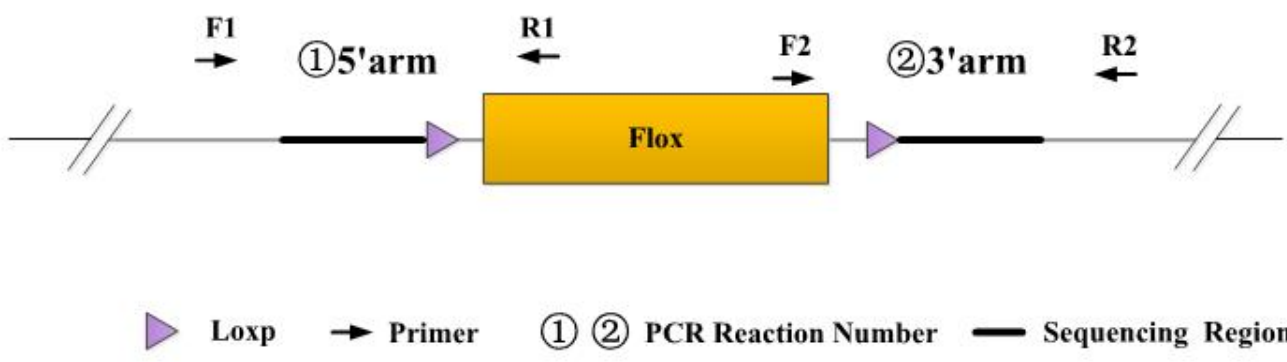


Genotyping Report

Strain ID	T052283	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Binjie Jiao	Gene Name	<i>Fgfr3</i>		

1. Strategy of Genotyping



▶ **Loxp** **→** **Primer** **① ②** **PCR Reaction Number** **—** **Sequencing Region**

Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.
 Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①(5'arm)	F1	T052283(P2)-F1	TTTGGCTACCAGGTTGTTGAGAG	WT: 379bp Targeted: 484bp
	R1	T052283(P2)-R1	CTACCATAGGACCATGTGCATCTG	
②(3'arm)	F2	T052283(P2)-F2	GAGGACTGAGCCTGCAGAAGTTTT	WT: 306bp Targeted: 412bp
	R2	T052283(P2)-R2	GGTTCCTGTTCCAAACAATCCC	

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 (μ l)
1	2 \times Rapid Taq Master Mix(Vazyme P222) or 2 \times Phanta Max Master Mix (Vazyme P515)		12.5
2	ddH ₂ O		9.5
3	Primer A(10pmol/ μ l)		1
4	Primer B(10pmol/ μ l)		1
5	Template(20~80ng/ μ l)		1
PCR program I priority selection			
Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	20 \times
2	98 $^{\circ}$ C	30s	
3	65 $^{\circ}$ C* (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	
5	98 $^{\circ}$ C	30s	15 \times
6	55 $^{\circ}$ C*	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	
PCR program II the second choice			
Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	35 \times
2	98 $^{\circ}$ C	30s	
3	58 $^{\circ}$ C*	30s	
4	72 $^{\circ}$ C	45s*	
5	72 $^{\circ}$ C	5min	
6	10 $^{\circ}$ C	hold	



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