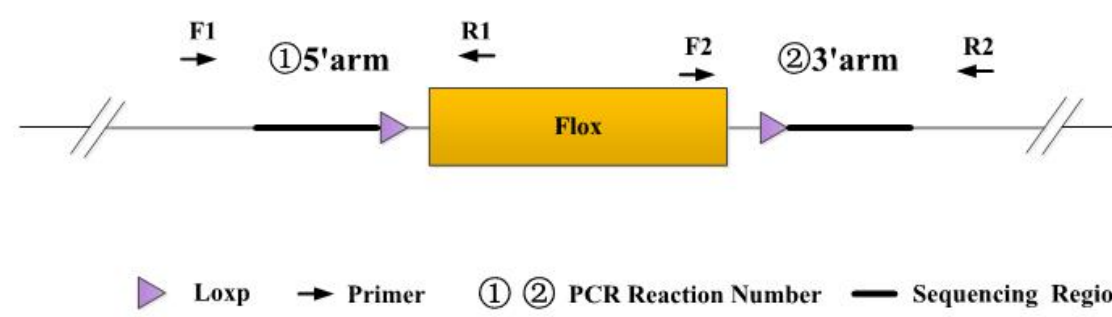


Genotyping Report

Strain ID	T024708	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Med23</i>		

1. Strategy of Genotyping



▶ Loxp ➔ Primer ① ② PCR Reaction Number — Sequencing Region

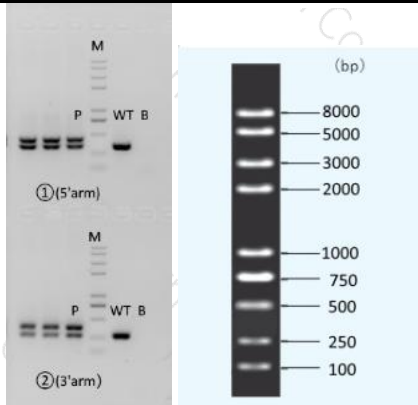
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.
 Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.
 Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T024708(P1)-F1A	ACAGTACTTGTGTACAGGTCAGAGGAC	WT: 311bp Targeted: 416bp
	T024708(P1)-R1A	AAAGCCATGCTCACACTGAAGC	
②(3'arm)	T024708(P1)-F2A	TGCAAACATGAGCTGCCACAC	WT: 265bp Targeted: 371bp
	T024708(P1)-R2A	ACAGTGCCTGGGCTACAGTTCAT	

3. Gel Image & Conclusion

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Note: P: Positive control; WT: Wildtype 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 × 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(≈100ng/μl)	1	
PCR program ① priority selection			
Seg.	Temp.	Time	Cycle
1	95 °C	5min	
2	98 °C	30s	20×
3	65 °C* (-0.5 °C/cycle)	30s	
4	72 °C	45s*	20×
5	98 °C	30s	
6	55 °C*	30s	
7	72 °C	45s*	
8	72 °C	5min	
9	10 °C	hold	
PCR program ② 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.