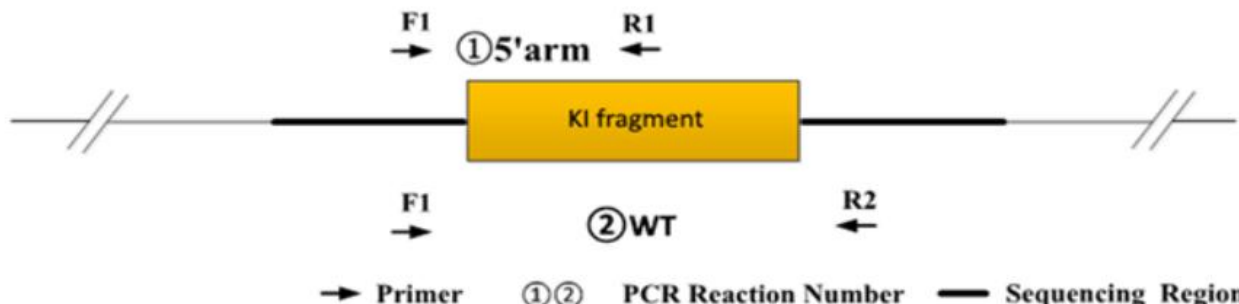


Genotyping Report

Strain ID	T051032	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Dongdong Zhang	Gene Name	<i>Msx2-P2A-iCre-P2A-EGFP</i>		

1. Strategy of Genotyping



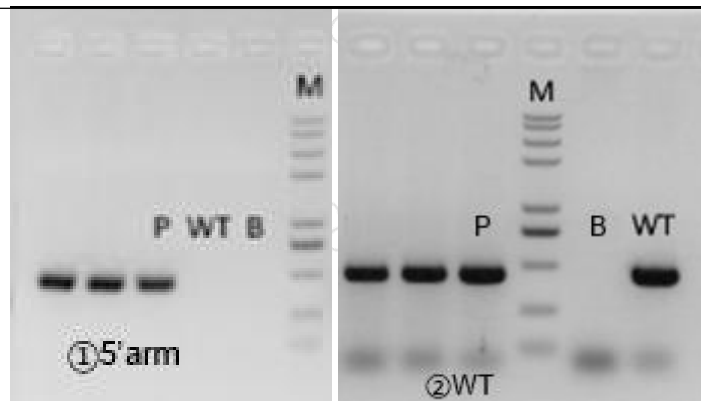
Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below. 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	T051032-F1	TCTGAACCTTACAGAGACCCAGGT	WT:0bp Targeted:429bp
	R1	T051032-R1	CTGACTTCATCAGAGGTGGCATC	
②WT	F1	T051032-F2	TCTGAACCTTACAGAGACCCAGGT	WT:451bp Targeted:2353bp
	R2	T051032-R2	AAACAGGTCTTAGTGCCTCTCACCT	

3. Gel Image & Conclusion

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Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH₂O); M: DNA Ladder

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	