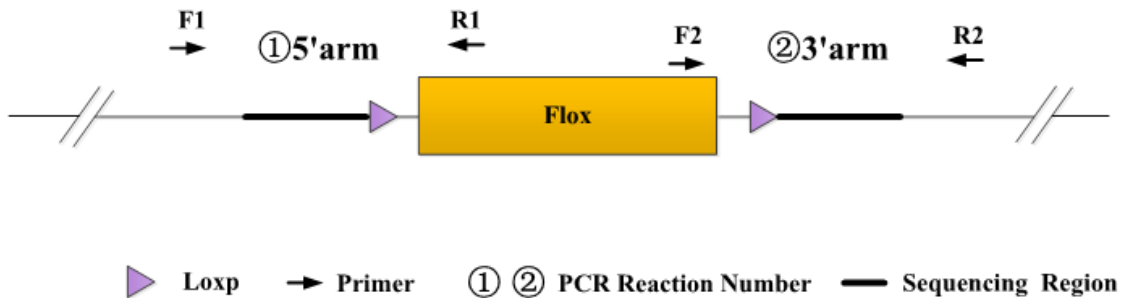


Genotyping Report

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|-----------|-----------|-------------|-------------|--------------------|-------------|
| Strain ID | T013326 | Strain Type | CKO(Cas9) | Genetic Background | C57BL/6JGpt |
| Designer | Ya'nan Xu | Gene Name | <i>Prnp</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 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) | T013326-F1 | ACTTCCTCATGCCTGGCTCTG | WT:254bp Targeted:356bp |
| | T013326-R1 | TGTGAGCCTCTACACAGAGGAGAAC | |
| ②(3'arm) | T013326-F2 | AGGGAGGAGTACACAGAAGGTAGTG | WT:327bp Targeted:430bp |
| | T013326-R2 | CGTTAAGGATTGATCCTGAAGGG | |

3. Gel Image & Conclusion

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4. PCR Condition

| PCR Reaction Component | | | |
|------------------------|-------------------------------------------------|-------------|-------|
| Seg. | reaction component | Volume (μl) | |
| 1 | 2 × Taq Master Mix , Dye Plus, (Vazyme P112-03) | 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 | | | |
| 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 | |
| 6 | 55°C | 30s | 20× |
| 7 | 72°C | 45s | |
| 8 | 72°C | 5min | |
| 9 | 10°C | hold | |

