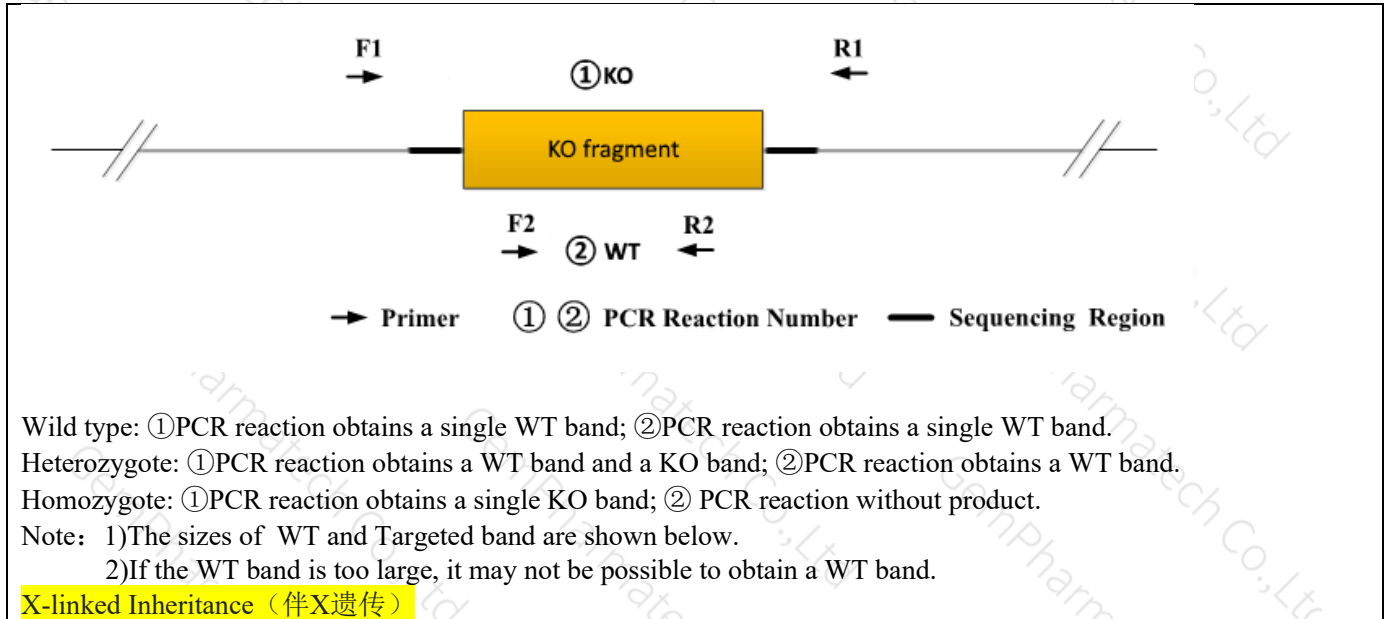


Genotyping Report

| | | | | | |
|-----------|-----------|-------------|--------------|--------------------|-------------|
| Strain ID | T052640 | Strain Type | KO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer | Zifan Lin | Gene Name | <i>Mecp2</i> | | |

1. Strategy of Genotyping



2. Primer Information

| PCR No. | Primer No. | Sequence | Band Size |
|---------|------------|--------------------------|--------------------------|
| PCR① | T052640-F1 | CTTGAGAAGTTGTGACTTTGCAGG | WT: 46371bp KO: 558bp |
| | T052640-R1 | GGTCAACAGCTTGTCTGGTCAGT | |
| PCR② | T052640-F2 | TGGGCTAAATGACTTGCAGGTC | WT: 508bp KO: 0bp |
| | T052640-R2 | TGCTAACTGGGGATCTCTGCTAC | |

3. Gel Image

tagcatagctgcaaaggacttggtgct-----45816bp+3bp-----TTTcgagaggttagctgactttacatagagcg



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

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

PCR Reaction Component

| Seg. | reaction component | Volume (μl) |
|------|--|-------------|
| 1 | 2 × Rapid Taq Master Mix (Vazyme P222) | 12.5 |
| 2 | ddH ₂ O | 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°C | 5min | |
| 2 | 98°C | 30s | 20× |
| 3 | 65°C* (-0.5°C/cycle) | 30s | |
| 4 | 72°C | 45s* | |
| 5 | 98°C | 30s | 20× |
| 6 | 55°C* | 30s | |
| 7 | 72°C | 45s* | |
| 8 | 72°C | 5min | |
| 9 | 10°C | hold | |

PCR program ② the second choice

| Seg. | Temp. | Time | Cycle |
|------|-------|------|-------|
| 1 | 95°C | 5min | |

| | | | |
|---|-------|------|-----|
| 2 | 98°C | 30s | 35× |
| 3 | 58°C* | 30s | |
| 4 | 72°C | 45s* | |
| 5 | 72°C | 5min | |
| 6 | 10°C | hold | |

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