**EMMA ID:** *EM:16998*
**Gene:** *C2cd4d*
**Common name:** *C2cd4d-em1_3*
**Allele:** *C2cd4d* $^{em1(IMPC)Hmgu}$

## 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.

**PCR primer pairs and expected size bands**

| Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|---|---|---|---|
| Wild type | C2cd4d_F2 | C2cd4d_wtF | 400 |
| Mutant | C2cd4d_F2 | C2cd4d_R3 | 516 |

**Primer sequences**

| Primer Name | Sequence 5' --> 3' |
|---|---|
| C2cd4d_F2 | CAGCAGCATCGATTCGCATC |
| C2cd4d_R3 | TCGCTTGGGGATGGACTCTA |
| C2cd4d_wtF | CAAAGTGCTGGACAGGGGC |

**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 |
| Taq Polymerase (5 U/µl) | 0,5 | 2,5 U/rxn |
| $H_2O$* | 10 | |
| Final volume | 25 | |

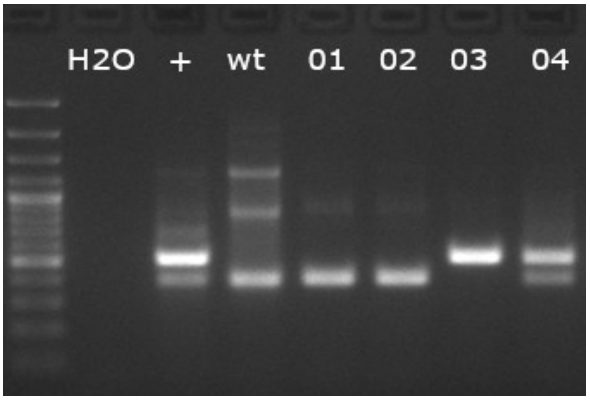
*** The amount of $H_2O$ is adjusted with the number of primer.**

**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>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 aneal at 55°C.These PCR conditions have been optimized for our methods and preparation

**Gel Image**




| | |
|---|---|
| + | **het control** |
| **1,2** | **wt** |
| **3** | **hom** |
| **4** | **het** |

Separated by gel electrophoresis on a 2% agarose gel.