

## Genotyping Report

|           |           |             |          |                    |             |
|-----------|-----------|-------------|----------|--------------------|-------------|
| Strain ID | T045209   | Strain Type | KO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | Znhit3   |                    |             |

### 1. Strategy of Genotyping



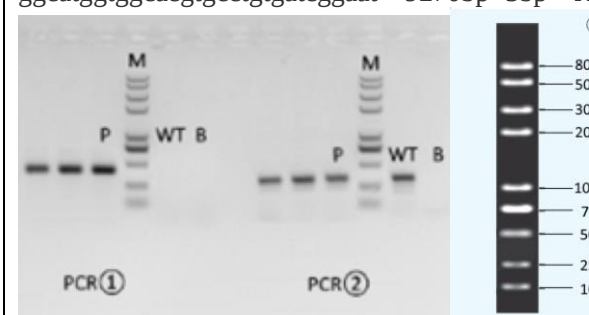
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a single KO band; ②PCR reaction without product.  
Note: 1)The sizes of WT and Targeted band are shown below.  
2)If the WT band is too large, it may not be possible to obtain a WT band.

### 2. Primer Information

| PCR No. | Primer No. | Sequence                 | Band Size               |
|---------|------------|--------------------------|-------------------------|
| PCR①    | T045209-F1 | GCCAAGCATTACCTAGAACCCTG  | WT: 9689bp<br>KO: 422bp |
|         | T045209-R1 | TGAAGCCAGTATGGGTACAGTGAG |                         |
| PCR②    | T045209-F2 | CTGCAACAGGGTCTCAGACCTTA  | WT: 331bp<br>KO: 0bp    |
|         | T045209-R2 | GGATGATTGGAAGACGGTCTCTTC |                         |

### 3. Gel Image

ggcatgggtggcagctgcctgtgatcggaat---9270bp+3bp---ATActtgggtggtattctgacaccatttcctct



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 |             |