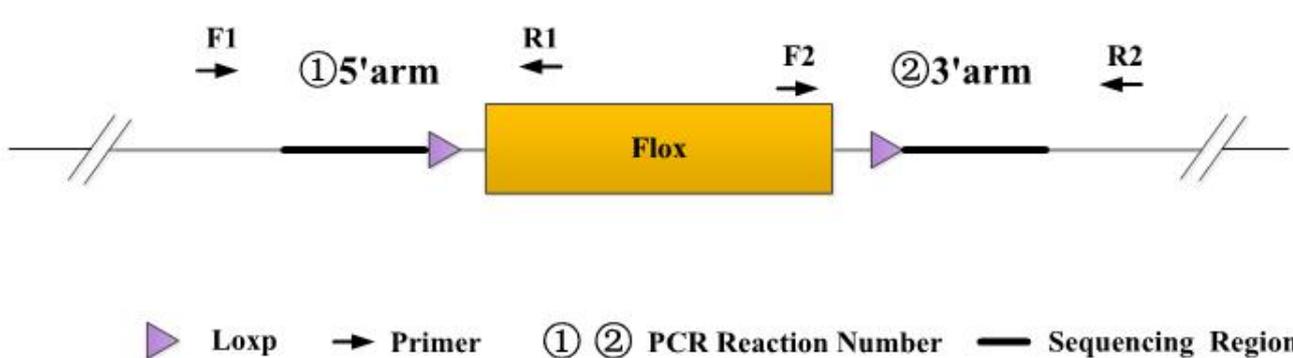


Genotyping Report

Strain ID	T062778	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Psmc3</i>		

1. Strategy of Genotyping



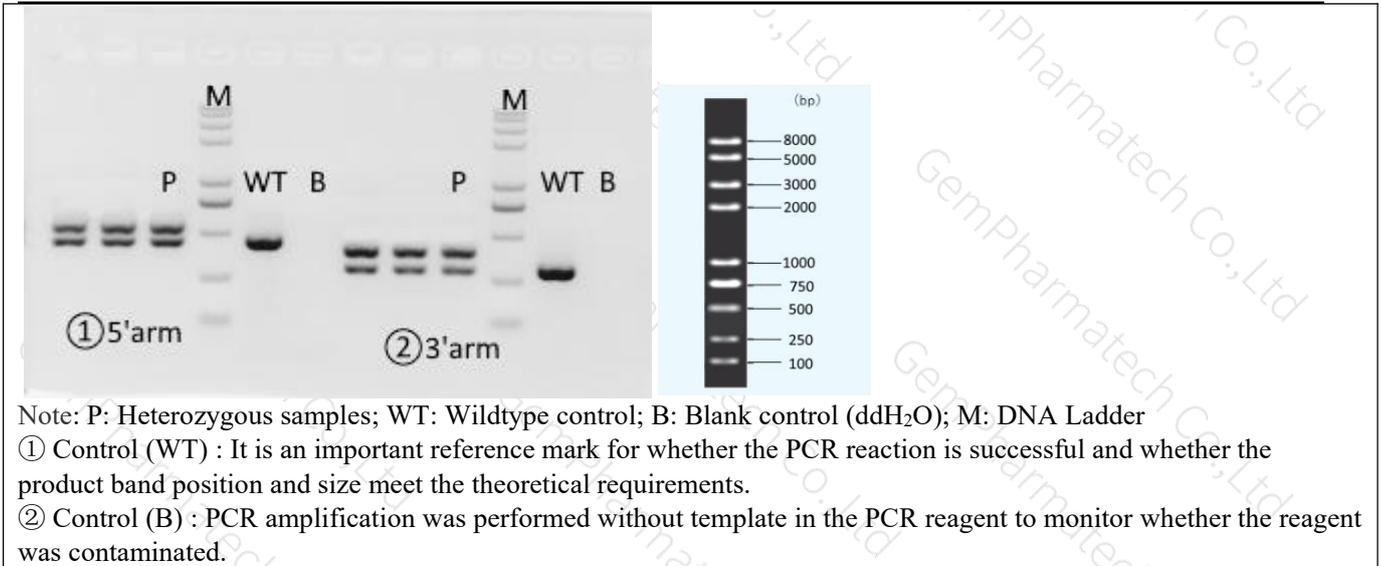
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single 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)	F1	T062778(P1)-F1	CACTCAGGCTCCCAGTTTTTGC	WT: 463bp
	R1	T062778(P1)-R1	GGCAAGACAATGGCTTCCAC	Targeted: 568bp
②(3'arm)	F2	T062778(P1)-F2	GAGCACAACCTCCAAGCTATGCTAAA	WT: 329bp
	R2	T062778(P1)-R2	AAACAATGGCTGGCAAACACC	Targeted: 435bp

3. Gel Image & Conclusion

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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 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℃	5min	20×
2	98℃	30s	
3	65℃* (-0.5℃/cycle)	30s	
4	72℃	45s*	15×
5	98℃	30s	
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	
PCR program II the second choice			

Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	35×
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.