

## RMRC Genotyping Protocol-PCR

|     |           |             |                                             |
|-----|-----------|-------------|---------------------------------------------|
| No. | RMRC13181 | Strain Name | C57BL/6- Tg(Flt3-RFP, -cre/ERT2)59Narl/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 <sub>2</sub> 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 (pmol/μl) | 0.5 μl  | 4    | 72              | 30        | 35               |
| Primer 2         | 10 μM (pmol/μl) | 0.5 μl  | 5    | 72              | 180       | 1                |
| gDNA             | 50-250 ng/μl    | 1 μl    |      |                 |           |                  |
| Taq              | 5 U/μl          | 0.2 μl  |      |                 |           |                  |
| Mg <sup>2+</sup> | 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

