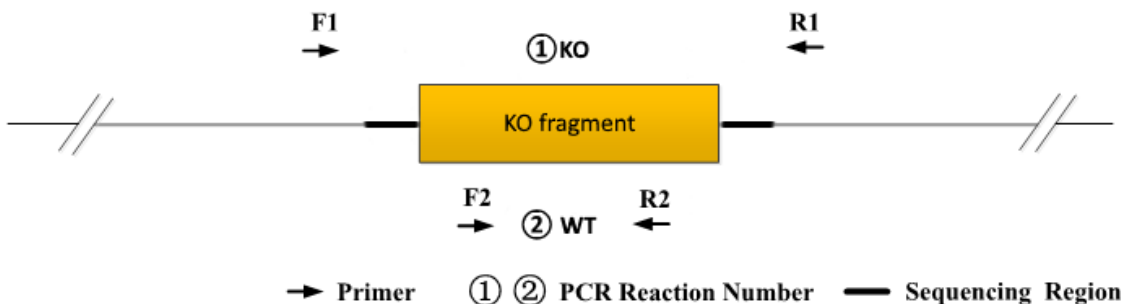


Genotyping Report

Strain ID	T011212	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tianjiao Wang	Gene Name	<i>Cdk2</i>		

1. Strategy of Genotyping



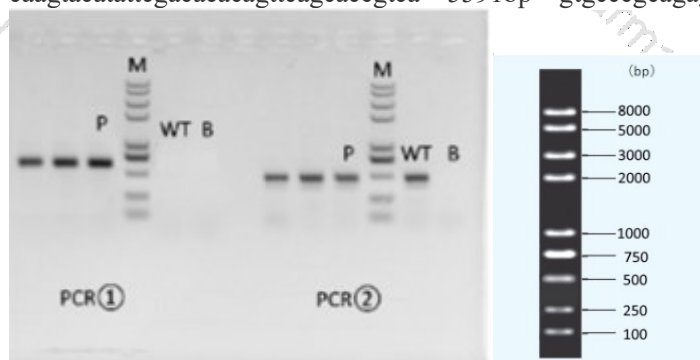
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note: 1)The sizes of WT and Targeted 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.	Sequence	Band Size
PCR①	T011212(P1)-F1A	AATTGAGGAGCTTCTATGAGCCGG	WT: 6249bp KO: 658bp
	T011212(P1)-R1A	GTGGTAGTCTGAAGACACTTTAGCCTCC	
PCR②	T011212(P1)-F2	GGAGTCCCTGTCCGAACCTTACA	WT: 441bp KO: 0bp
	T011212(P1)-R2	GCAACCAGTTTTCTGGCCTAG	

3. Gel Image

caagtacatattcgacacacagttcagaccgtca---5591bp---gtgccgcagaggccagaagagggcatatcatctc



Note: P: Positive control; 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	

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.