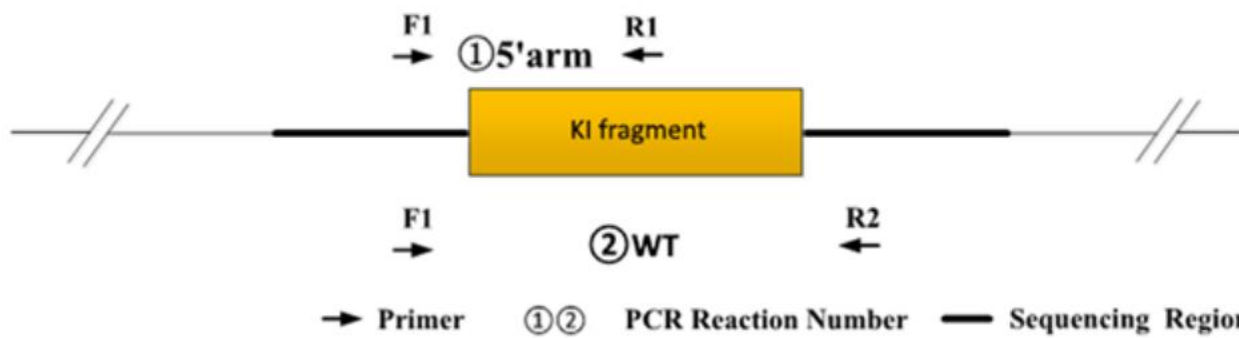


Genotyping Report

Strain ID	T052682	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Chen Chen	Gene Name	<i>Tac1-P2A-iCre</i>		

1. Strategy of Genotyping



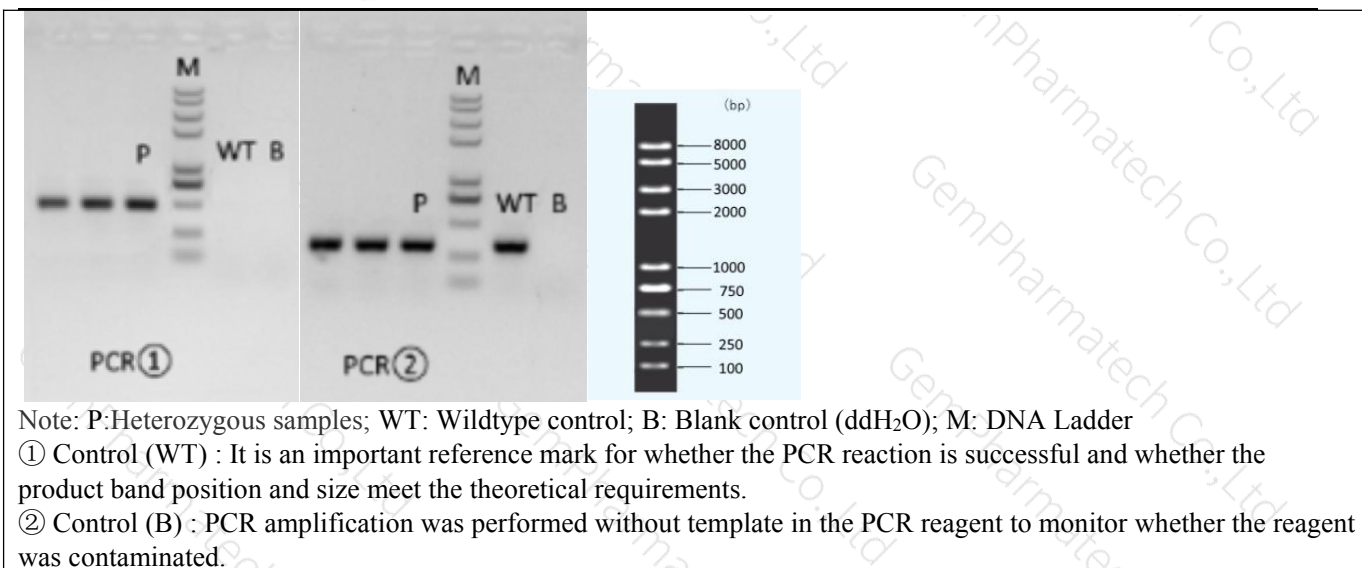
Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.
Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the 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	XM004148-Tac1-wt-tF1	AGCAGATGCCCAAAGAACTAGG	WT:0bp Targeted:500bp
	R1	IRES-iCre-5TR1	GAGCATCTTCCAGGTGTGTTCA	
②WT	F1	XM004148-Tac1-wt-tF1	AGCAGATGCCCAAAGAACTAGG	WT:336bp Targeted:1464bp
	R2	XM004148-Tac1-wt-tR1	TTTACCGTTCACCTGCTCACTGACAC	

3. Gel Image & Conclusion

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4. PCR Condition

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Rapid Taq Master Mix (Vazyme P222)		12.5
2	ddH2O		9.5
3	Primer A(10pmol/μl)		1
4	Primer B(10pmol/μl)		1
5	Template(20~80ng/μl)		1
PCR program I priority selection			
Seg.	Temp.	Time	Cycle
1	95℃	5min	20×
2	98℃	30s	
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	
5	98℃	30s	15×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program II the second choice			
Seg.	Temp.	Time	Cycle
1	95℃	5min	

2	98℃	30s	35×
3	58℃ *	30s	
4	72℃	45s*	
5	72℃	5min	
6	10℃	hold	

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.