

RMRC Genotyping Protocol-PCR

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|-----|-----------|-------------|---|
| No. | RMRC13328 | Strain Name | C57BL/6-Tg(Fgf1-RFP,-cre/ERT2)13Narl/Narl |
|-----|-----------|-------------|---|

A. Primers

| No. | Primer name | Sequence |
|-----|-------------|-------------------------|
| 1 | CreF | CTAAACATGCTTCATCGTCGGTC |
| 2 | CreR | TCTGACCAGAGTCATCCTTAGCG |

B. Reaction Conditions

| Component | Concentration | Volume | Step | Temperature, °C | Time, sec | Number of cycles |
|------------------|-----------------|---------|------|-----------------|-----------|------------------|
| H ₂ O | – | 15.4 μl | 1 | 94 | 180 | 1 |
| PCR buffer | 10 x | 2 μl | 2 | 94 | 30 | 35 |
| dNTP | 10 mM | 0.4 μl | 3 | 55 | 30 | 35 |
| Primer 1 | 10 μM (ρmol/μl) | 0.5 μl | 4 | 72 | 30 | 35 |
| Primer 2 | 10 μM (ρmol/μl) | 0.5 μl | 5 | 72 | 180 | 1 |
| gDNA | 50-250 ηg/μl | 1 μl | | | | |
| Taq | 5 U/μl | 0.2 μl | | | | |
| Mg ²⁺ | mM | μl | | | | |
| Total | | 20 μl | | | | |

C. PCR Product

| No. | Primer set | Product size | Target region |
|-----|----------------|--------------|---------------|
| 1 | CreF CreR | 560 bp | Cre |
| 2 | | bp | |

D. Gel Photo

