



PCR protocol

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RBRC 05384

Strain name B6.Cg-Tg(Prdm14-Venus)1Sait/SaitRbrc

| Primer | primer_name | primer_seq | length | Reference |
|--------|-------------|---------------------------|--------|-----------------------------------|
| 1 | intron 1 | AGAGCGCCTGAAGCCACTGC | 20 | RIKEN BRC |
| 2 | R | CACAGTCGAGGCTGATCTCG | 20 | Nat Genet., 40(8):1016-22 (2008). |
| 3 | oIMR0042 | CTAGGCCACAGAATTGAAAGATCT | 24 | JAX Genotyping Protocol |
| 4 | oIMR0043 | GTAGGTGGAAATTCTAGCATCATCC | 25 | JAX Genotyping Protocol |

Reaction Components

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

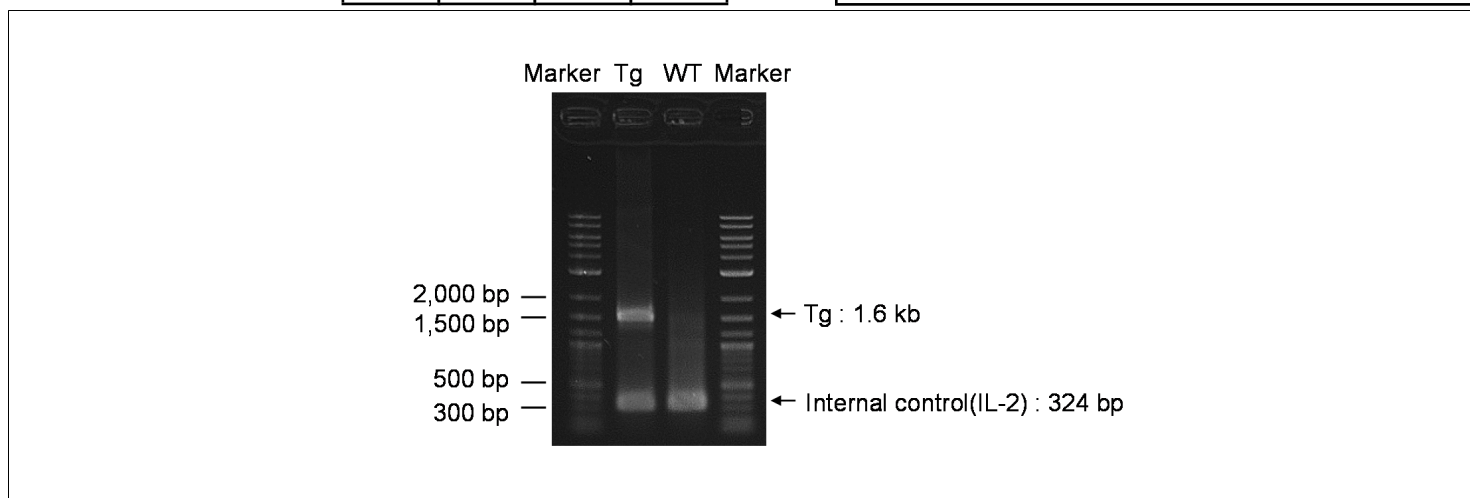
Reaction Conditions

| step | temp (°C) | time (sec) | note |
|------|-----------|------------|-----------------------|
| 1 | 94 | 120 | |
| 2 | 98 | 10* | |
| 3 | 60 | 30* | |
| 4 | 68 | 180* | *Repeat for 30 cycles |

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

(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 primer 2 | 1.6 kb | Tg |
| primer 3 primer 4 | 324 bp | Internal control (IL-2) |
| | | Non-Tg : 324 bp |
| | | Tg : 324 bp, 1.6 kb |

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

RBRC 05384

Strain name Prdm14-mVenus (Acc. No. CDB0461T)

| Primer | primer_name | primer_seq | length | Reference |
|--------|-------------|---------------------------|--------|-----------------------------------|
| 1 | primer 1 | ACTCATCTCAGAAGAGGATCTG | 22 | Nat Genet., 40(8):1016-22 (2008). |
| 2 | R | CACAGTCGAGGCTGATCTCG | 20 | Nat Genet., 40(8):1016-22 (2008). |
| 3 | oIMR0042 | CTAGGCCACAGAATTGAAAGATCT | 24 | JAX Genotyping Protocol |
| 4 | oIMR0043 | GTAGGTGGAAATTCTAGCATCATCC | 25 | JAX Genotyping Protocol |

Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

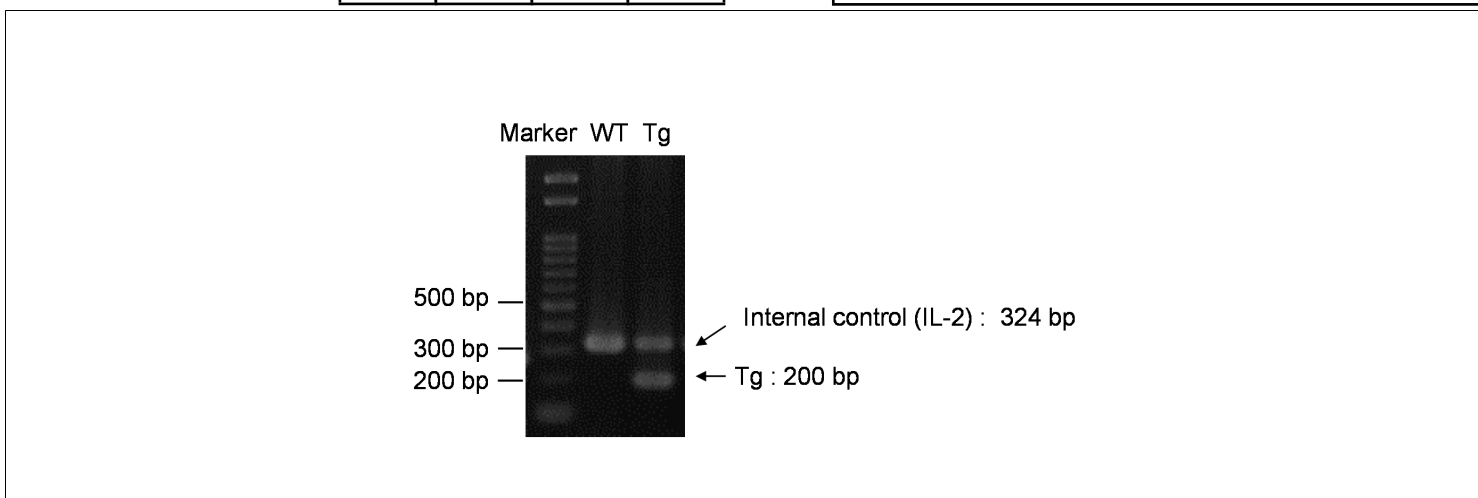
| Reagent | Conc | Reaction 1 | 2 | 3 | 4 |
|------------------|------------|------------|---|---|---|
| H ₂ O | | 11.3 | | | |
| PCR buffer | 10 X | 2.0 | | | |
| dNTP | 2.5 mM | 1.6 | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | |
| primer 4 | 10 pmol/μl | 1.0 | | | |
| DNA | | 1.0 | | | |
| Taq | 5 U/μl | 0.1 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| total | | 20 | | | |

Reaction Conditions

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

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 | 200 bp |
| primer 3 | primer 4 | 324 bp |
| | | Tg |
| | | Internal control (IL-2) |
| | | |
| | | |
| | | |

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