

## RMRC Genotyping Protocol-PCR

|     |           |             |                                                                       |
|-----|-----------|-------------|-----------------------------------------------------------------------|
| No. | RMRC13123 | Strain Name | C57BL/6- <i>Gskip</i> <sup>tm1a(GEMMS)<i>Narl</i></sup> / <i>Narl</i> |
|-----|-----------|-------------|-----------------------------------------------------------------------|

### A. Primers

| No. | Primer name | Sequence                 |
|-----|-------------|--------------------------|
| 1   | GskipLF     | TCTGCATTTCAGTCCCTAGTCAGC |
| 2   | GskipLR     | GAGAAGCACTGTGGGCATCAAC   |

### B. Reaction Conditions

| Component        | Concentration   | Volume  | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|-----------------|---------|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | –               | 10.8 µl | 1    | 94              | 180       | 1                |
| PCR buffer       | 10 x            | 1.5 µl  | 2    | 94              | 30        | 35               |
| Formamide        | –               | 0.5 µl  | 3    | 60              | 30        | 35               |
| dNTP             | 10 mM           | 0.3 µl  | 4    | 72              | 30        | 35               |
| Primer 1         | 20 µM (pmol/µl) | 0.3 µl  | 5    | 72              | 180       | 1                |
| Primer 2         | 20 µM (pmol/µl) | 0.3 µl  |      |                 |           |                  |
| gDNA             | 50-250 ng/µl    | 1 µl    |      |                 |           |                  |
| Taq              | 5 U/µl          | 0.3 µl  |      |                 |           |                  |
| Total            |                 | 15 µl   |      |                 |           |                  |

### C. PCR Product

| No. | Primer set |         | Product size | Target region |
|-----|------------|---------|--------------|---------------|
| 1   | GskipLF    | GskipLR | 377 bp       | Wildtype      |
| 2   | GskipLF    | GskipLR | 440 bp       | Mutant        |

### D. Gel Photo

