

## Genotyping Report

|           |           |             |           |                    |             |
|-----------|-----------|-------------|-----------|--------------------|-------------|
| Strain ID | T020009   | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | Gdf6      |                    |             |

### 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.

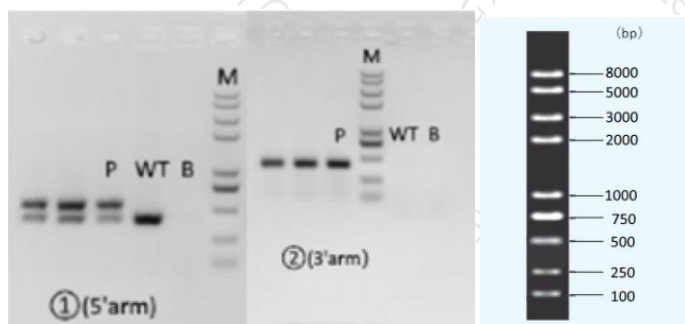
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a Targeted band.

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

### 2. Primer Information

| PCR No.  | Primer No. | Sequence                   | Band Size                    |
|----------|------------|----------------------------|------------------------------|
| ①(5'arm) | T020009-F1 | CAACCTGGGCTACATAGATTCTGTC  | WT: 446bp<br>Targeted: 548bp |
|          | T020009-R1 | GAAAACTGAGCTCTTGTATCCACACC |                              |
| ②(3'arm) | T020009-F2 | CATCGCATTGTCTGAGTAGGTG     | WT:0bp<br>Targeted:429bp     |
|          | T020009-R2 | CATGAGACATTGATTCCATTGGG    |                              |

### 3. Gel Image & Conclusion



Note: P:Positive control; WT: Wildtype control; B: Blank control (ddH<sub>2</sub>O); 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

| PCR Reaction Component           |                                        |      |             |
|----------------------------------|----------------------------------------|------|-------------|
| Seg.                             | reaction component                     |      | Volume (μl) |
| 1                                | 2 × Rapid Taq Master Mix (Vazyme P222) |      | 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 ① priority selection |                                        |      |             |
| 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 |             |
| PCR program ② the second choice  |                                        |      |             |
| Seg.                             | Temp.                                  | Time | Cycle       |
| 1                                | 95℃                                    | 5min | 35×         |
| 2                                | 98℃                                    | 30s  |             |
| 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 and amplification enzyme efficiency.



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