

## Genotyping Report

|           |           |             |              |                    |             |
|-----------|-----------|-------------|--------------|--------------------|-------------|
| Strain ID | T045829   | Strain Type | KO(Cas9)     | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | <i>Igsf6</i> |                    |             |

### 1. Strategy of Genotyping



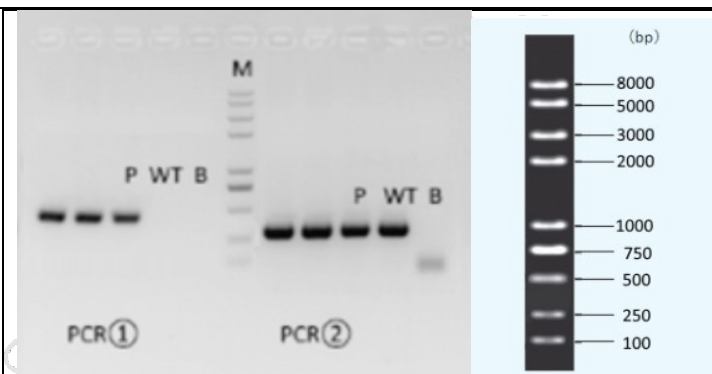
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.  
Note: 1)The sizes of WT and 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    | T045829-F1 | ATGGTTGAGGTCACACAACACGAG      | WT: 5560bp<br>KO: 436bp |
|         | T045829-R1 | AGTCAGAGGCAAGCGTGTCTTTGT      |                         |
| PCR②    | T045829-F2 | TGTGCCAGCTTAGTCCTCTTTCAGCT    | WT: 331bp<br>KO: 0bp    |
|         | T045829-R2 | GCTTCTTAGCTCAGGACACTTAGCAGAAC |                         |

### 3. Gel Image

|                                                                             |
|-----------------------------------------------------------------------------|
| gataaagagagaccatttgaccactgttttga---5124bp---gatccatcccagattcagtttttaaactaaa |
|-----------------------------------------------------------------------------|



Note: P: Positive control; WT: Wildtype control; B: Blank control (ddH<sub>2</sub>O); M: DNA Ladder

① Control (WT) : It is an important reference mark for whether the PCR reaction is successful and whether the product band position and size meet the theoretical requirements.

② Control (B) : PCR amplification was performed without template in the PCR reagent to monitor whether the reagent was contaminated.

#### 4. PCR Condition

| PCR Reaction Component           |                                        |      |             |
|----------------------------------|----------------------------------------|------|-------------|
| Seg.                             | reaction component                     |      | Volume (μl) |
| 1                                | 2 × Rapid Taq Master Mix (Vazyme P222) |      | 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 ① priority selection |                                        |      |             |
| 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 |             |
| PCR program ② the second choice  |                                        |      |             |
| Seg.                             | Temp.                                  | Time | Cycle       |
| 1                                | 95℃                                    | 5min | 35×         |
| 2                                | 98℃                                    | 30s  |             |
| 3                                | 58℃*                                   | 30s  |             |

|   |     |      |  |
|---|-----|------|--|
| 4 | 72℃ | 45s* |  |
| 5 | 72℃ | 5min |  |
| 6 | 10℃ | hold |  |

Note\*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.