



# PCR protocol

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RBRC 11271

Strain name C57BL/6N-Tg(Cd4-CADM1)#Kmori

| Primer | primer_name      | primer_seq                | length | Reference               |
|--------|------------------|---------------------------|--------|-------------------------|
| 1      | promoter         | GGCCTAGATTTCCCTTCTGAGC    | 22     | RIKEN BRC               |
| 2      | CD4-CADM1Tg-3'-R | GGGTCCATGGTGATACAAGG      | 20     | Depositor's primer      |
| 3      | oIMR0042         | CTAGGCCACAGAATTGAAAGATCT  | 24     | JAX Genotyping Protocol |
| 4      | oIMR0043         | GTAGGTGGAAATTCTAGCATCATCC | 25     | JAX Genotyping Protocol |
|        |                  |                           |        |                         |
|        |                  |                           |        |                         |
|        |                  |                           |        |                         |
|        |                  |                           |        |                         |
|        |                  |                           |        |                         |
|        |                  |                           |        |                         |

## Reaction Components

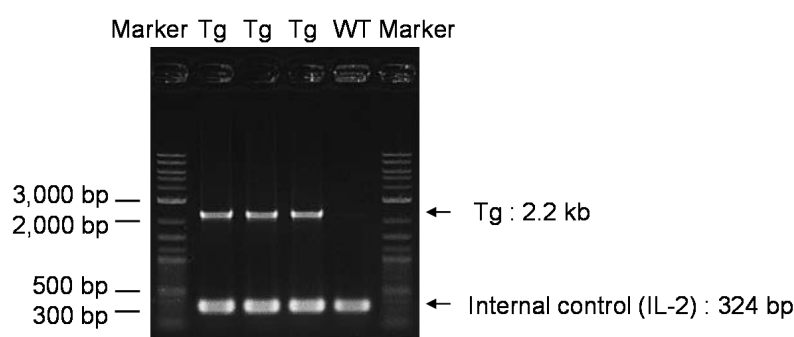
|        |                                   |
|--------|-----------------------------------|
| Enzyme | TOYOBO KOD FX (Code.No : KFX-101) |
|--------|-----------------------------------|

## 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        |   |   |   |
|            |            |            |   |   |   |
|            |            |            |   |   |   |
|            |            |            |   |   |   |
|            |            |            |   |   |   |
| total      |            | 20         |   |   |   |

|        |                                       |
|--------|---------------------------------------|
| 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     | 2.2 kb                |
| primer 3   | primer 4     | 324 bp                |
|            |              |                       |
|            |              |                       |
|            |              |                       |
|            |              |                       |

Comment [Standard PCR](#)

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