# PCR protocol

**RBRC** 02330 **Strain name** NOD.Cg-Fcer1g<tm1Rav> Fcgr2b<tm1Ttk>

**Primer**

| | primer_name | primer_seq | length | |
|---|---|---|---|---|
| 1 | Neo | CTCGTGCTTTACGGTATCGCC | 21 | |
| 2 | 5'EC1(F) | CCTACTCTACTGTCGACTCAAG | 22 | |
| 3 | 3'EC1(R) | GGCTGGCTATAGCTGCCTTTC | 21 | |
| 4 | 5'EC1(F) | AAACTCGACCCCCCGTGGATC | 21 | |
| 5 | 3'EC1(R) | TTGACTGTGGCCTTAAACGTGTAG | 24 | |
| | | | | |
| | | | | |
| | | | | |

**Reaction Components**

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

| | Conc | Run 1 | Run 2 | Run 3 |
|---|---|---|---|---|
| H2O | | 16.3 | 16.3 | |
| PCR buffer | 10 X | 2.5 | 2.5 | |
| dNTP | 2.5 mM | 2.0 | 2.0 | |
| primer 1 | 10 pmol／μ | 1.0 | 1.0 | |
| primer 2 | 10 pmol／μ | 1.0 | 0 | |
| primer 3 | 10 pmol／μ | 1.0 | 0 | |
| primer 4 | 10 pmol／μ | 0 | 1.0 | |
| primer 5 | 10 pmol／μ | 0 | 1.0 | |
| DNA | | 1.0 | 1.0 | |
| Taq | | 0.2 | 0.2 | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | total | 25 | 25 | |

**Reaction Conditions**

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 180 | |
| 2 | 94 | 30 | * |
| 3 | 63 | 30 | * |
| 4 | 72 | 60 | * *repeat for 30 cycles |
| 5 | 72 | 300 | |
| | | | |
| | | | |
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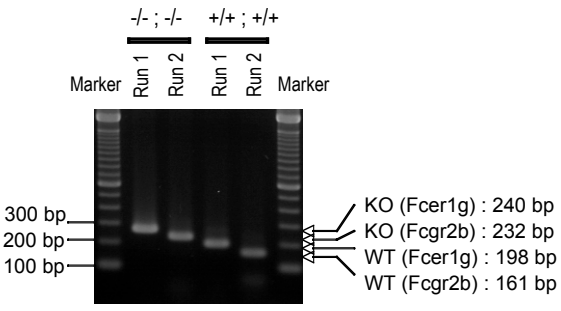


**Marker**

100 bp ladder (Invitrogen, Cat. 15628-050)

**Gel**

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

**PCR products**

| primer_set | | product_size | product |
|---|---|---|---|
| primer 2 | primer 3 | 198 bp | wild (Fcer1g) |
| primer 1 | primer 3 | 240 bp | KO (Fcer1g) |
| primer 4 | primer 5 | 161 bp | wild (Fcgr2b) |
| primer 1 | primer 5 | 232 bp | KO (Fcgr2b) |
| | | | |

**Comment**