

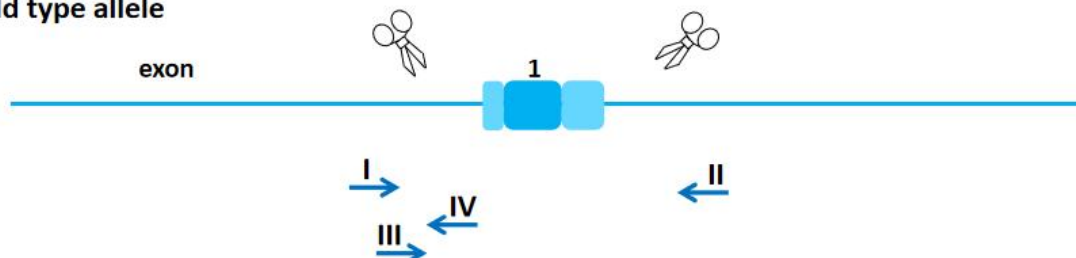
# Ch25h-KO Genotyping Protocol



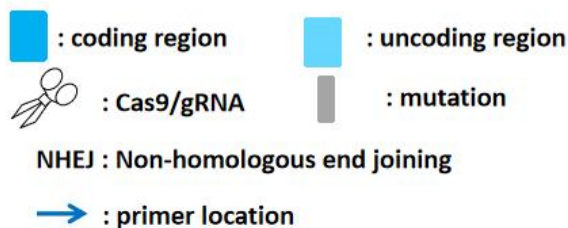
|                         |          |                 |              |
|-------------------------|----------|-----------------|--------------|
| <b>Common Name</b>      | Ch25h-KO | <b>Cat. NO.</b> | NM-KO-230396 |
| <b>Strain of Origin</b> | C57BL/6J | <b>Version</b>  | V1           |

## Genotyping strategy

### Wild type allele



### knockout allele



## Primers

| Primer    | Sequence (5'→3')       | Primer type |
|-----------|------------------------|-------------|
| <b>P1</b> | ACCAAATGGGAAGAGCCTGAA  | Forward     |
| <b>P2</b> | ATGCTGAGCCCACAATGCTA   | Reverse     |
| <b>P3</b> | GCTGAGGCCTTAGATAGAGAGC | Forward     |
| <b>P4</b> | GGCCATTCTCCCAGGGTTTC   | Reverse     |

## Expected results

|          |                                                                                                                                                                                                                                                                                                                      |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  | <p><b>P1/P2</b><br/>HE HE WT M<br/>3828bp<br/>717bp</p> <p><b>P3/P4</b><br/>HE HE WT M H<br/>304bp</p> <p>GeneRuler™ 1 kb DNA Ladder<br/>1% TopView™ LE Gel Agarose (#16491)<br/>0.5 µg/lane, 8 cm length gel,<br/>1x TBE, 7 V/cm, 45 min</p> <p>DL2000<br/>bp<br/>2000<br/>1000<br/>750<br/>500<br/>250<br/>100</p> |
| Genotype | <p>Knockout type: -3111bp</p> <p>Wild type: P1P2 =3828 bp; P3P4 =304 bp</p> <p>Heterozygote: P1P2 =3828 bp and 717 bp; P3P4 =304 bp</p> <p>Homozygote: P1P2 =717 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)     |
|                           | ddH <sub>2</sub> O                                       |      |        | 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   |                 |