

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

# PCR protocol

Sheet ID PS\_04728  
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RBRC 05454

Strain name B6;129B6(Cg)-Actb<tm1(tetO-ChR2\*C128S/EYFP)Kftnk>

| Primer | primer_name | primer_seq                | length |                         |
|--------|-------------|---------------------------|--------|-------------------------|
| 1      | Chr2PCRF    | CATCCTTATCCACCTGAGCAAC    | 22     | Depositor's primer      |
| 2      | Chr2PCRR    | ACCGACCCTTTGGCACAGTATG    | 22     | Depositor's primer      |
| 3      | oIMR0042    | CTAGGCCACAGAATTGAAAGATCT  | 24     | JAX Genotyping Protocol |
| 4      | oIMR0043    | GTAGGTGGAAATTCTAGCATCATCC | 25     | JAX Genotyping Protocol |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |

## Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

## Reaction Conditions

|            | Conc             | Run 1 | Run 2 | Run 3 | Run 4 |
|------------|------------------|-------|-------|-------|-------|
| H2O        |                  | 11.3  |       |       |       |
| PCR buffer | 10 X             | 2.0   |       |       |       |
| dNTP       | 2.5 mM           | 1.6   |       |       |       |
| 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        | 5 U/ $\mu$ l     | 0.1   |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
| total      |                  | 20    |       |       |       |

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

KI/? +/- Marker

Internal control  
(IL-2) : 324 bp →  
KI : ca 220 bp →



## Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

## Gel

2% agarose in 1 X TAE (100 V, 35 min)

## PCR products

| primer_set | product_size | product   |
|------------|--------------|-----------|
| primer 1   | primer 2     | ca 220 bp |
| primer 3   | primer 4     | 324 bp    |
|            |              |           |
|            |              |           |
|            |              |           |

Comment