

## RMRC Genotyping Protocol-PCR

|     |           |             |                                                                       |
|-----|-----------|-------------|-----------------------------------------------------------------------|
| No. | RMRC13314 | Strain Name | C57BL/6- <i>Smad4</i> <sup>tm1b(GEMMS)<i>Narl</i></sup> / <i>Narl</i> |
|-----|-----------|-------------|-----------------------------------------------------------------------|

### A. Primers

| No. | Primer name | Sequence                  |
|-----|-------------|---------------------------|
| 1   | Smad4LF     | TACCTTTAGTGCAGCAGTTTCAGCC |
| 2   | Smad4KR     | CCCAGGATCCATTAGGTGACACTA  |

### B. Reaction Conditions

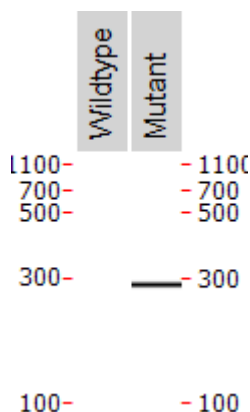
| Component        | Concentration   | Volume | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|-----------------|--------|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | –               | 5.9 μl | 1    | 94              | 110       | 1                |
| Primer 1         | 20 μM (pmol/μl) | 0.3 μl | 2    | 94              | 15        | 35               |
| Primer 2         | 20 μM (pmol/μl) | 0.3 μl | 3    | 60              | 15        | 35               |
| gDNA             | 50-250 ng/μl    | 1 μl   | 4    | 68              | 30        | 35               |
| OneTaq*          | 2X Master Mix   | 7.5 μl | 5    | 68              | 180       | 1                |
| Total            |                 | 15 μl  |      |                 |           |                  |

\*M0488 OneTaq Hot Start Quick-Load 2X Master Mix with Standard Buffer from New England Biolabs.

### C. PCR Product

| No. | Primer set |         | Product size | Target region |
|-----|------------|---------|--------------|---------------|
| 1   | Smad4LF    | Smad4KR | 280 bp       | Mutant        |

### D. Gel Photo



The above protocol CANNOT distinguish heterozygote from homozygote.

Only if you do both protocols (above and below) in the meantime are heterozygote and homozygote differentiated.

### A. Primers

| No. | Primer name | Sequence                    |
|-----|-------------|-----------------------------|
| 1   | Smad4DF     | TCAGGTGACACACCTGAGCAGCCTCTA |
| 2   | Smad4DR     | GTCTTCCTGGAGAGGCTATGGAGGT   |

### B. Reaction Conditions

| Component        | Concentration                    | Volume      | Step | Temperature, °C | Time, sec | Number of Cycles |
|------------------|----------------------------------|-------------|------|-----------------|-----------|------------------|
| H <sub>2</sub> O | –                                | 5.9 $\mu$ l | 1    | 94              | 110       | 1                |
| Primer 1         | 20 $\mu$ M ( $\mu$ mol/ $\mu$ l) | 0.3 $\mu$ l | 2    | 94              | 15        | 35               |
| Primer 2         | 20 $\mu$ M ( $\mu$ mol/ $\mu$ l) | 0.3 $\mu$ l | 3    | 60              | 15        | 35               |
| gDNA             | 50-250 ng/ $\mu$ l               | 1 $\mu$ l   | 4    | 68              | 30        | 35               |
| OneTaq*          | 2X Master Mix                    | 7.5 $\mu$ l | 5    | 68              | 180       | 1                |
| Total            |                                  | 15 $\mu$ l  |      |                 |           |                  |

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### C. PCR Product

| No. | Primer set |         | Product size | Target region |
|-----|------------|---------|--------------|---------------|
| 1   | Smad4DF    | Smad4DR | 251 bp       | Wildtype      |

### D. Gel Photo

