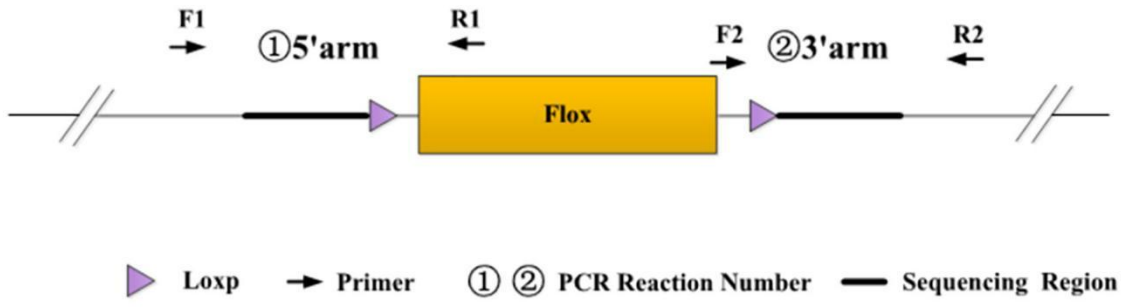
Genotyping Report

Strain ID	T051876	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	*Trim66*		

1. Strategy of Genotyping

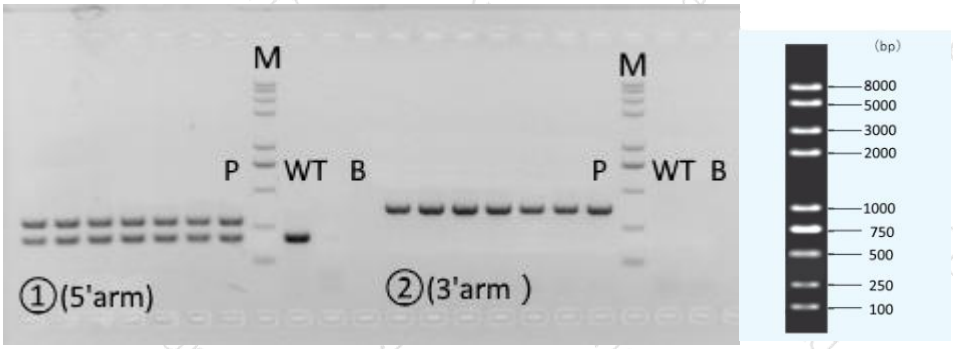


Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains none band.
Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a Targeted band.
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.
Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T051876-F1	CACAGATACCTTCCTCTCTCCAAG	WT:198bp Targeted:300bp
	T051876-R1	CACTTGTGAACAGCCTGACTCAC	
②(3'arm)	T051876-F2	CATCGCATTGTCTGAGTAGGTG	WT:0bp Targeted:388bp
	T051876-R2	TCTGTGCTTGACTGTGGTTTCC	

3. Gel Image & Conclusion



Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control (ddH_2O); 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% ≥ 60% or GC% ≤ 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	ddH_2O	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	
2	98℃	30s	20×
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.