

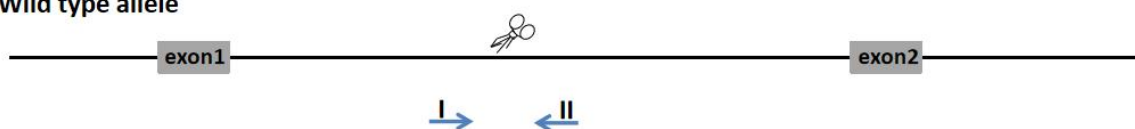
# R26-CAG-RSR-ZsGreen-Insulator-CAG-LSL-tdTomato Genotyping Protocol



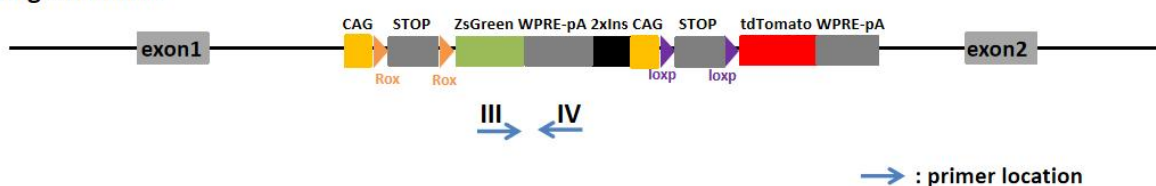
|                  |                             |          |              |
|------------------|-----------------------------|----------|--------------|
| Common Name      | R26-CAG-RSR-ZsGreen-In      | Cat. NO. | NM-KI-233483 |
|                  | ulator-CAG-LSL-tdToma<br>to |          |              |
| Strain of Origin | C57BL/6J                    | Version  | V1           |

### Genotyping strategy

#### Wild type allele



#### Targeted allele



### Primers

| Primer | Sequence (5' → 3' )    | Primer type |
|--------|------------------------|-------------|
| P1     | AGGCAGGGTCTCACTATGTAT  | Forward     |
| P2     | TTGATGGGGGAAAACCTGAATG | Reverse     |
| P3     | AGTCCAAGTTCTACGGCGTG   | Forward     |
| P4     | AGCAGCGTATCCACATAGCG   | Reverse     |

### Expected results

|          |                                                                                                                 |
|----------|-----------------------------------------------------------------------------------------------------------------|
| Results  | <p style="text-align: center;"><b>P3+P4</b></p>                                                                 |
| Genotype | <p>Wild type: P1P2 =451 bp;</p> <p>Heterozygote: P1P2 =451 bp; P3P4 =426 bp</p> <p>Homozygote: P3P4 =426 bp</p> |

### 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/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   |                 |