

EMMA ID: 09207

Gene: *Cenph*

Common name: *HEPD0530_5_A02*

Allele: *Cenph*^{tm1b(EUCOMM)Hmgu}

Allele Information

Further information about the allele can be found on IMPC website at (copy the link to web browser)

[http://www.mousephenotype.org/data/alleles/MGI:1349448/tm1b\(EUCOMM\)Hmgu](http://www.mousephenotype.org/data/alleles/MGI:1349448/tm1b(EUCOMM)Hmgu)

Links to the general information

About IKMC resource

<https://www.infrafrontier.eu/knowledgebase/protocols/ikmc-products>

IKMC allele types

<http://www.i-dcc.org/kb/entry/89/>

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice (assays infos available when required)

<http://www.mousephenotype.org/about-ikmc/targeting-strategies>

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.

PCR primer pairs and expected size bands

| Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|----------|----------------|----------------|-------------------------|
| Mutant | Cenph_Ef | KR 3277 | 346 |
| Wildtype | Cenph_Ef | Cenph_Er | 315 |

Primer sequences

| Primer Name | Sequence 5' --> 3' |
|-------------|-----------------------------|
| Cenph_Ef | ATTCCAGGCAGCTAGGATGTGGTTC |
| Cenph_Er | GAAGGGGTCTCACTGTGTAGTTCCTGA |
| KR 3277 | CTCCTACATAGTTGGCAGTGTTTGGG |

PCR setup (Qiagen, Hot Start Plus)

| Component | Volume (μ l) 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/ μ l) | 1 | 0,4 |
| Primer 2 (10 pmol/ μ l) | 1 | 0,4 |
| Taq Polymerase (5 U/ μ l) | 0,3 | 0,06 |
| H ₂ O* | 13,7 | |
| Final volume | 25 | |

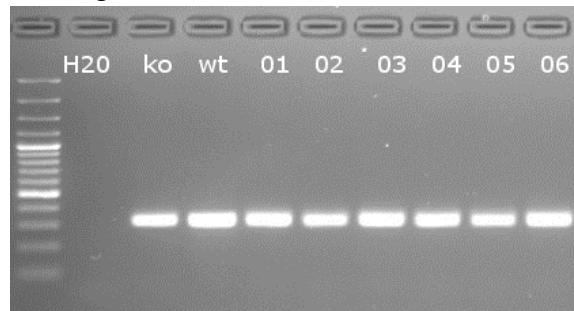
* The amount of H₂O is adjusted with the number of primer.

Amplification conditions

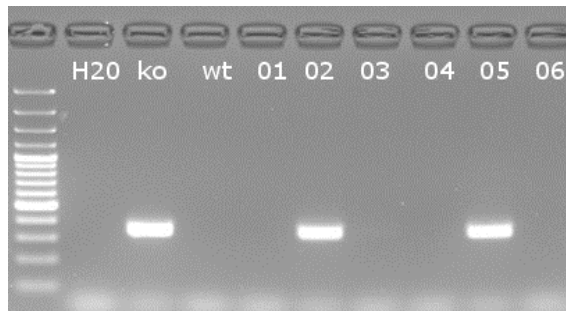
| PCR Settings | Temperature ($^{\circ}$ C) | Time | # of cycles |
|--|-----------------------------|--------|-------------|
| 1 Denaturation (Melting) | 95 $^{\circ}$ C | 5 min | 1 |
| 2 Amplification (Melting, Annealing, Polym.) | 94 $^{\circ}$ C | 30 sec | 39 |
| | 62 $^{\circ}$ C | 45 sec | |
| | 72 $^{\circ}$ C | 45 sec | |
| 3 Polymerisation | 72 $^{\circ}$ C | 10 min | 1 |
| 4 Cooling | 12 $^{\circ}$ C | hold | 1 |

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

Gel Image



WT-PCR



Mutant-PCR

Separated by gel electrophoresis on a 2% agarose gel.

Genotyping using PCR-assays for cassette detection

LacZ reporter, Neo selection cassettes are inserted into the Knockout-first mutant allele. Cassette changes by allele conversion can be found on: <http://www.mousephenotype.org/about-ikmc/targeting-strategies>. For example, tm1b allele contains still lacZ reporter cassette, Neo selection cassette is deleted (promotor-driven only).

Please note that these assays are with universal cassette primers other than gene-specific. The confirmation on gene identity performed by e.g. sr genespecific PCR as provided is suggested .

PCR primer pairs and expected size bands

| Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|-------|--------------------|--------------------|----------------------------|
| lacZ | LacZ_multi_Deen_2F | LacZ_multi_Deen_2R | mut 81 bp,wt without band |
| Neo | Neo_long_Deen_F1 | Neo_long_Deen_R1 | mut 186 bp,wt without band |

Primer sequences

| Primer Name | Sequence 5' --> 3' |
|--------------------|------------------------|
| LacZ_multi_Deen_2F | TACTGGAGGCTGAAGTTCAGAT |
| LacZ_multi_Deen_2R | GCGTTTCACCCTGCCATAA |
| Neo_long_Deen_F1 | TTGAACAAGATGGATTGCACGC |
| Neo_long_Deen_R1 | CCTCGTCTGCAGTTCATT |

PCR setup (Qiagen, Hot Start Plus)

| Component | Volume (µl) | 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 (25mM) | 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 | |

Amplification conditions

| PCR Settings | Temperature (°C) | Time | # of cycles |
|--|------------------|--------|-------------|
| Denaturation (Melting) | 95°C | 5 min | 1 |
| Amplification (Melting, Annealing, Polym.) | 94°C | 30 sec | 39 |
| | 58°C | 45 sec | |
| | 72°C | 45 sec | |
| Polymerisation | 72°C | 10 min | 1 |
| Cooling | 12°C | hold | 1 |

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

Tm1b Allele Conversion PCR-assays

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice

<http://www.mousephenotype.org/about-ikmc/targeting-strategies>

Tm1b allele is reporter-tagged deletion allele (post-Cre). Critical exon is deleted by creating a frame-shift using Cre method. Neo selection cassette is removed together in promoter-driven strains only. LacZ reporter cassette is kept for visualising gene expression.

| Assay | Forward Primer | Reverse Primer | Size Band (bp) | Allele |
|-------------------------|----------------|----------------|----------------|------------------------------|
| Tm1b Promotor-driven | tm1b_forw | Floxed LR | 380 bp | tm1b, Promotor-driven |
| | | | others | tm1a or partially conversion |
| Flox Promotorless | Floxed PNF | Floxed LR | 128 bp | tm1b, Promotorless |
| | | | ~ 1 kb | tm1a |

Primer sequences

| Primer Name | Sequence 5' --> 3' |
|-------------|-----------------------|
| tm1b_forw | CGGTCGCTACCATTACCAGT |
| Floxed LR | ACTGATGGCGAGCTCAGACC |
| Floxed PNF | ATCCGGGGGTACCGCGTCGAG |

PCR setup (Phire Hot Start II)

| Component | Volume (µl) 1x |
|----------------------|----------------|
| DNA (~ 50-100 ng) | 2,0 |
| H ₂ O | 12,7 |
| PCR-Buffer (5x) | 4,0 |
| DNTP mix (10 mM) | 0,4 |
| Primer mixed (10 µM) | 0,5 |
| Phire Tag (1 U/µl) | 0,4 |
| Final volume | 20 |

Amplification conditions

| PCR Settings | Temperature (°C) | Time |
|--------------------|------------------|--------|
| 1 | 98°C | 30 sec |
| 2 | 98°C | 5 sec |
| 3 | 58°C | 10 sec |
| 4 | 72°C | 10 sec |
| 5 to 2 + 34 cycles | | |
| 6 | 72°C | 1 min |
| 7 | 12°C | hold |

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