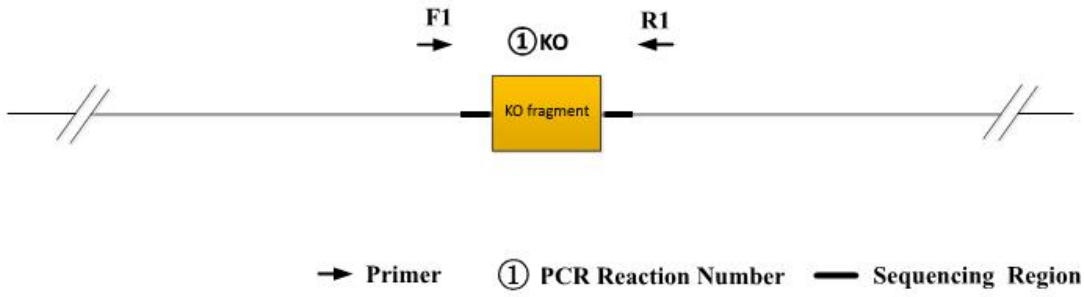
Genotyping Report

Strain ID	T003700	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	*Cfi*		

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.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR① Exon6	F1	100489-Cfi-5S-intF1	TGGAGACCAAAGCGATGAGCT	WT: 486bp KO: 478bp
	R1	100489-Cfi-5S-intR1	GCAGGGTTCAATAATGGAGTAGAGC	
PCR② intron6---7	F1	100489-Cfi-3S-intF1	GAGCACTGACTGCATTCCTGAACG	WT: 619bp KO:538bp
	R1	100489-Cfi-3S-intR2	GTCAGTCCATTCACATATGTTCAAC	

Sequencing protocol

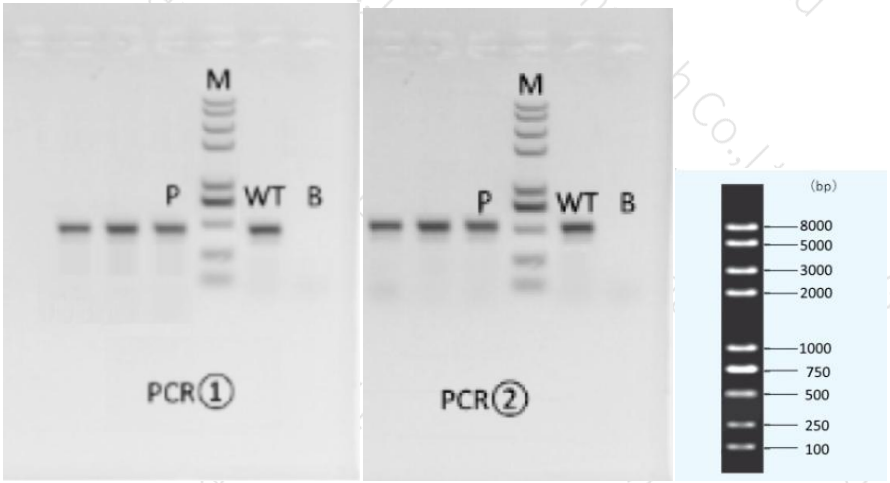
Primer Name	Sequence	Primer Description
100489-Cfi-5S-intF1	TGGAGACCAAAGCGATGAGCT	PCR①
100489-Cfi-3S-intR2	GTCAGTCCATTCACATATGTTCAAC	PCR②

3. Gel Image

Exon6：
aagtcgggagtttgcattccagaccaatacaagtg----------8bp----------aggtggactgcatcactggtgaagatgagagccgt

intron6---7：

agatggctctgtgggtaaaaggtgcttgctgctaa----------81bp----------acatcacacatatgtcataacacaacctcccaagt



Note:P: samples;WT: Wildtype control; B: Blank control (ddH$_2$O); 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 × Rapid Taq Master Mix (Vazyme P222)		12.5
2	ddH2O		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.