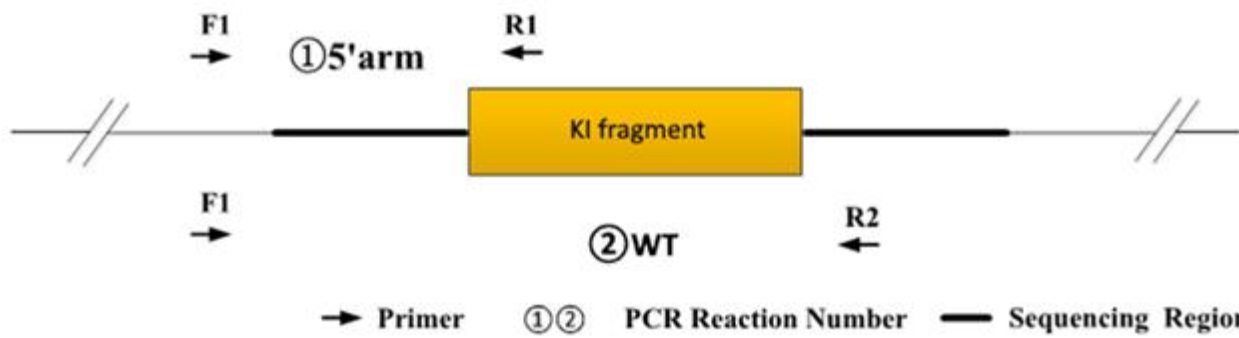


## Genotyping Report

|           |                |             |                        |                    |             |
|-----------|----------------|-------------|------------------------|--------------------|-------------|
| Strain ID | T053687        | Strain Type | KI(Cas9)               | Genetic Background | C57BL/6JGpt |
| Designer  | Dongdong Zhang | Gene Name   | <i>Npsr1-IRES-iCre</i> |                    |             |

### 1. Strategy of Genotyping



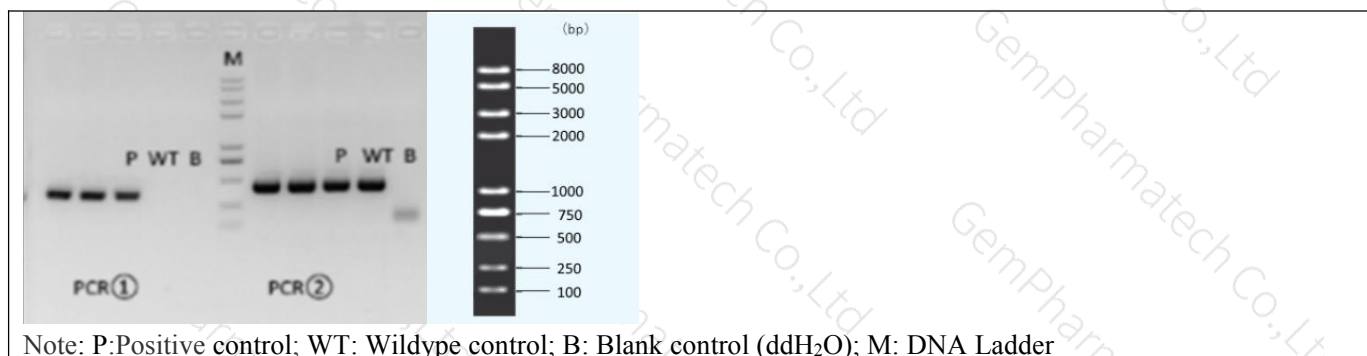
Legend: → Primer    ①② PCR Reaction Number    — Sequencing Region

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. | Primer Name | Sequence                 | Band Size                   |
|---------|------------|-------------|--------------------------|-----------------------------|
| ①5'arm  | F1         | T053687-F1  | GGAAGAGGTTTCATTGCTCTAGCC | WT:0bp<br>Targeted:337bp    |
|         | R1         | T053687-R1  | AAACGCACACCGGCCTTATT     |                             |
| ②WT     | F1         | T053687-F1  | GGAAGAGGTTTCATTGCTCTAGCC | WT:487bp<br>Targeted:2173bp |
|         | R2         | T053687-R2  | ACGGAAGAAGGTCTGGGAGACAT  |                             |

### 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 |             |