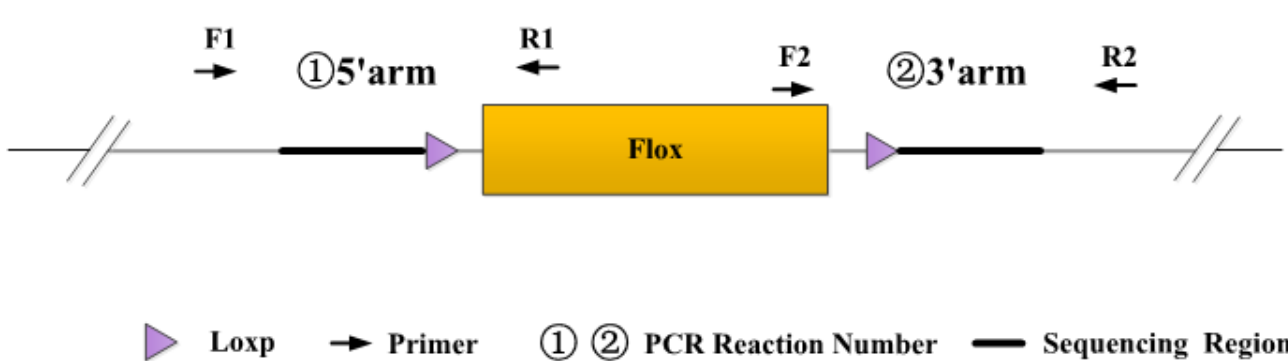


Genotyping Report

| | | | | | |
|-----------|-------------|-------------|-------------|--------------------|-------------|
| Strain ID | T018669 | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer | Binjie Jiao | Gene Name | <i>Mbd3</i> | | |

1. Strategy of Genotyping



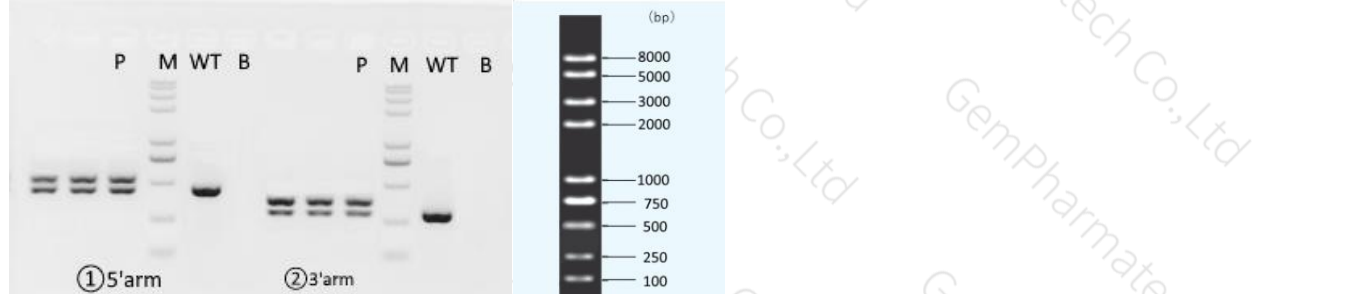
▶ **Loxp** **→** **Primer** ① ② **PCR Reaction Number** **—** **Sequencing Region**

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. | Primer Name | Sequence | Band Size |
|----------|------------|----------------|-------------------------|------------------------------|
| ①(5'arm) | F1 | T018669(P1)-F1 | GTCTCCAGTCAGTCCCATATCCT | WT: 443bp Targeted: 548bp |
| | R1 | T018669(P1)-R1 | TGGATGCTGTCATTCGACCT | |
| ②(3'arm) | F2 | T018669(P1)-F2 | TTCAGCCTCGTGGCTTTTAGCC | WT: 292bp Targeted: 398bp |
| | R2 | T018669(P1)-R2 | AGAGCAAGGGCTTTGGAAACG | |

3. Gel Image & Conclusion



Note: P: Heterozygous samples; 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

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as GC% \geq 60% or GC% \leq 40%, recommend to use Vazyme P515.)

| PCR Reaction Component | | | |
|------------------------------------|--|------|-------------------|
| Seg. | Reaction Component | | Volume (μ l) |
| 1 | 2 \times Rapid Taq Master Mix(Vazyme P222) or 2 \times Phanta Max Master Mix (Vazyme P515) | | 12.5 |
| 2 | ddH ₂ O | | 9.5 |
| 3 | Primer A(10pmol/ μ l) | | 1 |
| 4 | Primer B(10pmol/ μ l) | | 1 |
| 5 | Template(20~80ng/ μ l) | | 1 |
| PCR program I (priority selection) | | | |
| Seg. | Temp. | Time | Cycle |
| 1 | 95°C | 5min | |
| 2 | 98°C | 30s | 20 \times |
| 3 | 65°C*(-0.5°C/cycle) | 30s | |
| 4 | 72°C | 45s* | |
| 5 | 98°C | 30s | 15 \times |
| 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 | |
| 2 | 98°C | 30s | 35 \times |
| 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.