

## Genotyping Report

|           |           |             |          |                    |             |
|-----------|-----------|-------------|----------|--------------------|-------------|
| Strain ID | T012357   | Strain Type | KO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | Vps53    |                    |             |

### 1. Strategy of Genotyping



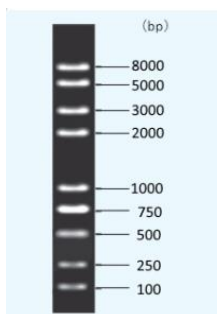
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.  
Homozygote: ①PCR reaction obtains a single KO band; ②PCR reaction without product.  
Note: 1)The sizes of WT and 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. | Primer Name           | Sequence                 | Band Size             |
|---------|------------|-----------------------|--------------------------|-----------------------|
| PCR①    | F1         | JS02258-Vps53-5wt-tF1 | CCAGCCTAGAACTCAAGAGACTCC | WT:1834bp<br>KO:250bp |
|         | R1         | JS02258-Vps53-3wt-tR1 | TTTGGAGCAACGTAGTCAAGCTAC |                       |
| PCR②    | F2         | JS12258-Vps53-wt-tF1  | GGCCAGTGTTTTACTGCCCATAC  | WT:319bp<br>KO:0bp    |
|         | R2         | JS12258-Vps53-wt-tR1  | CGTGGTTTATGGGTTTATGCCATG |                       |

### 3. Gel Image

|                                                                                    |
|------------------------------------------------------------------------------------|
| ccttgtagtgcctgtgtagcaaaactccataaa-----1584bp-----catcattaggtgcttctgcccagtggtctcttc |
|------------------------------------------------------------------------------------|



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

#### 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

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