

## Genotyping Report

|           |           |             |           |                    |             |
|-----------|-----------|-------------|-----------|--------------------|-------------|
| Strain ID | T014973   | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Ya'nan Xu | Gene Name   | Ryk       |                    |             |

### 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) | T014973-F1 | CCTCGTGCAGAGATAACAGCCA   | WT:396bp<br>Targeted:498bp |
|          | T014973-R1 | TTCTAGCAGTCAGCCGTGAGCAG  |                            |
| ②(3'arm) | T014973-F2 | ACATGGGCCTGGGTTGAACCA    | WT:381bp<br>Targeted:484bp |
|          | T014973-R2 | GCAAGCATATGGTGGTTCACAACC |                            |

### 3. Gel Image & Conclusion



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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