

## RMRC Genotyping Protocol-PCR

|     |           |             |                                |
|-----|-----------|-------------|--------------------------------|
| No. | RMRC13114 | Strain Name | FVB/N-Tg(CCL2-EGFP/UL23)12Klin |
|-----|-----------|-------------|--------------------------------|

### A. Primer

|   | Primer name | Sequence                  |
|---|-------------|---------------------------|
| 1 | MCP17s      | ACCGAGCTCGGATCCACTAGTAACG |
| 2 | pEGFP-C2    | CGTCGCCGTCCAGCTCGACCAG    |

### B. Reaction Conditions

| Component        | Concentration |             | Volume |    | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|---------------|-------------|--------|----|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | --            |             | 13.2   | μl | 1    | 95°C            | 4 min     | 35 cycles        |
| PCR buffer       | 10x           |             | 2      | μl | 2    | 95°C            | 35 sec    |                  |
| dNTP             | 10            | mM          | 0.4    | μl | 3    | 65°C            | 35 sec    |                  |
| Primer 1         | 10            | μM(μmol/μl) | 1      | μl | 4    | 72°C            | 50 sec    |                  |
| Primer 2         | 10            | μM(μmol/μl) | 1      | μl | 5    | 72°C            | 10 min    |                  |
| DNA              |               |             | 1      | μl | 6    | 10°C            | 5 min     |                  |
| Taq              | 5             | U/μl        | 0.2    | μl | 7    | 4°C             |           |                  |
| Mg <sup>2+</sup> | 50            | mM          | 1.2    | μl |      |                 |           |                  |
| Total            |               |             | 20     | μl |      |                 |           |                  |

### C. PCR Product

|   | Primer set |          | Product size | Target region |
|---|------------|----------|--------------|---------------|
| 1 | MCP17s     | pEGFP-C2 | 812 bp       |               |

### D. Gel Photo

