

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



# PCR protocol

Sheet ID PS\_00811  
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RBRC 01759

Strain name CAG-CAT-Bmp4

| Primer | primer_name | primer_seq                | length | Reference                                          |
|--------|-------------|---------------------------|--------|----------------------------------------------------|
| 1      | CAT3        | ACTGGTGAAACTCACCCA        | 18     | Depositor's primer                                 |
| 2      | CAT2        | CAGTCAGTTGCTCAATGTACC     | 21     | Depositor's primer                                 |
| 3      | oIMR0042    | CTAGGCCACAGAATTGAAAGATCT  | 24     | Internal control (IL-2)<br>JAX Genotyping Protocol |
| 4      | oIMR0043    | GTAGGTGGAAATTCTAGCATCATCC | 25     | Internal control (IL-2)<br>JAX Genotyping Protocol |
|        |             |                           |        |                                                    |
|        |             |                           |        |                                                    |
|        |             |                           |        |                                                    |
|        |             |                           |        |                                                    |
|        |             |                           |        |                                                    |
|        |             |                           |        |                                                    |
|        |             |                           |        |                                                    |

## Reaction Components

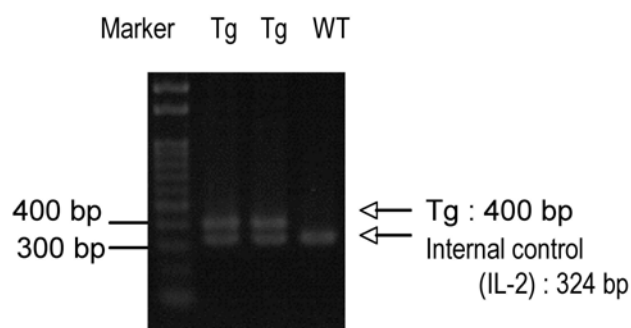
|        |                                 |
|--------|---------------------------------|
| Enzyme | TaKaRa Ex Taq (Cat. No. RR001A) |
|--------|---------------------------------|

| Reagent    | Conc             | Reaction 1 | 2 | 3 | 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         |   |   |   |

## Reaction Conditions

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 180        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 55        | 30*        |                       |
| 4    | 72        | 60*        | *Repeat for 30 cycles |
| 5    | 72        | 300        |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

|        |                                        |
|--------|----------------------------------------|
| Marker | Gene Ladder 100 (Wako, Cat. 316-06951) |
| Gel    | 2% agarose in 1 X TAE (100 V, 35 min)  |



| primer_set | product_size | product (allele type) |
|------------|--------------|-----------------------|
| primer 1   | primer 2     | 400 bp                |
| primer 3   | primer 4     | 324 bp                |
|            |              |                       |
|            |              |                       |
|            |              |                       |
|            |              |                       |

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