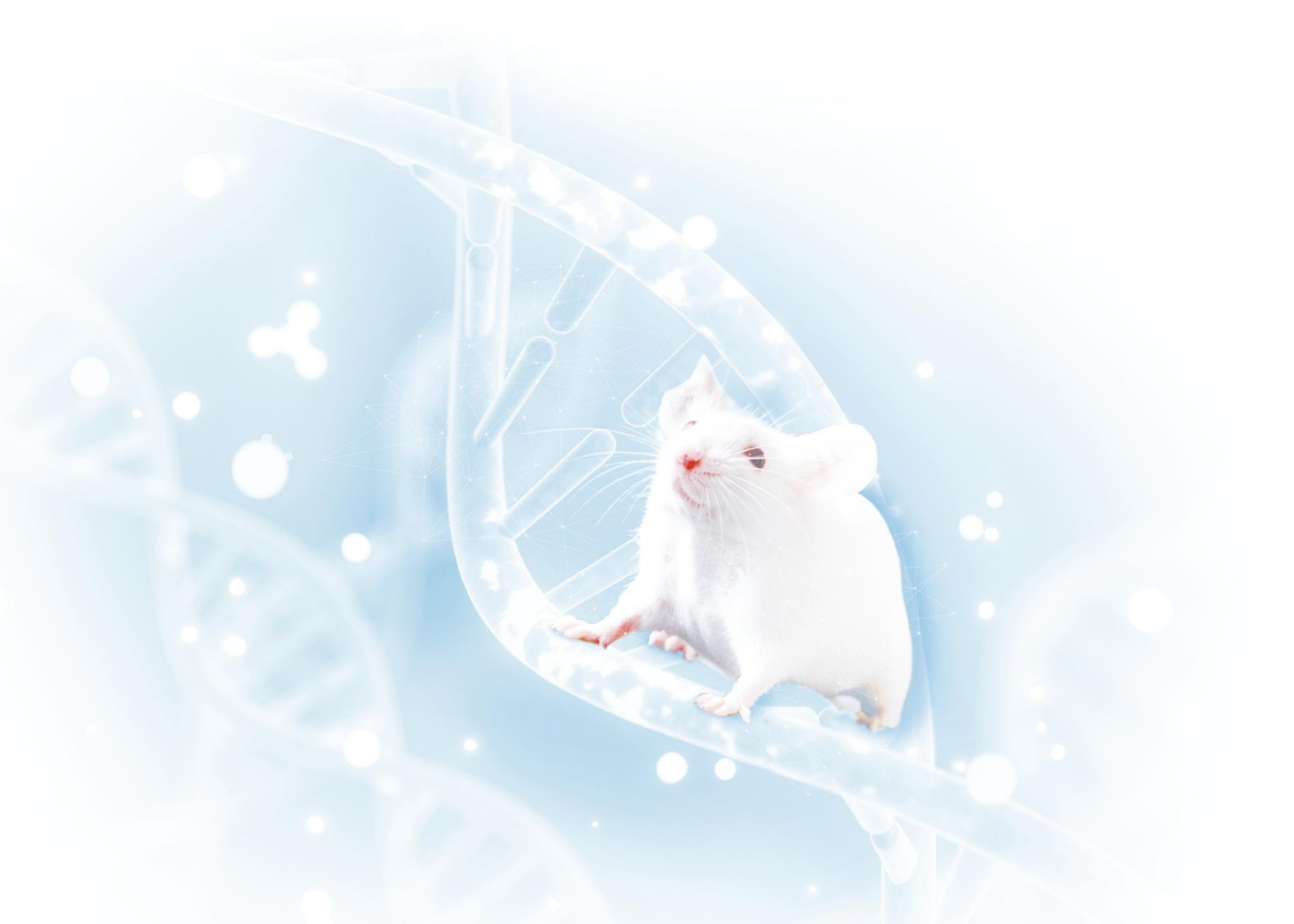
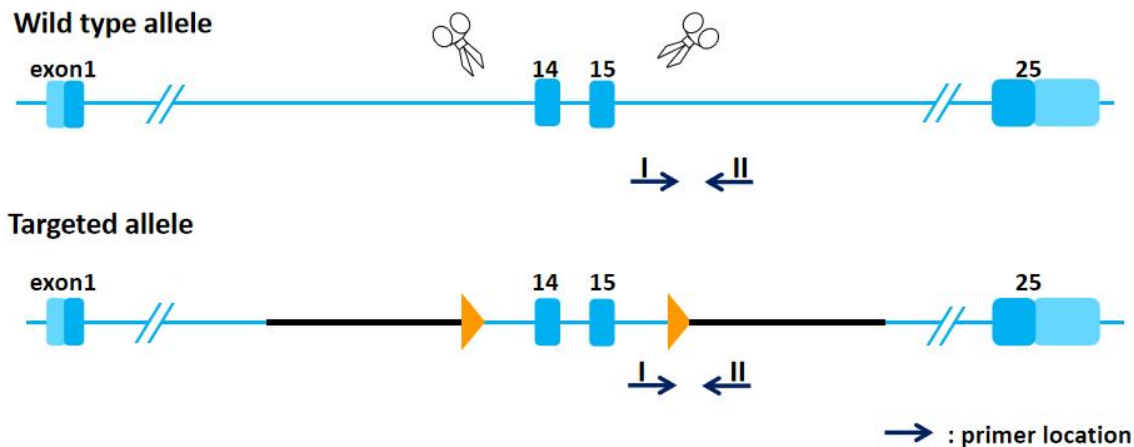


# Ace-Flox Genotyping Protocol



|                  |          |          |               |
|------------------|----------|----------|---------------|
| Common Name      | Ace-Flox | Cat. NO. | NM-CK0-233983 |
| Strain of Origin | C57BL/6J | Version  | V1            |

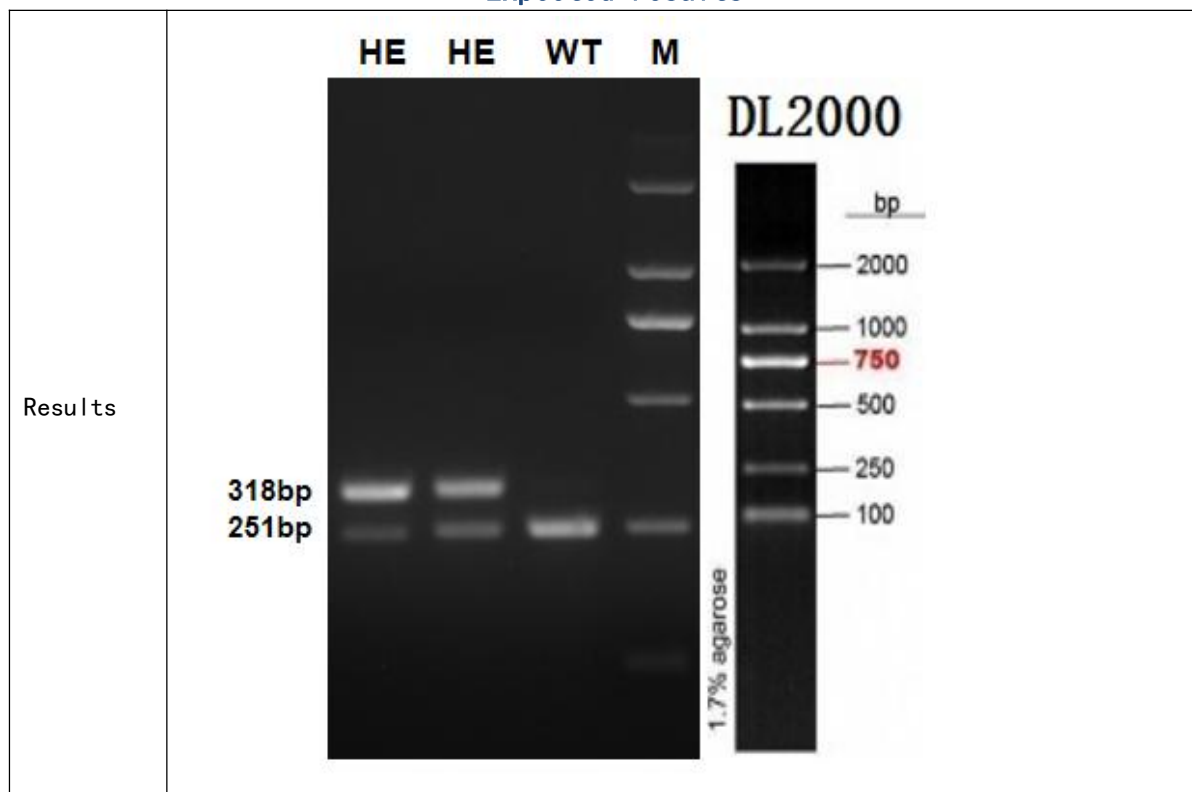
### Genotyping strategy



### Primers

| Primer | Sequence (5' → 3' ) | Primer type |
|--------|---------------------|-------------|
| P1     | TGTAAGGTTGTCTGCCAG  | Forward     |
| P2     | GCTAGGAACAGGAGGCAAG | Reverse     |

### Expected results



|          |                                                                                                |
|----------|------------------------------------------------------------------------------------------------|
| Genotype | Wild type: P1P2 =251 bp;<br>Heterozygote: P1P2 =318 bp and 251 bp;<br>Homozygote: P1P2 =318 bp |
|----------|------------------------------------------------------------------------------------------------|

### Reaction &Cycling

|                           |                                                         |       |        |                 |
|---------------------------|---------------------------------------------------------|-------|--------|-----------------|
| PCR<br>Reaction<br>System | Reaction Component                                      |       |        | Volume (μl)     |
|                           | ddH2O                                                   |       |        | 8.0             |
|                           | 2×Taq Plus Master Mix                                   |       |        | 10.0            |
|                           | P1 (10 pmol/μl)                                         |       |        | 0.5             |
|                           | P2 (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   |                 |