

## RMRC Genotyping Protocol-PCR

|     |           |             |                             |
|-----|-----------|-------------|-----------------------------|
| No. | RMRC13141 | Strain Name | B6.129-Grid1<tm2a(LacZ)Vda> |
|-----|-----------|-------------|-----------------------------|

### A. Primer

|   | Primer name | Sequence              |
|---|-------------|-----------------------|
| 1 | For437      | CCATGCTGATCTCTTCCTGTC |
| 2 | Rev438      | GGTCGCTACAGACGTTGTTTG |
| 3 | Rev440      | GAGCATGACATCATTGAGACG |

### B. Reaction Conditions

| Component        | Concentration |             | Volume |    | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|---------------|-------------|--------|----|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | --            |             | 19     | μl | 1    | 94              | 180       | 1                |
| PCR buffer       | 10x           |             | 2.5    | μl | 2    | 94              | 30        | 30               |
| dNTP             |               | mM          |        | μl | 3    | 56              | 30        | 30               |
| Primer 1         | 10            | μM(μmol/μl) | 1      | μl | 4    | 68              | 120       | 30               |
| Primer 2         | 10            | μM(μmol/μl) | 1      | μl | 5    | 68              | 600       | 1                |
| DNA              | --            |             | 1      | μl | 6    |                 |           |                  |
| Taq              | AccuPrime     | U/μl        | 0.5    | μl | 7    |                 |           |                  |
| Mg <sup>2+</sup> |               | mM          |        | μl | 8    |                 |           |                  |
| Total            |               |             | 25     | μl | 9    |                 |           |                  |

### C. PCR Product

|   | Primer set |        | Product size | Target region |
|---|------------|--------|--------------|---------------|
| 1 | For437     | Rev438 | 363 bp       | GFP           |
| 2 | For437     | Rev440 | 266 bp       |               |

### D. Gel Photo

