

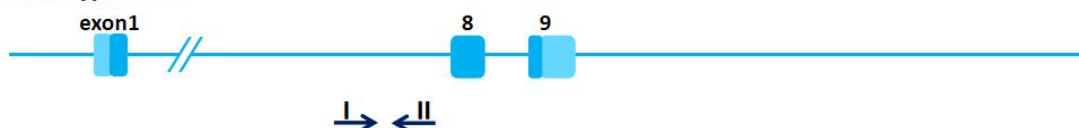
# Calr-del-flox Genotyping Protocol



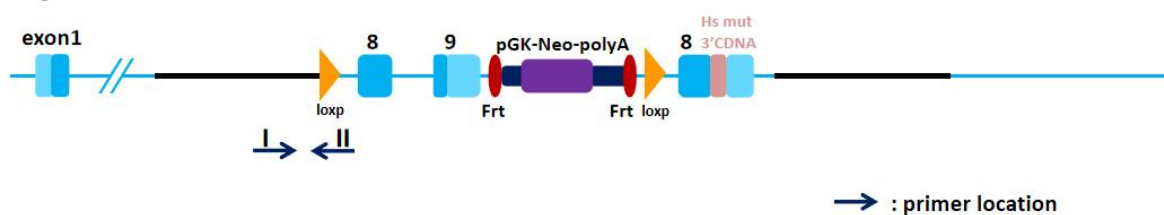
|                  |               |          |              |
|------------------|---------------|----------|--------------|
| Common Name      | Calr-del-flox | Cat. NO. | NM-KI-231939 |
| Strain of Origin | C57BL/6J      | Version  | V1           |

### Genotyping strategy

#### Wild type allele



#### Targeted allele



### Primers

| Primer | Sequence (5' → 3' )  | Primer type |
|--------|----------------------|-------------|
| P1     | AATGCTCCCAGGATCACAGC | Forward     |
| P2     | CAAAATGTGTGGGGCAGCAG | Reverse     |

### Expected results

|          |                                                                                                                                                                                                     |
|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Results  | <p><b>P1+P2</b></p> <p>HE HE HE HE WT M</p> <p><b>DL2000</b></p> <p>bp</p> <p>2000</p> <p>1000</p> <p><b>750</b></p> <p>500</p> <p>250</p> <p>100</p> <p>1.7% agarose</p> <p>337bp</p> <p>278bp</p> |
| Genotype | <p>Wild type: P1P2 =278 bp</p> <p>Heterozygote: P1P2 =337 bp and 278 bp</p> <p>Homozygote: P1P2 =337 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)                                           | 0.5         |
|                           | P2 (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   |                 |