

**EMMA ID:** *EM:16585*

**Gene:** *Tmem91*

**Common name:** *Tmem91-em1\_1*

**Allele:** *Tmem91*<sup>*m1(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 | Tmem91 for2    | Tmem91_wtR2    | 255                     |
| Mutant    | Tmem91 for2    | Tmem91 rev2    | 368                     |

### Primer sequences

| Primer Name | Sequence 5' --> 3'      |
|-------------|-------------------------|
| Tmem91 for2 | AGATAACCTTGAGTTCAGATCAC |
| Tmem91 rev2 | TTTCTCCAGCCCTAAATACCTTT |
| Tmem91_wtR2 | CCACACAGATACACACAGGT    |

| 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             |             |

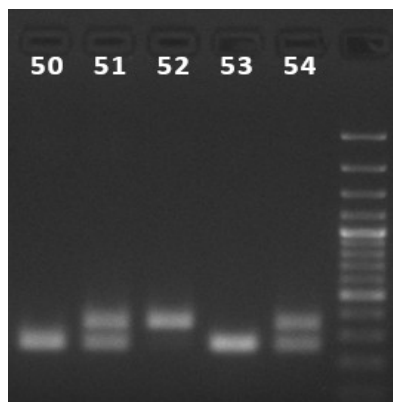
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

#### Amplification conditions

| PCR Settings                                 | Temperature (°C) | Time   | # of cycles |
|----------------------------------------------|------------------|--------|-------------|
| 1 Denaturation (Melting)                     | 95°C             | 5 min  | 1           |
| 2 Amplification (Melting, Annealing, Polym.) | 94°C             | 30 sec | 39          |
|                                              | 61°C             | 45 sec |             |
|                                              | 72°C             | 45 sec |             |
| 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



50,53 wt  
51,54 het  
52 hom

Separated by gel electrophoresis on a 2% agarose gel.