# PCR protocol

Sheet ID PS_05409
Release date : 4/5/2018
Print date : 4/5/2018

**RBRC** 05762 **Strain name** B6.Cg-Trafd1<tm1Ayos>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | gFLN29wa F14225 | ATGCTGGGCCTCGCCTACAGGCCTCA | 26 | | Depositor's primer |
| 2 | gFLN29 neo2-2F | GGCTGCTAAAGCGCATGCTCCAGACTG | 27 | | Depositor's primer |
| 3 | gFLN29 R-176 | CAGGCTTGCTTGCTGCAACGAGGCTC | 26 | | Depositor's primer |

**Reaction Components**

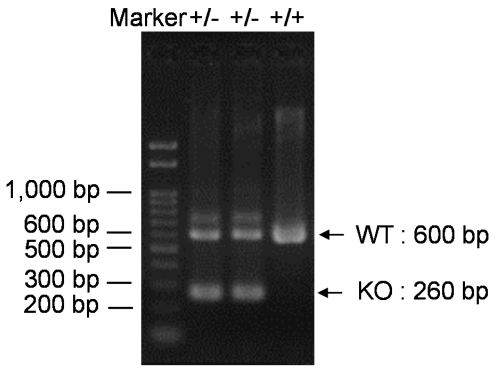
**Enzyme** TOYOBO KOD FX (Code.No : KFX-101)

| | Conc | Run 1 | Run 2 | Run 3 | Run 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 | | | |
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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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Standard PCR




**Marker**

Gene Ladder 100 (Wako, Cat. 316-06951)

**Gel**

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

| PCR products | primer_set | product_size | product |
|---|---|---|---|
| primer 1 | primer 3 | 600 bp | WT |
| primer 2 | primer 3 | 260 bp | KO |
| | | | |
| | | | |
| | | | |
| | | | |

**Comment**

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