTCTGAGTTGTTA	WT:0bp Targeted:375bp
	R1	T052778-R1	TGGCGTTACTATGGGAACATACGTC	
② WT	F1	T052778-F2	CCCAAAGTCGCTCTGAGTTGTTA	WT:479bp Targeted:0bp
	R2	T052778-R2	TCGGGTGAGCATGTCTTTAATCT	

3. Gel Image & Conclusion



4. PCR Condition

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Taq Master Mix , Dye Plus, (Vazyme P112-03)		12.5
2	ddH2O		9.5
3	Primer A(10pmol/μl)		1
4	Primer B(10pmol/μl)		1
5	Template(≈100ng/μl)		1
PCR program			
Seg.	Temp.	Time	Cycle
1	95℃	5min	20×
2	98℃	30s	
3	65℃ （-0.5℃/cycle）	30s	
4	72℃	45s	
5	98℃	30s	20×
6	55℃	30s	
7	72℃	45s	
8	72℃	5min	
9	10℃	hold	