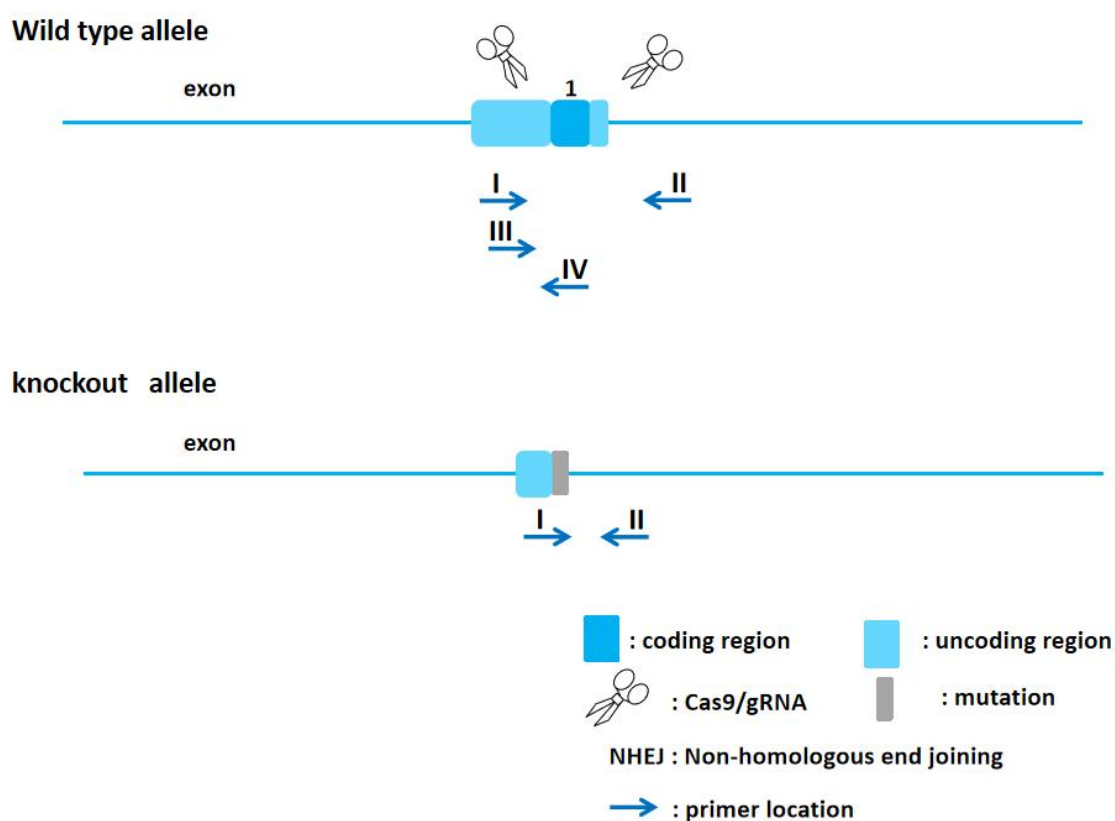


# Cs1-K0 Genotyping Protocol



|                  |          |          |              |
|------------------|----------|----------|--------------|
| Common Name      | CsI-K0   | Cat. NO. | NM-K0-240268 |
| Strain of Origin | C57BL/6J | Version  | V1           |

### Genotyping strategy

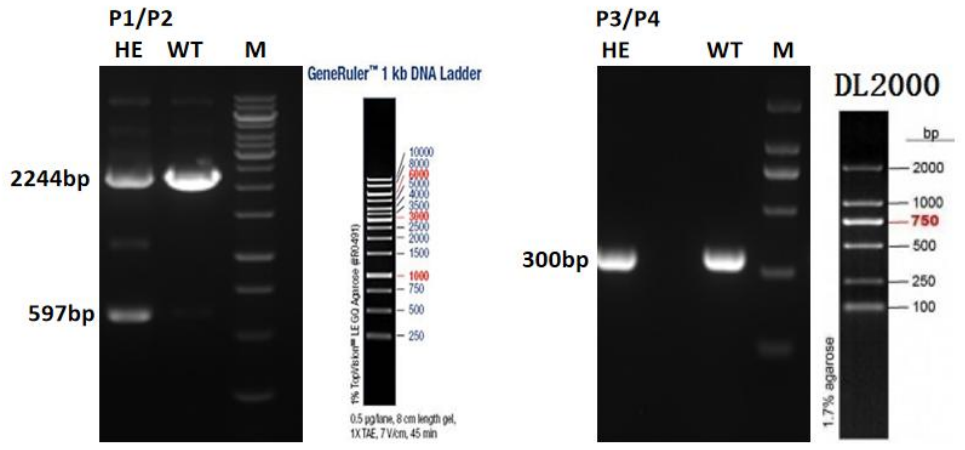


### Primers

| Primer | Sequence (5' → 3' )  | Primer type |
|--------|----------------------|-------------|
| P1     | AGGGCTCAGACATCAGGACA | Forward     |
| P2     | ACCATGCCACTTACGGTCAC | Reverse     |
| P3     | CCTTTCAACATCACTGGCGG | Forward     |

|    |                     |         |
|----|---------------------|---------|
| P4 | CCACAACGTCTTCCCGTGT | Reverse |
|----|---------------------|---------|

### Expected results

|          |                                                                                                                                                                          |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  |                                                                                        |
| Genotype | <p>Knockout type: -1647 bp</p> <p>Wild type: P1P2 =2244 bp; P3P4 =300 bp</p> <p>Heterozygote: P1P2 =2244 bp and 597 bp; P3P4 =300 bp</p> <p>Homozygote: P1P2 =597 bp</p> |

**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 Component       | Volume (μl) |
|----------|--------------------------|-------------|
| Reaction | ddH2O                    | 8.0         |
| System   | 2 × Rapid Taq Master Mix | 10.0        |

|                     |                                                             |       |        |                 |
|---------------------|-------------------------------------------------------------|-------|--------|-----------------|
|                     | P1 (10 pmol/μl) or P3 (10 pmol/μl)                          |       |        | 0.5             |
|                     | P2 (10 pmol/μl) or P4 (10 pmol/μl)                          |       |        | 0.5             |
|                     | Genomic DNA                                                 |       |        | 1.0             |
|                     | Total                                                       |       |        | 20              |
|                     | 2 × Rapid Taq Master Mix from Vazyme (Code Number: P222-01) |       |        |                 |
| Cycling<br>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   |                 |