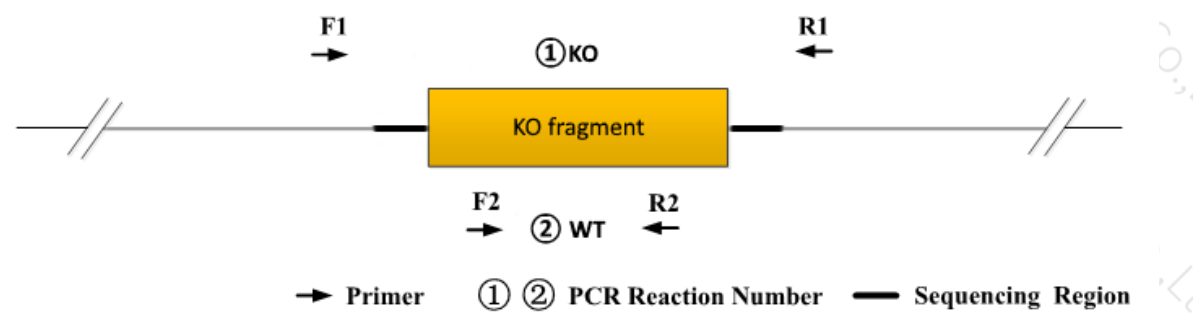


Genotyping Report

| | | | | | |
|-----------|-------------|-------------|--------------|--------------------|-------------|
| Strain ID | T029594 | Strain Type | KO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer | Binjie Jiao | Gene Name | <i>Cd160</i> | | |

1. Strategy of Genotyping

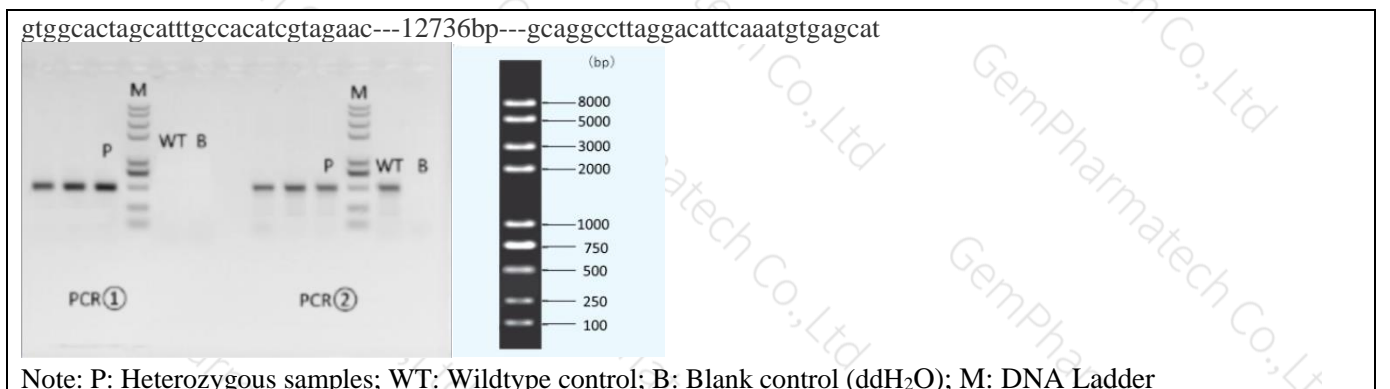


Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note: 1)The sizes of WT and Targeted 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 | JS20527-Cd160-5wt-tF1 | CAGGCAACTGTAACACAACAGCA | WT:13227bp KO:491bp |
| | R1 | JS20527-Cd160-3wt-tR1 | TTGTTGGATTAGCTGGCTGTAGC | |
| PCR② Alternative | F2 | JS30527-Cd160-wt-tF1A | CCCTTCAAGGTATGTCCAGAAGAGA | WT:502bp KO:0bp |
| | R2 | JS30527-Cd160-wt-tR1A | CAAGGACAGGTGAACTTGCATC | |

3. Gel Image



- 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

(Generally recommend to use Vazyme P222; If the sequences contain special structures such as GC% \geq 60% or GC% \leq 40%, recommend to use Vazyme P515.)

| PCR Reaction Component | | | |
|------------------------------------|---|-------------------|-------------|
| Seg. | reaction component | Volume (μ l) | |
| 1 | 2 \times Rapid Taq Master Mix (Vazyme P222) | 12.5 | |
| 2 | ddH ₂ O | 9.5 | |
| 3 | Primer A(10pmol/ μ l) | 1 | |
| 4 | Primer B(10pmol/ μ l) | 1 | |
| 5 | Template(20~80ng/ μ l) | 1 | |
| PCR program I (priority selection) | | | |
| Seg. | Temp. | Time | Cycle |
| 1 | 95 $^{\circ}$ C | 5min | |
| 2 | 98 $^{\circ}$ C | 30s | 20 \times |
| 3 | 65 $^{\circ}$ C* (-0.5 $^{\circ}$ C/cycle) | 30s | |
| 4 | 72 $^{\circ}$ C | 45s* | |
| 5 | 98 $^{\circ}$ C | 30s | 15 \times |
| 6 | 55 $^{\circ}$ C* | 30s | |
| 7 | 72 $^{\circ}$ C | 45s* | |
| 8 | 72 $^{\circ}$ C | 5min | |
| 9 | 10 $^{\circ}$ C | hold | |
| PCR program II (the second choice) | | | |
| Seg. | Temp. | Time | Cycle |
| 1 | 95 $^{\circ}$ C | 5min | |
| 2 | 98 $^{\circ}$ C | 30s | 35 \times |
| 3 | 58 $^{\circ}$ C* | 30s | |
| 4 | 72 $^{\circ}$ C | 45s* | |
| 5 | 72 $^{\circ}$ C | 5min | |
| 6 | 10 $^{\circ}$ C | hold | |

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.

