# PCR protocol

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RBRC 11206

Strain name B6.129S2-Ramp1<tm1.1Tsuj>

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | F1 | CAGAATGGAGAAACTGAGTAGAGC | 24 | | Depositor's primer |
| 2 | R1 | AGGAAGGAACGTAACACAGGTGG | 23 | | Depositor's primer |

**Reaction Components**

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

| Reagent | Conc | Reaction 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| H2O | | 2.8 | | | |
| 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 | | | |
| DNA | | 1.0 | | | |
| Taq | 1 U/μl | 0.2 | | | |
| | | | | | |
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| | | | | | |
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| | | | | | |
| | | | | | |
| | | | | | |
| | total | 20 | | | |

(μL)

**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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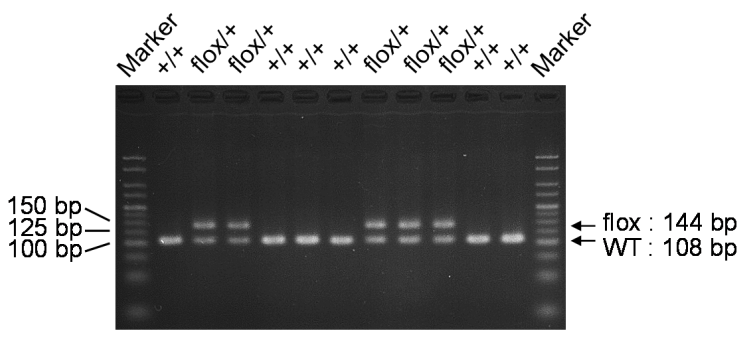
Marker: 25bp DNA Step Ladder (Wako, Cat. 292-69001)

Gel: 4% Nusieve in 0.5 X TBE (100 V, 60 min)




| primer_set | | product_size | product (allele type) |
|---|---|---|---|
| primer 1 | primer 2 | 108 bp | WT |
| primer 1 | primer 2 | 144 bp | flox |
| | | | |
| | | | |
| | | | |
| | | | |

+/+ (wild-type): 108 bp
flox/+ (heterozygous): 108 bp and 144 bp

**Comment** Standard PCR

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