# PCR protocol

Sheet ID PS_07162
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**RBRC** 09661 **Strain name** B6.Cg-Gt(ROSA)26Sor<tm12.1(Nphs1-EGFP)Maoh>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | M273 | TCAGTAAGGGAGCTGCAGTGG | 21 | | Nucleic Acids Res., 38, e198 (2010). |
| 2 | | GGCAAGCTGACCCTGAAGTTC | 21 | | RIKEN BRC |
| 3 | M274 | CGATGGAAAATACTCCGAGGC | 21 | | Nucleic Acids Res., 38, e198 (2010). |

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

**Reaction Conditions**

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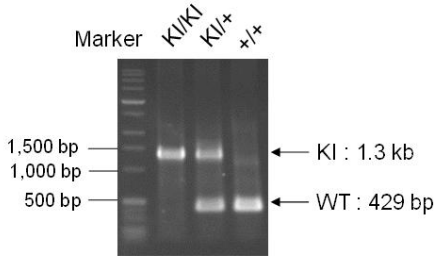


**Marker**

DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

**Gel**

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

| PCR products | primer_set | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 1.3 kb | KI |
| primer 1 | primer 3 | 429 bp | WT |
| | | | |
| | | | |
| | | | |

**Comment**

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