

## Genotyping Report

|           |           |             |           |                    |             |
|-----------|-----------|-------------|-----------|--------------------|-------------|
| Strain ID | T020661   | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | Rasgrp3   |                    |             |

### 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.

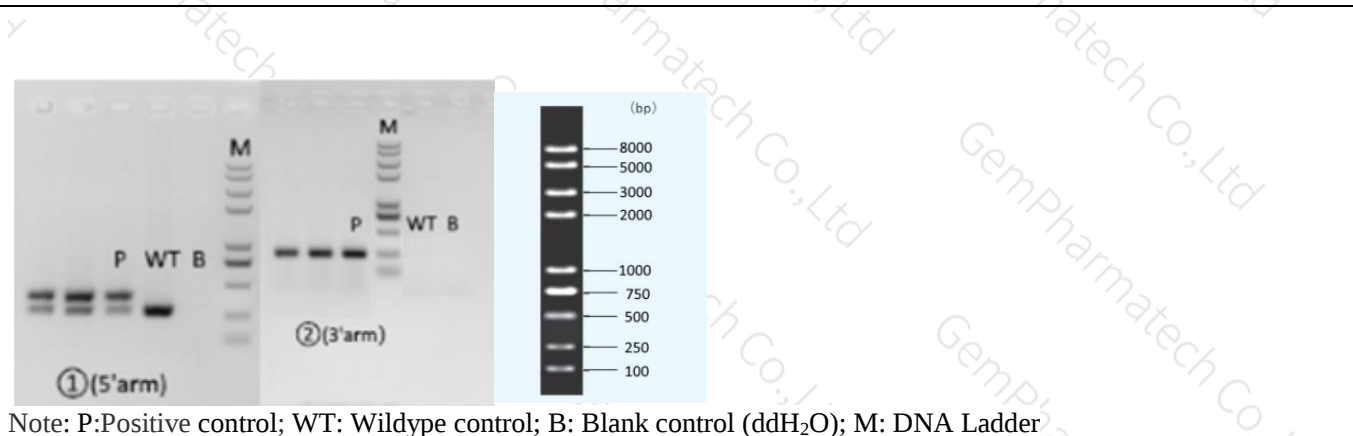
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.

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

### 2. Primer Information

| PCR No.  | Primer No. | Sequence                  | Band Size                   |
|----------|------------|---------------------------|-----------------------------|
| ①(5'arm) | T020661-F1 | ATAGTAACGACAGTTGACCTTGGGA | WT:274bp<br>Targeted: 376bp |
|          | T020661-R1 | AGAGATGCCAGGGTTGGTCTTT    |                             |
| ②(3'arm) | T020661-F2 | CATCGCATTGTCTGAGTAGGTG    | WT:0bp<br>Targeted:250bp    |
|          | T020661-R2 | TCTGACACTCACCCTCCTAAGCA   |                             |

### 3. Gel Image & Conclusion



#### 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 |             |