

## Genotyping protocol

EMMA ID: EM:10556

Gene symbol: Wnt4

Strain designation: B6;129-Wnt4<sup>tm3(mCherry)Svo</sup>/Oulu

### Primers:

| Primer name | Primer sequence (5'- 3')       |
|-------------|--------------------------------|
| JDPU 032    | ACT CCT CGT CTT CGC CGT GTT CT |
| JDPL 044    | CAG ACG CAC TGC CAG CCC        |
| JDPU017     | CGG GAG GCG GCC TTT GTA T      |
| JDPL019     | GGC TTT AGA TGT CTT GTT GC     |

### Products:

| Allele    | Primer 1 | Primer 2 | Product size (bp) |
|-----------|----------|----------|-------------------|
| Wild-type | JDPU 032 | JDPL 044 | 257               |
| Mutant    | JDPL017  | JDPL019  | 593               |

### Reaction mix:

| Component                             | Volume (µl) |
|---------------------------------------|-------------|
| Genomic DNA (~50-100 ng)              | 1.0         |
| Primer 1 (10 µM)                      | 1.0         |
| Primer 2 (10 µM)                      | 1.0         |
| 10x Buffer (15 mM MgCl <sub>2</sub> ) | 3.0         |
| MgCl <sub>2</sub> (25 mM)             | 1.0         |
| dNTPs (25 mM)                         | 0.25        |
| DMSO                                  | 1.5         |
| DNA polymerase (5 U/µl)               | 0.1         |
| H <sub>2</sub> O                      | 20.15       |
| <b>Total</b>                          | <b>30.0</b> |

### PCR program:

| Time   | Temperature (+ °C) | Cycles |
|--------|--------------------|--------|
| 5 min  | 94                 |        |
| 30 sec | 94                 | 35     |
| 30 sec | 60                 |        |
| 1 min  | 72                 |        |
| 7 min  | 72                 |        |
| ∞      | 4                  |        |