# PCR protocol

Sheet ID PS_08118
Release date : 1/13/2021
Print date : 1/13/2021

RBRC 01400

**Strain name** B6D2-Tg(CAG-Ptafr)1Tksh/TkshRbrc

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | 5CAG | CCTACAGCTCCTGGGCAACGTGC | 23 | | RIKEN BRC |
| 2 | GP-2 | TCAGGATCAGGTCATGAT | 18 | | Depositor's primer |
| 3 | oIMR0042 | CTAGGCCACAGAATTGAAAGATCT | 24 | | JAX Genotyping Protocol |
| 4 | oIMR0043 | GTAGGTGGAAATTCTAGCATCATCC | 25 | | JAX Genotyping Protocol |

**Reaction Components**

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

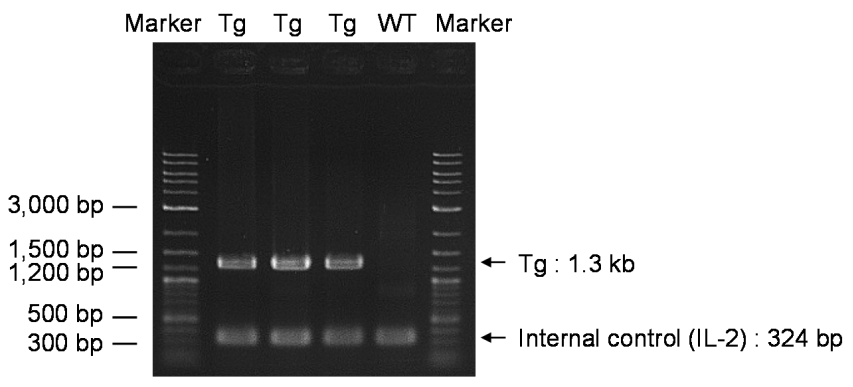
| Reagent | Conc | Reaction 1 | 2 | 3 | 4 | |
|---|---|---|---|---|---|---|
| H2O | | 0.8 | | | | (μL) |
| PCR buffer | 2 X | 10.0 | | | | |
| dNTP | 2.0 mM | 4.0 | | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | | |
| primer 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 | 55 | 30* | |
| 4 | 68 | 180* | *Repeat for 30 cycles |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

**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 | primer 2 | 1.3 kb | Tg |
| primer 3 | primer 4 | 324 bp | Internal control (IL-2) |
| | | | |
| | | | |
| | | | |
| | | | |

**Comment** Standard PCR

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

# PCR protocol

Sheet ID PS_00344
Release date : 6/1/2006
Print date : 1/13/2021

RBRC 01400

Strain name B6D2-Tg(CAG-Ptafr)1Tksh/TkshRbrc

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | GP-1 | ACCACACTCCTGTCAATC | 18 | | Depositor's primer |
| 2 | GP-2 | TCAGGATCAGGTCATGAT | 18 | | Depositor's primer |
| 3 | oIMR0042 | CTAGGCCACAGAATTGAAAGATCT | 24 | Internal control (IL-2) | JAX Genotyping Protocol |
| 4 | oIMR0043 | GTAGGTGGAAATTCTAGCATCATCC | 25 | Internal control (IL-2) | JAX Genotyping Protocol |

## Reaction Components

Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

| Reagent | Conc | Reaction 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| H2O | | 15.3 | | | |
| PCR buffer | 10 X | 2.5 | | | |
| dNTP | 2.5 mM | 2.0 | | | |
| 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 | | 0.2 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | total | 25 | | | |

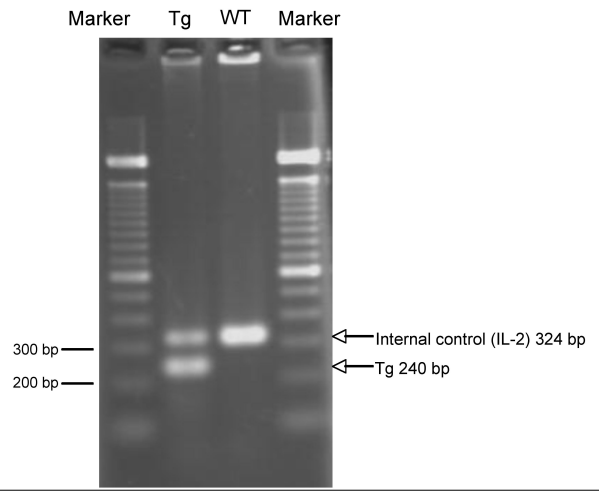
(μL)

## Reaction Conditions

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

Marker: 100 bp ladder (Invitrogen, Cat. 15628-050)

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




| primer_set | | product_size | product (allele type) |
|---|---|---|---|
| primer 1 | primer 2 | 240 bp | Tg |
| primer 3 | primer 4 | 324 bp | Internal control (IL-2) |
| | | | |
| | | | |
| | | | |
| | | | |

Comment Standard PCR