

**EMMA ID:** *EM:16576*

**Gene:** *Rtp3*

**Common name:** *Rtp3 em1\_1*

**Allele:** *Rtp3<sup>em1(IMPC)Hmgu</sup>*

## Allele Information

For more information on production, guides and mutation, search for gene/project, go to project summary, go to production plan, go to production outcome and "more details"

<https://www.gentar.org>

IMPC mouse phenotype data, search by the gene name  
<http://www.mousephenotype.org/>

## Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on wild type allele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: mutant positive, wild type positive = Heterozygous. In addition to the expected product, the mutant assay may also amplify the endogenous wild type sequence, which will appear as a larger band on an agarose gel. The presence of this extra band will depend on the size of the original deletion.

### PCR primer pairs and expected size bands

| Assay     | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|-----------|----------------|----------------|-------------------------|
| Wild type | Rtp3_F         | Rtp3_wtR       | 418                     |
| Mutant    | Rtp3_F         | Rtp3_R         | 301                     |

### Primer sequences

| Primer Name | Sequence 5' --> 3'     |
|-------------|------------------------|
| Rtp3_F      | CACTGGGCTTAGCAAATGCC   |
| Rtp3_R      | TCTGGTAGTCTCCACGAGGA   |
| Rtp3_wtR    | AGAGCAGGTTGTTCAAGATCCT |

| Component                 | Volume (µl) 1x | Final conc. |
|---------------------------|----------------|-------------|
| DNA (~ 50-100 ng)         | 2              |             |
| Q-Solution (5x)           | 2,5            | 0,5         |
| PCR-Buffer (5x)           | 5              | 1           |
| DNTP mix (10 mM)          | 0,5            | 0,2         |
| MgCl <sub>2</sub> (25 mM) | 1,5            | 1,5         |
| Primer 1 (10 pmol/µl)     | 1              | 0,4         |
| Primer 2 (10 pmol/µl)     | 1              | 0,4         |
| Primer 3 (10 pmol/µl)     | 1              | 0,4         |
| Taq Polymerase (5 U/µl)   | 0,5            | 2,5 U/rxn   |
| H <sub>2</sub> O*         | 10             |             |
| Final volume              | 25             |             |

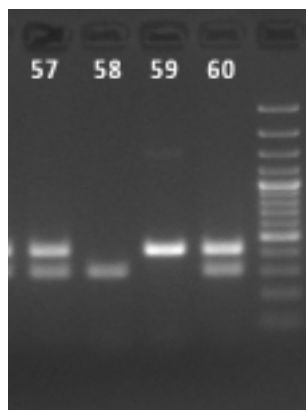
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

#### Amplification conditions

| PCR Settings                                 | Temperature (°C)         | Time                       | # of cycles |
|----------------------------------------------|--------------------------|----------------------------|-------------|
| 1 Denaturation (Melting)                     | 95°C                     | 5 min                      | 1           |
| 2 Amplification (Melting, Annealing, Polym.) | 94°C<br>65-55 TD<br>72°C | 30 sec<br>45 sec<br>45 sec | 39          |
| 3 Polymerisation                             | 72°C                     | 10 min                     | 1           |
| 4 Cooling                                    | 4°C                      | hold                       | 1           |

Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles anneal at 55°C. These PCR conditions have been optimized for our methods and preparation

#### Gel Image



59 wt  
57;60 het  
58 hom

Separated by gel electrophoresis on a 2% agarose gel.