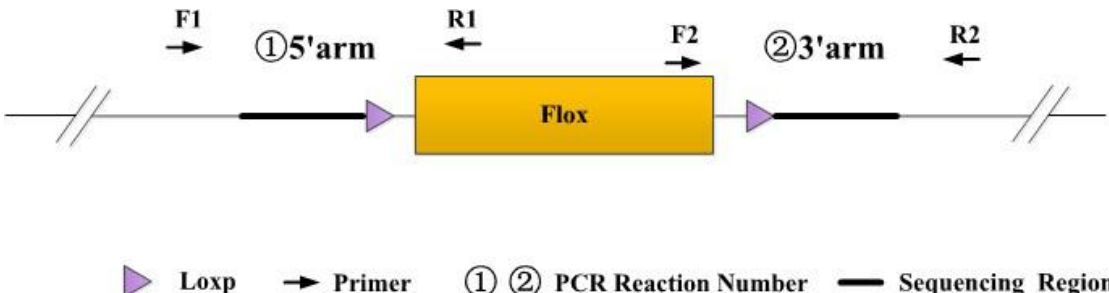


## Genotyping Report

|           |           |             |              |                    |                    |
|-----------|-----------|-------------|--------------|--------------------|--------------------|
| Strain ID | T059306   | Strain Type | CKO(Cas9)    | Genetic Background | D000750&C<br>SS750 |
| Designer  | Chen Chen | Gene Name   | <i>Hdac4</i> |                    |                    |

### 1. Strategy of Genotyping



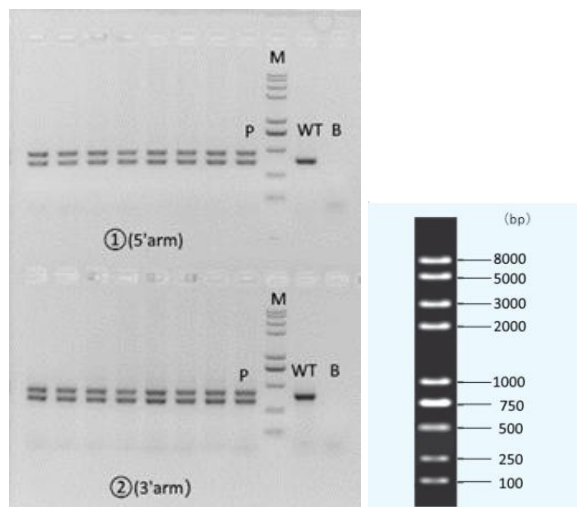
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.  
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.  
Note: The sizes of WT and Targeted band are shown below.

### 2. Primer Information

| PCR No.   | Primer No. | Primer Name    | Sequence                   | Band Size                    |
|-----------|------------|----------------|----------------------------|------------------------------|
| ①(5'arm)  | F1         | T059306(P1)-F1 | ACCTTTCAGCAACAGTCACCATATC  | WT:366 bp<br>Targeted: 456bp |
|           | R1         | T059306(P1)-R1 | GCATGAGAACGGCTTCTGTTAGCT   |                              |
| ②(3'arm ) | F2         | T059306(P1)-F2 | AGTGAGGACCTCTGGCTCAGTCAT   | WT:348 bp<br>Targeted:439 bp |
|           | R2         | T059306(P1)-R2 | AAGGATTGCTAAAAGCCTACAGTCAG |                              |

### 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 × 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 |             |

