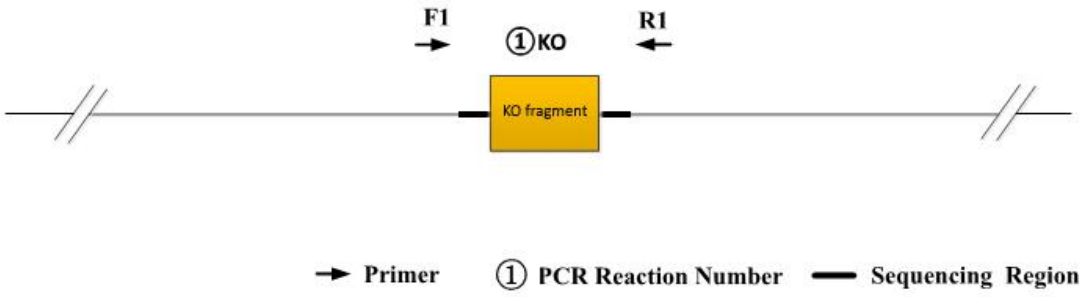
Genotyping Report

Strain ID	T003319	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	*Scap*		

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 ≤20bp，the genotype should be confirmed by Sequencing.
Sequence：tcccgctcgtgaccacctacatcatcctgtttgcctacatActacttctccacacgtaggtcgcaagatgagctgctccat

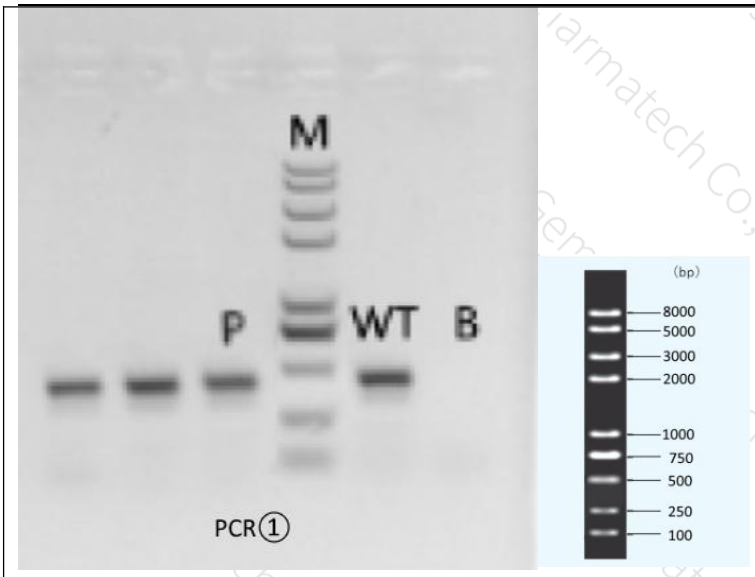
2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	T003319-F1	3310-Scap-3S-intF1	ACCTACAGGTTTCTGAGCAGCCT	WT: 418bp KO: 419bp
	T003319-R1	3310-Scap-3S-intR1	AAGGTCCTGCTTAACCGGAAG	

Sequencing protocol

Primer Name	Sequence	Primer Description
3310-Scap-3S-intF1	ACCTACAGGTTTCTGAGCAGCCT	PCR①

3. Gel Image



Note:P: 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

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	