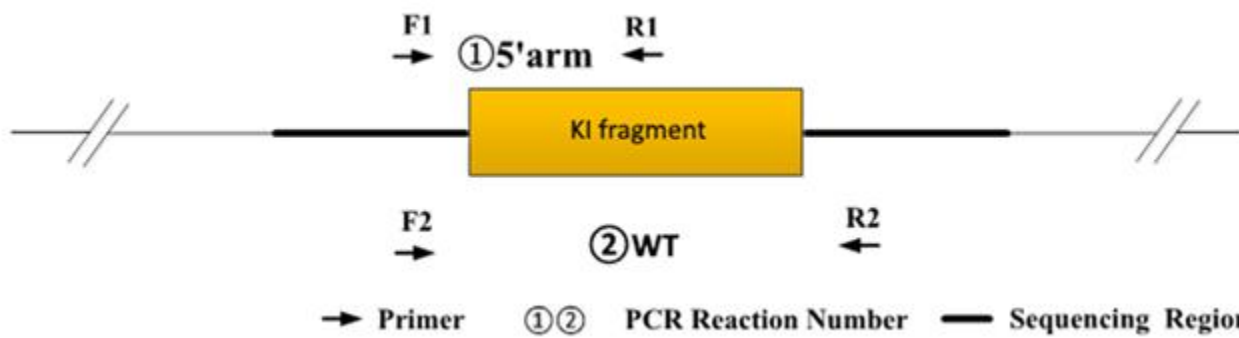


Genotyping Report

Strain ID	T055741	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Rosa26-CAG-Frt-ZsGreen-Stop-Frt-tdTomato</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	Rosa26-S4-tF2	GTGGGATACAGAAGACCAATGCAG	WT:0bp Targeted:555bp
	R1	CAG-tR1	TGGCGTTACTATGGGAACATACGTC	
② WT	F2	Rosa26-S4-tF1	AATGTAGGGCCAGAGTTTAGCCAG	WT:460bp Targeted:6349bp
	R2	Rosa26-S4-tR1	TGAAAGATTTCCCAACCCAC	

3. Gel Image & Conclusion



Note: P:Heterozygous samples; 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

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(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	35×
2	98℃	30s	
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.