

**EMMA ID:** 02232

**Gene:** *Col4a1*

**Common name:** *Col4a1*<sup>ENU911</sup>, *ENU911*

**Allele:** *Col4a1*<sup>ENU911</sup>

## Allele Information

The *Col4a1*<sup>ENU911</sup> mutation is a point mutation (Nucleotide cG2866T; Codon GGC --> GTC; Amino acid Gly912Val), referring to table 3 from the following paper.

Genetics. 2007 Feb; 175(2): 725–736. doi: 10.1534/genetics.106.064733 PMID: PMC1800636

**Type IV Procollagen Missense Mutations Associated With Defects of the Eye, Vascular Stability, the Brain, Kidney Function and Embryonic or Postnatal Viability in the Mouse, *Mus musculus*: An Extension of the *Col4a1* Allelic Series and the Identification of the First Two *Col4a2* Mutant Alleles**

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<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1800636/#>

## Genotyping Information

Genotyping was done by end-point PCR. The PCR-products were analysed by Hi-Res Melting with a Lightscanner (Idaho Technology Inc.). The meltingpoint of the products is at around 88°C.

### PCR primer pairs and expected size bands

| Assay    | Forward Primer | Reverse Primer | Expected Size Band (bp) | Codon |
|----------|----------------|----------------|-------------------------|-------|
| Wildtype | Col4a1_F       | Col4a1_R       | 135                     | GGC   |
| Mutant   | Col4a1_F       | Col4a1_R       | 135                     | GTC   |

### Primer sequences

| Primer Name | Sequence 5' --> 3' |
|-------------|--------------------|
| Col4a1_F    | CACAGCTGGGTCTGGAT  |
| Col4a1_R    | ATGCCAGGAAGCCCAAC  |

### PCR setup (Phire Hot Start II DNA polymerase)

| Component               | Volume (µl) 1x |  |
|-------------------------|----------------|--|
| DNA (~ 50 ng)           | 1µl            | (DNA was dried before the PCR was performed) |
| PCR-Buffer (5 fach)     | 2              |  |
| DNTP mix (10 mM)        | 0,4            |  |
| LC green                | 1              |  |
| Primer1                 | 0,5            |  |
| Primer2                 | 0,5            |  |
| 100% DMSO               | 0,5            |  |
| Taq Polymerase (1 U/µl) | 0,2            |  |
| H <sub>2</sub> O*       | 4,9            |  |
| Final volume            | 10             |  |

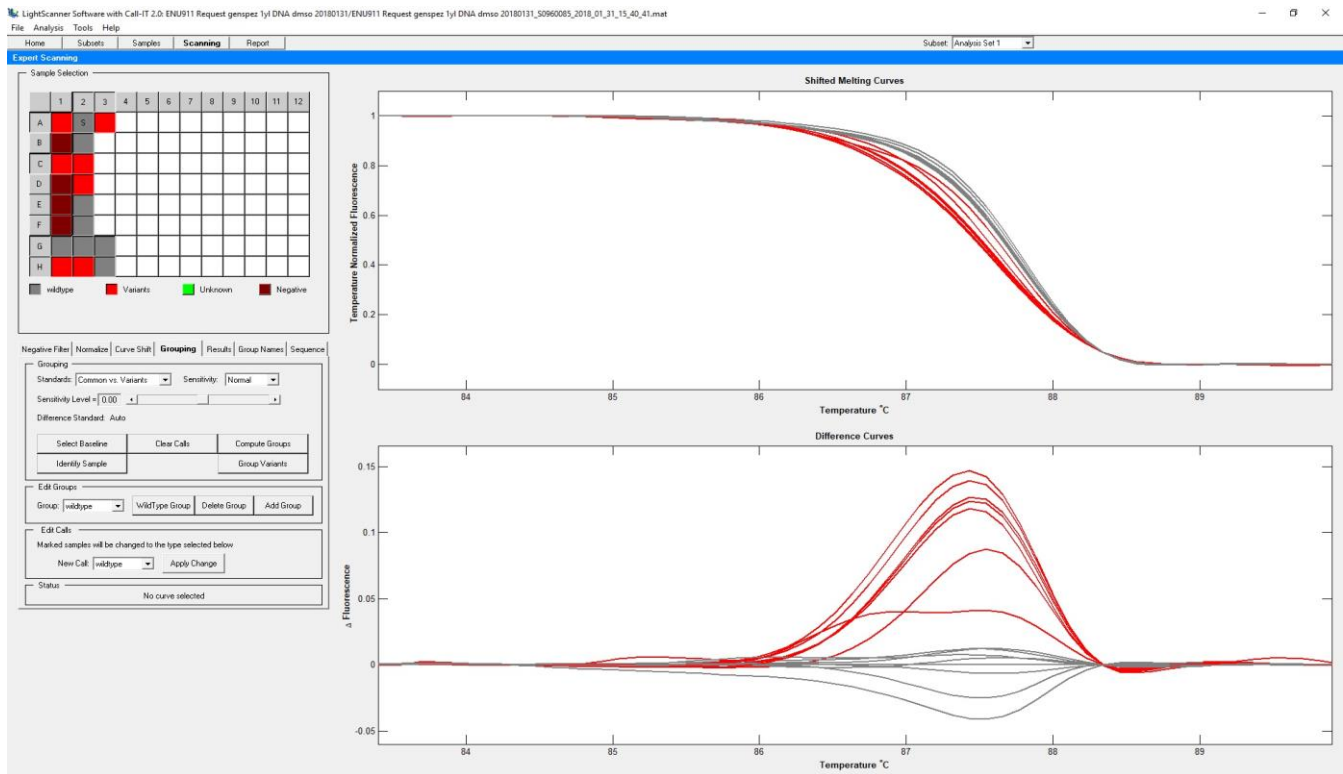
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### Amplification conditions

| PCR Settings                                       | Temperature (°C) | Time   | # of cycles |
|--|------------------|--------|-------------|
| 1 Denaturation<br>(Melting)                        | 98°C             | 30 sec | 1           |
| 2 Amplification<br>(Melting, Annealing,<br>Polym.) | 98°C             | 5 sec  | 35          |
|  | 64°C             | 5 sec  |             |
|  | 72°C             | 5 sec  |             |
| 3 Polymerisation                                   | 72°C             | 1 min  | 1           |
| 4 Hybridisation                                    | 98°C             | 30 sec | 1           |
|  | 20°C             | 30 sec | 1           |
| 5 Cooling  | 12°C             | hold   | 1           |

These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

## Lightscanner Image



- > red sample group are heterozygous mice, melting before wildtype
- > grey sample group are wildtype mice, sample G3/H3 are wildtype controls