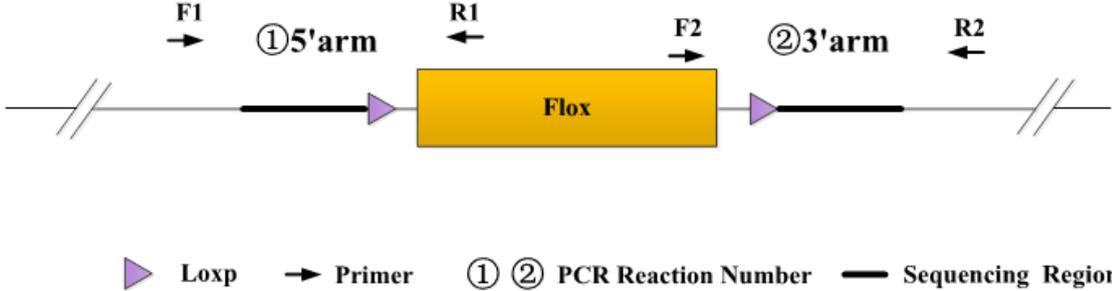


## Genotyping Report

|           |           |             |             |                    |             |
|-----------|-----------|-------------|-------------|--------------------|-------------|
| Strain ID | T038308   | Strain Type | CKO(Cas9)   | Genetic Background | C57BL/6JGpt |
| Designer  | Ya'nan Xu | Gene Name   | <i>Usf1</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 Targeted band; ②PCR reaction obtains a WT band and a Targeted band.  
Homozygote: ①PCR reaction obtains a single 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. | Sequence                  | Band Size                   |
|----------|------------|---------------------------|-----------------------------|
| ①(5'arm) | T038308-F1 | GACTAAGAACATTGGATTTCGGGAG | WT: 239bp<br>Targeted:344bp |
|          | T038308-R1 | AGTCCTGGAAC TTGTTGTGTGGAG |                             |
| ②(3'arm) | T038308-F2 | TGGTTACCCTGCCACTCAGTCTAT  | WT: 348bp<br>Targeted:454bp |
|          | T038308-R2 | ATGGAGGTCAGAGAAGGACACTTGA |                             |

### 3. Gel Image & Conclusion



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                                | ddH <sub>2</sub> O                     | 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°C                                   | 5min        |       |
| 2                                | 98°C                                   | 30s         | 20×   |
| 3                                | 65°C* (-0.5°C/cycle)                   | 30s         |       |
| 4                                | 72°C                                   | 45s*        |       |
| 5                                | 98°C                                   | 30s         |       |
| 6                                | 55°C*                                  | 30s         | 20×   |
| 7                                | 72°C                                   | 45s*        |       |
| 8                                | 72°C                                   | 5min        |       |
| 9                                | 10°C                                   | hold        |       |
| PCR program ② the second choice  |  |             |       |
| Seg.                             | Temp.                                  | Time        | Cycle |
| 1                                | 95°C                                   | 5min        |       |
| 2                                | 98°C                                   | 30s         | 35×   |
| 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.

