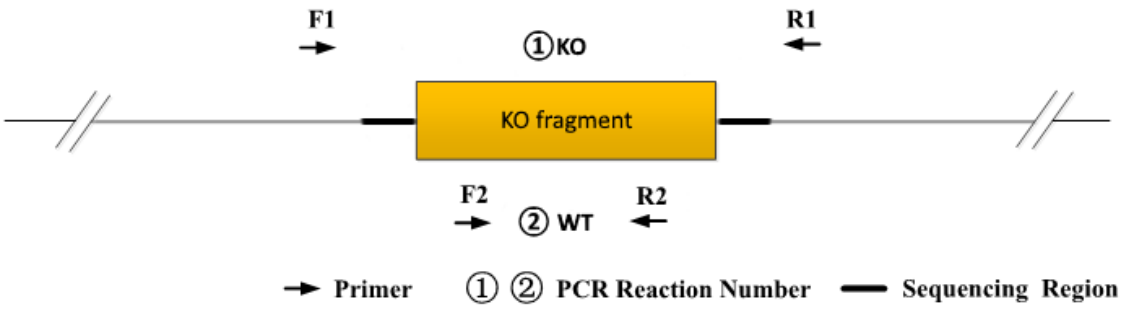
Genotyping Report

Strain ID	T045540	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	*Ttc28*		

1. Strategy of Genotyping



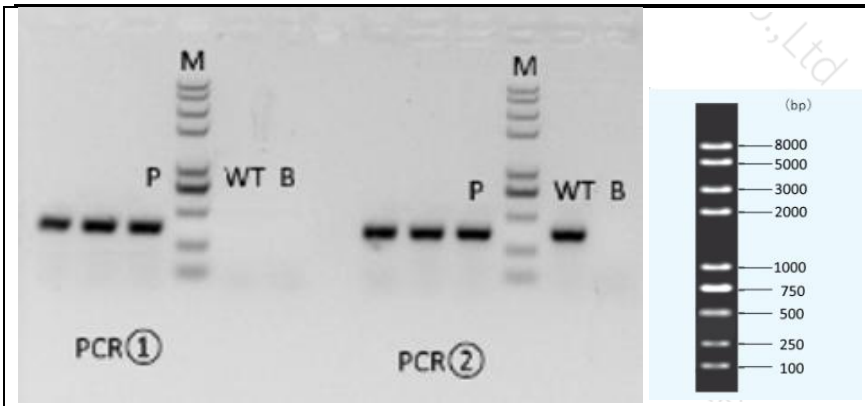
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note：1)The sizes of WT and Targeted band are shown below.
2)If the WT band is too large, it may not be possible to obtain a WT band.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
PCR①	T045540-F1	TGTCACACAACAGAGAAGAAGCAACC	WT: 2822bp Targeted: 353bp
	T045540-R1	GGTGTTGATTTGTTCACCATGTTCC	
PCR②	T045540-F2	ACTCATAGCCCTGATAAAGAGTCCCT	WT: 340bp
	T045540-R2	ATCTGCCCTCGTTAGACAGTTCATC	

3. Gel Image

tgttgttaatgtgccatactaggca---*2469bp*---tctgagcggttagctctctagagaa



Note: P: Positive control; WT: Wildype 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	
2	98℃	30s	20×
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	