

RBRC 10251

Strain name C.129-KIre1<tm1.1Tgi>

| Primer | primer_name | primer_seq             | length |  | Reference                  |
|--------|-------------|------------------------|--------|--|----------------------------|
| 1      | J03KO1      | GATGGATGAAGCACCTGTAAC  | 21     |  | Blood 104, 781-783 (2004). |
| 2      | J03KO3      | TCAGAAACCCATCAGACCAACC | 22     |  | Blood 104, 781-783 (2004). |
| 3      |             | GCAGAAGAACGGCATCAAGGTG | 22     |  | RIKEN BRC                  |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |
|        |             |                        |        |  |                            |

**Reaction Components**

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

**Reaction Conditions**

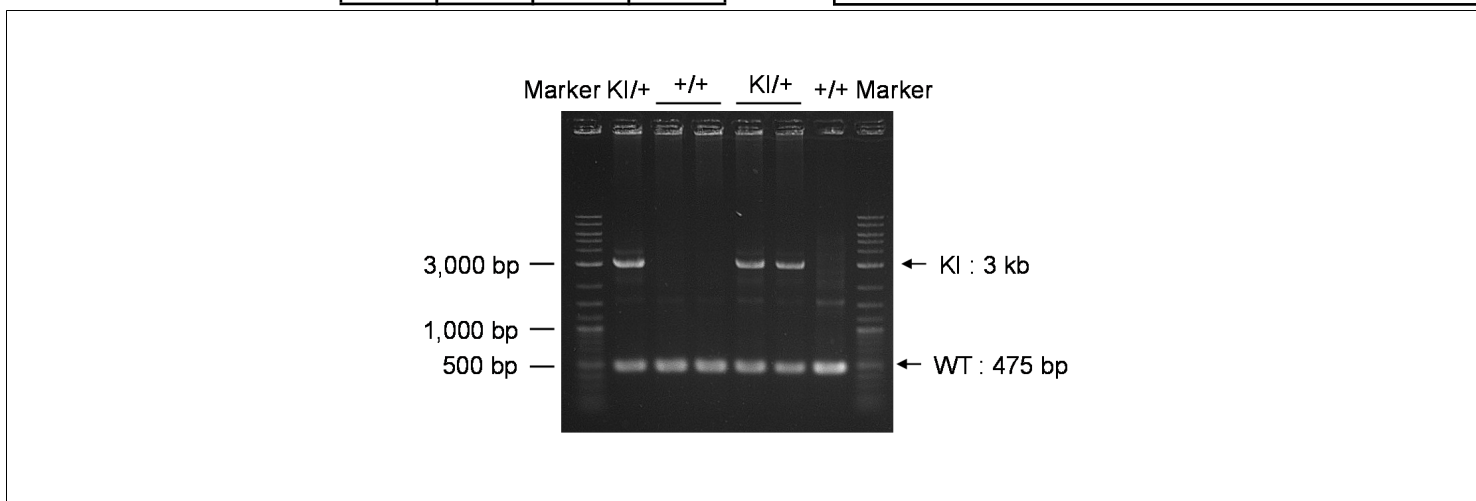
| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 120        |                       |
| 2    | 98        | 10*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 68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

| Reagent      | Conc       | Reaction 1 | 2 | 3 | 4 |
|--------------|------------|------------|---|---|---|
| H2O          |            | 1.8        |   |   |   |
| PCR buffer   | 2 X        | 10.0       |   |   |   |
| dNTP         | 2 mM       | 4.0        |   |   |   |
| primer 1     | 10 pmol/µl | 1.0        |   |   |   |
| primer 2     | 10 pmol/µl | 1.0        |   |   |   |
| primer 3     | 10 pmol/µl | 1.0        |   |   |   |
| DNA          |            | 1.0        |   |   |   |
| Taq          | 1 U/µl     | 0.2        |   |   |   |
|              |            |            |   |   |   |
|              |            |            |   |   |   |
|              |            |            |   |   |   |
|              |            |            |   |   |   |
|              |            |            |   |   |   |
| <b>total</b> |            | 20         |   |   |   |

(µL)

**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   | 475 bp       | WT                    |
| primer 2   | 3 kb         | KI                    |
| primer 2   |              |                       |
|            |              |                       |
|            |              |                       |
|            |              |                       |
|            |              |                       |
|            |              |                       |
|            |              |                       |

+ / + : 475 bp  
KI / + : 475 bp, 3 kb

**Comment** Standard PCR

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