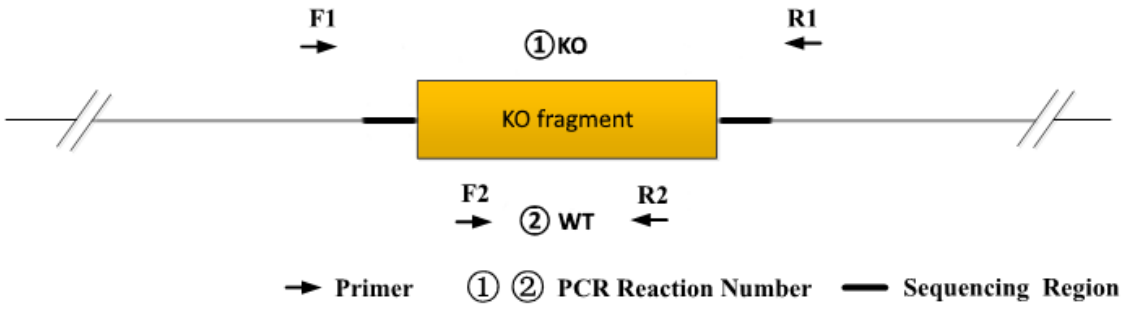
江苏集萃药康生物科技股份有限公司
GemPharmatech Co.,Ltd

Genotyping Report

Strain ID	T034556	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Minghui Wang	Gene Name	*Ddhd2*		

1. Strategy of Genotyping



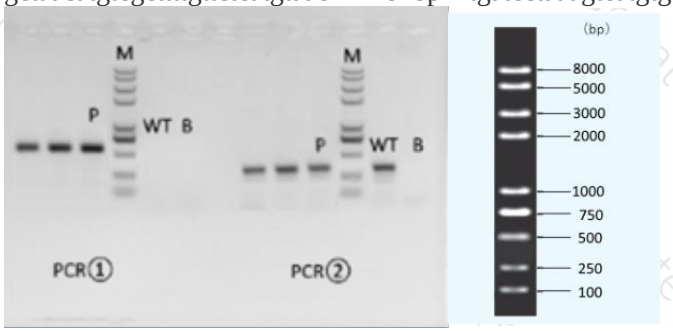
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note：1)The sizes of WT and Targeted 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	T034556(P1)-F1	GGTACTAGATTCCAAGGAGCTCTGGAT	WT: 1834bp KO: 647bp
	T034556(P1)-R1	CTGGTAGTTAAGAGCACTTGCTGCTCT	
PCR②	T034556(P1)-F2	ATAAGTATGTCCCCTACTCGGAGAGCT	WT: 362bp KO: 0bp
	T034556(P1)-R2	TTGATGTCCTGGGTCTCACTCTGTAGA	

3. Gel Image

gctaacatgtcgcttttgttctcatgtaac---1187bp---tgatcctaaagtcatgtgccacgatgccta



Note: P: Positive control; WT: Wildype 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	
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	