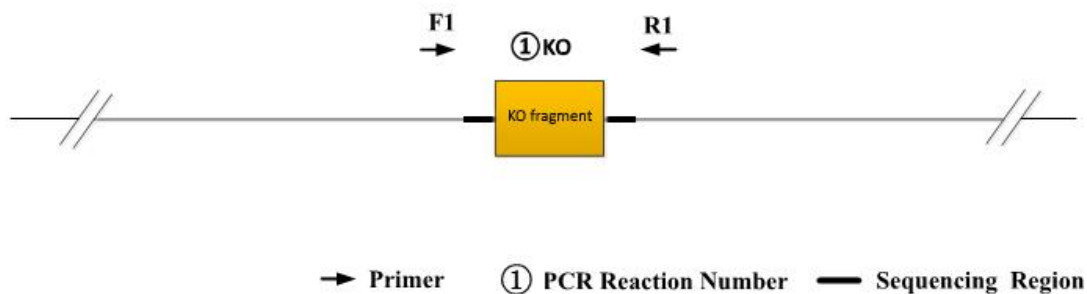


Genotyping Report

Strain ID	T001878	Strain Type	KO(Cas9)	Genetic Background	C57BL/6NGpt
Designer	Zifan Lin	Gene Name	Usp40		

1. Strategy of Genotyping



Wild type: ①PCR reaction obtains a single WT band.

Heterozygote: ①PCR reaction obtains a WT band and a KO band.

Homozygote: ①PCR reaction obtains a single KO band.

Note: 1)The sizes of WT and Targeted band are shown below.

2)If the KO fragment $\leq 20\text{bp}$, the genotype should be confirmed by Sequencing.

Sequence: TTTTGTGTT AGAAG-----26bp-----CTGGG TTCTTTAGAG GATA

2. Primer Information

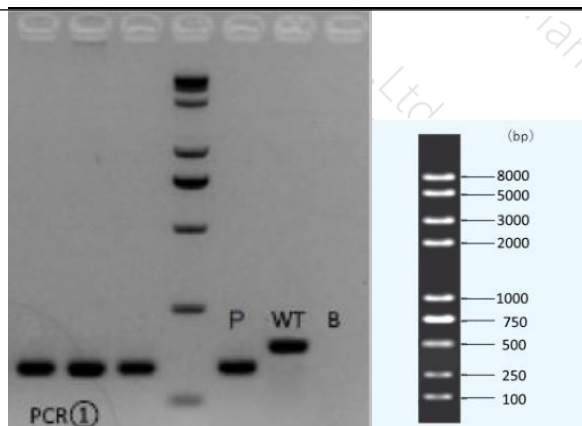
PCR No.	Primer Name	Sequence	Band Size
PCR①	T001878-F1	CTAAGAAGAAGCCGTATCAC	WT: 162bp KO: 136bp
	T001878-R1	GTCCCAAATATTCTAAGAGAC	

Sequencing protocol

Primer Name	Sequence	Primer Description
T001878-F1	CTAAGAAGAAGCCGTATCAC	PCR①

3. Gel Image

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Note: P: 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

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	20×
2	98℃	30s	
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	