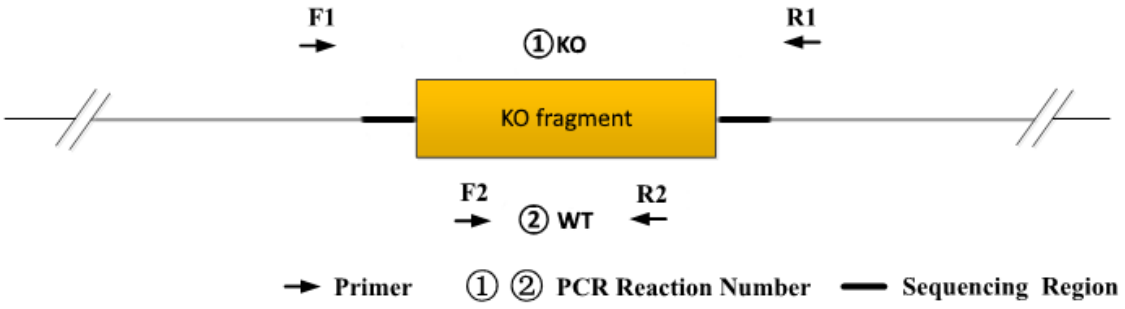
江苏集萃药康生物科技股份有限公司
GemPharmatech Co.,Ltd

Genotyping Report

Strain ID	T003672	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	*Cd226*		

1. Strategy of Genotyping



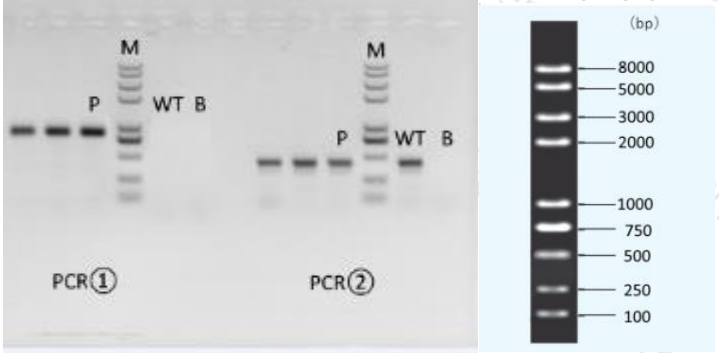
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note：1)The sizes of WT and Targeted 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	T003672-F1	GTCTTATTGACTACCTCCTTTGCC	WT:5024bp KO:953bp
	T003672-R1	CAAGACTTCTACCACAAACTCTGC	
PCR②	T003672-F2	GATCATTTGGCACTAGTTTCAGC	WT:330bp KO:0bp
	T003672-R2	TGGGTTGTTCTGTTACTTCAGTG	

3. Gel Image

ataattgtgtgtttgtttggtatgtgtggtttc---4071bp---caacctgtatgagtactataaggatagcagcatag



Note: P: Heterozygous samples; 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

(Generally recommend to use Vazyme P222;If the sequences contain special structures such as GC% ⩾ 60% or GC% ⩽ 40%， recommend to use Vazyme P515.)

PCR Reaction Component

Seg.	reaction component	Volume (μl)
1	2 × Rapid Taq Master Mix(Vazyme P222) or 2 × Phanta Max Master Mix (Vazyme P515)	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	

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