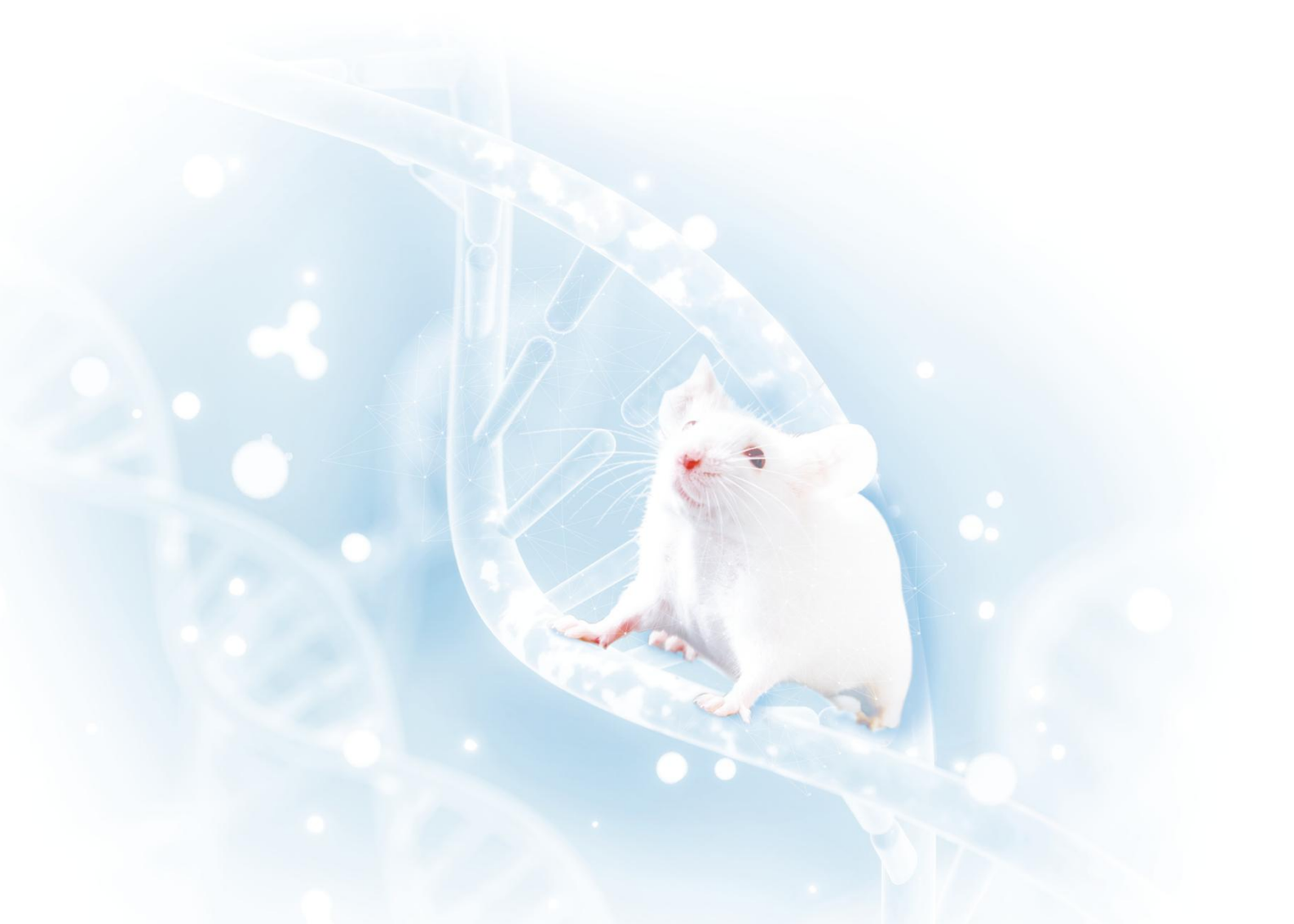


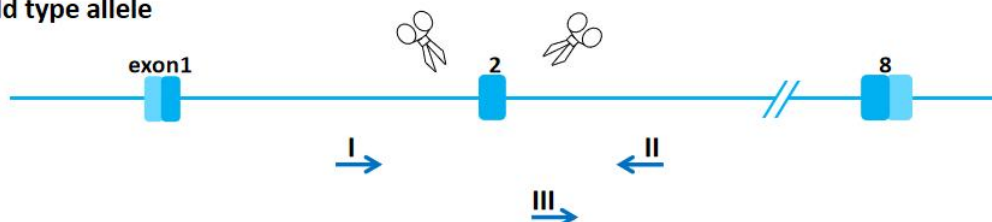
# U2af1-K0 Genotyping Protocol



|                  |          |          |              |
|------------------|----------|----------|--------------|
| Common Name      | U2af1-KO | Cat. NO. | NM-KO-205051 |
| Strain of Origin | C57BL/6J | Version  | 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 |
|--------|------------------------|-------------|
| P1     | CGTCCACCATGTGACCTTCA   | Forward     |
| P2     | CTCAGGATGCCTTGTGGTGT   | Reverse     |
| P3     | CTTGTGCTTTCAGGAGCGATTA | Forward     |

### Expected results

|         |        |    |    |                  |   |                                                |
|---------|--------|----|----|------------------|---|------------------------------------------------|
| Results | P1P2P3 |    |    |                  |   |                                                |
|         | HE     | HE | WT | H <sub>2</sub> O | M | DL2000                                         |
|         |        |    |    |                  |   | bp<br>2000<br>1000<br>750<br>500<br>250<br>100 |

|          |                                         |
|----------|-----------------------------------------|
| Genotype | Knockout type: -756bp                   |
|          | Wild type: P3P2=354 bp                  |
|          | Heterozygote: P1P2 =626 bp; P3P2=354 bp |
|          | Homozygote: P1P2 =626 bp                |

**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                                      |       |        | 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   |                 |