

EMMA ID: 13579
Gene: *Echs1*
Common name: *Echs1-em1_3*
Allele: *Echs1*^{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 | Echs1_F | Echs1_wtR | 510/1722 |
| Mutant | Echs1_F | Echs1_R | 602 |

Primer sequences

| Primer Name | Sequence 5' --> 3' |
|-------------|-----------------------|
| Echs1_F | gaatttgagtggctgaccaac |
| Echs1_R | aggtgaacttgccatcaagag |
| Echs1_wtR | gccttgaggactgagaagttc |

PCR setup (Qiagen, Hot Start Plus)

| Component | Volume (µl) 1x | Final conc. |
|---------------------------|----------------|-------------|
| DNA (~ 50-100 ng) | 2 | |
| Q-Solution (5x) | 2,5 | 0,5 |
| PCR-Buffer (10x) | 2,5 | 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 |
| Taq Polymerase (5 U/µl) | 0,3 | 0,06 |
| H ₂ O* | 13,7 | |
| Final volume | 25 | |

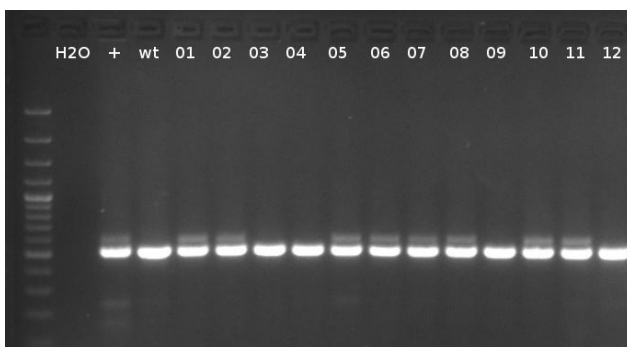
* The amount of H₂O is adjusted with the number of primer.

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 |
| | 65-55 (↓1°C/Cycle) | 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 kits. Adaptions may be required.

Gel Image



Separated by gel electrophoresis on a 2% agarose gel.