# PCR protocol

Sheet ID PS_06208
Release date : 3/3/2017
Print date : 3/3/2017

**RBRC** 06506 **Strain name** ICR.Cg-Tg(CAG-cat,-3xFLAG/Dazl)4

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | A | GCTCTAGAGCCTCTGCTAACC | 21 | | FEBS Letters, 500, 7-11, (2001). |
| 2 | | GTCTGTTCTCAGGGTTAAACAGACAAG | 27 | | RIKEN BRC |
| 3 | oIMR0042 | CTAGGCCACAGAATTGAAAGATCT | 24 | | JAX Genotyping Protocol |
| 4 | oIMR0043 | GTAGGTGGAAATTCTAGCATCATCC | 25 | | JAX Genotyping Protocol |

## Reaction Components

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

| | Conc | Run 1 | Run 2 | Run 3 | Run 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 | | | |

## Reaction Conditions

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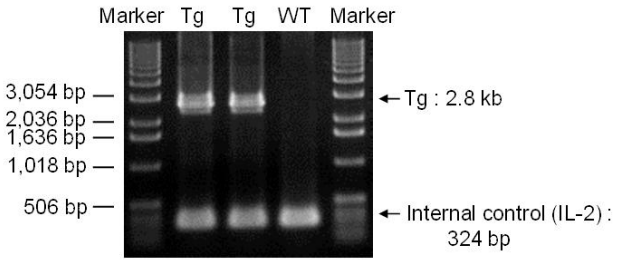


**Marker**
1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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

| PCR products | primer_set | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 2.8 kb | Tg |
| primer 3 | primer 4 | 324 bp | Internal control (IL-2) |
| | | | |
| | | | |
| | | | |

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