

RBRC 10636

Strain name C57BL/6J-Tg(Chat-neo,-tTA)803Sleep

| Primer | primer_name     | primer_seq                | length | Reference               |
|--------|-----------------|---------------------------|--------|-------------------------|
| 1      | exon 3          | TTCAGGCTCTGTGTGATGCTGAG   | 23     | RIKEN BRC               |
| 2      | tTA-Ad-386bp-rv | CCAGGCCACATATGATTAGTTCCAG | 25     | Depositor's primer      |
| 3      | oIMR0042        | CTAGGCCACAGAATTGAAAGATCT  | 24     | JAX Genotyping Protocol |
| 4      | oIMR0043        | GTAGGTGGAAATTCTAGCATCATCC | 25     | JAX Genotyping Protocol |
|        |                 |                           |        |                         |
|        |                 |                           |        |                         |
|        |                 |                           |        |                         |
|        |                 |                           |        |                         |
|        |                 |                           |        |                         |

**Reaction Components**

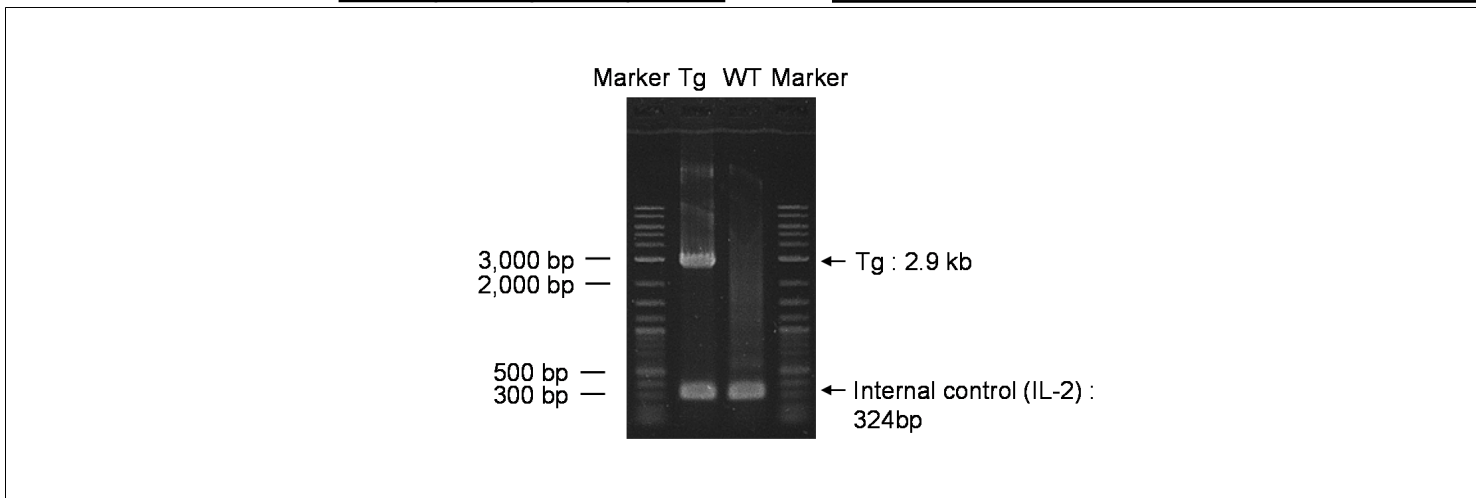
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

| Reagent      | Conc             | Reaction 1 | 2 | 3 | 4 | ( $\mu$ L) |
|--------------|------------------|------------|---|---|---|------------|
| H2O          |                  | 0.8        |   |   |   |            |
| PCR buffer   | 2 X              | 10.0       |   |   |   |            |
| dNTP         | 2 mM             | 4.0        |   |   |   |            |
| primer 1     | 10 pmol/ $\mu$ l | 1.0        |   |   |   |            |
| primer 2     | 10 pmol/ $\mu$ l | 1.0        |   |   |   |            |
| primer 3     | 10 pmol/ $\mu$ l | 1.0        |   |   |   |            |
| primer 4     | 10 pmol/ $\mu$ l | 1.0        |   |   |   |            |
| DNA          |                  | 1.0        |   |   |   |            |
| Taq          | 1 U/ $\mu$ l     | 0.2        |   |   |   |            |
|              |                  |            |   |   |   |            |
|              |                  |            |   |   |   |            |
|              |                  |            |   |   |   |            |
|              |                  |            |   |   |   |            |
|              |                  |            |   |   |   |            |
| <b>total</b> |                  | <b>20</b>  |   |   |   |            |

**Reaction Conditions**

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 120        |                       |
| 2    | 98        | 10*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 68        | 240*       | *Repeat for 30 cycles |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

**Marker** 2-Log DNA Ladder (NEB, Cat. N3200)  
**Gel** 1% agarose in 1 X TAE (100 V, 30 min)



| primer_set          | product_size | product (allele type)   |
|---------------------|--------------|-------------------------|
| primer 1 / primer 2 | 2.9 kb       | Tg                      |
| primer 3 / primer 4 | 324 bp       | Internal control (IL-2) |
|                     |              |                         |
|                     |              |                         |
|                     |              |                         |

**Comment** Standard PCR

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