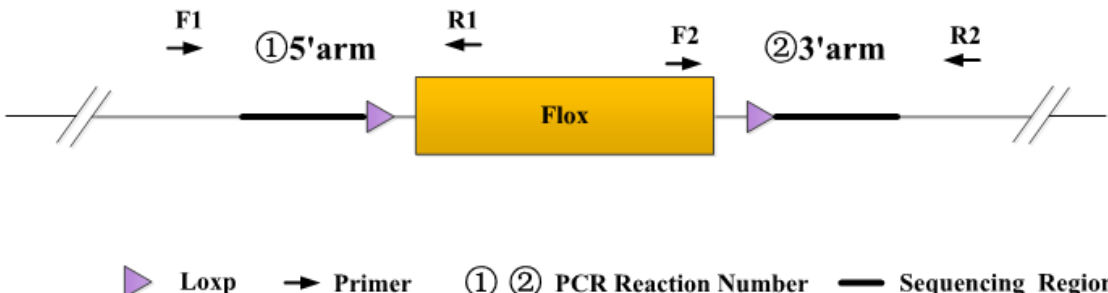


Genotyping Report

Strain ID	T009594	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Ya'nan Xu	Gene Name	<i>Muc1</i>		

1. Strategy of Genotyping

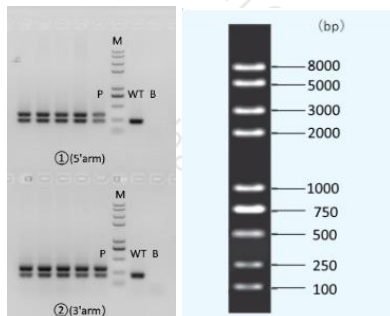


Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted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

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T009594-F1	AAAGAGGCTAAGGAAGGAGGCTGAG	WT: 231bp Targeted:336bp
	T009594-R1	AGTATTCATACTGTAGCCTAAGCTGGCC	
②(3'arm)	T009594-F2	AAAGCCTCGCCGTCTACCTGTAC	WT: 225bp Targeted:331bp
	T009594-R2	ACCCTTGCTTTCTAAACTCCC	

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 Component			
Seg.	reaction component		Volume (μ l)
1	2 \times Rapid Taq Master Mix (Vazyme P222)		12.5
2	ddH ₂ O		9.5
3	Primer A(10 μ mol/ μ l)		1
4	Primer B(10 μ mol/ μ l)		1
5	Template(\approx 100ng/ μ l)		1
PCR program ① priority selection			
Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	20 \times
3	65 $^{\circ}$ C* (-0.5 $^{\circ}$ C/cycle)	30s	
4	72 $^{\circ}$ C	45s*	
5	98 $^{\circ}$ C	30s	
6	55 $^{\circ}$ C*	30s	
7	72 $^{\circ}$ C	45s*	
8	72 $^{\circ}$ C	5min	
9	10 $^{\circ}$ C	hold	
PCR program ② the second choice			
Seg.	Temp.	Time	Cycle
1	95 $^{\circ}$ C	5min	
2	98 $^{\circ}$ C	30s	35 \times
3	58 $^{\circ}$ C*	30s	
4	72 $^{\circ}$ C	45s*	
5	72 $^{\circ}$ C	5min	
6	10 $^{\circ}$ C	hold	

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