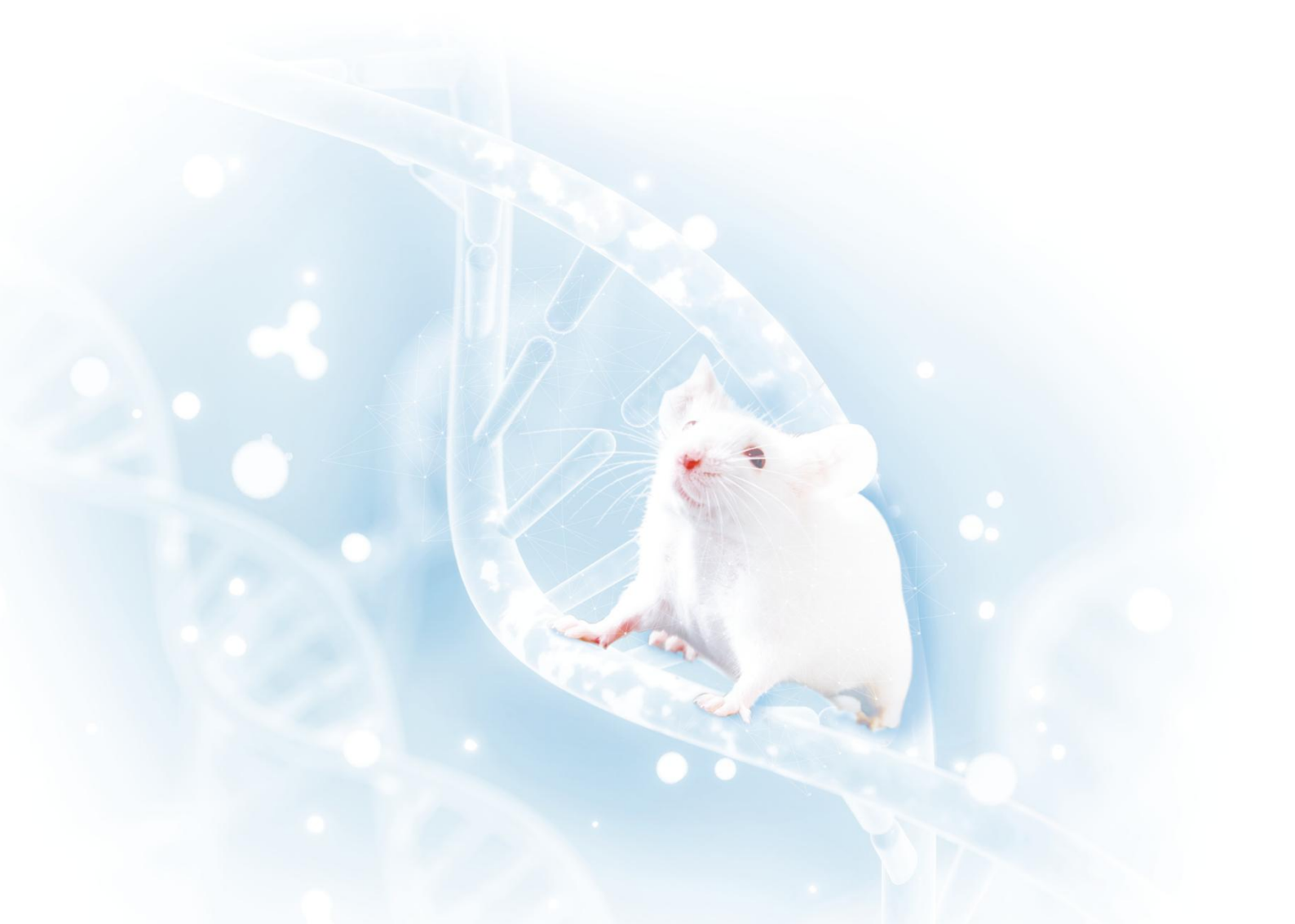


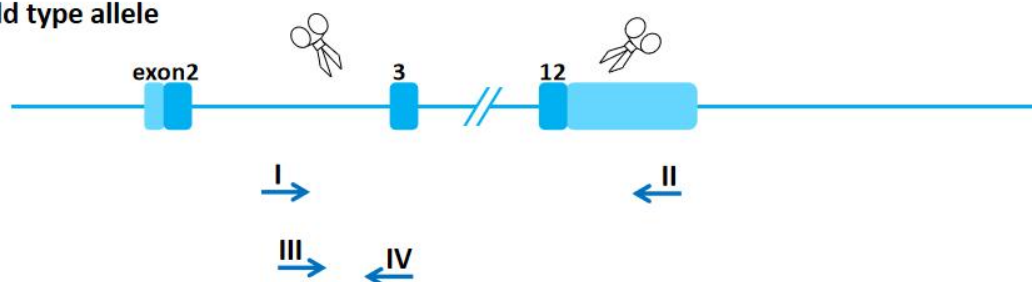
# Bora-KO Genotyping Protocol



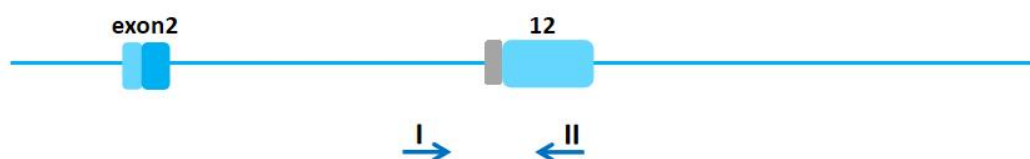
|                  |          |          |              |
|------------------|----------|----------|--------------|
| Common Name      | Bora-K0  | Cat. NO. | NM-K0-240291 |
| Strain of Origin | C57BL/6J | Version  | V1           |

### Genotyping strategy

#### Wild type allele



#### knockout allele



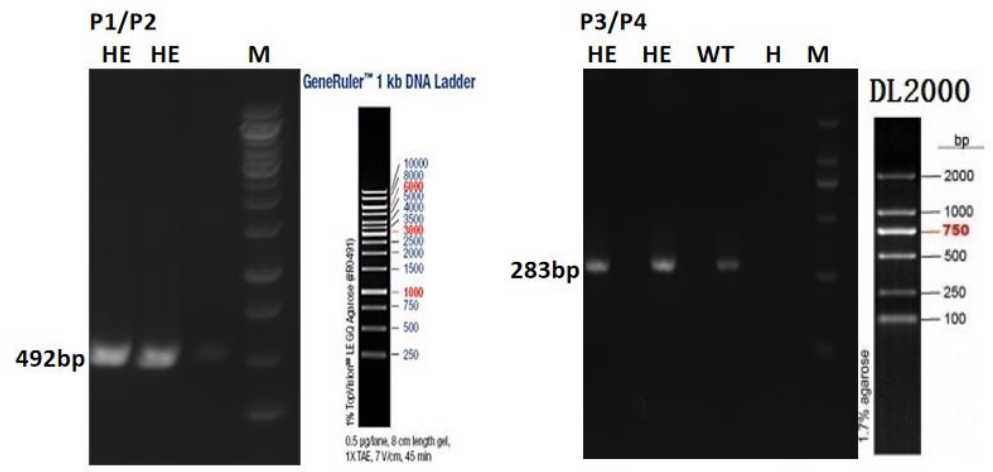
- : coding region    
  : uncoding region  
 : Cas9/gRNA    
 : mutation  
 NHEJ : Non-homologous end joining  
 : primer location

### Primers

| Primer | Sequence (5' → 3' )   | Primer type |
|--------|-----------------------|-------------|
| P1     | GGAGCTGTTGGAGCTACTTGT | Forward     |
| P2     | CCTGTAGGACAGGAGAAAGCC | Reverse     |
| P3     | GCAGCAGGTTGACTTAGTGG  | Forward     |

|    |                      |         |
|----|----------------------|---------|
| P4 | GCCTTAGGTAGGAAGCCTGC | Reverse |
|----|----------------------|---------|

### Expected results

|          |                                                                                                                                                                                                                                                                                                                                                                            |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  |  <p>The image shows two gel electrophoresis results. The left gel is for P1/P2 primers, showing bands at 492bp for HE and HE lanes, and a ladder (M) lane. The right gel is for P3/P4 primers, showing bands at 283bp for HE, HE, WT, H, and M lanes. A DL2000 ladder is also shown.</p> |
| Genotype | <p>Knockout type: -23855 bp</p> <p>Wild type: P3P4 =283 bp</p> <p>Heterozygote: P1P2 =492 bp; P3P4 =283 bp</p> <p>Homozygote: P1P2 =492 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 K0 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   |                 |