

## Genotyping Report

|           |           |             |               |                    |             |
|-----------|-----------|-------------|---------------|--------------------|-------------|
| Strain ID | T026002   | Strain Type | CKO(Cas9)     | Genetic Background | C57BL/6JGpt |
| Designer  | Ya'nan Xu | Gene Name   | <i>Sema5a</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.

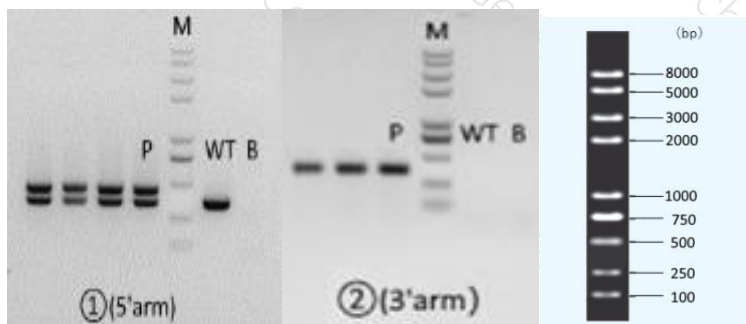
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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

### 2. Primer Information

| PCR No.                   | Primer No.             | Sequence                | Band Size                   |
|---------------------------|------------------------|-------------------------|-----------------------------|
| ①(5'arm)<br>GC%(wt):39.6% | JS26296-Sema5a-5wt-tF1 | CACAAGGCTACCTCTGCTTCATG | WT:351bp<br>Targeted: 453bp |
|                           | JS26296-Sema5a-5wt-tR1 | TGTCTGGACACAGGGTCTCATTG |                             |
| ②(3'arm)                  | ZMK-2F4                | GCATCGCATTGTCTGAGTAGGTG | WT:0bp<br>Targeted:346bp    |
|                           | JS26296-Sema5a-3wt-tR1 | TCAGAACCTACAGGTGAGCAGTG |                             |

### 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

| PCR Reaction Component           |                                        |      |             |
|----------------------------------|----------------------------------------|------|-------------|
| Seg.                             | reaction component                     |      | Volume (μl) |
| 1                                | 2 × Rapid Taq Master Mix (Vazyme P222) |      | 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.