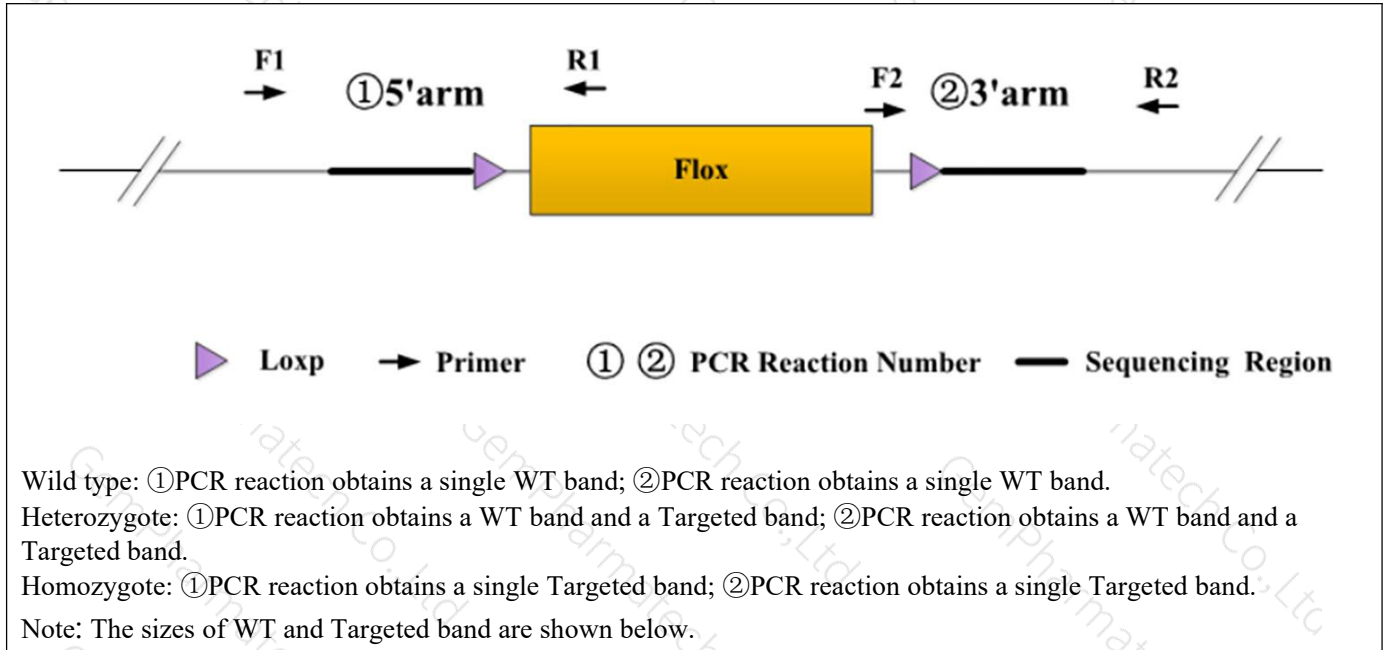


Genotyping Report

| | | | | | |
|-----------|--------------|-------------|---------------|--------------------|-------------|
| Strain ID | T018733 | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer | Tiantian Sun | Gene Name | <i>Anp32a</i> | | |

1. Strategy of Genotyping

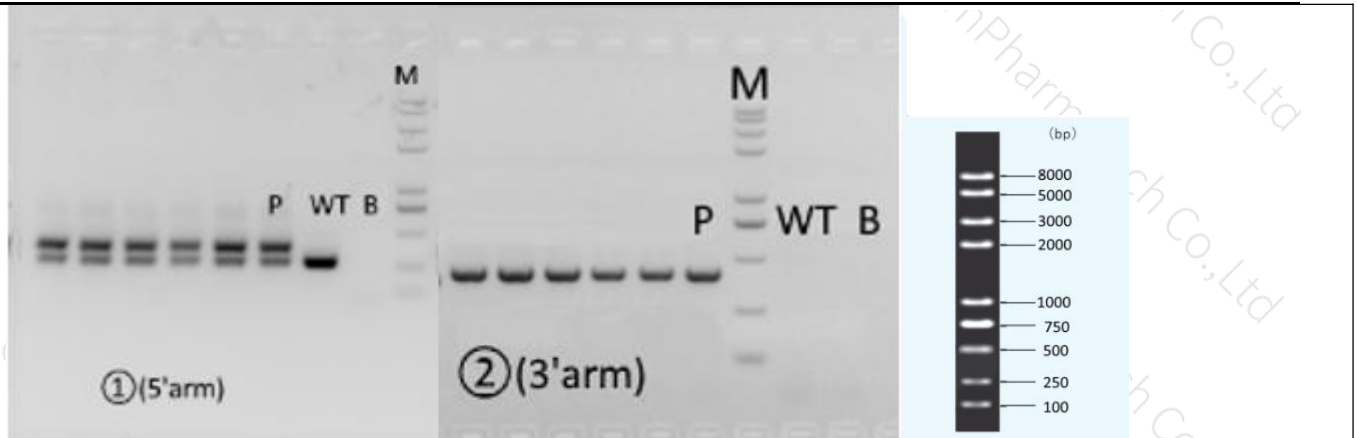


2. Primer Information

| PCR No. | Primer No. | Sequence | Band Size |
|----------|------------|--------------------------|----------------|
| ①(5'arm) | T018733-F1 | ATGAGGATGTGGACGAGGGTTCT | WT:287bp |
| | T018733-R1 | ACCTGGTATCCCTGCCTACGAATG | Targeted:389bp |
| ②(3'arm) | T018733-F2 | CATCGCATTGTCTGAGTAGGTG | WT:0bp |
| | T018733-R2 | CAACACCATACTGTGTGCAACAGG | Targeted:409bp |

3. Gel Image & Conclusion

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Note: P: Positive control; 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 | 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℃ | 5min | 20× |
| 2 | 98℃ | 30s | |
| 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.