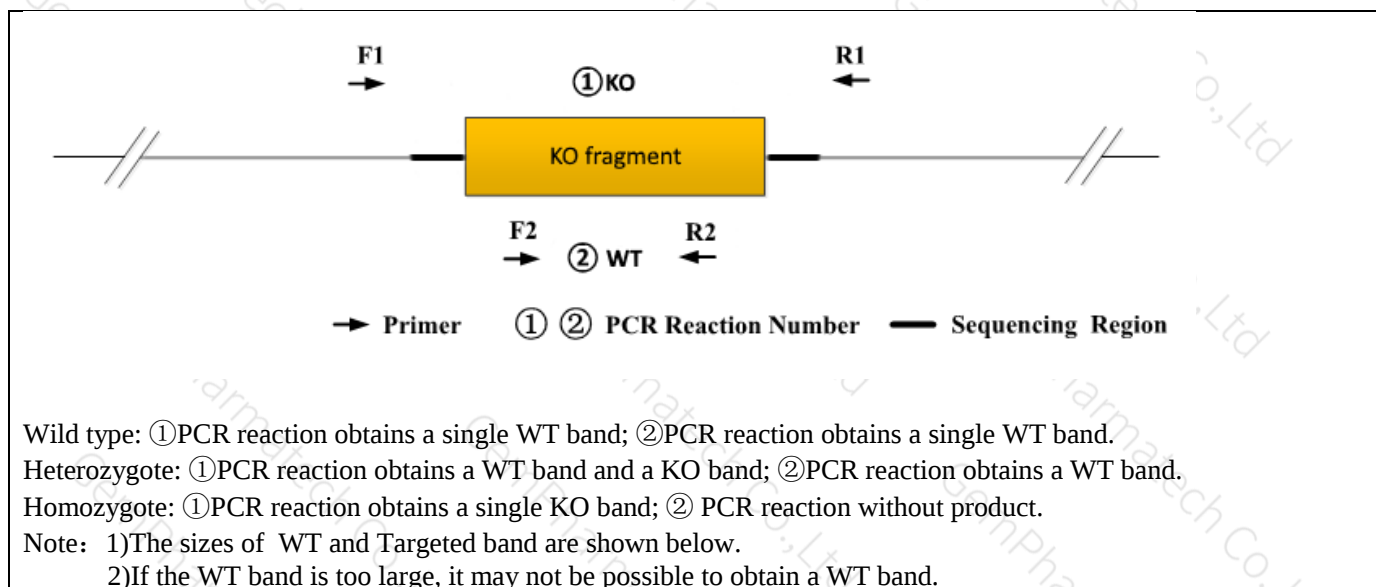


## Genotyping Report

|           |           |             |          |                    |             |
|-----------|-----------|-------------|----------|--------------------|-------------|
| Strain ID | T032801   | Strain Type | KO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | Evx1     |                    |             |

### 1. Strategy of Genotyping

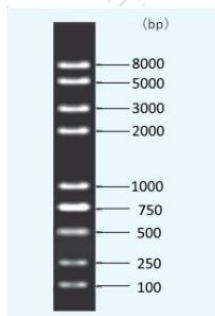
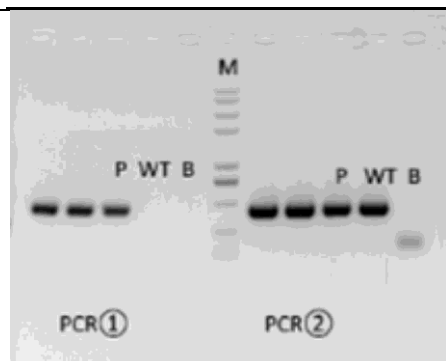


### 2. Primer Information

| PCR No. | Primer No. | Sequence                 | Band Size  |
|---------|------------|--------------------------|------------|
| PCR①    | T032801-F1 | GGATAGCTCAGAAGGGAGTCTCTC | WT: 4173bp |
|         | T032801-R1 | TTACCTGTCGCAAGCAATCTCTG  | KO: 407bp  |
| PCR②    | T032801-F2 | TCCGATGCTCTGGGTAGCAGTC   | WT: 402bp  |
|         | T032801-R2 | CAGCAGTGGCTGACTATGGACTG  | KO: 0bp    |

### 3. Gel Image

acctctaggcctaggcagccaacaccacct---3869bp+103bp---GGGATTGTCTGAGTACGTGATAACTTCGTATAGCATA  
CATTATACGAAGTTATGGGCCAACAGAATTTCGATATCCTGCAGGGACTAACAGAAGAACGCGTTGTgcgtg  
gaaccttctaccagcaactgtgct



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 |             |