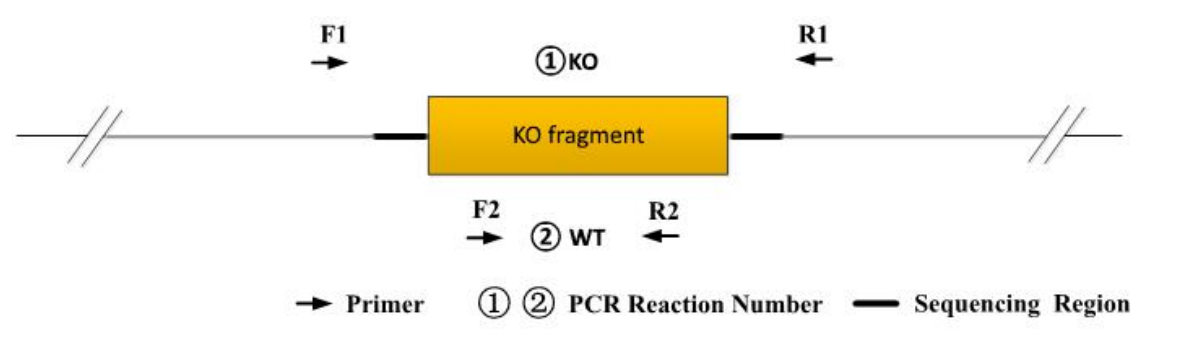


Genotyping Protocol

Strain ID	T003746	Strain Type	KO(Cas9)	Genetic Background	C57BL/6J
Designer	Hao Yan	Gene Name	<i>Il1b</i>		

1. Strategy of Genotyping



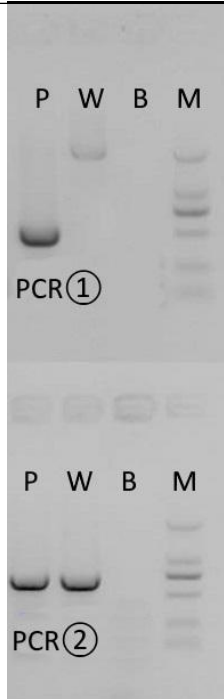
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ②PCR reaction without product.
Note: 1)The sizes of WT and Targeted 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.	Primer Name	Sequence	Band Size
PCR①	F1	100352-II1b-5s-OuttF1	CCAGAACATGGCTTTCCCTG	WT: 2142bp Ko: 526bp
	R1	100352-II1b-5s-OuttR1	ACCTGGACCAGAGTAGACAGCT	
PCR②	F2	100352-II1b-5s-OuttF1	CCAGAACATGGCTTTCCCTG	WT: 811bp
	R2	100352-II1b-5s-intR1	GAAGGTGCTCATGTCCTCATC	

3. Gel Image

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Note: P: Positive control; W: Wildtype 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	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	

Reviewer:Ting Sun

Date:2019-12-25