# PCR protocol

Sheet ID PS_06637
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**RBRC** 09434 **Strain name** B6CD1-Tg(Pax2-cre)1Akg

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | intron 1 | GCGGCACACTGGTGTGAGAG | 20 | | RIKEN BRC |
| 2 | Cre | CTAATCGCCATCTTCCAGCAGG | 22 | | Depositor's primer |
| 3 | intron 2 | TAGCCAGACCCTATTCCACCCTC | 23 | | RIKEN BRC |

## Reaction Components

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

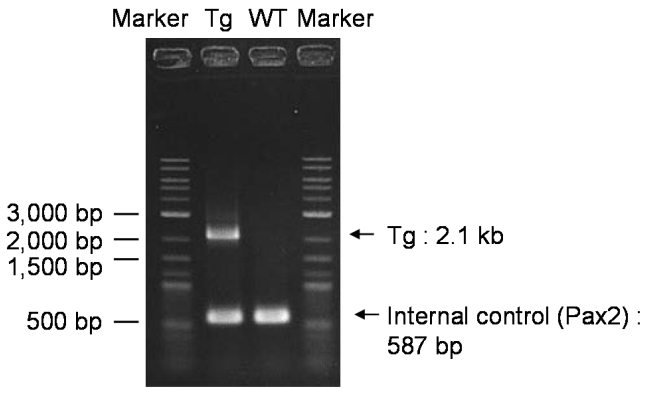
| Reagent | Conc | Reaction 1 | 2 | 3 | 4 | |
|---|---|---|---|---|---|---|
| H2O | | 1.8 | | | | (μL) |
| PCR buffer | 2 X | 10.0 | | | | |
| dNTP | 2.0 mM | 4.0 | | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | | |
| DNA | | 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 | 120 | * *Repeat for 30 cycles |

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

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




| primer_set | | product_size | product (allele type) |
|---|---|---|---|
| primer 1 | primer 2 | 2.1 kbp | Tg |
| primer 1 | primer 3 | 587 bp | Internal control ( Pax2 ) |
| | | | |
| | | | |
| | | | |
| | | | |

Non-Tg : 587 bp
Tg : 587 bp, 2.1 kb

**Comment** Standard PCR

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