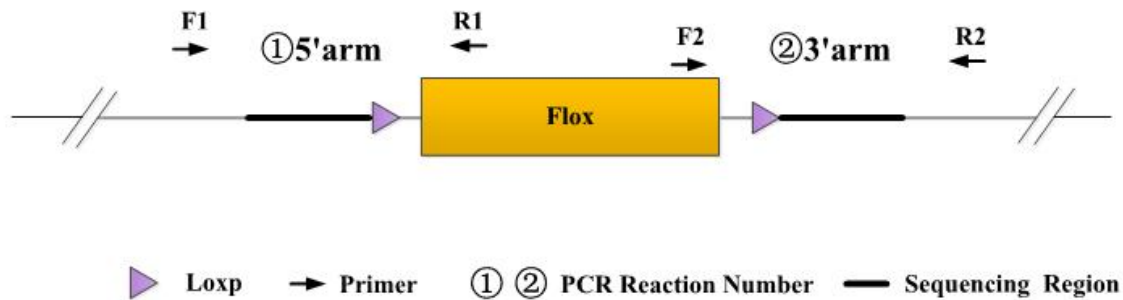


## Genotyping Report

|           |           |             |             |                    |             |
|-----------|-----------|-------------|-------------|--------------------|-------------|
| Strain ID | T009920   | Strain Type | CKO(Cas9)   | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | <i>Ahi1</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.

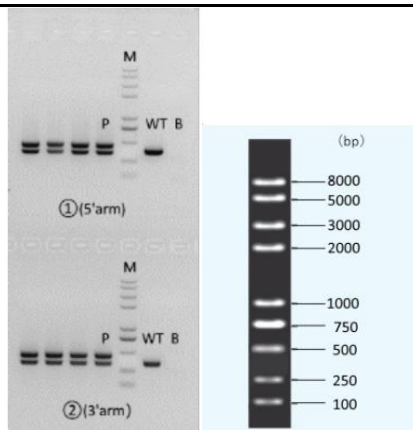
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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)<br>GC%:36.0% | F1         | T009920(P1)-F1 | CTCACAGAGAAGGAGATCCTTGAAC | WT:381bp<br>Targeted:486bp |
|                       | R1         | T009920(P1)-R1 | AGCCTGGACCAGAAGCTATCCTAT  |                            |
| ②(3'arm)              | F2         | T009920(P1)-F2 | AGCAGGAAACTCAGTGTGGAGATC  | WT:335bp<br>Targeted:436bp |
|                       | R2         | T009920(P1)-R2 | ATTTCCAGCATGTCCCACATG     |                            |

### 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 |             |

Reviewer:Ting Sun

Date:2020-5-12