# PCR protocol

Sheet ID PS_04248
Release date : 08/01/2012
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**RBRC** 04950 **Strain name** B6;129P2-Traf6<tm1Jino>

**Primer**

| | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | COM | CGTGCCATGTAATGCATTCTG | 21 | | Depositor's primer |
| 2 | Ex4_Rv | CTCGAGATGTCTCAGTTCCATC | 22 | | RIKEN BRC |
| 3 | NEO | TACAGACACTCAGCACCATTT | 21 | | Depositor's primer |

**Reaction Components**

**Enzyme** TaKaRa Ex Taq (Cat. No. RR001A)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 12.3 | | | |
| PCR buffer | 10 X | 2.0 | | | |
| dNTP | 2.5 mM | 1.6 | | | |
| 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 | 5 U/μl | 0.1 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | total | 20 | | | |

**Reaction Conditions**

| step | temp (℃) | time (sec) | | note |
|---|---|---|---|---|
| 1 | 94 | 180 | | |
| 2 | 94 | 30 | * | |
| 3 | 55 | 30 | * | |
| 4 | 72 | 60 | * | *Repeat for 30 cycles |
| 5 | 72 | 300 | | |
| | | | | |
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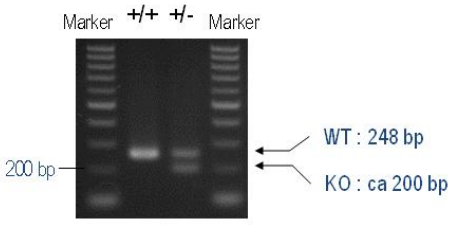


**Marker**

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 2 | 248 bp | WT |
| primer 1 | primer 3 | ca 200 bp | KO |
| | | | |
| | | | |
| | | | |

**Comment**