

RBRC 06270

Strain name C57BL/6N-Tgm3<tm1a(KOMP)Wtsi>/B12

| Primer | primer_name | primer_seq               | length |           |
|--------|-------------|--------------------------|--------|-----------|
| 1      | 14997-15020 | GACAGTTACACTATCACCTCCATC | 24     | RIKEN BRC |
| 2      |             | GCCAAGCATAGTCAATAACGGAGC | 24     | RIKEN BRC |
| 3      |             | GGCACATGGCTGAATATCGACGG  | 23     | RIKEN BRC |
|        |             |                          |        |           |
|        |             |                          |        |           |
|        |             |                          |        |           |
|        |             |                          |        |           |
|        |             |                          |        |           |
|        |             |                          |        |           |
|        |             |                          |        |           |
|        |             |                          |        |           |

### Reaction Components

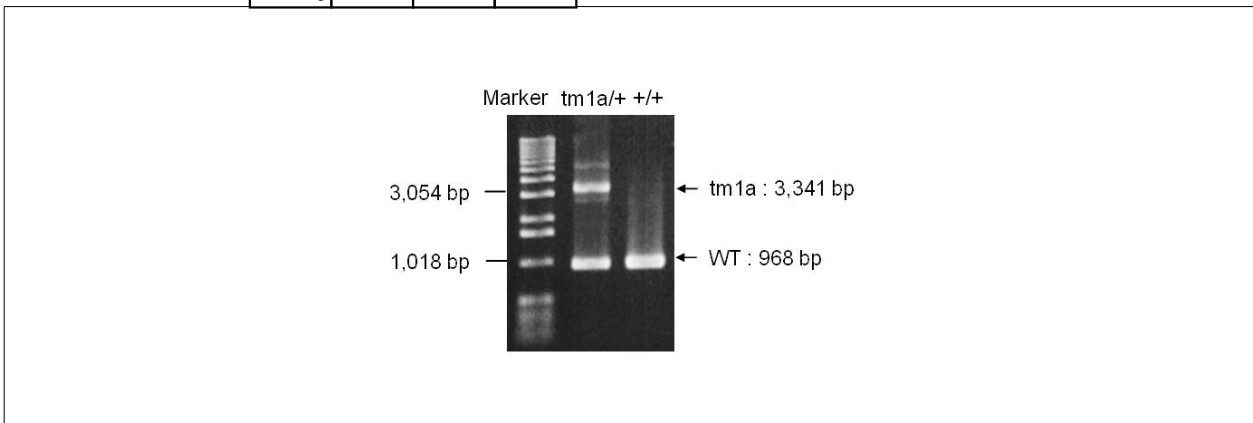
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

### Reaction Conditions

|              | Conc             | Run 1     | Run 2 | Run 3 | Run 4 |
|--------------|------------------|-----------|-------|-------|-------|
| H2O          |                  | 1.8       |       |       |       |
| PCR buffer   | 2 X              | 10.0      |       |       |       |
| dNTP         | 2.0 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       |       |       |       |
| DNA          |                  | 1.0       |       |       |       |
| Taq          | 1 U/ $\mu$ l     | 0.2       |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
|              |                  |           |       |       |       |
| <b>total</b> |                  | <b>20</b> |       |       |       |

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

### Standard PCR



### 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   | 968 bp       | WT      |
| primer 2     | primer 3   | 3,341 bp     | tm1a    |
| primer 2     | primer 3   | 603 bp       | tm1b    |
|              |            |              |         |
|              |            |              |         |
|              |            |              |         |
|              |            |              |         |

### Comment

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