

# PCR protocol

RBRC 06583

Strain name C57BL/6-Tg(Gt(ROSA)26Sor-eNpHR3/EYFP)1Imayo

| Primer | primer_name   | primer_seq                | length |                    |
|--------|---------------|---------------------------|--------|--------------------|
| 1      | RCE-Rosa1     | CCCAAAGTCGCTCTGAGTTGTTATC | 25     | Depositor's primer |
| 2      | RCE-Rosa2     | GAAGGAGCGGGAGAAATGGATATG  | 24     | Depositor's primer |
| 3      | LFcheck-HR-rv | TGAAGGGCCACGGCACTCTCGGTC  | 24     | Depositor's primer |
|        |               |                           |        |                    |
|        |               |                           |        |                    |
|        |               |                           |        |                    |
|        |               |                           |        |                    |
|        |               |                           |        |                    |
|        |               |                           |        |                    |
|        |               |                           |        |                    |
|        |               |                           |        |                    |

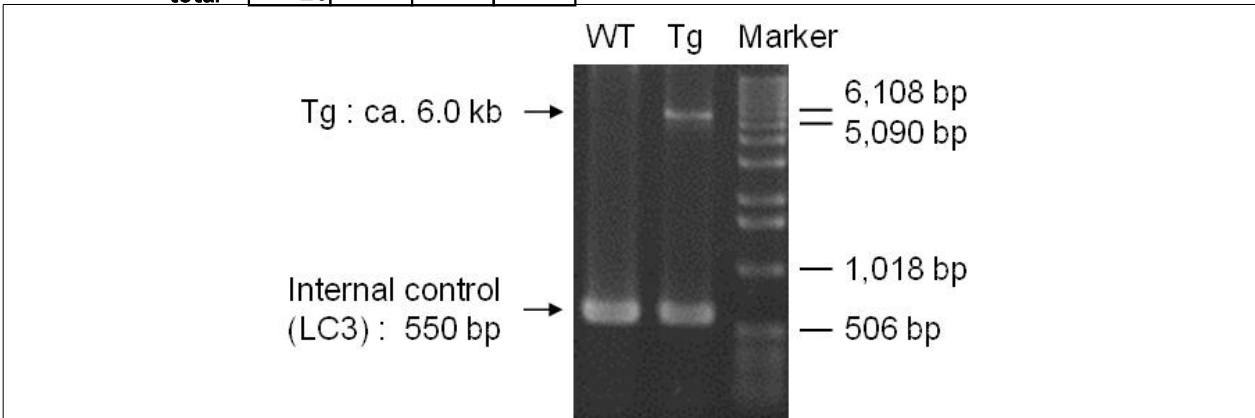
**Reaction Components**

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

**Reaction Conditions**

|                  | Conc             | Run 1 | Run 2 | Run 3 | Run 4 |
|------------------|------------------|-------|-------|-------|-------|
| H <sub>2</sub> O |                  | 1.6   |       |       |       |
| PCR buffer       | 2 X              | 10.0  |       |       |       |
| dNTP             | 2.0 mM           | 4.0   |       |       |       |
| primer 1         | 10 pmol/ $\mu$ l | 1.5   |       |       |       |
| primer 2         | 10 pmol/ $\mu$ l | 0.5   |       |       |       |
| primer 3         | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| DNA              |                  | 1.0   |       |       |       |
| Taq              | 1 U/ $\mu$ l     | 0.4   |       |       |       |
| total            |                  | 20    |       |       |       |

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 120        |                       |
| 2    | 98        | 10*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 68        | 420*       | *Repeat for 30 cycles |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |



**Marker**  
1 kb DNA ladder (Invitrogen, Cat. 15615-024)

**Gel**  
1% agarose in 1 X TAE (100 V, 30 min)

**PCR products**

| primer_set        | product_size | product |
|-------------------|--------------|---------|
| primer 1 primer 2 | 550 bp       | WT      |
| primer 1 primer 3 | ca. 6.0 kb   | Tg      |
|                   |              |         |
|                   |              |         |
|                   |              |         |
|                   |              |         |

**Comment**