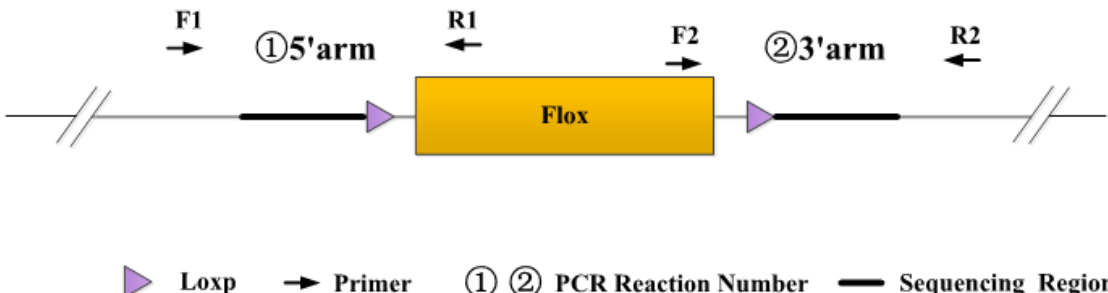


## Genotyping Report

|           |           |             |           |                    |             |
|-----------|-----------|-------------|-----------|--------------------|-------------|
| Strain ID | T021599   | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Ya'nan Xu | Gene Name   | Pygb      |                    |             |

### 1. Strategy of Genotyping



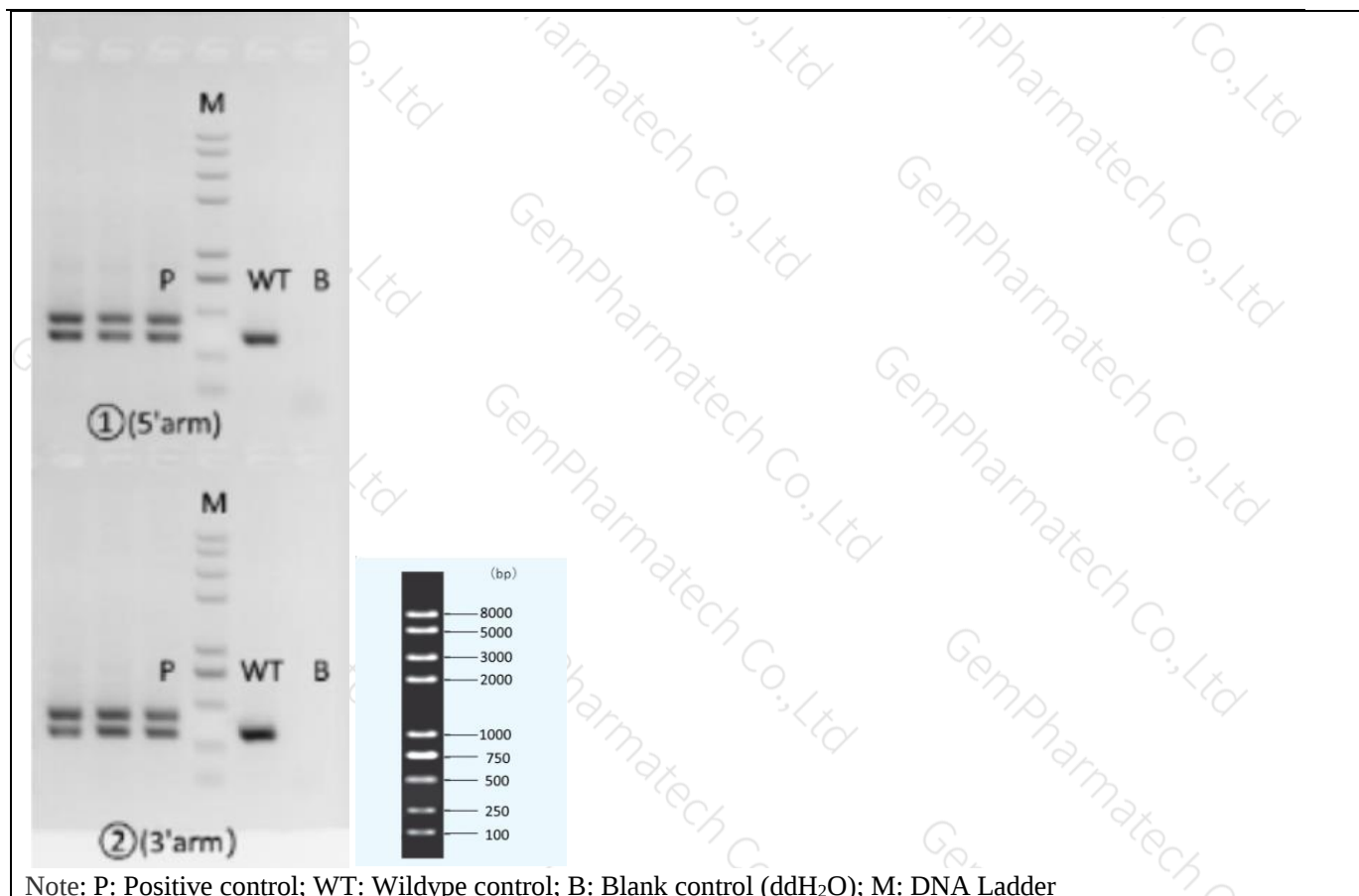
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
Heterozygote: ①PCR reaction obtains a WT band and a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.  
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single 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) | F1         | JS21893-Pygb-5wt-tF1 | TAGGTCAGGGCCTCACACAATCC  | WT: 350bp<br>Targeted: 454bp |
|          | R1         | JS21893-Pygb-5wt-tR1 | TGGTGCTTAGTAGGGAGGCTGAG  |                              |
| ②(3'arm) | F2         | JS21893-Pygb-3wt-tF1 | CTCACTCTTCAGCTACTCTTCGCC | WT: 320bp<br>Targeted: 421bp |
|          | R2         | JS21893-Pygb-3wt-tR1 | ATACCGACCAATCAAGCCATCC   |                              |

### 3. Gel Image & Conclusion

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|  |
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#### 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