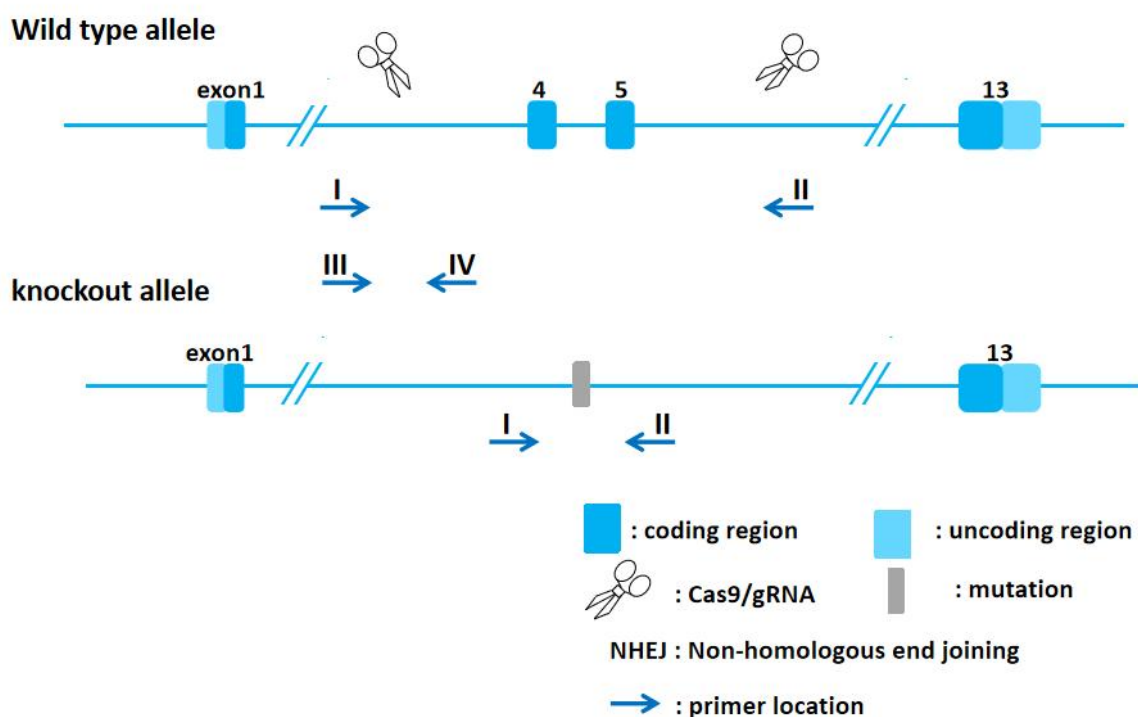


## Bmpr2-KO Genotyping Protocol



|                  |          |          |              |
|------------------|----------|----------|--------------|
| Common Name      | Bmpr2-KO | Cat. NO. | NM-KO-231426 |
| Strain of Origin | C57BL/6J | Version  | V1           |

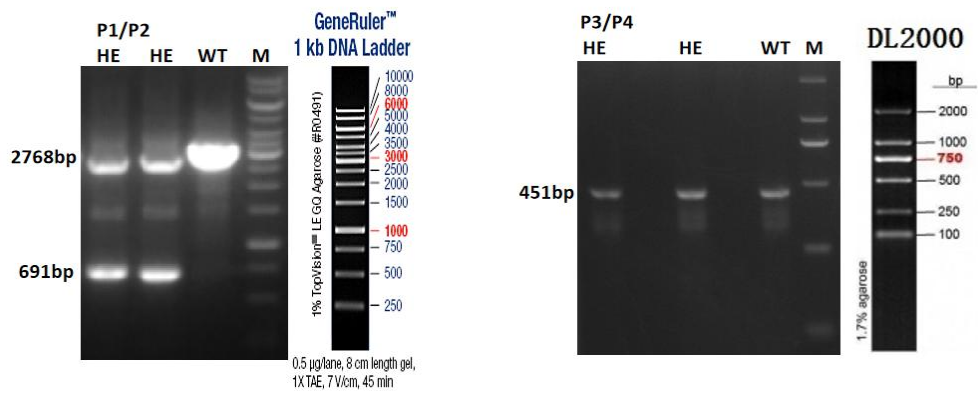
### Genotyping strategy



### Primers

| Primer | Sequence (5' → 3' )       | Primer type |
|--------|---------------------------|-------------|
| P1     | ACCTCAAAGCTTCCCCACAG      | Forward     |
| P2     | GTGCACTGGAATCAAAGGCG      | Reverse     |
| P3     | TACCTCAAAGCTTCCCCACAG     | Forward     |
| P4     | CAAACTAGCTTTTAGGAAACCTGTA | Reverse     |

## Expected results

|          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  |  <p>GeneRuler™<br/>1 kb DNA Ladder</p> <p>P1/P2<br/>HE HE WT M</p> <p>2768bp<br/>691bp</p> <p>P3/P4<br/>HE HE WT M</p> <p>451bp</p> <p>DL2000</p> <p>bp</p> <p>10000<br/>8000<br/>6000<br/>5000<br/>4000<br/>3500<br/>3000<br/>2500<br/>2000<br/>1500<br/>1000<br/>750<br/>500<br/>250</p> <p>1% TopView™ LE GQ Agarose #R0.491</p> <p>0.5 µg/lane, 8 cm length gel,<br/>1X TAE, 7 V/cm, 45 min</p> <p>1.7% agarose</p> |
| Genotype | <p>Knockout type: -2077 bp</p> <p>Wild type: P1P2 =2768 bp; P3P4 =451 bp</p> <p>Heterozygote: P1P2 =2768 bp and 691 bp; P3P4 =451 bp</p> <p>Homozygote: P1P2 =691 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/ $\mu$ l) or P4 (10 pmol/ $\mu$ l)                     |       |        | 0.5             |
|                     | Genomic DNA                                                        |       |        | 1.0             |
|                     | Total                                                              |       |        | 20              |
|                     | 2 $\times$ 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   |                 |