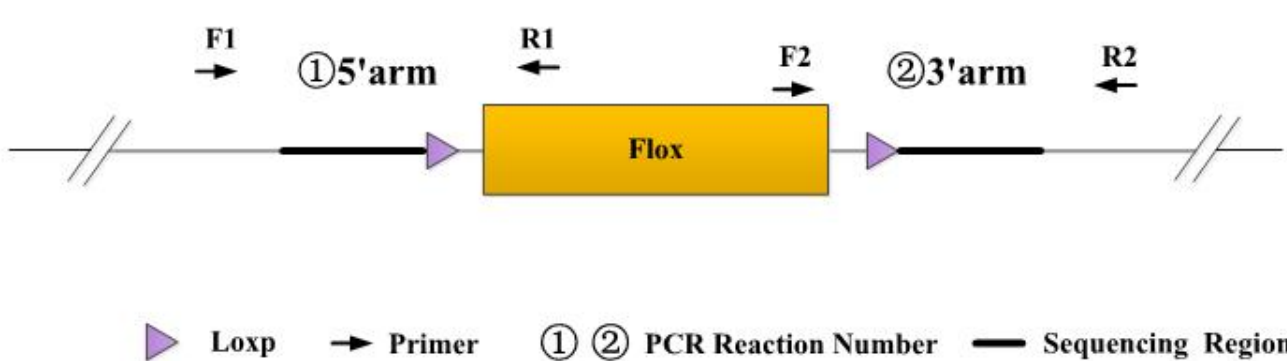


Genotyping Report

Strain ID	T039534	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Megf6</i>		

1. Strategy of Genotyping



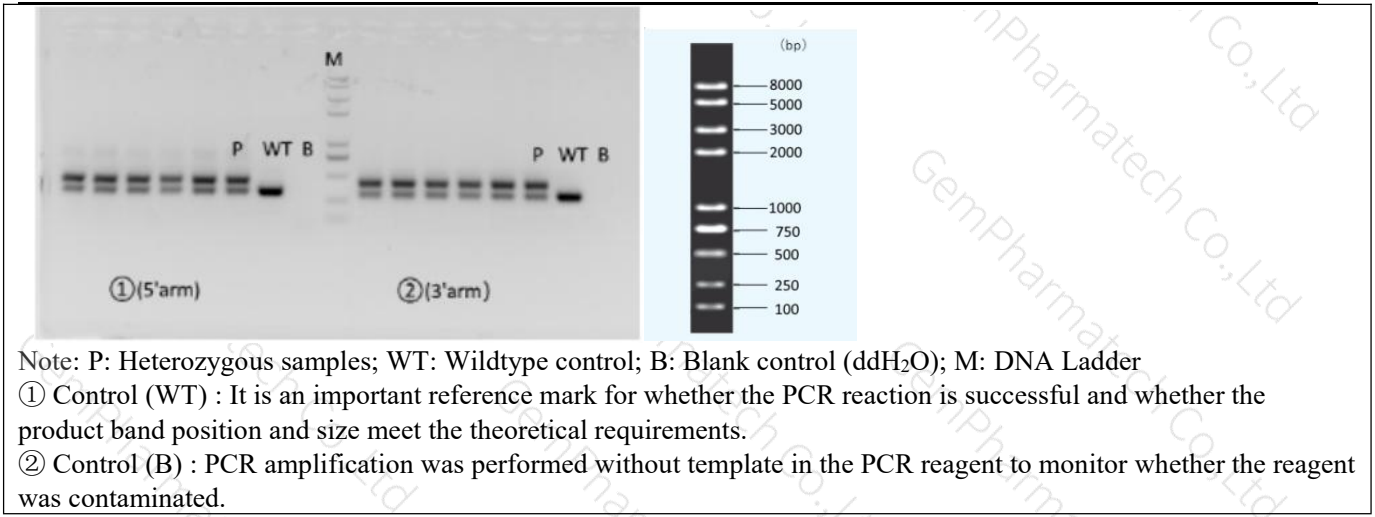
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single 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) Alternative	F1	T039534-5tF1A	TGGTGGAAACTCTATGTCCGTCC	WT: 307bp Targeted:412bp
	R1	T039534-5tR1A	AGCTGTGGAGCGTCTACAAATGG	
②(3'arm) Alternative	F2	T039534-3tF1A	TGCACAATCCTCCTGGTGCAT	WT: 293bp Targeted: 399bp
	R2	T039534-3tR1A	GGGTAGACCTGAAGCCATGTATGTA	

3. Gel Image & Conclusion

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4. PCR Condition

PCR Reaction Component			
Seg.	reaction component	Volume (μl)	
1	2 × Rapid Taq Master Mix (Vazyme P222)	12.5	
2	ddH ₂ O	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°C	5min	20×
2	98°C	30s	
3	65°C* (-0.5°C/cycle)	30s	
4	72°C	45s*	
5	98°C	30s	15×
6	55°C*	30s	
7	72°C	45s*	
8	72°C	5min	
9	10°C	hold	
PCR program II the second choice			
Seg.	Temp.	Time	Cycle
1	95°C	5min	35×
2	98°C	30s	

3	58°C*	30s	
4	72°C	45s*	
5	72°C	5min	
6	10°C	hold	

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