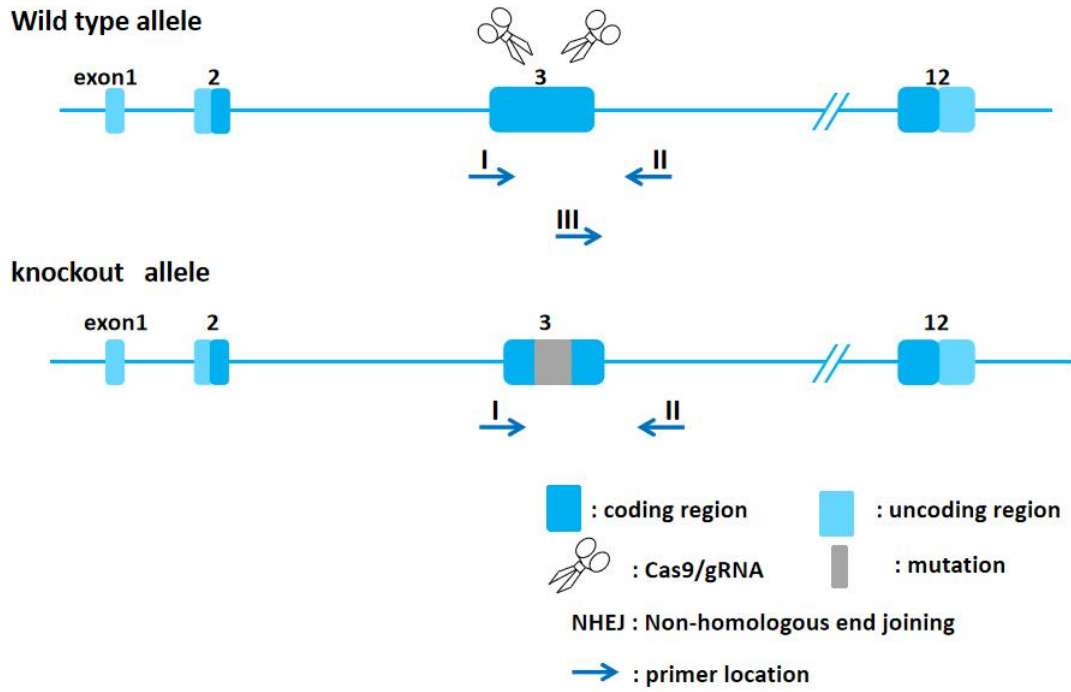


Capn15-KO Genotyping Protocol



| | | | |
|-------------------------|-----------|-----------------|--------------|
| Common Name | Capn15-K0 | Cat. NO. | NM-K0-233293 |
| Strain of Origin | C57BL/6J | Version | V1 |

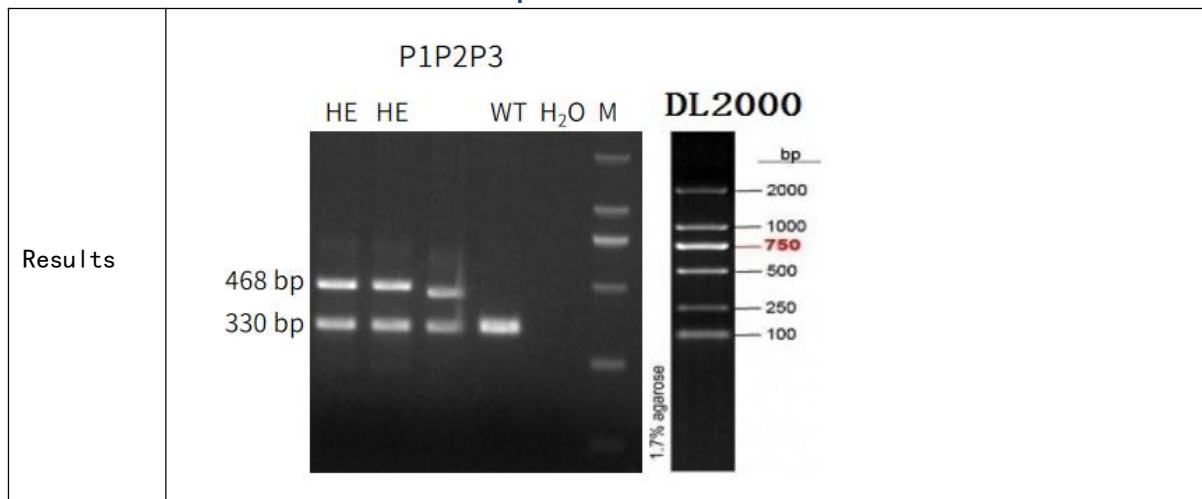
Genotyping strategy



Primers

| Primer | Sequence (5' → 3') | Primer type |
|--------|----------------------|-------------|
| P1 | GCAGCCACACTCATTCTCCT | Forward |
| P2 | CCATGCTGCCCTGTGTAGAA | Reverse |
| P3 | TGCAGCCTGCCATACACCA | Forward |

Expected results



| | |
|----------|---|
| Genotype | Knockout type: -1213bp |
| | Wild type: P3P2=330 bp |
| | Heterozygote: P1P2 =468 bp; P3P2=330 bp |
| | Homozygote: P1P2 =468 bp |

Note: In both wild-type and heterozygous mice, whether the P1 and P2 primers can amplify larger bands does not affect the interpretation of the results, because the purpose of designing this pair of primers is to amplify KO band

Reaction & Cycling

| PCR Reaction System | Reaction Component | | Volume (μl) | | |
|---------------------------|---|-------|-------------|------|-----------------|
| | ddH ₂ O | | 7.5 | | |
| | 2×Taq Plus Master Mix | | 10.0 | | |
| | P1 (10 pmol/μl) | | 0.5 | | |
| | P2 (10 pmol/μl) | | 0.5 | | |
| | P3 (10 pmol/μl) | | 0.5 | | |
| | Genomic DNA | | 1.0 | | |
| | Total | | 20 | | |
| | 2×Taq Plus Master Mix from Vazyme (Code Number: P222-1) | | | | |
| Cycling Reaction | Step | Temp | Time | Note | |
| | 1 | 95° C | 5 min | | |
| | 2 | 95° C | 20 sec | | |
| | 3 | 60° C | 20 sec | | |
| | 4 | 72° C | 20 sec | | 35 repeats to 2 |
| | 5 | 72° C | 5 min | | |
| | 6 | 12° C | Hold | | |