

## Genotyping Report

|           |           |             |             |                    |             |
|-----------|-----------|-------------|-------------|--------------------|-------------|
| Strain ID | T052464   | Strain Type | KO(Cas9)    | Genetic Background | C57BL/6JGpt |
| Designer  | Zifan Lin | Gene Name   | <i>Hrh3</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; ②PCR 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.     | Sequence                  | Band Size               |
|---------|----------------|---------------------------|-------------------------|
| PCR①    | T052464(P2)-F1 | GCAGCCAAGAACCCTTTAAGCCAAG | WT: 1523bp<br>KO: 432bp |
|         | T052464(P2)-R1 | TCCTTCCTCTCACTACTGGAGCTGG |                         |
| PCR②    | T052464(P2)-F2 | GCCAGGCTCTGCCCTTTCTCTAT   | WT: 422bp<br>KO: 0bp    |
|         | T052464(P2)-R2 | AGCCAACCTCCTGCCTCATGCTAG  |                         |

### 3. Gel Image

agcccagggtttgtgttcagaggtccctat---1091bp---ttttggccctcctgccacccttccccttg



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.