**EMMA ID:** *13439*
**Gene:** *Prox2*
**Common name:** *Prox2-em1_5*
**Allele:** *Prox2* $^{em1(IMPC)Hmgu}$

## 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.

**Prox2-em1_5 contains a -1bp deletion and therefore needs to be sequenced.**

**PCR primer pairs and expected size bands**

| Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|---|---|---|---|
| Wild type | Prox1 for1 | Prox1 rew1 | 597 |
| Mutant | same as wt | same as wt | 596 |

**Primer sequences**

| Primer Name | Sequence 5' --> 3' |
|---|---|
| Prox1 for1 | tcctcagagtctccaccaaggcccgtg |
| Prox1 rew1 | ccgcagaggcaggcagagactcgtttc |

**PCR setup (LongAmp® Taq DNA Polymerase)**

| Component | Volume (µl) 1x |
|---|---|
| DNA (~ 50-100 ng) | 2-4 |
| 100% DMSO | 0,4 |
| PCR-Buffer (5x) | 4 |
| DNTP mix (10 mM) | 0,5 |
| Primer 1 (10 pmol/µl) | 1 |
| Primer 2 (10 pmol/µl) | 1 |
| Primer 3 (10 pmol/µl) | 1 |
| Taq Polymerase (2,5U/µl | 0,5 |
| H2O* | 7,6 |
| Final volume | 20 |

*** The amount of H2O is adjusted with the number of primer.**

**Amplification conditions**

| PCR Settings | Temperature (°C) | Time | # of cyles |
|---|---|---|---|
| 1 Denaturation (Melting) | 94°C | 3 min | 1 |
| 2 Amplification (Melting, Annealing, Polym.) | 94°C<br>68-58 (↓1°C/Cycle)<br>65°C | 30 sec<br>20 sec<br>1 min | 39 |
| 3 Polymerisation | 65°C | 10 min | 1 |
| 4 Cooling | 4°C | hold | 1 |

**Touch-Down cycling protocol: first 10 cycles anneal at 68°C, decreasing 1°C per cycle, next 30 cycles aneal at 58°C.**
**These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.**

**Sequence / mutation**

**Wt:**

ccgggctttactgctccacagaggtccttgctagaactctggtgaggctcgttctccacggtccaggcaccagatgagcagctgttcctcggccttgccctcggggagcttcttggctc
agttcctcctgggctctgtgctggtgctctg

**Mut:**

ccgggctttactgctccacagaggtccttgctagaactctggtgaggctcgttctccacgtccaggcaccagatgagcagctgttcctcggccttgccctcggggagcttcttggctca
gttcctcctgggctctgtgctggtgctctg