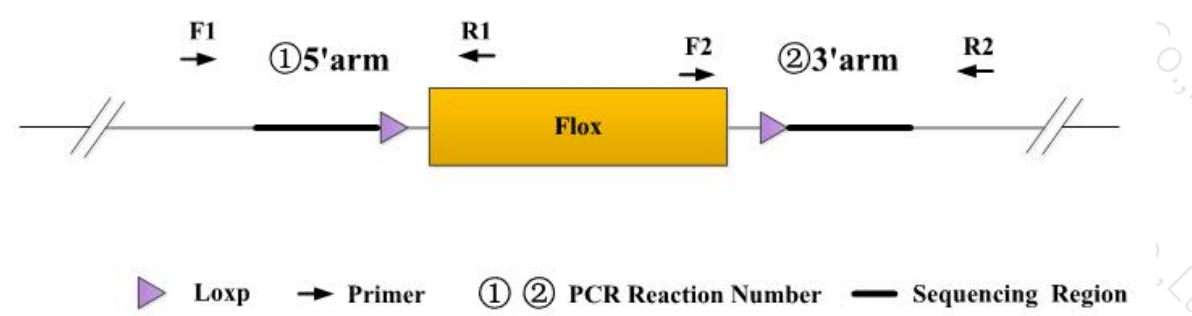


Genotyping Report

| | | | | | |
|-----------|-----------|-------------|--------------|--------------------|-------------|
| Strain ID | T052231 | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer | Ya'nan Xu | Gene Name | <i>Ncoa3</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) | T052231-F1 | TTAGTAGCTGGGCCAGCAGCTT | WT: 329bp Targeted:434bp |
| | T052231-R1 | CACACCTCATGCACATACATGCA | |
| ②(3'arm) | T052231-F2 | TTAGGTTGGGTCTCAGAACCTTCTG | WT: 278bp Targeted:384bp |
| | T052231-R2 | CCACCCTCACAGTTACCTGACATTA | |

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% ≥ 60% or GC% ≤ 40%, recommend to use Vazyme P515.)

| 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℃ | 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 | |
| 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

