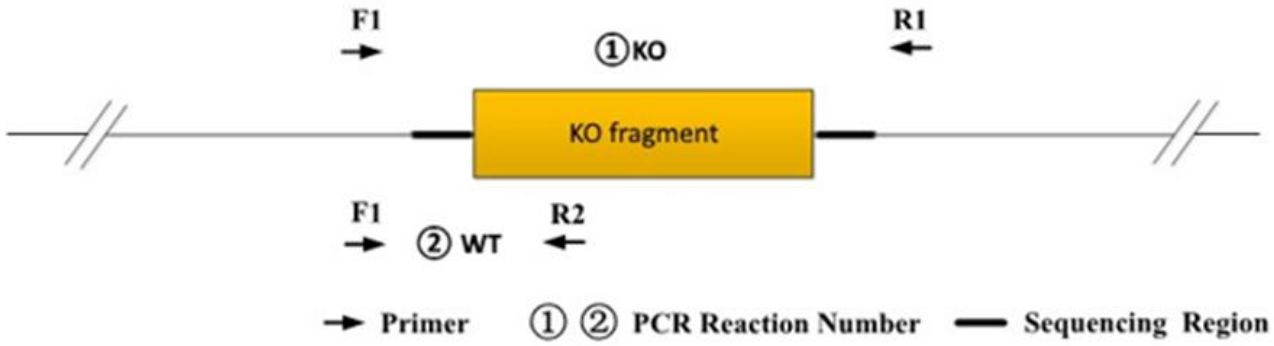
# Genotyping Report

| Strain ID | T003591 | Strain Type | KO(Cas9) | Genetic Background | C57BL/6JGpt |
|---|---|---|---|---|---|
| Designer | Zifan Lin | Gene Name | *Cd28* | | |

## 1. Strategy of Genotyping



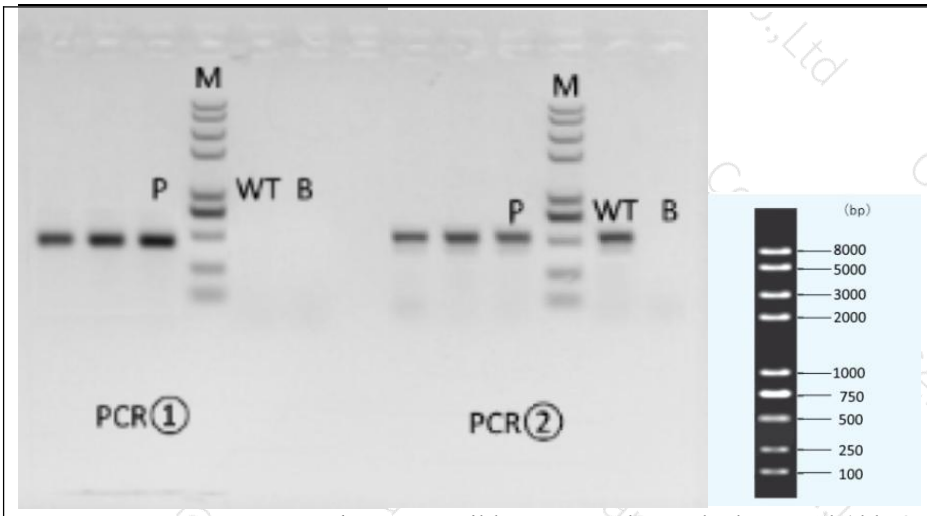

Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note：1)The sizes of WT and Targeted 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. | Primer Name | Sequence | Band Size |
|---|---|---|---|---|
| PCR① | F1 | 3496-huCD28-KO-TF1 | CCATTCAGAGAAAGATGCCA | WT: 2780bp<br>KO: 480bp |
| | R1 | 3496-huCD28-KO-TR1 | GGGAAACAGAACTCACATCAGATC | |
| PCR② | F1 | 3496-huCD28-KO-TF1 | CCATTCAGAGAAAGATGCCA | WT: 654bp<br>KO:0bp |
| | R2 | 3496-huCD28-wt-TR1 | CGAGAGCCCCAGCTTCACTTA | |

## 3. Gel Image

gaaaacaagattttggtaaagcagtcgcccctgc------2300bp------ctgttttgggcactggtcgtggttgctggagtcctg




Note: P: Heterozygous samples; WT: Wildtype 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(20~80ng/μl) | | 1 |
| **PCR program Ⅰ (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 | 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 | |
| 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.