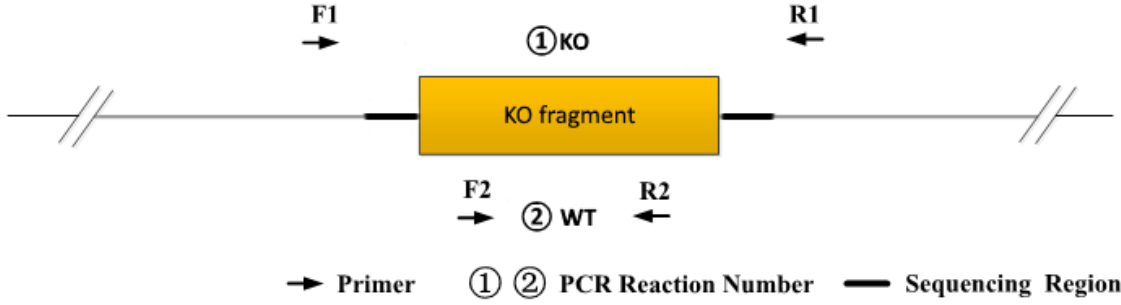


## Genotyping Report

|           |           |             |              |                    |             |
|-----------|-----------|-------------|--------------|--------------------|-------------|
| Strain ID | T012604   | Strain Type | KO(Cas9)     | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | <i>Itgal</i> |                    |             |

### 1. Strategy of Genotyping



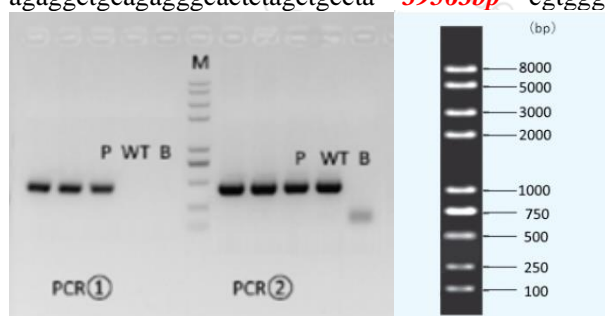
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.  
 Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.  
 Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.  
 Note: 1)The sizes of WT 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         | T012604-F1  | ATGGTTGGAAGGACTCAGGATG     | WT:40008bp<br>KO:445bp |
|         | R1         | T012604-R1  | CCTGCTGACCACAGAAGAGTATTAG  |                        |
| PCR②    | F2         | T012604-F2  | GCTCAGCAATACTTCCTACCAGGTAC | WT:417bp<br>KO:0bp     |
|         | R2         | T012604-R2  | TACTACTGGACAGGGTTAGCTCGAC  |                        |

### 3. Gel Image

agaggctgcagagggcactctagctgccta---39563bp---cgtgggctatttgaccaaactaaagagag



Note: P: Heterozygous samples; 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

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as GC%  $\geq$  60% or GC%  $\leq$  40%, recommend to use Vazyme P515.)

| PCR Reaction Component           |  |      |                   |
|----------------------------------|--|------|-------------------|
| Seg.                             | Reaction Component   |      | Volume ( $\mu$ l) |
| 1                                | 2 $\times$ Rapid Taq Master Mix(Vazyme P222)<br>or<br>2 $\times$ Phanta Max Master Mix (Vazyme P515) |      | 12.5              |
| 2                                | ddH <sub>2</sub> O   |      | 9.5               |
| 3                                | Primer A(10pmol/ $\mu$ l)  |      | 1                 |
| 4                                | Primer B(10pmol/ $\mu$ l)  |      | 1                 |
| 5                                | Template(20~80ng/ $\mu$ l)   |      | 1                 |
| PCR program I priority selection |  |      |                   |
| Seg.                             | Temp.  | Time | Cycle             |
| 1                                | 95°C   | 5min | 20 $\times$       |
| 2                                | 98°C   | 30s  |                   |
| 3                                | 65°C*(-0.5°C/cycle)  | 30s  |                   |
| 4                                | 72°C   | 45s* |                   |
| 5                                | 98°C   | 30s  | 15 $\times$       |
| 6                                | 55°C*  | 30s  |                   |
| 7                                | 72°C   | 45s* |                   |
| 8                                | 72°C   | 5min |                   |
| 9                                | 10°C   | hold |                   |
| PCR program II the second choice |  |      |                   |
| Seg.                             | Temp.  | Time | Cycle             |
| 1                                | 95°C   | 5min | 35 $\times$       |
| 2                                | 98°C   | 30s  |                   |
| 3                                | 58°C*  | 30s  |                   |
| 4                                | 72°C   | 45s* |                   |
| 5                                | 72°C   | 5min |                   |
| 6                                | 10°C   | hold |                   |

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

