

## Genotyping Report

|           |           |             |           |                    |             |
|-----------|-----------|-------------|-----------|--------------------|-------------|
| Strain ID | T019058   | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | Rasgrf1   |                    |             |

### 1. Strategy of Genotyping



Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains none band.

Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a Targeted band.

Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.

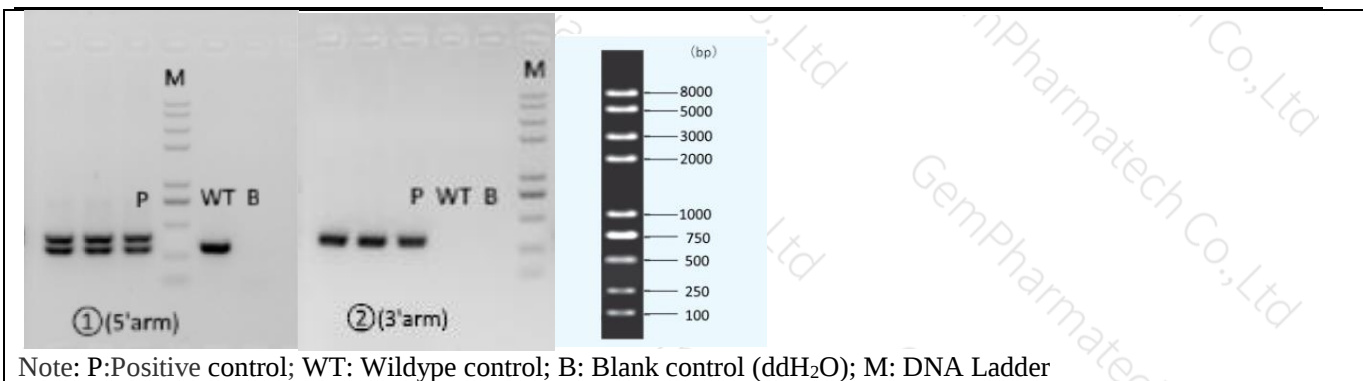
Note: The sizes of WT and Targeted band are shown below.

### 2. Primer Information

| PCR No.  | Primer No. | Primer Name             | Sequence                 | Band Size                  |
|----------|------------|-------------------------|--------------------------|----------------------------|
| ①(5'arm) | F1         | JS09352-Rasgrf1-5wt-tF1 | GCCAAGAAAGAGCTACATCCTCC  | WT:292bp<br>Targeted:394bp |
|          | R1         | JS09352-Rasgrf1-5wt-tR1 | TTTGGGACCTGACCTTTGCCA    |                            |
| ②(3'arm) | F2         | ZMK2F4                  | CATCGCATTGTCTGAGTAGGTG   | WT:0bp<br>Targeted:285bp   |
|          | R2         | JS09352-Rasgrf1-3wt-tR1 | CGATAAGGCTCATCTTGGATGACC |                            |

### 3. Gel Image & Conclusion

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#### 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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