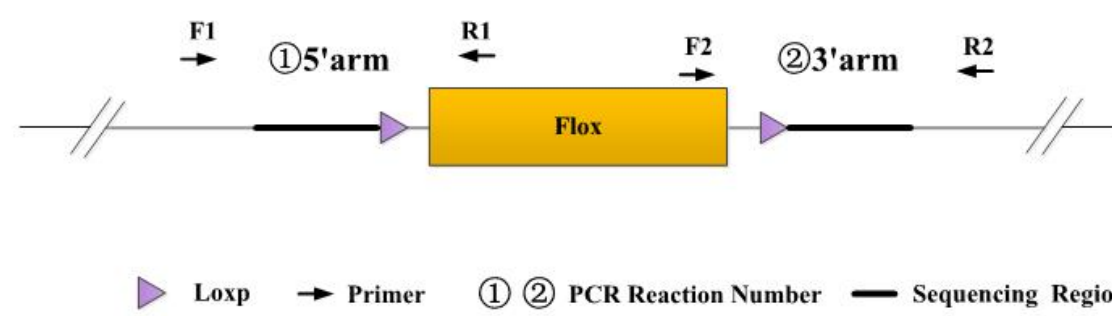


Genotyping Report

| | | | | | |
|-----------|-----------|-------------|---------------|--------------------|-------------|
| Strain ID | T005147 | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer | Ya'nan Xu | Gene Name | <i>Tsg101</i> | | |

1. Strategy of Genotyping



Legend: ▶ 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) | T005147-F1 | CCCATTAGAAACAGTTTTTGGATGG | WT:289bp Targeted:370bp |
| | T005147-R1 | CCTCAGGCTCGCCTTTCTTAATCTA | |
| ②(3'arm) | T005147-F2 | AAGGGTCAGTAAGATGGTGTCACAC | WT:282bp Targeted:360bp |
| | T005147-R2 | TGACGCTTCCAAAGGCTGTTTTAGG | |

3. Gel Image & Conclusion



Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH₂O); M: DNA Ladder

4. PCR Condition

| PCR Reaction Component | | | |
|------------------------|--|------|-------------|
| Seg. | reaction component | | Volume (μl) |
| 1 | 2 × Taq Master Mix , Dye Plus , (Vazyme P112-03) | | 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 | | | |
| Seg. | Temp. | Time | Cycle |
| 1 | 95℃ | 5min | |
| 2 | 98℃ | 30s | 20× |
| 3 | 65℃ (-0.5℃/cycle) | 30s | |
| 4 | 72℃ | 45s | |
| 5 | 98℃ | 30s | |
| 6 | 55℃ | 30s | 20× |
| 7 | 72℃ | 45s | |
| 8 | 72℃ | 5min | |
| 9 | 10℃ | hold | |

Reviewer:Ting Sun

Date:2021-6-9