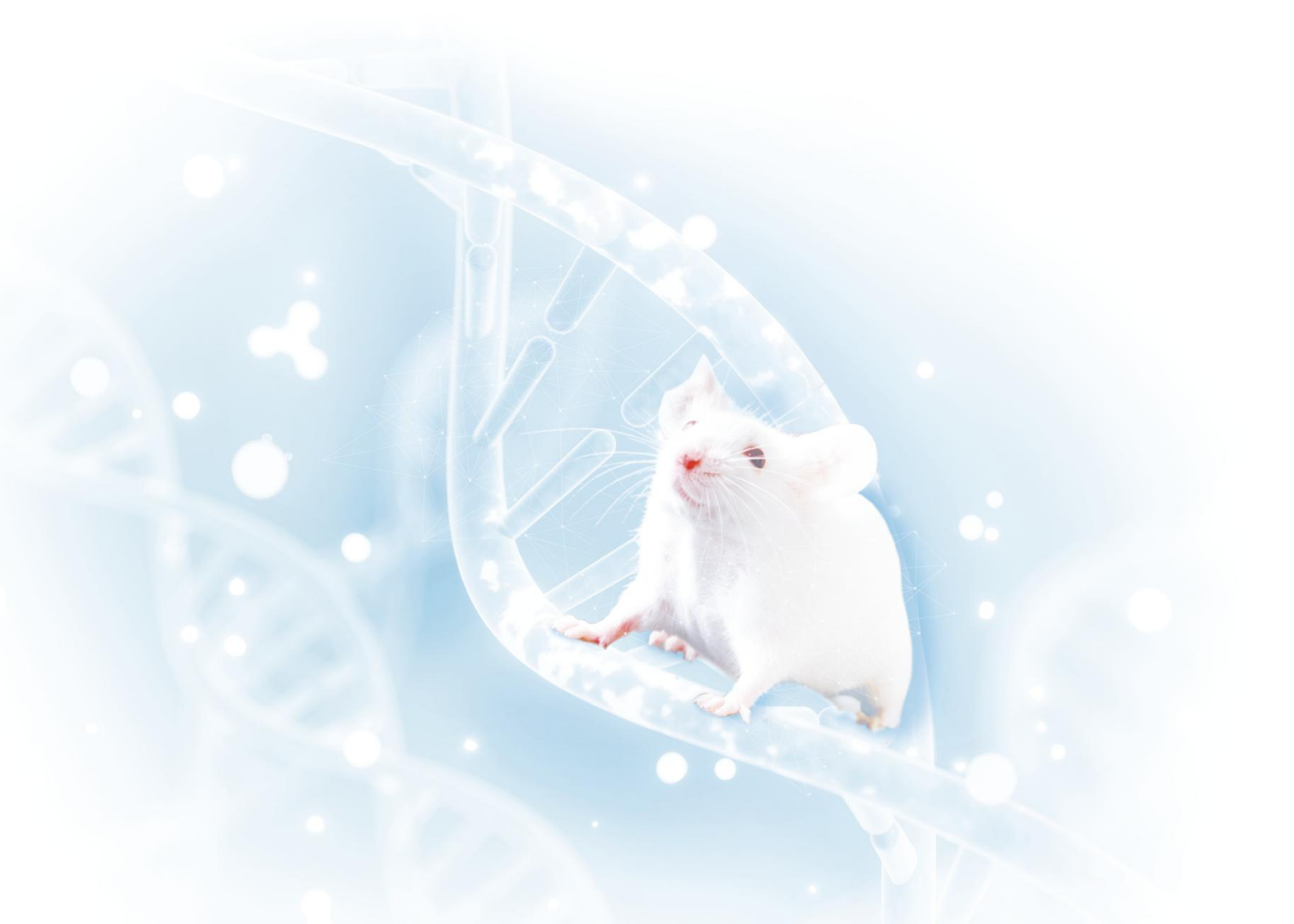


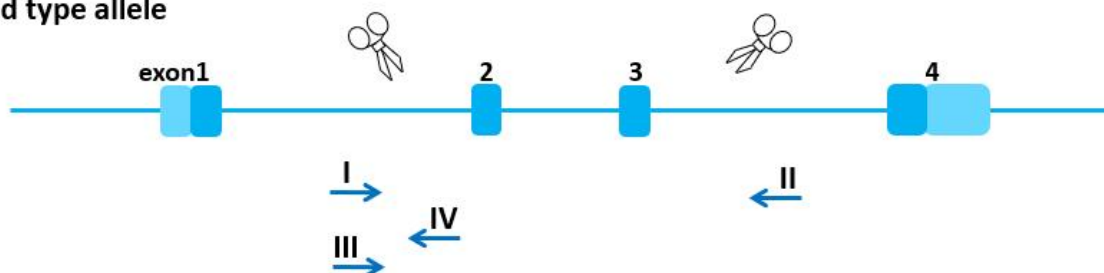
# Cybrd1-KO Genotyping Protocol



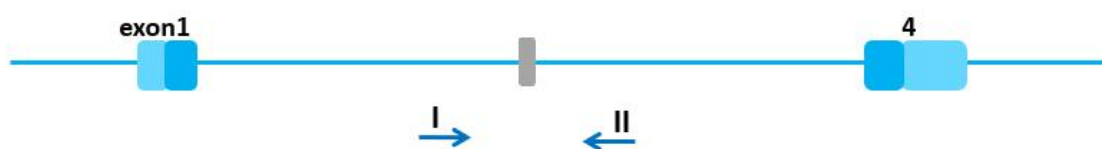
|                         |           |                 |              |
|-------------------------|-----------|-----------------|--------------|
| <b>Common Name</b>      | Cybrd1-KO | <b>Cat. NO.</b> | NM-KO-241245 |
| <b>Strain of Origin</b> | C57BL/6J  | <b>Version</b>  | V1           |

## Genotyping strategy

### Wild type allele



### knockout allele



 : coding region       : uncoding region

 : Cas9/gRNA       : mutation

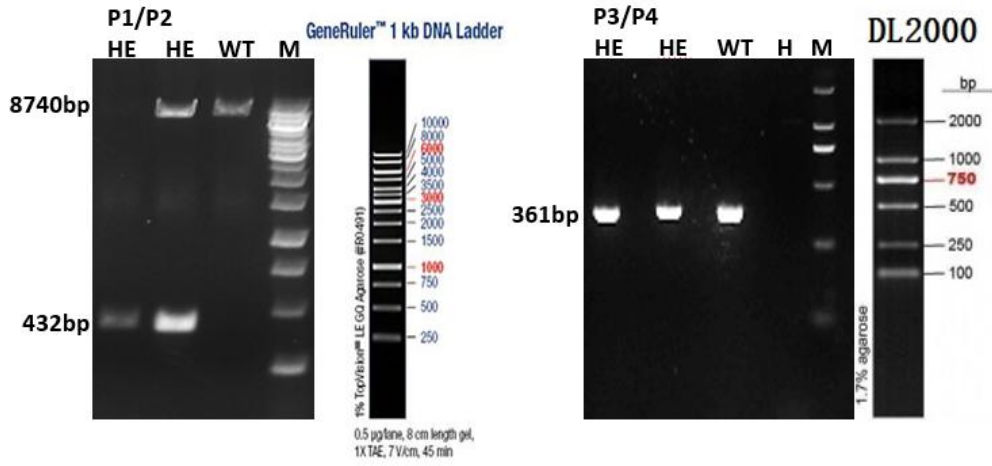
NHEJ : Non-homologous end joining

 : primer location

## Primers

| Primer    | Sequence (5'→3')      | Primer type |
|-----------|-----------------------|-------------|
| <b>P1</b> | CAGGGCCCAATTGTCCAGAT  | Forward     |
| <b>P2</b> | ACCCAATAATTTCAACACACA | Reverse     |
| <b>P3</b> | AGGACATCAGTTTGGTGGC   | Forward     |
| <b>P4</b> | AACCCGGCATGGATGGATTT  | Reverse     |

## Expected results

|          |                                                                                                                                                                                                |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  |  <p>GeneRuler™ 1 kb DNA Ladder</p> <p>DL2000</p> <p>0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min</p> |
| Genotype | <p>Knockout type: -8308bp</p> <p>Wild type: P1P2 =8740 bp; P3P4 =361 bp</p> <p>Heterozygote: P1P2 =8740 bp and 432 bp; P3P4 =361 bp</p> <p>Homozygote: P1P2 =432 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   |                 |