# Genotyping Report

| Strain ID | T024623 | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
|---|---|---|---|---|---|
| Designer | Ya'nan Xu | Gene Name | *Tjp2* | | |

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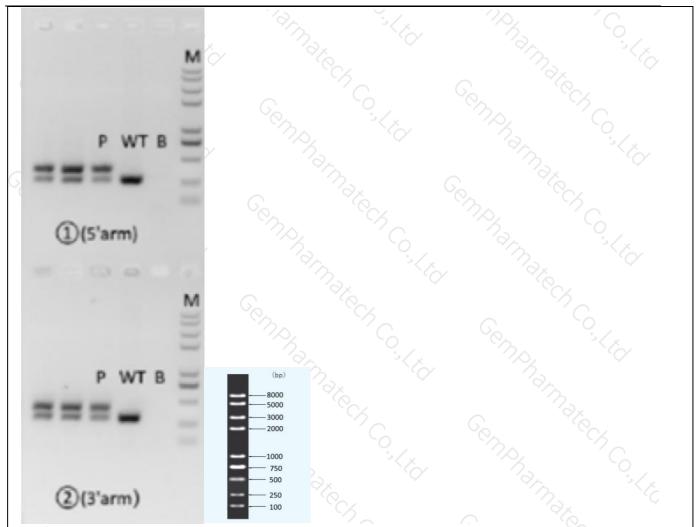
Homozygote: ①PCR reaction obtains a single Targeted band; ②PCR reaction obtains a single Targeted band.

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

## 2. Primer Information

| PCR No. | Primer No. | Sequence | Band Size |
|---|---|---|---|
| ①(5'arm) | T024623-F1 | CATCTTGTTTGAACACTGGGACAC | WT: 290bp<br>Targeted:395bp |
| | T024623-R1 | AGGAAACTGGTGTTTATGGAGCTG | |
| ②(3'arm) | T024623-F2 | GCGGATCCTATTCATCAATCTTTCTC | WT: 325bp<br>Targeted:431bp |
| | T024623-R2 | TTTCCCACACTGAGAAGCACCA | |

## 3. Gel Image & Conclusion

Note: P: Positive control; WT: Wildype control; B: Blank control (ddH₂O); 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(≈100ng/µl) | 1 |
| **PCR program ① priority selection** | | |

| Seg. | Temp. | Time | Cycle |
|---|---|---|---|
| 1 | 95℃ | 5min | |
| 2 | 98℃ | 30s | 20× |
| 3 | 65℃＊（-0.5℃/cycle） | 30s | |
| 4 | 72℃ | 45s＊ | |
| 5 | 98℃ | 30s | 20× |
| 6 | 55℃＊ | 30s | |
| 7 | 72℃ | 45s＊ | |
| 8 | 72℃ | 5min | |
| 9 | 10℃ | hold | |

**PCR program ②　the second choice**

| Seg. | Temp. | Time | Cycle |
|---|---|---|---|
| 1 | 95℃ | 5min | |
| 2 | 98℃ | 30s | 35× |
| 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.