# PCR protocol

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**RBRC** 06172 **Strain name** ICR;FVB-Invs<inv> Tg(EF1-Invs/EGFP)B27Hmd

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | | CATGTGATAGTCACTCCAGGGGTTG | 25 | | RIKEN BRC |
| 2 | | CATTGATCCAGATTCATACTGGGTC | 25 | | RIKEN BRC |
| 3 | | CAGCTGCAAAGCCTTGTTGCAGTGC | 25 | | RIKEN BRC |
| 4 | | GTGCTAGGAGCTGTCGAGACCAC | 23 | | RIKEN BRC |

**Reaction Components**

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

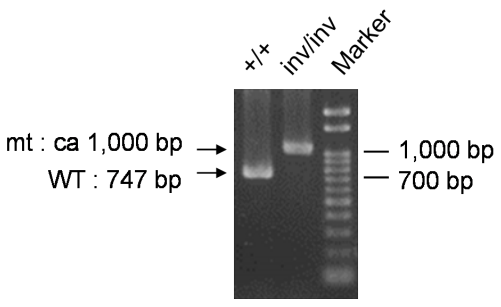
| Reagent | Conc | Reaction 1 | 2 | 3 | 4 | |
|---|---|---|---|---|---|---|
| H2O | | 0.8 | | | | (μL) |
| PCR buffer | 2 X | 10.0 | | | | |
| dNTP | 2.0 mM | 4.0 | | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | | |
| primer 4 | 10 pmol/μl | 1.0 | | | | |
| DNA | | 1.0 | | | | |
| Taq | 1 U/μl | 0.2 | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | total | 20 | | | | |

**Reaction Conditions**

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

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

**Gel** 2% agarose in 1 X TAE (100 V, 30 min)




| primer_set | | product_size | product (allele type) |
|---|---|---|---|
| primer 1 | primer 2 | ca 1000 bp | mutant(inv) |
| primer 3 | primer 4 | 747 bp | WT |
| | | | |
| | | | |
| | | | |
| | | | |

**Comment** Standard PCR

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