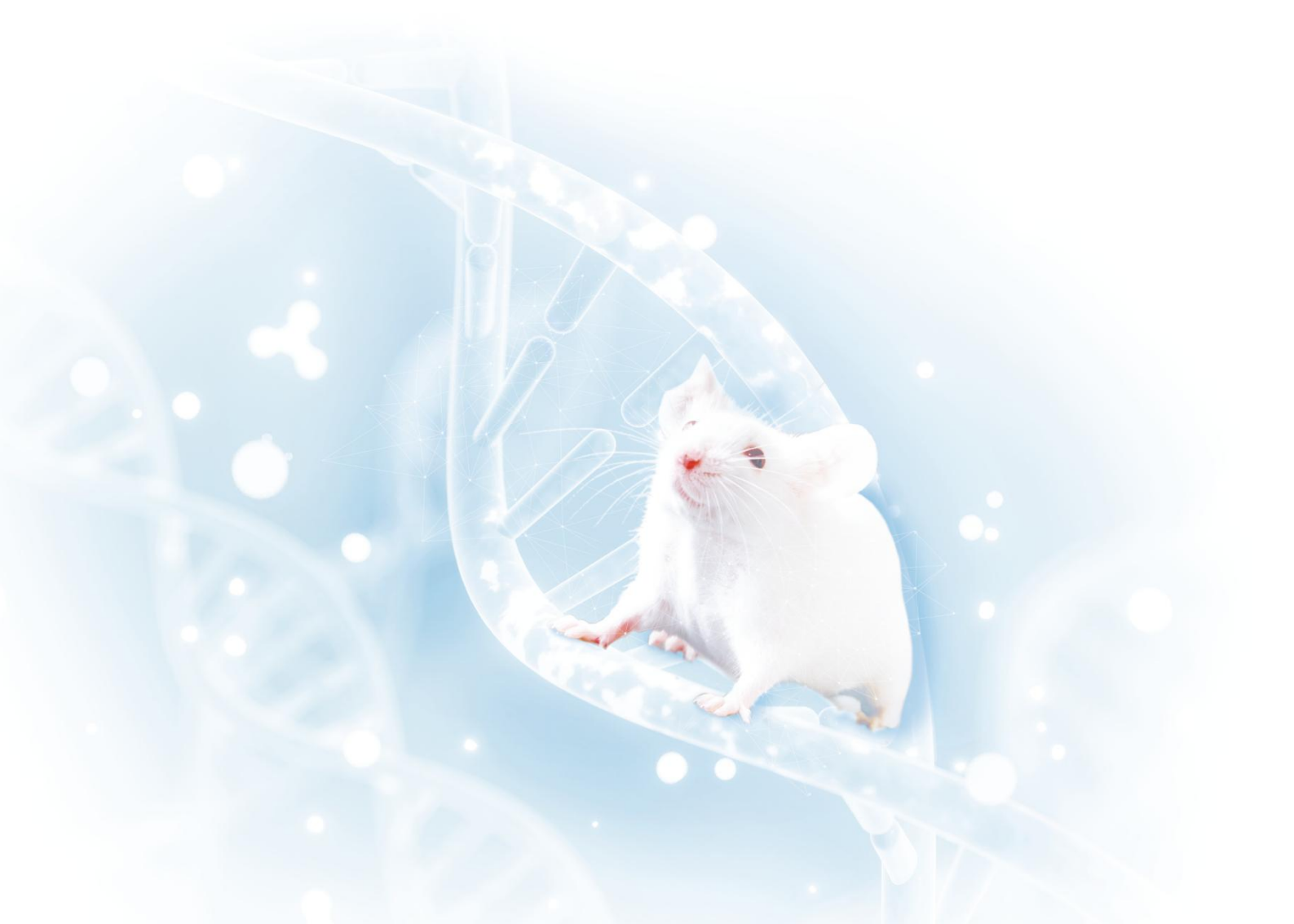


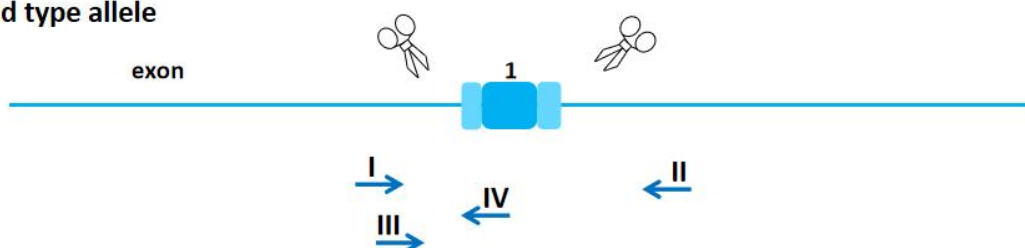
# Znrf4-KO Genotyping Protocol



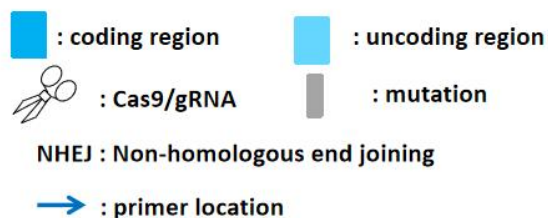
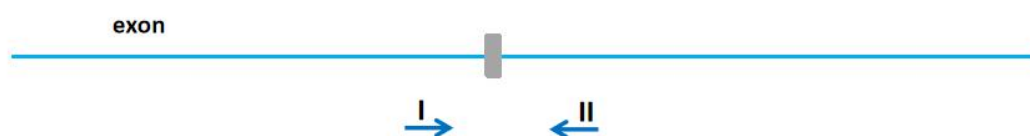
|                  |          |          |              |
|------------------|----------|----------|--------------|
| Common Name      | Znrf4-KO | Cat. NO. | NM-KO-240542 |
| Strain of Origin | C57BL/6J | Version  | V1           |

### Genotyping strategy

#### Wild type allele



#### knockout allele

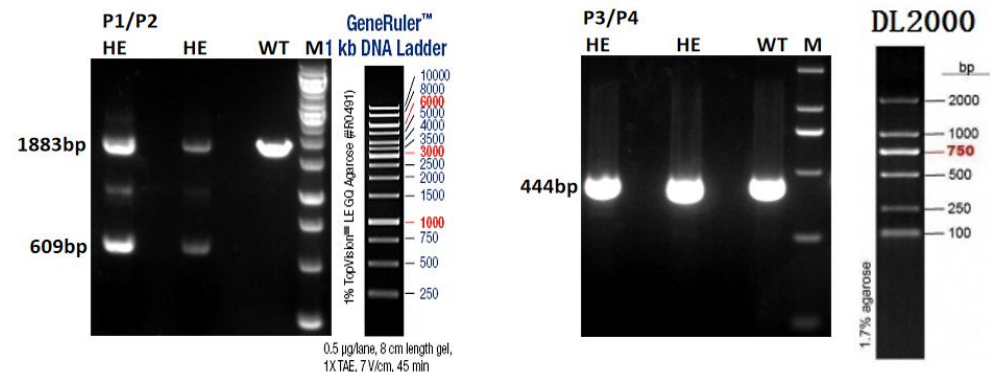


### Primers

| Primer | Sequence (5' → 3' )  | Primer type |
|--------|----------------------|-------------|
| P1     | TGAGAGGCTGCTCTGTCAAC | Forward     |
| P2     | AGCCCGTTTAACAGATGGGA | Reverse     |
| P3     | AATGAGGCGAATGACCTGGG | Forward     |

|    |                      |         |
|----|----------------------|---------|
| P4 | AGTTAACCTGAGCGTCCGTG | Reverse |
|----|----------------------|---------|

### Expected results

|          |                                                                                                                                                                          |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  |  <p>0.5 µg/lane, 8 cm length gel,<br/>1X TAE, 7 V/cm, 45 min</p>                       |
| Genotype | <p>Knockout type: -1274 bp</p> <p>Wild type: P1P2 =1883 bp; P3P4 =444 bp</p> <p>Heterozygote: P1P2 =1883 bp and 609 bp; P3P4 =444 bp</p> <p>Homozygote: P1P2 =609 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<br>Reaction<br>System | Reaction Component                 | Volume (µl) |
|                           | ddH2O                              | 8.0         |
|                           | 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   |                 |