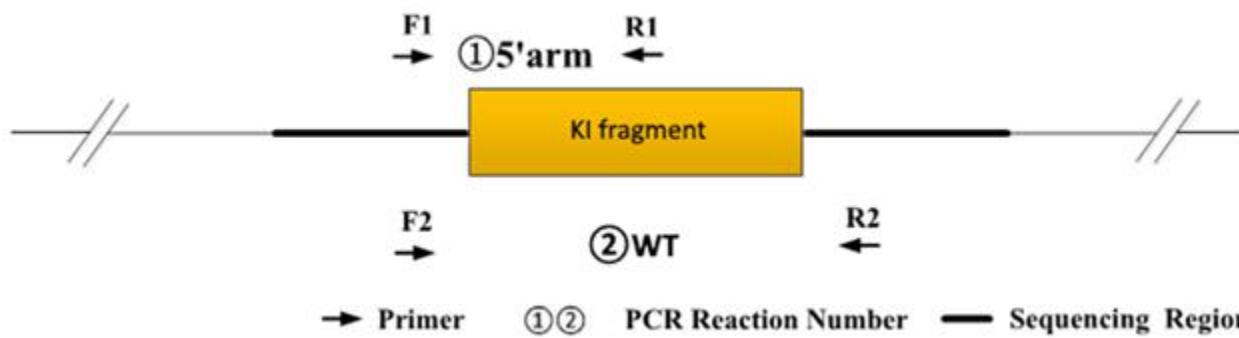


## Genotyping Report

|           |                |             |                                                     |                    |             |
|-----------|----------------|-------------|-----------------------------------------------------|--------------------|-------------|
| Strain ID | T054998        | Strain Type | KI(Cas9)                                            | Genetic Background | C57BL/6JGpt |
| Designer  | Dongdong Zhang | Gene Name   | <i>H11-CAG-LSL-mmu_circ_0000953-IRES-EGFP-PolyA</i> |                    |             |

### 1. Strategy of Genotyping

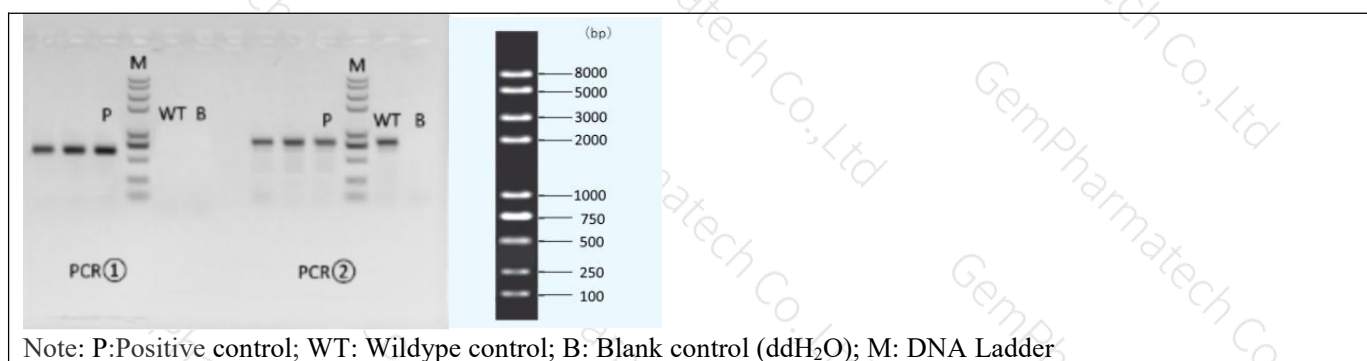


Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.  
Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.  
Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the target band, it is likely to produce dominant amplification, the reaction is only used to judge whether there is a WT allele.

### 2. Primer Information

| PCR No. | Primer No. | Sequence                 | Band Size                   |
|---------|------------|--------------------------|-----------------------------|
| ① 5'arm | T054998-F1 | AGTCTTTCCTTGCTCTGCT      | WT:0bp<br>Targeted:628bp    |
|         | T054998-R1 | AGGCGGGCCATTTACCGTAAGTTA |                             |
| ② WT    | T054998-F2 | AGTCTTTCCTTGCTCTGCT      | WT:825bp<br>Targeted:9607bp |
|         | T054998-R2 | GGGTCTCCACCTTCTTCAG      |                             |

### 3. Gel Image & Conclusion



#### 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 |             |