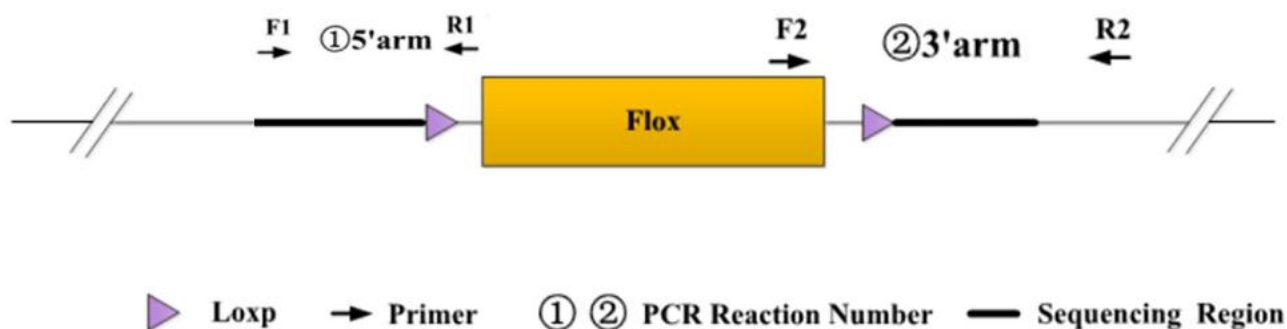


## Genotyping Report

|           |            |             |              |                    |             |
|-----------|------------|-------------|--------------|--------------------|-------------|
| Strain ID | T060483    | Strain Type | CKO(Cas9)    | Genetic Background | C57BL/6JGpt |
| Designer  | Yuting Liu | Gene Name   | <i>Ube3c</i> |                    |             |

### 1. Strategy of Genotyping



Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a single WT band.

Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.

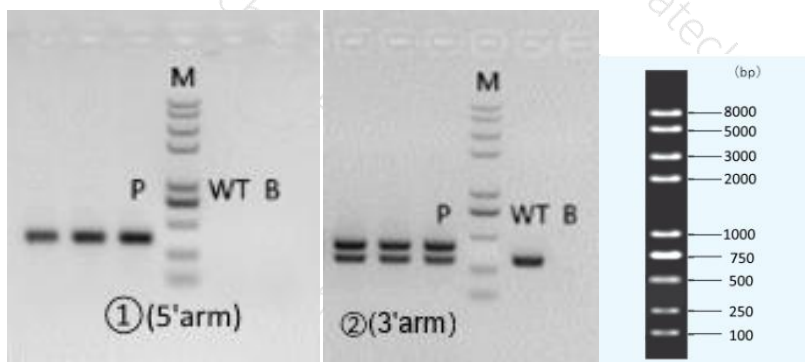
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a single Targeted band.

Note: The sizes of WT and Targeted band are shown below.

### 2. Primer Information

| PCR No.  | Primer No. | Primer Name     | Sequence                | Band Size                    |
|----------|------------|-----------------|-------------------------|------------------------------|
| ①(5'arm) | F1         | ES-1R5          | ATTTGTCACGTCCTGCACGA    | WT: 0bp<br>Targeted: 329bp   |
|          | R1         | T060483(P1)-R1A | AAACAGTGCTCACGTGGCTAC   |                              |
| ②(3'arm) | F2         | T060483(P1)-F2  | GATGTGGGTTATCCCTCAGCCCT | WT: 318bp<br>Targeted: 424bp |
|          | R2         | T060483(P1)-R2  | AGTGTTTAAGCCCCACAGACCCC |                              |

### 3. Gel Image & Conclusion



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 (μl) |
| 1                                | 2 × Rapid Taq Master Mix(Vazyme P222)<br>or<br>2 × Phanta Max Master Mix (Vazyme P515) |      | 12.5        |
| 2                                | ddH2O                                                                                  |      | 9.5         |
| 3                                | Primer A(10pmol/μl)                                                                    |      | 1           |
| 4                                | Primer B(10pmol/μl)                                                                    |      | 1           |
| 5                                | Template(20~80ng/μl)                                                                   |      | 1           |
| PCR program I 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  | 15×         |
| 6                                | 55℃*                                                                                   | 30s  |             |
| 7                                | 72℃                                                                                    | 45s* |             |
| 8                                | 72℃                                                                                    | 5min |             |
| 9                                | 10℃                                                                                    | hold |             |
| PCR program II 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.