**EMMA ID:** *EM:16732*
**Gene:** *Tg(Cnp-tdTomato)*
**Common name:** *Tg(Cnp-tdTomato*)AJhi/leg*
**Allele:** Cnp-tdTomato* / Cnp-tdTomato-CAAX

## Allele Information

Transgenic mouse line expressing a membrane-targeted variant of the red fluorescent protein tdTomato in myelinating glial cells (oligodendrocytes, Schwann cells) driven by the mouse Cnp-promoter.

For membrane association a CAAX-membrane targeting sequence coding the human H-Ras prenylation signal was fused to the 3'-end of the tdTomato open reading frame.

## Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short ran PCR using primers on wild type allele and a mutant allele-specific short range PCR. T 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 also amplify the endogenous wild type sequence, which will appear as a larger band 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 | JH865 (wt) | JH866 (wt) | 721 (Slc1a3 as internal control) |
| Mutant | JH863 (mut) | JH864 (mut) | 548 |

**Primer sequences**

| Primer Name | Sequence 5' --> 3' |
|---|---|
| JH863 (mut) | GAG CAG TAT GAA CGG AGC GA |
| JH864 (mut) | GGA TAC AGC CGT TCA GTG CT |
| JH865 (wt) | GAG GCA CTT GGC TAG GCT CTG AGG A |
| JH866 (wt) | GAG GAG ATC CTG ACC GAT CAG TTG G |

**PCR setup (AllTaq)**

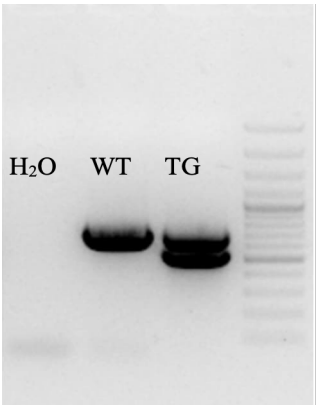
| Component | Volume (µl) 1x | Final conc. |
|---|---|---|
| DNA (~ 50-100 ng) | 2 | |
| Q-Solution (5x) | 2,5 | 0,5 |
| PCR-Buffer (5x) | 5 | 1 |
| DNTP mix (10 mM) | 0,5 | 0,2 |
| $MgCl_2$ (25 mM) | 1,5 | 1,5 |
| Primer 1 (10 pmol/µl) | 1 | 0,4 |
| Primer 2 (10 pmol/µl) | 1 | 0,4 |
| Primer 3 (10 pmol/µl) | 1 | 0,4 |
| Primer 4 (10 pmol/µl) | 1 | 0,4 |
| Taq Polymerase (5 U/µl) | 0,5 | 2,5 U/rxn |
| $H_2O$* | 9 | |
| Final volume | 25 | |

**Amplification conditions**

| PCR Settings | Temperature (°C) | Time | # of cyles |
|---|---|---|---|
| 1 Denaturation (Melting) | 95°C | 5 min | 1 |
| 2 Amplification (Melting, Annealing, Polym.) | 94°C<br>60°C<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 aneal at 55°C.These PCR conditions have been optimized for our methods and preparation

**Gel Image**




The CNPMTO primers (JH863, JH864) amplify a 548 bp sized product within the tdTomato open reading frame. Therefore, in CNPMTO transgenic animals the band at 548 bp will be present, while in wildtype animals this band is lacking The PCR for Slc1a3 is used as internal control. The corresponding band at 721 bp should be present in all samples (except for negative controls without template DNA) to indicate DNA integrity and successful PCR reaction

Separated by gel electrophoresis on a 2% agarose gel.