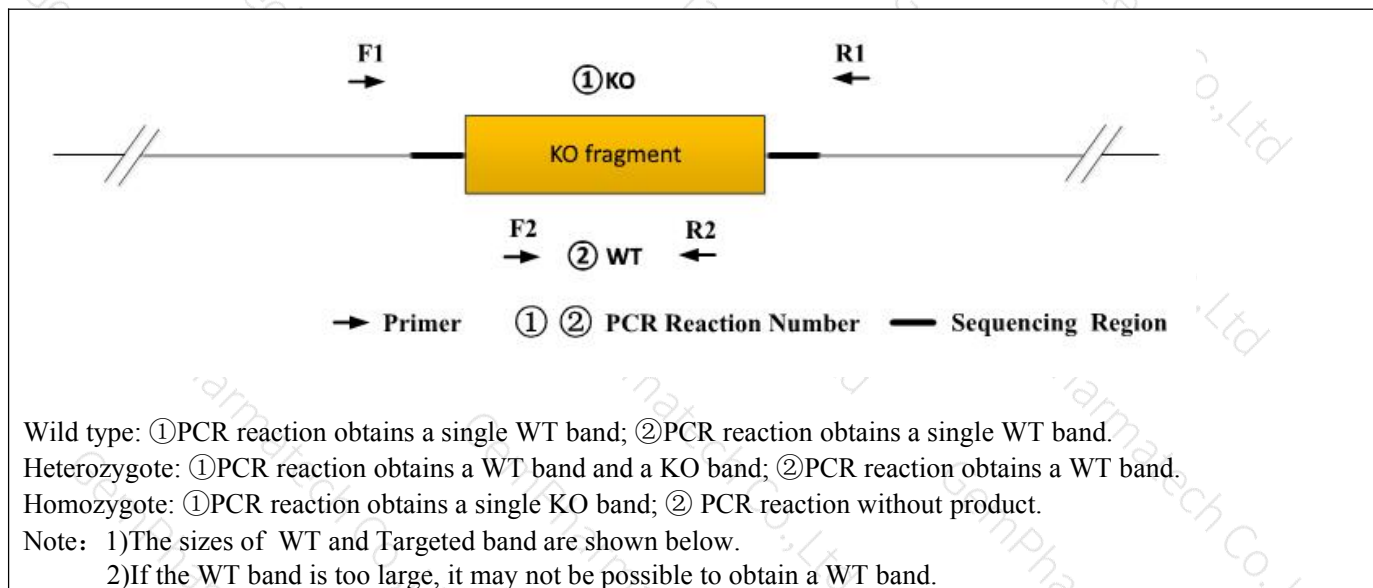


## Genotyping Report

|           |           |             |               |                    |             |
|-----------|-----------|-------------|---------------|--------------------|-------------|
| Strain ID | T002576   | Strain Type | KO(Cas9)      | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | <i>Pfkfb3</i> |                    |             |

### 1. Strategy of Genotyping

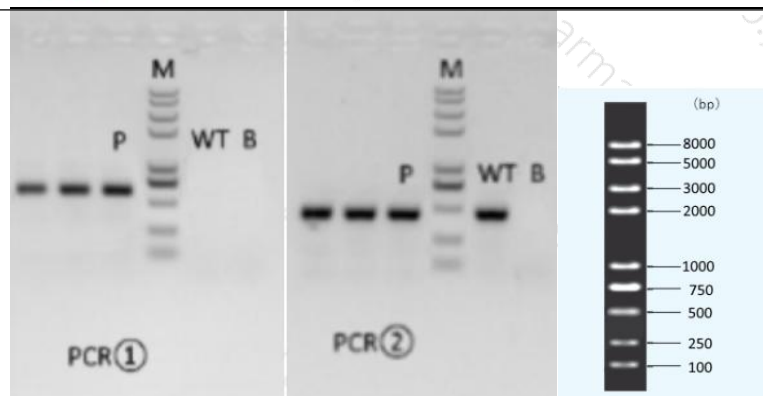


### 2. Primer Information

| PCR No. | Primer No. | Primer Name | Sequence               | Band Size              |
|---------|------------|-------------|------------------------|------------------------|
| PCR①    | F1         | T002576-F1  | GGTCTCACTCAAGTCTATTT   | WT:2960bp<br>KO: 660bp |
|         | R1         | T002576-R1  | AGAGAGGCCGAAGACTTGTC   |                        |
| PCR②    | F2         | T002576-F2  | CCCAGCACCTGTCTAGGTTATG | WT:412bp<br>KO:0bp     |
|         | R2         | T002576-R2  | TCTCAGTGACCAGCCAGCCTT  |                        |

### 3. Gel Image

|                                                                          |
|--------------------------------------------------------------------------|
| ctcaggccaaagggagtggccattaggtgg---2296bp---atctggacgttaatgagtaggttagattgg |
|--------------------------------------------------------------------------|



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 |             |