

## Genotyping Report

|           |           |             |          |                    |             |
|-----------|-----------|-------------|----------|--------------------|-------------|
| Strain ID | T031576   | Strain Type | KO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | Adcy7    |                    |             |

### 1. Strategy of Genotyping



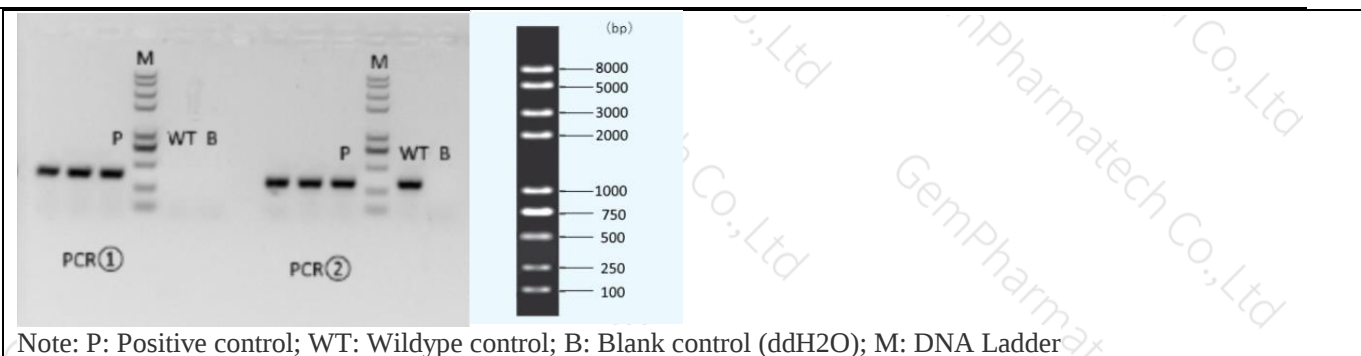
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.  
Note: 1)The sizes of WT and Targeted band are shown below.  
2)If the WT band is too large, it may not be possible to obtain a WT band.

### 2. Primer Information

| PCR No. | Primer No. | Sequence                  | Band Size               |
|---------|------------|---------------------------|-------------------------|
| PCR①    | T031576-F1 | AAGGTTGCAGCTACAACCTAGATGC | WT: 3649bp<br>KO: 384bp |
|         | T031576-R1 | TTTCCAGTGGTCTTAGGTGGTC    |                         |
| PCR②    | T031576-F2 | GCTGAGATGGCTGCTCACTTACTAG | WT: 340bp<br>KO: 0bp    |
|         | T031576-R2 | CCTAGACCACCTTGCTCATCATC   |                         |

### 3. Gel Image

|                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------|
| ctgttctttgtgtccctccaaagccctcct---3326bp+61bp---<br>ATCCTCTGGGTACTTAAGCAAGGCTTTGCCTGAGAAGAGCCTGGTTCTGGCTCCTGTGGTCaggccatggaacata<br>gtgaggcctgagggc |
|----------------------------------------------------------------------------------------------------------------------------------------------------|



#### 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 |             |