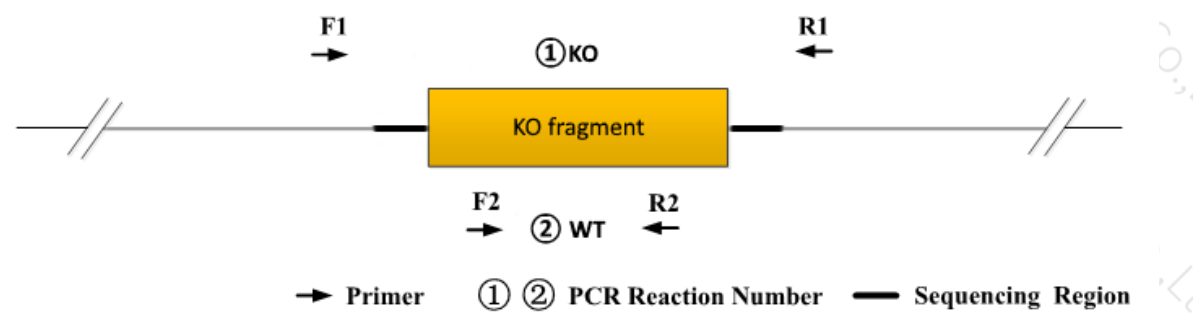


Genotyping Report

Strain ID	T012000	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	<i>Flt3</i>		

1. Strategy of Genotyping



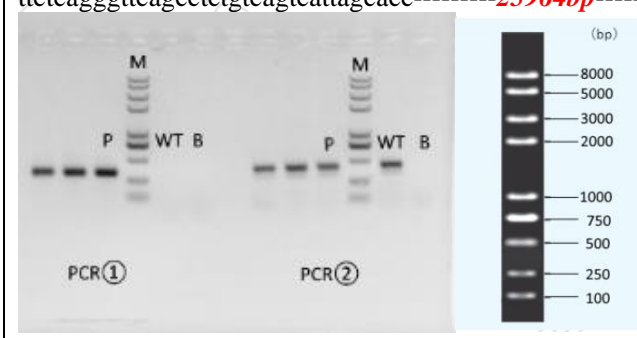
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
 Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
 Note: 1)The sizes of WT and Targeted 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.	Primer Name	Sequence	Band Size
PCR①	F1	04089-Flt3-5wt-tF1	AGACTCCTAACCATCCATCCACG	WT:26265bp KO:301bp
	R1	04089-Flt3-3wt-tR1	TCGCACCTACTCCGTCAGGG	
PCR②	F2	14089-Flt3-wt-tF1	AGGGATTCAACTGAGCACCTC	WT:430bp KO:0bp
	R2	14089-Flt3-wt-tR1	TTCCATACACCAGGCTGGCC	

3. Gel Image

ttctcagggttcagcctctgtcagtcattagcacc-----**25964bp**-----attagtgagccacattggccttgcaactctctttt



Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH2O); M: DNA Ladder

4. PCR Condition



PCR Reaction Component			
Seg.	reaction component		Volume (μ l)
1	2 \times Taq Master Mix , Dye Plus, (Vazyme P112-03)		12.5
2	ddH ₂ O		9.5
3	Primer A(10pmol/ μ l)		1
4	Primer B(10pmol/ μ l)		1
5	Template(\approx 100ng/ μ l)		1
PCR program			
Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	20 \times
3	65 $^{\circ}$ C (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s	
5	98 $^{\circ}$ C	30s	20 \times
6	55 $^{\circ}$ C	30s	
7	72 $^{\circ}$ C	45s	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	

Reviewer:Ting Sun

Date:2020-7-1