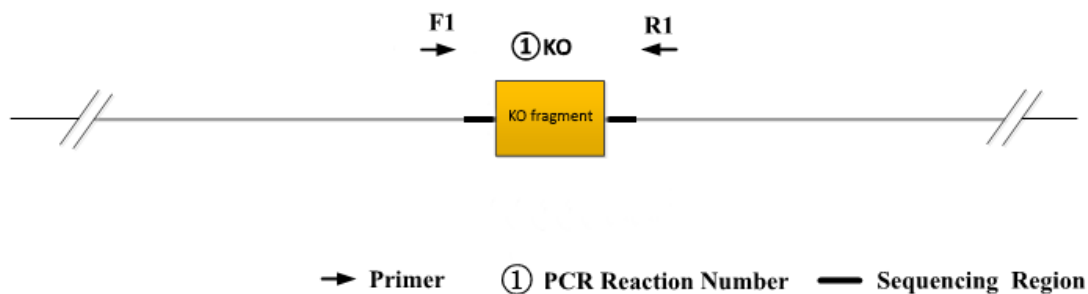


## Genotyping Report

|           |           |             |               |                    |             |
|-----------|-----------|-------------|---------------|--------------------|-------------|
| Strain ID | T001674   | Strain Type | KO(Cas9)      | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | <i>Fbxl20</i> |                    |             |

### 1. Strategy of Genotyping



Wild type: ①PCR reaction obtains a single WT band.

Heterozygote: ①PCR reaction obtains a WT band and a KO band.

Homozygote: ①PCR reaction obtains a single KO band.

Note: 1)The sizes of WT and Targeted band are shown below.

2)If the KO fragment  $\leq 20\text{bp}$ , the genotype should be confirmed by Sequencing.

### 2. Primer Information

| PCR No. | Primer Name | Sequence               | Band Size              |
|---------|-------------|------------------------|------------------------|
| PCR①    | T001674-F1  | GTAAGTGTATGAGGGTACAAAG | WT: 827bp<br>KO: 636bp |
|         | T001674-R1  | GACTATATGCAGACAACATG   |                        |

### 3. Gel Image



Note: WT: Wildtype control; N: 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 |             |