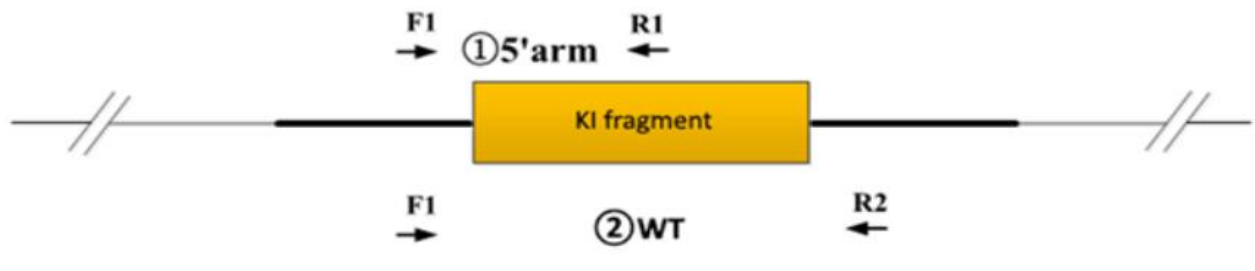


Genotyping Report

Strain ID	T052687	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Foxg1-P2A-iCre</i>		

1. Strategy of Genotyping

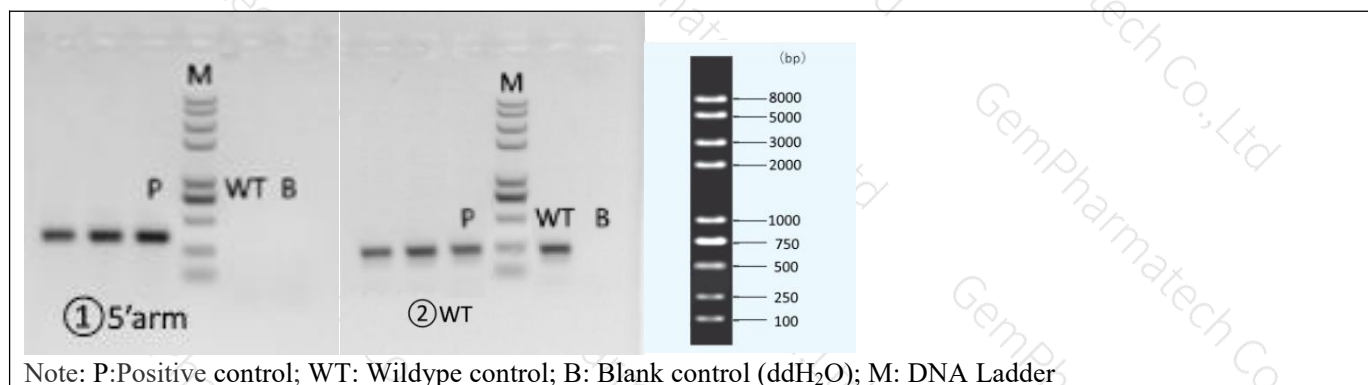


Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the 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	T052687-F1	GTCAATGACTTCGCAGACCAG	WT:0bp Targeted:348bp
	R1	T052687-R1	CTGACTTCATCAGAGGTGGCATC	
②WT	F2	T052687-F2	GTCAATGACTTCGCAGACCAG	WT: 229bp Targeted:1339bp
	R2	T052687-R2	ACGTTCACTTACAGTCTGGTCCC	

3. Gel Image & Conclusion



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 (μl)
1	2 × Rapid Taq Master Mix(Vazyme P222) or 2 × Phanta Max Master Mix (Vazyme P515)		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	