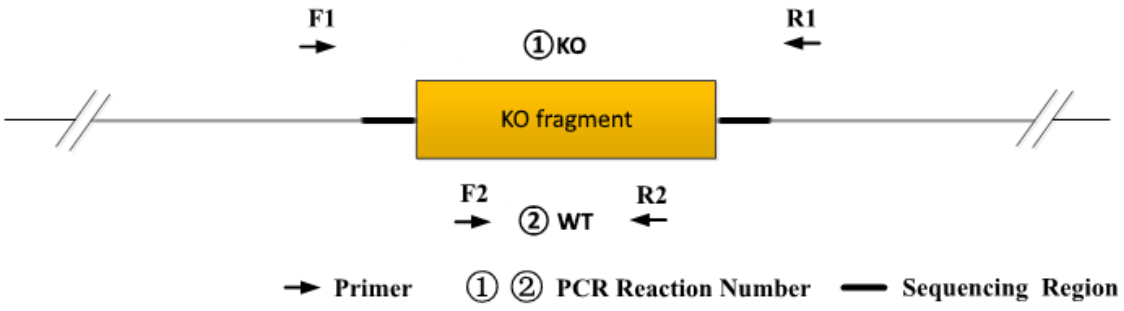
江苏集萃药康生物科技有限公司
GemPharmatech Co.,Ltd

Genotyping Report

Strain ID	T027391	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Dongdong Zhang	Gene Name	*C9orf72*		

1. Strategy of Genotyping



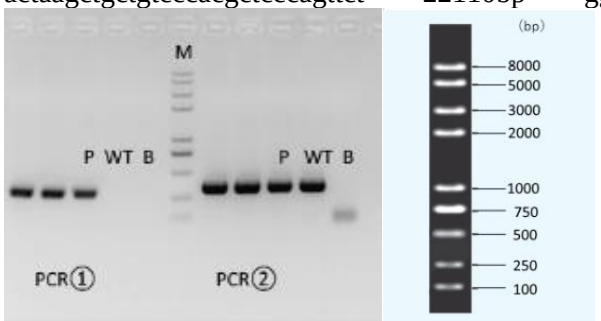
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note：1)The sizes of WT and Targeted 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	JS08324-C9orf72-5wt-tF1	GAGTTTGTTCTGGAAGCTGTGAC	WT: 22396bp KO: 286bp
	R1	JS08324-C9orf72-3wt-tR1	CAGCAGTTTACTGTGGCTGCT	
PCR②	F2	JS18324-C9orf72-wt-tF1	CTAGGCAAGCAGAACTGAAGGTC	WT: 355bp KO: 0bp
	R2	JS18324-C9orf72-wt-tR1	GATGATGAGCTGTGATGTAGACGC	

3. Gel Image

actaagctgctgtcccacgctcccagttct------22110bp------ggttggagggctttaggaagcagtgaccct



Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control (ddH_2O); 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	ddH_2O	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.