

RBRC 01515

Strain name C57BL/6-Tg(SLC18A3-cre)KMisa/MisaRbrc

| Primer | primer_name | primer_seq                  | length | Reference                              |
|--------|-------------|-----------------------------|--------|--|
| 1      | promoter    | GCGGAGATCGCAAGCCTCTGG       | 21     | RIKEN BRC                              |
| 2      |             | CGAGTTGATAGCTGGCTGG         | 19     | Genesis., 37, 44-50 (2003).            |
| 3      | mLC3ex4AG   | GTTAGCATTGAGCTGCAAGCGCCGTCT | 27     | Mol. Biol. Cell., 15, 1101-1111(2004). |
| 4      | mLC3ex3GT   | TGAGCGAGCTCATCAAGATAATCAGGT | 27     | Mol. Biol. Cell., 15, 1101-1111(2004). |
|        |             |                             |        |  |
|        |             |                             |        |  |
|        |             |                             |        |  |
|        |             |                             |        |  |
|        |             |                             |        |  |
|        |             |                             |        |  |

**Reaction Components**

Enzyme

**Reaction Conditions**

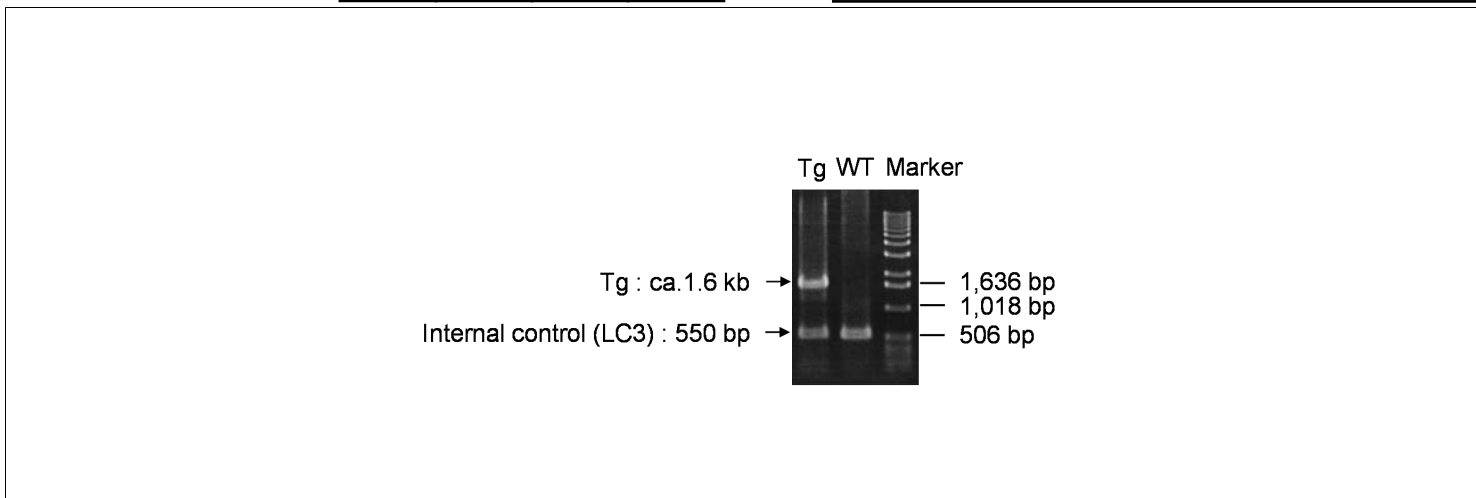
| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 120        |                       |
| 2    | 98        | 10*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 68        | 180*       | *Repeat for 30 cycles |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

| Reagent      | Conc       | Reaction 1 | 2 | 3 | 4 |
|--------------|------------|------------|---|---|---|
| H2O          |            | 0.8        |   |   |   |
| PCR buffer   | 2 X        | 10.0       |   |   |   |
| dNTP         | 2.0 mM     | 4.0        |   |   |   |
| primer 1     | 10 pmol/μl | 1.0        |   |   |   |
| primer 2     | 10 pmol/μl | 1.0        |   |   |   |
| primer 3     | 10 pmol/μl | 1.0        |   |   |   |
| primer 4     | 10 pmol/μl | 1.0        |   |   |   |
| DNA          |            | 1.0        |   |   |   |
| Taq          | 1 U/μl     | 0.2        |   |   |   |
|              |            |            |   |   |   |
|              |            |            |   |   |   |
|              |            |            |   |   |   |
|              |            |            |   |   |   |
| <b>total</b> |            | 20         |   |   |   |

(μL)

**Marker**

**Gel**



| primer_set | product_size | product (allele type)  |
|------------|--------------|------------------------|
| primer 1   | primer 2     | ca 1.6 kb              |
| primer 3   | primer 4     | 550 bp                 |
|            |              | Tg                     |
|            |              | Internal control (LC3) |
|            |              |                        |
|            |              |                        |
|            |              |                        |
|            |              |                        |

**Comment** [Standard PCR](#)

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

RBRC 01515

Strain name VChT-Cre.Fast

| Primer | primer_name        | primer_seq                | length | Reference               |
|--------|--------------------|---------------------------|--------|-------------------------|
| 1      | VChT-Cre transgene | CAGAAACCAAAGGGATTTGGAG    | 22     | Depositor's primer      |
| 2      |                    | GAAACCTGTGAGTGTGGTGGC     | 21     | Depositor's primer      |
| 3      | Cre-coding region  | ACCTGATGGACATGTTTCAGG     | 20     | Depositor's primer      |
| 4      |                    | CGAGTTGATAGCTGGCTGG       | 19     | Depositor's primer      |
| 5      |                    | ACCAAGAAGTGGGAAGCATC      | 20     | Depositor's primer      |
| 6      |                    | TCCAGCAGGCCAGAGTCTC       | 20     | Depositor's primer      |
| 7      | oIMR0042           | CTAGGCCACAGAATTGAAAGATCT  | 24     | JAX Genotyping Protocol |
| 8      | oIMR0043           | GTAGGTGGAAATTCTAGCATCATCC | 25     | JAX Genotyping Protocol |

### Reaction Components

Enzyme

| Reagent      | Conc       | Reaction 1 | 2         | 3 | 4 |
|--------------|------------|------------|-----------|---|---|
| H2O          |            | 16.3       | 15.3      |   |   |
| PCR buffer   | 10 X       | 2.5        | 2.5       |   |   |
| dNTP         | 2.5 mM     | 2.0        | 2.0       |   |   |
| primer 1     | 10 pmol/μl | 0.5        | 0         |   |   |
| primer 2     | 10 pmol/μl | 0.5        | 0         |   |   |
| primer 3     | 10 pmol/μl | 1.0        | 0         |   |   |
| primer 4     | 10 pmol/μl | 1.0        | 0         |   |   |
| primer 5     | 10 pmol/μl | 0          | 1.0       |   |   |
| primer 6     | 10 pmol/μl | 0          | 1.0       |   |   |
| primer 7     | 10 pmol/μl | 0          | 1.0       |   |   |
| primer 8     | 10 pmol/μl | 0          | 1.0       |   |   |
| DNA          |            | 1.0        | 1.0       |   |   |
| Taq          |            | 0.2        | 0.2       |   |   |
| <b>total</b> |            | <b>25</b>  | <b>25</b> |   |   |

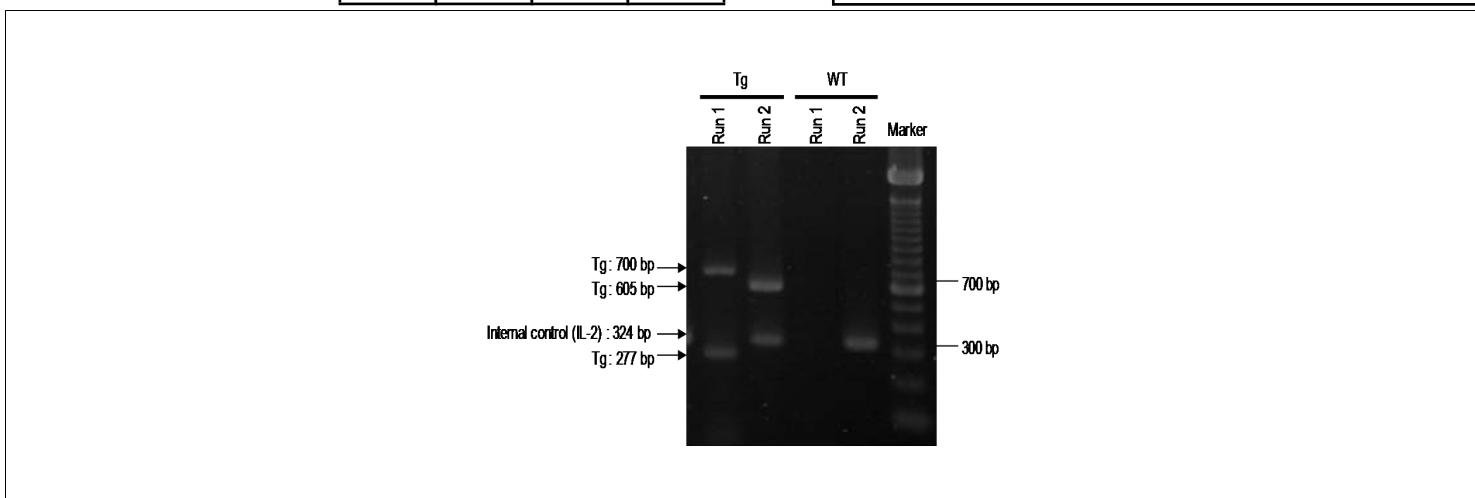
(μL)

### Reaction Conditions

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 180        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 72        | 60*        | *Repeat for 35 cycles |
| 5    | 72        | 300        |                       |

Marker

Gel



| primer_set | product_size | product (allele type)          |
|------------|--------------|--------------------------------|
| primer 1   | primer 2     | 277 bp Tg (VChT)               |
| primer 3   | primer 4     | 700 bp Tg (Cre)                |
| primer 5   | primer 6     | 605 bp Tg (CHAT)               |
| primer 7   | primer 8     | 324 bp Internal control (IL-2) |
|            |              |                                |
|            |              |                                |

Comment [Separated PCR](#)