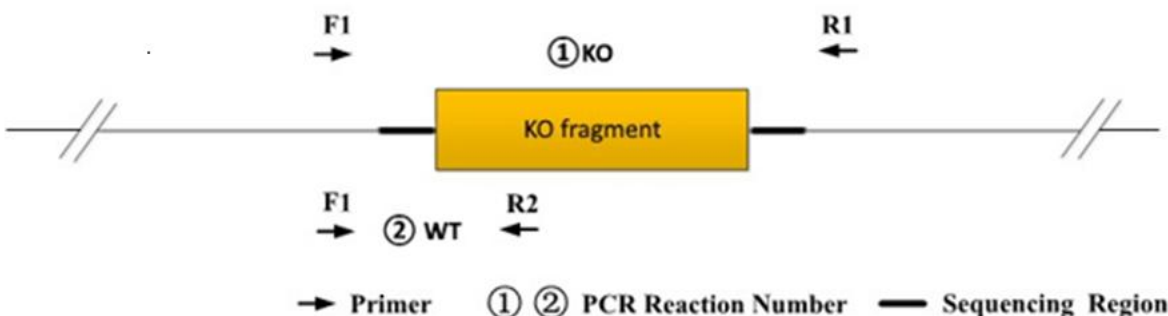


## Genotyping Report

|           |           |             |              |                    |             |
|-----------|-----------|-------------|--------------|--------------------|-------------|
| Strain ID | T002033   | Strain Type | KO(Cas9)     | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | <i>Ctla4</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. | Primer Name                 | Sequence                  | Band Size             |
|---------|------------|-----------------------------|---------------------------|-----------------------|
| PCR①    | F1         | Ctla4-Cas9-KO(B5S3/B3S3)-F2 | GAAGTTAAAGCAGGGTTGTCTCA   | WT:2645bp<br>KO:417bp |
|         | R1         | Ctla4-Cas9-KO(B5S3/B3S3)-R2 | AAATGCCCAAGATAGTTGGCTAGAT |                       |
| PCR②    | F1         | Ctla4-Cas9-KO(B5S3/B3S3)-F2 | GAAGTTAAAGCAGGGTTGTCTCA   | WT:494bp<br>KO:0bp    |
|         | R2         | Ctla4-wt-tR1                | GTCAGTCACTCTCCAAGATCCCCT  |                       |

### 3. Gel Image

gaagcttactttgagagatgag---2228bp---gctggctgactagacatccattgt



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

Date:2020-7-1