# PCR protocol

Sheet ID PS_08231
Release date : 8/31/2021
Print date : 8/31/2021

RBRC 10606

Strain name C57BL/6J-Tg(Slc32a1-cre)65Kzy

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | promoter | AGTGACGAAGACCACTCTGTCTG | 23 | | RIKEN BRC |
| 2 | Cre | CTAATCGCCATCTTCCAGCAGG | 22 | | 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 | | | |
| PCR buffer | 2 X | 10.0 | | | |
| dNTP | 2 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)

**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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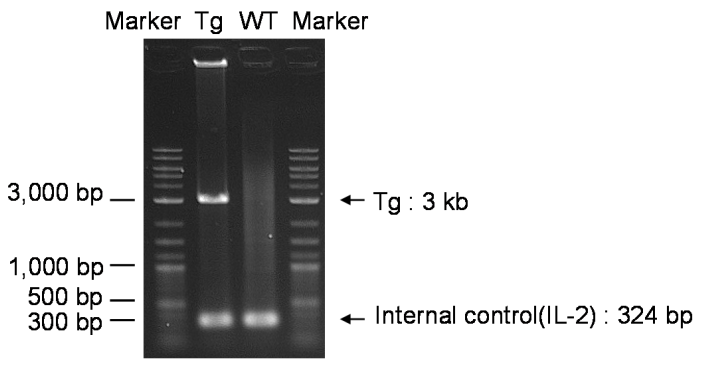
| | | | |

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

Non-Tg : 324 bp bp
Tg : 324 bp, 3 kb

**Comment** Standard PCR

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

# PCR protocol

Sheet ID PS_07881
Release date : 11/14/2019
Print date : 8/31/2021

RBRC 10606

Strain name C57BL/6J-Tg(Slc32a1-cre)65Kzy

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | promoter | AGTGACGAAGACCACTCTGTCTG | 23 | | RIKEN BRC |
| 2 | exon 1 | CACGGCCTGGTCCTTGGAGC | 20 | | RIKEN BRC |
| 3 | CW-Cre2 | ACCTGATGGACATGTTCAGGGATCG | 25 | | Nature, 406(6797): 726-731 (2000). |

## Reaction Components

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

| Reagent | Conc | Reaction 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| H2O | | 1.8 | | | |
| 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 | | | |
| DNA | | 1.0 | | | |
| Taq | 1 U/μl | 0.2 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | total | 20 | | | |

(μL)

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 120 | |
| 2 | 98 | 10* | |
| 3 | 63 | 30* | |
| 4 | 68 | 240* | *Repeat for 30 cycles |
| | | | |
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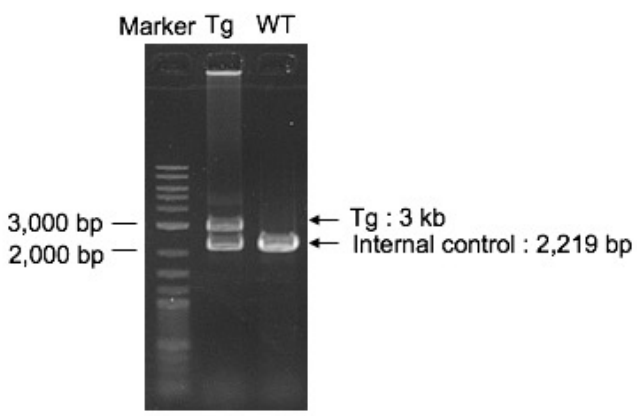
| | | | |

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 | 2,219 bp | Internal control |
| primer 2 | primer 3 | 3 kb | Tg |
| | | | |
| | | | |
| | | | |
| | | | |

Non-Tg : 2,219 bp
Tg : 2,219 bp, 3 kb

Comment Standard PCR

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