**EMMA ID:** *04052*
**Gene:** *Setdb1*
**Common name:** *EUC BL6-FP00042B07 / EPD0028_1_B07*
**Allele:** *Setdb1* $^{tm1a(EUCOMM)Wtsi}$

## Allele Information

Further information about the allele can be found on IMPC website at (copy the link to web browser)
https://www.mousephenotype.org/data/alleles/MGI:1934229/tm1a%2528EUCOMM%2529Wtsi?

## 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 gene-specific 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 | FP42B07-F2 | LAR3 | 291 |
| Wildtype | FP42B07-F2 | FP42B07-R | 532 |

**Primer sequences**

| Primer Name | Sequence 5' --> 3' |
|---|---|
| FP42B07-F2 | CTGGTGAGATGGCTTAGTGAGTAA |
| FP42B07-R | TGTATCTAGAGAACAAGAGCTAGG |
| LAR3 | CAACGGGTTCTTCTGTTAGTCC |

**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 |
| $H_2O$* | 13,7 | |
| Final volume | 25 | |

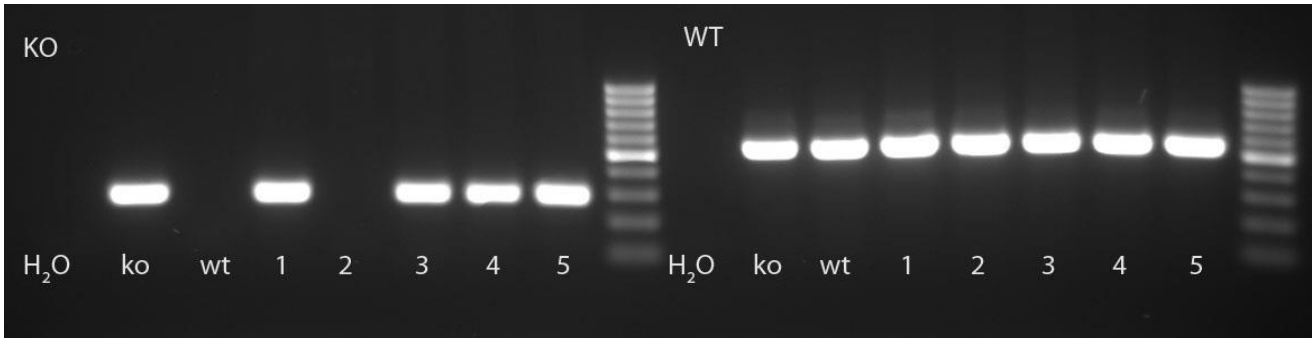
*** The amount of $H_2O$ is adjusted with the number of primer.**

**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°C<br>72°C | 30 sec<br>45 sec<br>45 sec | 39 |
| 3 Polymerisation | 72°C | 10 min | 1 |
| 4 Cooling | 12°C | hold | 1 |

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

**Gel Image**




**Mutant-PCR** **WT-PCR**

Separated by gel electrophoresis on a 2% agarose gel.
ko/wt separate

## 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 | CCTCGTCCTGCAGTTCATT |

**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 |
| $H_2O$ | 13,7 | |
| Final volume | 25 | |

**Amplification conditions**

| PCR Settings | Temperature (°C) | Time | # of cyles |
|---|---|---|---|
| Denaturation (Melting) | 95°C | 5 min | 1 |
| Amplification (Melting, Annealing, Polym.) | 94°C<br>58°C<br>72°C | 30 sec<br>45 sec<br>45 sec | 39 |
| 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.**