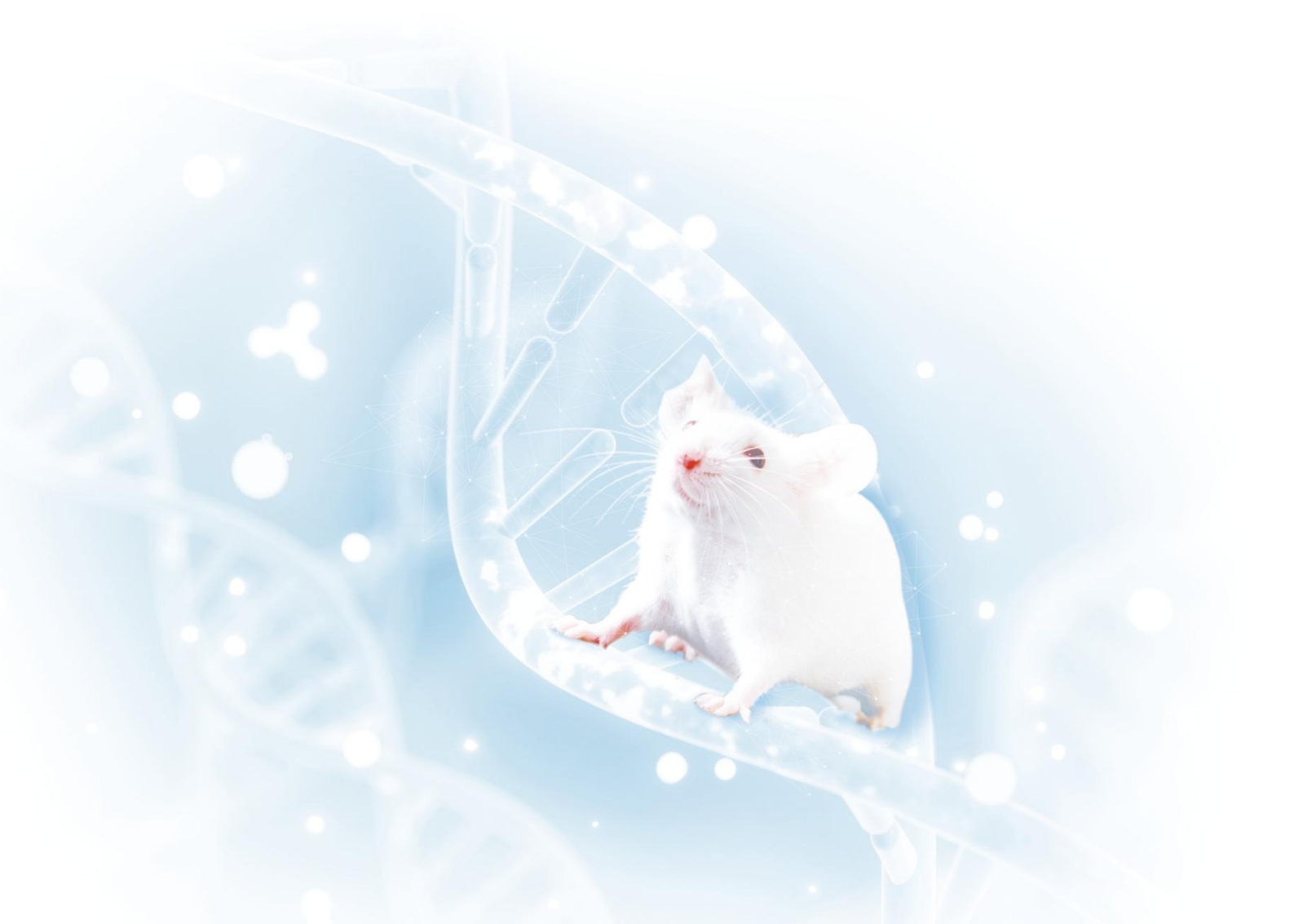
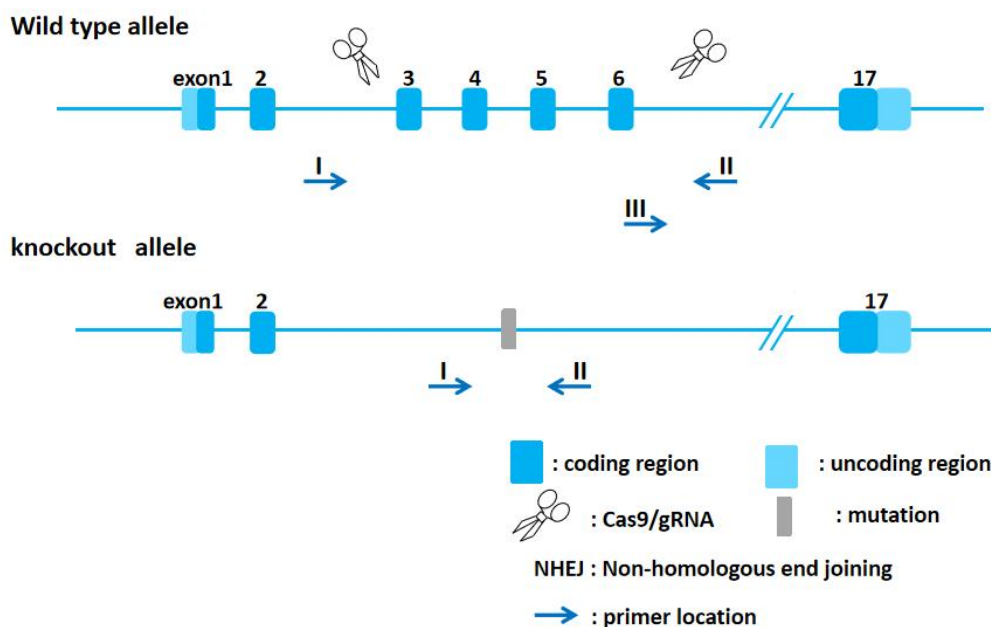


# Rasgrp1-K0 Genotyping Protocol



|                  |            |          |              |
|------------------|------------|----------|--------------|
| Common Name      | Rasgrp1-KO | Cat. NO. | NM-KO-232693 |
| Strain of Origin | C57BL/6J   | Version  | V1           |

### Genotyping strategy



### Primers

| Primer | Sequence (5' → 3' )  | Primer type |
|--------|----------------------|-------------|
| P1     | TGCAAATCATCATGGCCCT  | Forward     |
| P2     | TGAAGGGTATGTGTGCCCTC | Reverse     |
| P3     | CGCAAAGTGTCCCTGCTGTT | Forward     |

### Expected results

|         |                                                                                                                                              |  |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------|--|
| Results | <p style="text-align: center;"><b>P1P2P3</b></p>                                                                                             |  |
|         | <p>Knockout type: -7733 bp</p> <p>Wild type: P2P3 =414 bp</p> <p>Heterozygote: P1P2 =580 bp; P2P3=414 bp</p> <p>Homozygote: P1P2 =580 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<br>Reaction<br>System | Reaction Component                                      |       |        | Volume (μl)     |
|                           | ddH2O                                                   |       |        | 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<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   |                 |