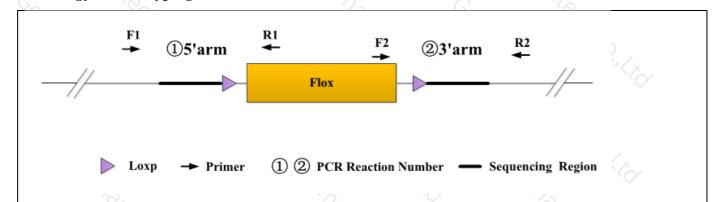
Genotyping Report

Strain ID	T024691	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	3	Mcm5	0)

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

1 1/2	/		7/1
PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T024691-F1	CTGGATGTAACCTGAGCTCACTGA	WT: 405bp Targeted:510bp
	T024691-R1	CACAGATACGGAATCTACAGCTCCTG	
②(3'arm)	T024691-F2	TTGGATCTCTGAATTTAAGG	WT: 328bp
	T024691-R2	TTTGGGCTCAAATCTGAAGCAAA	Targeted:434bp

3. Gel Image & Conclusion





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 Comp	onent	Px	°C/
Seg.	reaction component		Volume (μl)
1 9/2	2 × Rapid Taq Master Mix (Vazyme P2	12.5	
2	ddH2O	34%	9.5
3	Primer A(10pmol/μl)	1	
4 6	Primer B(10pmol/μl)	1 6	
5	Template(≈100ng/μl)	1 3	
PCR program ① pri	ority selection	**************************************	7/2
Seg.	Temp.	Time	Cycle
1	95°C	5min	**************************************
2	98℃	30s	20×
3	65°C* (-0.5°C/cycle)	30s	3/2
4	72℃	45s*	
5	98℃	30s	20×
6	55℃*	30s	16
7	72°C	45s*	19/2 'X
80. 27	72℃	5min	79%
9	10℃	hold	3
PCR program ② th	e second choice	7, 7	
Seg.	Temp.	Time	Cycle
1	95℃	5min	19X
2	98℃	30s	35×
3	58℃*	30s	3/x
4	72℃	45s*	
5	72℃	5min	3/2
6	10℃	hold	3×

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

