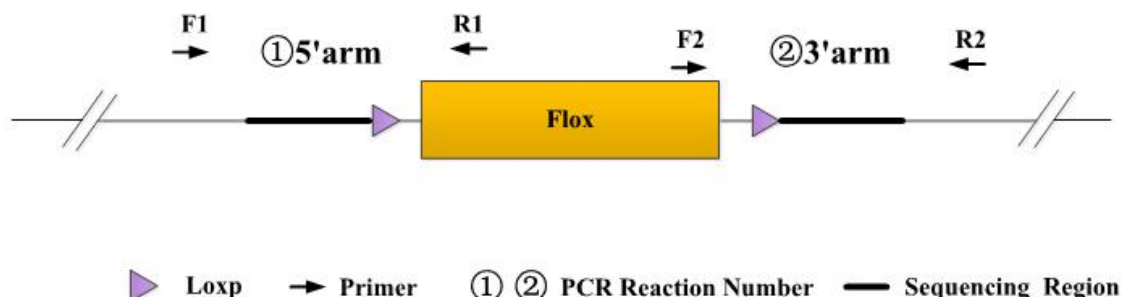


## Genotyping Report

|           |           |             |             |                    |             |
|-----------|-----------|-------------|-------------|--------------------|-------------|
| Strain ID | T051992   | Strain Type | CKO(Cas9)   | Genetic Background | C57BL/6JGpt |
| Designer  | Ya'nan Xu | Gene Name   | <i>Htr4</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.

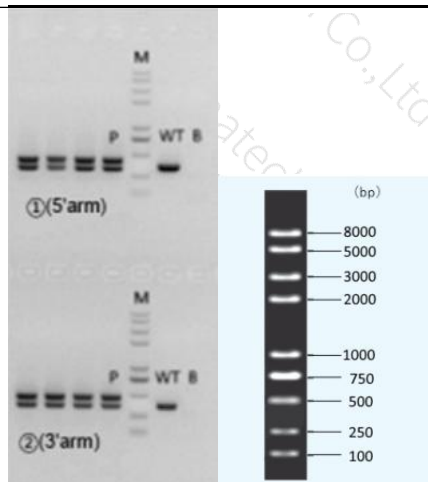
Note: The sizes of WT and Targeted band are shown below.

### 2. Primer Information

| PCR No.    | Primer No.  | Sequence               | Band Size                     |
|------------|-------------|------------------------|-------------------------------|
| ①(5'arm)备用 | T051992-F1A | GAGCAGCCTGCAAATACTGTGC | WT: 323bp<br>Targeted: 403bp  |
|            | T051992-R1A | GGAACCAAGGTCTGCATGAACA |                               |
| ②(3'arm)   | T051992-F2  | ATGTCAAGAGAGCAGGGACC   | WT: 378 bp<br>Targeted: 432bp |
|            | T051992-R2  | GTGAAGGATGAAGTGCCTGG   |                               |

### 3. Gel Image & Conclusion





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 |             |