

## Genotyping Report

|           |           |             |           |                    |             |
|-----------|-----------|-------------|-----------|--------------------|-------------|
| Strain ID | T009256   | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Ya'nan Xu | Gene Name   | Robo1     |                    |             |

### 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) | T009256-F1 | ACTAGGTGTCGGGATTCTCCTTTC  | WT:342bp<br>Targeted:446bp |
|          | T009256-R1 | CATCACAGGTGCCTTTACTGACTG  |                            |
| ②(3'arm) | T009256-F2 | GAAGCACTCAGTTCAAAGACAAGTG | WT:294bp<br>Targeted:395bp |
|          | T009256-R2 | ATACCAATAGCTGGTCAGGAGACAC |                            |

### 3. Gel Image & Conclusion

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Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH<sub>2</sub>O); 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                      | ddH2O                                           |      | 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 | 20×         |
| 2                      | 98℃                                             | 30s  |             |
| 3                      | 65℃ (-0.5℃/cycle)                               | 30s  |             |
| 4                      | 72℃                                             | 45s  |             |
| 5                      | 98℃                                             | 30s  | 20×         |
| 6                      | 55℃                                             | 30s  |             |

|   |     |      |  |
|---|-----|------|--|
| 7 | 72℃ | 45s  |  |
| 8 | 72℃ | 5min |  |
| 9 | 10℃ | hold |  |

Reviewer:Ting Sun

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