

Genotyping Report

Strain ID	T025432	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	<i>Pfkfb4</i>		

1. Strategy of Genotyping



Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains none band.

Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a Targeted band.

Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.

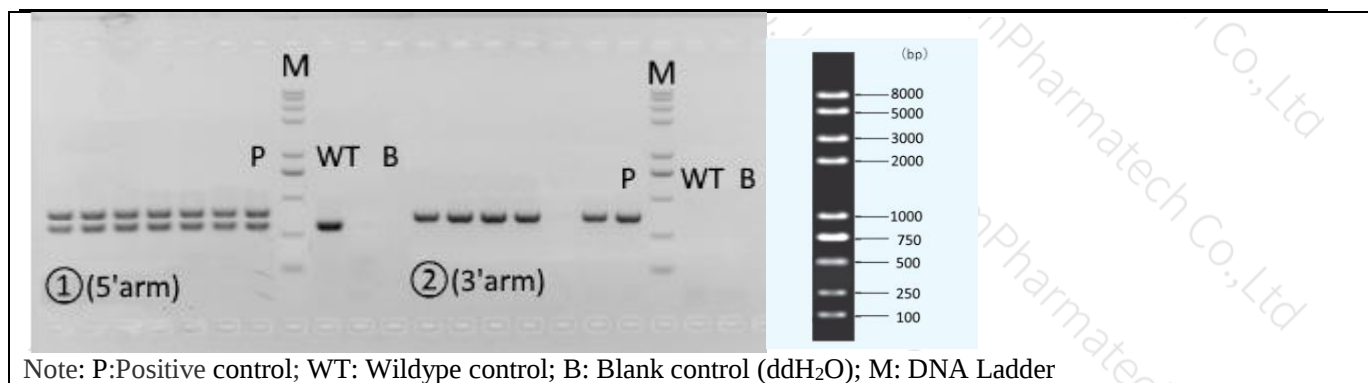
Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
①(5'arm)	F1	JS25726-Pfkfb4-5wt-tF1	CTGATTGACATCCCCGACTTGGTAG	WT:292bp Targeted:397bp
	R1	JS25726-Pfkfb4-5wt-tR1	GCTTAGACTGAACTTGTGGAGCTG	
②(3'arm)	F2	ZMK-2F4	GCATCGCATTGTCTGAGTAGGTG	WT:0bp Targeted:335bp
	R2	JS25726-Pfkfb4-3wt-tR1	CTACTCATTCCACAGCATCCAAGC	

3. Gel Image & Conclusion

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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	

Reviewer:Ting Sun

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