# PCR protocol

Sheet ID PS_07843
Release date : 4/15/2020
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**RBRC** 10624 **Strain name** B6.Cg-Tlk2<tm1.1Chum>

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | Tlk2KO-Fwd | GCATGTCTGGCTGCTCATAACTGC | 24 | | Depositor's primer |
| 2 | intron 13 | CCAGCCTTTCCAGCTCTGCC | 20 | | RIKEN BRC |
| 3 | Tlk2KO-Rev | GGCATTCTCAAGACAGGCAACCAC | 24 | | Depositor's primer |

**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 | | | | |
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| | | | | | | |
| total | | 20 | | | | |

**Reaction Conditions**

| step | temp (℃) | time (sec) | | note |
|---|---|---|---|---|
| 1 | 94 | 120 | | |
| 2 | 98 | 10 | * | |
| 3 | 60 | 30 | * | |
| 4 | 68 | 60 | * | *Repeat for 30 cycles |
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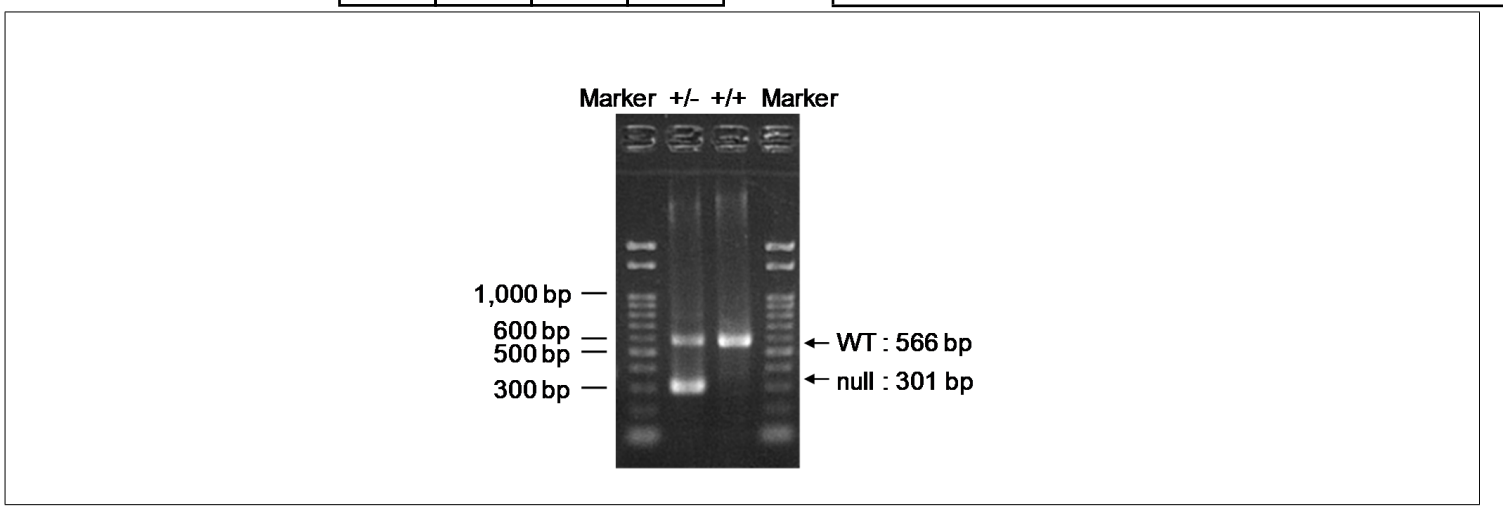
**Marker** Gene Ladder 100 (Wako, Cat. 316-06951)

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




| primer_set | | product_size | product (allele type) | |
|---|---|---|---|---|
| primer 1 | primer 2 | 566 bp | WT | +/+ (wild-type): 566 bp<br>+/- (heterozygous): 566 bp and 301 bp<br>-/- (homozygous): 301 bp |
| primer 1 | primer 3 | 301 bp | null | |
| | | | | |
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**Comment** Standard PCR

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