

## RMRC Genotyping Protocol-PCR

|     |           |             |                                   |
|-----|-----------|-------------|-----------------------------------|
| No. | RMRC13287 | Strain Name | C57BL/6-Rictor <sup>em1cSct</sup> |
|-----|-----------|-------------|-----------------------------------|

### A. Primer

|   | Primer name | Sequence               |
|---|-------------|------------------------|
| 1 | RictorI35F  | TGCTAGACTCTGAGCAACCAAG |
| 2 | RictorI33B  | AGACTGACCTCTTCCCTCGAC  |
| 3 |             | T                      |

### B. Reaction Conditions

| Component        | Concentration |             | Volume |    | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|---------------|-------------|--------|----|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | --            |             | 40     | μl | 1    | 95              | 120       | 1                |
| PCR buffer       | 10x           |             | 5      | μl | 2    | 95              | 20        | 35               |
| dNTP             | 10            | mM          | 1      | μl | 3    | 60              | 20        | 35               |
| Primer 1         | 20            | μM(μmol/μl) | 1      | μl | 4    | 72              | 45        | 35               |
| Primer 2         | 20            | μM(μmol/μl) | 1      | μl | 5    | 72              | 600       | 1                |
| Primer 3         | 20            | μM(μmol/μl) |        | μl | 6    |                 |           |                  |
| DNA              | 0.1 μg/μl     |             | 1      | μl | 7    |                 |           |                  |
| Taq              | 5             | U/μl        | 1      | μl | 8    |                 |           |                  |
| Mg <sup>2+</sup> |               | mM          |        | μl | 9    |                 |           |                  |
| Total            |               |             | 50     | μl | 10   |                 |           |                  |

### C. PCR Product

|   | Primer set |            | Product size | Target region    |
|---|------------|------------|--------------|------------------|
| 1 | RictorI35F | RictorI33B | 521 bp       | Wild type allele |
| 2 | RictorI35F | RictorI33B | 550 bp       | Rictor 1c allele |

### D. Gel Photo

